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McDERMOTT, WILL & EMERY

March 17, 2003

VIA HAND DELIVERY

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

Re: In the Matter of Notice of Hearing: Proposal to Withdraw Approval of New Animal Drug Application for Enrofloxacin for Poultry ("Enrofloxacin Hearing)
FDA Docket: 00N-1571

Dear Sir/Madam:

Enclosed for filing please find an original and one copy of Respondent Bayer Corporation's and Participant Animal Health Institute's Joint Proposed Findings of Fact.

Please call with any questions.

Sincerely,

Robert B. Nicholas

Enclosure

cc: Kent D. McClure
Nadine Steinberg

00N-1571

FF2

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

1227 '03 MAR 17 P4:42

In the Matter of:

Enrofloxacin for Poultry:
Withdrawal of Approval of
New Animal Drug Application
NADA 140-828

FDA DOCKET: 00N-1571

Date: March 17, 2003

RESPONDENT BAYER CORPORATION'S AND PARTICIPANT ANIMAL HEALTH INSTITUTE'S JOINT PROPOSED FINDINGS OF FACT

Pursuant to the April 10, 2002 Order and Schedule of Due Dates in this proceeding, Respondent Bayer Corporation and Participant Animal Health Institute hereby jointly submit the following findings of fact established by the evidence. The support for each fact is provided by a citation to Written Direct Testimony and/or to documents in the evidentiary record.

1. *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, rodents, cattle, sheep, pigs, companion animals, wild birds and poultry. [Newell (B-1908) P.3 L.14-17; B-205 P.2; G-253 P.1; G-385 P.1-3; Nachamkin (G-1470) P.3 L.28-29; Patterson (B-1910) P.4 L.1-4; Feldman (B-1902) P.15 L.5-10]

2. *Campylobacter* are very fragile organisms which can normally only reproduce in the intestinal tract of a host animal. [G-457 P.3; Newell (B-1908) P.22 L.4-6]

3. *Campylobacter* require a reduced oxygen environment to grow. [Meng (G-1466) P.2 L.10, P.2, L.40-43]

4. *Campylobacters* are susceptible to stresses such as heat and atmospheric oxygen. [Newell (B-1908) P.22 L.5-6; G-457 P.3; B-205 P.1]

5. The source of *Campylobacter* infection in broiler flocks is unclear. The source is thought to be predominantly horizontal, primarily from environments contaminated

with feces from domestic and wild animals and birds. [Newell (B-1908) P.17 L.7-9]

6. A NCCLS approved method for animal-origin *Campylobacter* susceptibility testing was not available until May 2002 when NCCLS published M31-A2, "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals." [Joint Stipulation 29]

7. The "agar dilution" method is the only NCCLS-approved method for testing susceptibility in *Campylobacter* isolates. [Joint Stipulation 30]

8. Most evidence, serotyping and case-control studies suggest that the primary source of introduction of *Campylobacter* into the poultry flock is the external environment. As *Campylobacters* are ubiquitous this hypothesis is intuitive. [Tompkin (A-204) P.45 L.2-4]

9. All the current evidence from epidemiological studies in Europe indicates that it is most likely that *Campylobacters* enter the broiler house from the external environment. [Newell (B-1908) P.8 L.17-18; B-1336]

10. The majority of flocks are infected by strains external to the poultry house environment. [Newell (B-1908) P.9 L.4-5]

11. Most broiler houses are closed but tend to be located in rural settings within environments occupied by wild life including rabbits, rodents and wild birds. Most farms have a rodent control policy but the effectiveness of this can vary. In houses open to the environment, wild birds, especially passiformes and columiformes, and rodents may readily enter the broiler houses scavenging for feed. Even with closed houses, maintained under good order, rodents and even wild birds, can readily overcome barriers and enter the house coming into contact with the poultry. [Newell (B-1908) P.9 L.9-15]

12. Fecal contamination by rodents, wild birds and pets of the internal broiler house environment could result in flock colonization with *Campylobacter*. [Newell (B-1908) P.9 L.22-23]

13. Farm-workers play an interesting role in the epidemiology of flock colonization. Case-control studies have demonstrated farm staff as a risk factor and external contamination of a flock by catchers has been demonstrated. [Tompkin (A-204) P.45 L.13-15]

14. Fecal waste from rodents, wild birds and pets may contaminate the external environment of the poultry house and be transported into the poultry house via farm workers boots and clothing. [Newell (B-1908) P.9 L.23 – P.10 L.2]

15. Few *Campylobacters* from environmental sources have been investigated but fluoroquinolone-resistant organisms have been recovered from wild birds including sparrows. [Patterson (B-1910) P.10 L.11-13; Newell (B-1908) P.17 L.10-11]

16. Chickens, including those in the United States, are easily colonized by *Campylobacter*, predominately *C. jejuni*, which is a commensal organism in chickens. [Jacobs-Reitsma (G-1459) P.7 L.27-28; Tauxe (G-1475) P.10 L.26-30; White (G-1484) P.2 L.42-45]

17. Colonization with *Campylobacter* normally occurs in chickens after two weeks of age. [Jacobs-Reitsma (G-1459) P.7 L.28]

18. Turkeys, including those in the United States, are naturally colonized by *Campylobacter*, which is a commensal organism in turkeys. [Newell (B-1908) P.4 L.1-12, P.10 L.13-14]

19. Evidence shows that turkeys are preferentially colonized by *Campylobacter coli* compared to *Campylobacter jejuni* . [Gonder (A-201) P.12 L.17-23; G-727; Newell (B-1908) P.4 L.7-8]

20. Studies suggest that *Campylobacter* colonization in broilers and turkeys may have significant host specific differences. [Newell (B-1908) P.4 L.11-12]

21. *Campylobacter* is not considered an essential component of gut flora in poultry. [Newell (B-1908) P.5 L.8-9]

22. Poultry may acquire *Campylobacter* horizontally from many possible sources including; feed, water, husbandry staff, as well as via an environment contaminated by wild life and domestic animals. [Newell (B-1908) P.7 L.1-3; Robach (B-1911) P.6 (water); B-1532 (water); B-591 (water)]

23. Poultry may acquire *Campylobacter* from flies and other insects, as insects can act as carriers. [G-1612 P.6; G-1719 P.3; G-572]

24. The prevalence of flock infection varies from 10% to over 90% (Newell & Wagenaar, 2000), but because of differences in sampling and culture methodology accurate comparison of such surveys is difficult. [Newell (B-1908) P.3 L.19-21]

25. Prevalence of flock colonization with *Campylobacter* is seasonal and the seasonal peaks can vary between countries. [Newell (B-1908) P.3 L.22-23]

26. *Campylobacter* is rarely isolated from intact eggs, and epidemiological evidence indicates that vertical transmission occurs rarely in chickens, if at all. [Newell (B-1908) P.6 L.12-19]

27. *Campylobacters* can be isolated from many species of wild animals including, field mice, foxes, rabbits, badgers, and wild birds including passiformes and

columiformes. [Newell (B-1908) P.9 L.18-29; Jacobs-Reitsma (G-1459) P.3 L.21-23; B-263 (wild birds)]

28. *Campylobacter* is found in the environment, including in water and at beaches. [Jacobs-Reitsma (G-1459) P.3 L.21-23; Patterson (B-1910) P.4 L.4-6; G-75 (beaches)]

29. *Campylobacter*, including fluoroquinolone-resistant *Campylobacter* are frequently isolated in surface and ground waters, including drinking water supplies. [Patterson (B-1910) P.4 L.9-10]

30. Studies suggest there is little or no carry-over or persistence of *Campylobacter* from one flock to the subsequent flock, and that the majority of flocks are infected by strains from external sources. [Newell (B-1908) P.8 L.17 – P.9 L.8; Tompkin (A-204) P.44 L.20-22]

31. There are no reports of *Campylobacters* being isolated from fresh bedding or feed. This is not surprising as these organisms are very susceptible to dessication. [Newell (B-1908) P.7 L.4-6 citing B-673, (Luechtefeld *et al.*, 1981) (Doyle & Roman, 1982)]

32. There is little or no persistence of *Campylobacter* strains within the poultry house and that the majority of flocks are infected by strains from external sources. [Newell (B-1908) P.8 L.15-16]

33. The 1994 Joint Advisory Committee meeting was held to discuss the use of fluoroquinolones in food animal medicine. The Joint Advisory Committee discussed the relative benefits and risks to animals and humans of the use of fluoroquinolones in food animals in light of possible concerns about microbial resistance to fluoroquinolones. [Joint Stipulation 35]

34. Fluoroquinolone resistance develops in *Campylobacter* as a spontaneous genetic mutation within a *Campylobacter* population and is not as a result of exposure to fluoroquinolones. Fluoroquinolone exposure then can select for resistant *Campylobacter*. [Joint Stipulation 1]

35. All of the quinolones physically interact with DNA gyrase, an enzyme essential for bacterial replication, and prevent it from functioning normally. [Barrett (G-1453) P.2 L.7-9]

36. 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. [Barrett (G-1453) P.2 L.9-11 Walker (G-1482) P.8 L.5-13]

37. 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. [Barrett (G-1453) P.2 L.12-14]

38. It is not necessary that bacteria be exposed to nalidixic acid to become resistant to nalidixic acid. Bacteria with mutations in the DNA gyrase gene can be selected for by the use of fluoroquinolones as well as nalidixic acid. [Barrett (G-1453) P.2 L.14-16]

39. Some strains of quinolone-resistant bacteria do not have altered DNA gyrase genes, but are intrinsically resistant to nalidixic acid or other quinolones because the drugs are unable to enter the cell, or because the cell is able to pump them out before they can inhibit cell replication. These strains are typically less resistant than bacteria that have acquired mutations in the DNA gyrase gene, but still have a selective advantage in an environment where quinolones are present. [Barrett (G-1453) P.2 L.18-25]

40. Resistant *Campylobacter* can be present in poultry or on chicken products as a consequence of factors other than the treatment of domestic flocks. [Newell (B-1908) P.15 L.12-13]

41. Treatment is not the only source of fluoroquinolone-resistant *Campylobacters* in poultry. Gaunt and Piddock, in 1993/4, before enrofloxacin was licensed for use in the UK, undertook a small survey of retail domestic and foreign produced poultry products. Ciprofloxacin-resistant *Campylobacters* were found in one of 64 UK-produced chickens. This indicates that resistant *Campylobacter* can be acquired by broiler flocks, other than by treatment. [Newell (B-1908) P.16 L.24 – P.17 L.6 citing B-609 and Gaunt and Piddock (1996)]

42. Fluoroquinolone use in chickens and turkeys is not the only cause of the development of fluoroquinolone-resistant *Campylobacter* species in chickens and turkeys. [CVM Response to Bayer's Interrogatory 4]

43. Fluoroquinolone-resistant *Campylobacter* (*C. jejuni* and *C. coli*) existed in chickens and turkeys in the United States prior to 1995. [CVM Response to Bayer's Interrogatory 81]

44. The horizontal transfer of genes conferring fluoroquinolone resistance in *Campylobacter* has not been demonstrated. [Joint Stipulation 40]

45. 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 (G-1478) P.2 L.29-32]

46. 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 (G-1453) P.2 L.16-18]

47. Fluoroquinolone use in chickens and turkeys can act as a selection pressure for fluoroquinolone-resistant bacteria in the chicken and turkey digestive tract. [Joint Stipulation 7]

48. The use of enrofloxacin in chickens and turkeys can exert a selection pressure that can lead to fluoroquinolone resistance. [Joint Stipulation 45]

49. A large variety of drug residues are found in raw sewage, in sewage treatment plant effluents and in their associated receiving waters. [Patterson (B-1910) P.12 L.20-21]

50. Antibiotic residues such as fluoroquinolones or tetracycline in sewage treatment plants may select for resistance in bacterial strains entering or residing within the sewage treatment plants. [Patterson (B-1910) P.12 L.22 – P.13 L.1]

51. Exposure to the array of drug residues present in sewage treatment plants may select for resistant strains at the expense of more susceptible organisms. [Patterson (B-1910) P.13 L.2-3, citing to B-1807]

52. Because sewage treatment plants discharge into waters used for recreation and drinking water sources, they likely constitute a major source of resistant bacteria, including fluoroquinolone-resistant *Campylobacter*, to human populations, both in the United States and abroad. [Patterson (B-1910) P.13 L.12-14; Burkhart (B-1900) P.4, L.4-9]

53. Poultry production (including farm runoff) or processing facilities cannot be a dominant source of fluoroquinolone-resistant *Campylobacter* into sewage treatment plants, given the widespread geographical occurrence of antibiotic-resistant pathogens influent to (and effluent from) major municipal sewage treatment plants and that the vast majority of major municipal sewage treatment plants are outside of the geographically localized poultry raising and processing regions within the U.S. [Patterson (B-1910) P.13 L.15-19]

54. 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 (B-1908) P.13 L.13-16]

55. Although isolation percentages of *Campylobacter* within positive-tested poultry flocks are generally close to 100%, not all broiler flocks become colonized during the production cycle. [Jacobs-Reitsma (G-1459) P.4 L.34-36]

56. Long-term *Campylobacter* negative farms, as well as long-term-positive farms are relatively rare. [Jacobs-Reitsma (G-1459) P.4 L.35-37]

57. The percentage of broiler flocks that are colonized with *Campylobacter* varies by country and by season. [Jacobs-Reitsma (G-1459) P.4 L.38-39]

58. Differences in *Campylobacter* flock prevalence may also reflect differences in culture methods and sample quality. [Jacobs-Reitsma (G-1459) P.4 L.41-43]

59. *Campylobacter* flock colonization data is not easily compared between countries. [Jacobs-Reitsma (G-1459) P.4 L.43-45]

60. The prevalence of susceptible *Campylobacter* far exceeds that of fluoroquinolone-resistant *Campylobacter* in the poultry population. (Prucha (A-203) P.14 L.3-4)

61. In 1994, Jacobs-Reitsma conducted an experimental lab study on the use of Baytril in chickens. Chickens treated with either 15 ppm and 50 ppm enrofloxacin were all cross-resistant to nalidixic acid, flumequine, and enrofloxacin as determined using a disc diffusion method. [Jacobs-Reitsma (G-1459) P.7 L.7-24; G-315]

62. In 2002, based on pooled samples of 5 individual birds, McDermott conducted an experimental lab study on the use of fluoroquinolones in chickens. Birds were treated with sarafloxacin at 40 ppm for 5 days. Within 24 hours of treatment, 100% of *C. jejuni* isolates were resistant to ciprofloxacin. However, three weeks after ending treatment, 72% of the isolates tested still displayed high-level ciprofloxacin MICs (32 mg/l or higher) while 28% were again susceptible isolates (cipro MICs of 0.125 mg/l). Hence, there exists a limited persistence of fluoroquinolone resistance after the discontinuation of the fluoroquinolone. [B-868]

63. McDermott acknowledges in his 2002 article that the results of his study were essentially the same as those found by Jacobs-Reitsma in 1994. [McDermott (G-1465) P.4 L.11-12; B-868]

64. Although Jacobs-Reitsma's method did not quantify the magnitude of change in resistance in the study and the McDermott study was able to do so, the results of the McDermott study are the same as the results of the Jacobs-Reitma study: in both experiments fluoroquinolone treatment did not eliminate *Campylobacter* from the intestinal tract of chickens, but rather, rapidly selected for fluoroquinolone-resistant isolates. [McDermott (G-1465) P.4 L.15-23; B-868]

65. In 2001, Luo conducted an experimental lab study on the use of fluoroquinolones in chickens. Birds were treated with enrofloxacin at 25 ppm and 40 ppm for 5 days. Within three days after treatment, 100% of *C. jejuni* isolates were resistant to fluoroquinolones. However, at 8 days after treatment, only 50% of the population treated at 25 ppm were fluoroquinolone-resistant, and only 33% of the population treated at 25 ppm were fluoroquinolone-resistant after 12 and 15 days after treatment. [A-190]

66. Although use of fluoroquinolones will select for fluoroquinolone-resistant *Campylobacter*, current evidence most relevant to actual usage conditions (i.e. 25 ppm), demonstrates that fluoroquinolone-resistant *Campylobacter* do not persist and that fluoroquinolone-susceptible *Campylobacter* recolonize the boiler gut, particularly at the 25 ppm dose. [B-868; A-190]

67. In 1992, Jacobs-Reitsma studied the susceptibility of 116 strains of *Campylobacter* and found 13% of the *Campylobacter* isolates from non-treated laying hens from The Netherlands showed complete cross-resistance to the quinolones tested. [Jacobs-Reitsma (G-1459) P.6 L.26-40; B-36]

68. The presence of fluoroquinolone-resistant *Campylobacter* in untreated flocks demonstrates that there are potential selective pressures in poultry other than enrofloxacin usage. [B-36 P.2-3; G-62 1-2; Hanninen (G-1458) P.4, ¶ 3; Jacobs-Reitsma (G-1459) P.6 L.36-37; Newell (B-1908) P.17 L.1-6]

69. In the United Kingdom, Piddock (1995) investigated strains from 64 retail chicken carcasses prior to the licensing of enrofloxacin in 1993/4 and found 2.7% resistance. [Newell (B-1908) P.14 L.15-17]

70. Chlorine and organic acids may exert selective pressures for gyr-A mutations in enteric bacteria. [Silley (B-1913); Attachment 1 P.53 ¶ 1 & P.52 ¶ 3; B-983]

71. Baytril 3.23% Concentrate Antimicrobial Solution (hereinafter "Baytril") is not used to treat *Campylobacter* in poultry, Baytril is used only to treat *E. coli* infections and fowl cholera, both life threatening diseases. [Glisson (B-1903) at P.5 L.21 – P.6 L.1; Smith (B-1914) P.18 L.8-9]

72. In 2001, there were 8.6 billion broilers (chickens) raised for slaughter in the United States. [Joint Stipulation 43]

73. In 2000, there were 270 million turkeys raised for slaughter in the United States. [Joint Stipulation 44]

74. Baytril is used sparingly in poultry, in broilers it is used in approximately 1-2% of the total yearly flock production population. [Gonder (A-201) P.20 L.9; A-192]

75. In the United States, enrofloxacin is approved for use only by prescription and only under veterinary supervision. [Joint Stipulation 15]

76. In the United States, enrofloxacin is approved for therapeutic use only and is not approved for growth promotion. [Joint Stipulation 16]

77. In the United States, extra-label use of enrofloxacin is prohibited by law for food producing animals. [Joint Stipulations 17 & 46]

78. Baytril is administered via drinking water and FDA acknowledges water medication as a safe and effective means to administer therapeutic animal drugs. [Joint Stipulation 18]

79. The vast majority of broilers in the United States who are treated with enrofloxacin are treated at a dose of 25 ppm for three days. [Hofacre (A-202) P.20 L.22 – P.21 L.1, P.23 L.7-11; Glisson (B-1903) P.5 L.10-12; Smith (B-1914) P.27 L.4-7]

80. Many turkeys in the United States are treated with enrofloxacin at a dose of 25 ppm, although the labeled dosage is 25-50 ppm. [Gonder (A-201) P.27 L.6-9; Wages (B-1917) P.18 L.12]

81. Epidemiology is the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems. [Feldman (B-1902) P.5 L.14-16, citing Gregg, Field Epidemiology, 2nd ed. Oxford Press (2002) B-1902 Attachment 1]

82. Epidemiologists study the frequency and pattern of health events in a population. *Frequency* means not only the number of health events in a population but the relationship of that number to the size of that population, that is, the number of events divided by the study population. The resulting ratio allows epidemiologists to compare disease occurrence between different populations. [Feldman (B-1902) P.5 L.26-31, citing B-1902 Attachment 1 (Gregg 2002)]

83. Epidemiology is premised on the fact that the disease and other health events do not occur randomly in a population--that disease is more likely to occur in some members of the population than others. One important use of epidemiology is to identify the reasons (risk factors) that increase some members' risk of disease above others. [Feldman (B-1902) P.7 L1-5, citing B-1902 Attachment 1 (Gregg 2002)]

84. A number of models of disease causation have been proposed. Among the simplest of these is the epidemiologic triad or triangle, the traditional model for infectious disease. The triad comprises an external *agent*, a susceptible *host*, and an *environment* that brings together the host and the agent. In this model, disease results from the interaction between the agent and the susceptible host in an environment that supports transmission of the agent from a source to that host. [Feldman (B-1902) P.7 L.7-13, citing B-1902 Attachment 1 (Gregg 2002)]

85. "Agent factors" include bacterium, such as *Campylobacter* and "generally the agent must be present for disease to occur, that is, they are necessary but not always sufficient to cause disease. A variety of factors influence whether exposure to an organism will result in disease, including the organism's pathogenicity (ability to cause disease) and dose." [Feldman (B-1902) P.7 L.15-18, citing B-1902 Attachment 1 (Gregg 2002)]

86. "Host factors" are "intrinsic factors that influence an individual's exposure, susceptibility, or response to a causative agent. Age, gender, and behaviors (smoking, drug abuse, lifestyle, sexual practices, contraception, and eating habits) are just some of the many host factors that affect a person's likelihood of exposure. Age, genetic composition, nutritional and immunologic status, anatomic structure, presence of disease or medications, and psychological makeup are some of the host factors that affect a person's susceptibility and response to an agent." [Feldman (B-1902) P.7 L.18-24, citing B-1902 Attachment 1 (Gregg 2002)]

87. "Environmental factors" are "extrinsic factors that affect the agent and host and the opportunity for exposure." Environmental factors generally include " physical factors such as geology and climate; biologic factors such as insect vectors that transmit the agent; and socioeconomic factors such as crowding, sanitation and the availability of health services." [Feldman (B-1902) P.7 L.24-28, citing B-1902 Attachment 1 (Gregg 2002)]

88. An important tenet of epidemiologic analysis is to identify, control for, and correct for confounding variables. "Confounding is the distortion of an exposure-disease association by the effect of some third factor (a 'confounder'). A third factor may be a confounder and distort the exposure-disease association if it is: associated with the outcome independent of the exposure - that is, even in the nonexposed group. (In other words, it must be an independent "risk factor."); or associated with the exposure but not a consequence of it." [Feldman (B-1902) P.8 L.1-7, citing B-1902 Attachment 1 (Gregg 2002)]

89. Confounding variables should be controlled or corrected for to eliminate inappropriate inferences concerning risk factors. [Feldman (B-1902) P.16 L.5-6]

90. In undertaking infectious disease epidemiologic evaluations (such as studying the risk and transmission of campylobacteriosis) it is important to consider the presence of confounders (or other factors that may influence the outcome) and the appropriateness of the study methodology. [Feldman (B-1902) P.8 L.26-29]

91. Host factors such as immunity, age, gender, and behaviors (such as eating undercooked meats) are some of the many host factors that affect a person's likelihood of exposure to *Campylobacter* and which have been demonstrated to effect the incidence of campylobacteriosis in epidemiologic studies. [Feldman (B-1902) P.9 L.4-7]

92. Acquired immunity is a potentially important host factor in *Campylobacter* epidemiologic investigations. [Feldman (B-1902) P.9 L.8-9]

93. Age is not only a host factor but also a potential confounder for enteric disease investigations, because human immune response varies by age. Young children and the elderly will likely have different disease outcomes after exposure to enteric pathogens than healthy adults. Age is a pervasive determinant of disease. Age represents three important determinants of disease risk including: host condition (individuals of different ages often differ in susceptibility or predisposition to disease); intensity of exposure (for example, infants and small children may be at a far greater risk of exposure to organisms spread through fecal-oral than through exposure to undercooked foods, which is of greater importance for older individuals), and passage of time (older individuals have a greater over-all time of exposure and may have developed protective immunity). [Feldman (B-1902) P.9 L.20 – P.21 L.7, citing B-1902 Attachment 1 (Gregg 2002)]

94. Age is an important consideration in *Campylobacter* epidemiology because the incidence of campylobacteriosis varies with age. The age distribution peaks in infancy and in the early twenties. [Feldman (B-1902) P.10 L.8-10]

95. Environmental factors such as "seasonality" and "place" are two of the many environmental factors that affect a person's likelihood of exposure to *Campylobacter* and which have been demonstrated to effect the incidence of campylobacteriosis in epidemiologic studies. [Feldman (B-1902) P.10 L.11-14]

96. Time patterns or "seasonality" is important in infectious disease epidemiologic evaluations. [Feldman (B-1902) P.10 L.15-16]

97. Geographic location is another important variable to examine in epidemiologic investigations. This is particularly important in *Campylobacter* cases since an infection acquired outside the country may be by a strain of bacteria with different virulence from those acquired in the United States. [Feldman (B-1902) P.16 L.7-10]

98. The ecology of *Campylobacter* differs throughout regions of the world. [Nachamkin (G-1470) P.5 L.29-30]

99. Typical epidemiologic studies are either "cohort studies" or "case-control studies." [Feldman (B-1902) P.13 L.2-3, citing B-1902 Attachment 1 (Gregg 2002)]

100. A cohort study is a follow-up study in which enrollment of the study group is based on exposure characteristics or membership in a particular group. The occurrence of disease or outcome is determined and the rate or frequency in the Cohort group is compared among other exposure groups. [Feldman (B-1902) P.13 L.5-8, citing B-1902 Attachment 1 (Gregg 2002)]

101. A case-control study, enrollment of the study group is based on the presence ("case") or absence ("control") of the disease being studied. The frequency of exposures is compared between those with the disease (cases) and those without (controls). (Gregg P.118). [Feldman (B-1902) P.13 L.9-11, citing B-1902 Attachment 1 (Gregg 2002)]

102. Cohort studies are appropriate in a well-defined population (persons on a cruise ship, workers in a plant) but not well suited for nationwide epidemics where the population at risk is not known. The scientifically valid way to analyze a nationwide epidemic is through a case-control study. [Feldman (B-1902) P.13 L.16-19, citing B-1902 Attachment 1 (Gregg 2002)]

103. The definition of controls is vital to case-control studies because misclassification and bias may result. [Feldman (B-1902) P.13 L.20-21, citing B-1902 Attachment 1 (Gregg 2002)]

104. Two typical types of bias are "selection bias" and "information bias." "Selection bias is a systematic error in choosing the study groups to be enrolled, or enrollment of study participants that results in a mistaken estimate of the an exposure-disease association." [Feldman (B-1902) P.14 L.4-5 , citing B-1902 Attachment 1 (Gregg 2002)]

105. Information bias is a systematic error in the collection of exposure or outcome data about the study participants that results in a mistaken estimate of an exposure's effect on the risk of disease. [Feldman (B-1902) P.14 L.11-13 , citing B-1902 Attachment 1 (Gregg 2002)]

106. An example of information bias is "recall bias" in which one part of an enrolled study group is more likely to remember and report an exposure compared to another part of the enrolled study group. [Feldman (B-1902) P.14 L.13-15 , citing B-1902 Attachment 1 (Gregg 2002)]

107. An example of recall bias is persons who develop severe diarrhea; they are much more likely to have thought about all the preceding meals and foods eaten, while healthy controls are not. [Feldman (B-1902) P.14 L.15-17, citing B-1902 Attachment 1 (Gregg 2002)]

108. An Odds Ratio (OR) is the ratio of the odds of disease among the exposed compared to the odds of the disease among the unexposed, and is often used in reporting the results of a case control study. The Odds Ratios do not describe the incidence (rates) of the disease in the general population, nor do they provide data on the number of people potentially affected in any region or any country. The Odds Ratio is only relevant to the exposure to a factor in the study population. However, to the extent that the group studied is of the general population, it may be possible to identify vehicles of infections in the general population. (Gregg, P.121-127, 142-144, 162-165). [Feldman (B-1902) P.15 L.17 – P.16 L.1, citing B-1902 Attachment 1 (Gregg 2002)]

109. FDA is committed to following well recognized principles of epidemiology. [Joint Stipulation 28]

110. The most important natural reservoirs of *Campylobacter* include the intestinal tract of humans, and of warm-blooded wild and domesticated animals (dogs and cats), rodents (field mice, foxes, rabbits, badgers), deer, pets, swine, cattle, sheep, and birds including wild starlings, gulls, sparrows, and geese. [Patterson (B-1910) P.3 L.22 – P.4 L.3; Newell (B-1908) P.9 L.18-21, P.19 L.18-20; Feldman (B-1902) P.15 L.5-10; Nachamkin (G-1470) P.4 L.608; Wegener (G-1483) P.8 L.15-17]

111. Nearly all animals, wild and domesticated, harbor *Campylobacter* as a normal inhabitant of the gastrointestinal tract. [Wegener (G-1483) P.4 L.14-15]

112. Sources of *Campylobacter* infection other than poultry, such as domestic pets, are known. [Joint Stipulation 32]

113. *Campylobacter* contaminate the water environment via wild and domestic animal excretions, urban and agricultural drainage, and sewage and industrial wastewater discharges. [Patterson (B-1910) P.4 L.12-13; Newell (B-1908) P.8 L.1-3]

114. *Campylobacter* has been demonstrated to be ubiquitous in the water environment, present both in surface waters and ground waters. [Patterson (B-1910) P.4 L.4-6; Newell (B-1908) P.7 L.24 – P.8 L.1; CVM Response to Bayer's Interrogatory 1]

115. *Campylobacter*, including fluoroquinolone-resistant *Campylobacter*, are frequently isolated in surface and ground waters, including drinking water supplies. *Campylobacter jejuni* and *Campylobacter coli* have been reported present as cohorts in both source water and in municipal drinking water treatment plants. [Patterson (B-1910) P.4 L.8-12]

116. Non-poultry risk factors for campylobacteriosis are identified in many case-control studies, suggesting that chicken is not the only source of human campylobacteriosis. Other factors include drinking unpasteurized milk, drinking milk contaminated by birds, contact with animals (farm animals as well as cats or dogs), drinking untreated water (non-chlorinated), taking medication, having an underlying disease, eating pork and eating meat prepared at a barbecue. [Wegener (G-1483) P.15 L.13-18]

117. In developed countries, non-poultry risk factors for acquiring campylobacteriosis include other foods of animal origin, inadequately treated water, raw milk, contact with farm animals and pets, and foreign travel. [Tauxe (G-1475) P.5 L.43 – P.6 L.1; G-1743; Newell (B-1908) P.21 L.16-19; Burkhart (B-1900) P.9 L.28-30; Nachamkin (G-1470) P.4 L.22-29]

118. *Campylobacter* infection has also been associated with direct contact with infected animals that may or may not be ill, and is well recognized as a cause of traveler's diarrhea, most likely because of exposure to contaminated food or water overseas. [Tauxe (G-1475) P.6 L.3-6]

119. Campylobacteriosis outbreaks in the United States have been caused by a variety of non-poultry foods, including beef, fruit, and other foods, but the most common single food is unpasteurized milk. [Tauxe (G-1475) P.6 L.27-29]

120. Milk may be subject to fecal contamination either during the milking process or when mixed in the raw milk tank at the farm. *Campylobacter* survives well in refrigerated raw milk, whereas they are readily killed by pasteurization of the milk. [Wegener (G-1483) P.8 L.8-10]

121. Raw milk contaminated with *Campylobacter* as well as water contaminated with *Campylobacter* constitutes a risk of infection. [Wegener (G-1483) P.10 L.27-28]

122. Contact with faeces from cats and dogs are a potential risk factor for human infection. [Wegener (G-1483) P.10 L.30-31; Nachamkin (G-1470) P.4 L.23-26]

123. The putative sources of human *Campylobacter* infections are direct animal contacts, food, water, environment, and human contacts. [Wegener (G-1483) P.10 L.38-39]

124. Foreign travel is a significant risk factor for acquiring *Campylobacter* infections. [Wegener (G-1483) P.13 L.12; Kassenborg (G-1460) P.9 L.10-11; G-1452 Attachment 1 P.46; Nachamkin (G-1470) P.4 L.25-26]

125. In the United States, summer is a traditional time to take vacations, and this may result in increased international travel and more eating outside the home, both risk factors for *Campylobacter* infection. [Angulo (G-1452) Attachment 1 P.55]

126. Epidemiological investigations in the United States and in other developed nations to determine risk factors for sporadic *Campylobacter* infections consistently indicate that a dominant source of infection is transmission from pets and other animals. [Angulo (G-1452) Attachment 3 P.82]

127. Epidemiological investigations in the United States and in other developed nations to determine risk factors for sporadic *Campylobacter* infections consistently indicate that a dominant source of infection is consumption of raw milk. [Angulo (G-1452) Attachment 3 P.82]

128. Humans can serve as sources of *Campylobacter* infection in other humans, by fecal-oral transmission. [Wegener (G-1483) P.20 L.11-12]

129. Transmission of campylobacteriosis from ill food handlers occurs. [Angulo (G-1452) P.9 L.28-29]

130. *Campylobacter* is common in the intestinal tracts of both wild and domestic animals and, as a result of fecal contact during processing, frequently contaminates foods derived from animals. [White (G-1484) P.2 L.44-46]

131. The sources and routes of transmission of campylobacteriosis, and the relative contribution of all these potential sources, remain unclear. [Newell (B-1908) P.21 L.19-20]

132. Humans can come into contact with *Campylobacters* from a range of hosts and via a range of routes. [Newell (B-1908) P.23 L.3-4]

133. The fact that most studies have focused on chicken combined with a variety of other factors, which may vary from study to study, might contribute to the frequent detection of poultry as a risk factor for *Campylobacter* infection. [Wegener (G-1483) P.15 L.28-30]

134. In order to accurately assess the relative significance of alternative routes of transmission of *Campylobacter* to humans, it is important to evaluate each probable route; foodborne, waterborne and perhaps other (e.g., occupational). [Patterson (B-1910) P.4 L.20-22]

135. Ascribing most nonforeign-travel-related *Campylobacter* infections in humans to the handling and consumption of raw or undercooked poultry is problematic and/or unfounded in light of convincing recent molecular and other evidence that non-poultry sources have been significantly underestimated. Among the sources most seriously underestimated are drinking water and recreational contact waters. [Patterson (B-1910) P.5 L.15-19]

136. It remains impossible to determine the contribution of poultry as a source of human campylobacteriosis because representative populations from structured surveys have not yet been undertaken. However, it seems likely that the role of poultry has been overestimated, on the basis of these studies, as contributing disproportionally to human campylobacteriosis. The importance of other potential sources, such as sheep, cattle and pets, and environmental contamination is now increasingly recognized. [Newell (B-1908) P.36 L.18-24]

137. The assumption that poultry is a major source of fluoroquinolone-resistant *Campylobacters* is now questioned, and alternative sources are being actively sought. [Newell (B-1908) P.40 L.20-22; Feldman (B-1902) P.35 L.1 – P.36 L.11]

138. It is important to bear in mind that in a questionnaire-based case-control investigation you do not get information about things you do not ask about, and furthermore, it is extremely difficult to identify risk factors if they are very common. [Wegener (G-1483) P.12 L.32-34]

139. Effler recognizes that his questionnaire was skewed toward chicken; "Finally, because it is a well-recognized source of *C. jejuni*, the histories pertaining to poultry were intentionally very detailed. Resources did not permit obtaining such comprehensive information on all other food items included in the questionnaire and it is possible that associations between illness and other food items were missed as a result." [Effler (G-185) P.4; Feldman (B-1902) P.26 L.11-17]

140. Epidemiological investigations in the United States and in other developed nations to determine risk factors for sporadic *Campylobacter* infections consistently indicate that a dominant source of infection is consumption of contaminated drinking water. [Angulo (G-1452) Attachment 3 P.82]

141. Bacterial pathogens are the leading cause of pollution in water quality-impaired U. S. rivers and streams. [Patterson (B-1910) P.3 L.10-11]

142. Emerging recognition of the significance of bacterial pathogens in drinking and recreational contact waters has become increasingly important during the past two decades. These include newly recognized pathogens from fecal sources, such as *Campylobacter spp.* [Patterson (B-1910) P.3 L.12-14]

143. Water, both open water resources such as lakes and rivers as well as closed resources such as ground water reservoirs may become contaminated with

Campylobacter. In all instances, the source of the contamination is believed to be fecal material. [Wegener (G-1483) P.9 L.1-4; Newell (B-1908) P.7 L.24 – P.8 L.3]

144. Waterborne pathogens of emerging concern in surface waters include *Campylobacter*. [Patterson (B-1910) P.14 L.15-16]

145. *Campylobacter spp.*, particularly *C. jejuni,* are gastroenteric pathogens of environmental concern. [Patterson (B-1910) P.3 L.20-21]

146. *Campylobacter* can survive for several weeks to months in aqueous environments at low temperatures. For example, *Campylobacter jejuni* inoculated into autoclaved mountain stream water remained viable for 33 days at 4° C. [Patterson (B-1910) P.3 L.16-19; Newell (B-1908) P.3 L.14-15, P.7 L.8-11]

147. Survival times for *Campylobacter spp.* in aquatic environments range from days to weeks, and even months. [Patterson (B-1910) P.15 L.15-16]

148. Survival mechanisms for *Campylobacter* in the environment include formation of a viable, but non-culturable stage as a strategy to survive under stressed conditions until favorable environmental conditions arise. [Patterson (B-1910) P.15 L.18-19]

149. The viable, but non-culturable stage of *Campylobacter* is defined as involving bacteria that are metabolically active, while being incapable of undergoing the cellular division required for growth (and for isolation and enumeration by culturing). [Patterson (B-1910) P.15 L.20-22]

150. Campylobacteriosis outbreaks have been caused by contamination of untreated or poorly disinfected water supplies, and by drinking water from unprotected sources such as lakes or streams. [Tauxe (G-1475) P.6 L.38-42]

151. *Campylobacter spp.* including fluoroquinolone-resistant *Campylobacter* have been widely documented in human, agricultural, and industrial (including food products processing) wastewaters and in the treated wastewater effluents discharged to the environment. [Patterson (B-1910) P.6 L.20-22]

152. The U.S. population is routinely exposed to the pathogen *Campylobacter* via waterborne routes, and there is, at minimum, parity in the incidences of campylobacteriosis in the U.S. between all foodborne routes and the waterborne route. [Patterson (B-1910) P.7 L.20-22]

153. The predominant routes of fluoroquinolone-resistant *Campylobacter* to humans are other than associated with poultry. [Patterson (B-1910) P.8 L.3-4]

154. *Campylobacter* are found in high concentrations in raw sewage, and they occur in fecally contaminated surface and ground waters. [Patterson (B-1910) P.9 L.18-19]

155. *Campylobacter* found in water most probably originate from wild and domestic animals, urban and rural drainage including rainfall or seasonal snowmelt,

industrial effluents including food products processing, and sewage including that containing hospital and other health care facility discharges. [Patterson (B-1910) P.9 L.20 – P.10 L.2; Newell (B-1908) P.8 L.1-3]

156. Domestic and wild animal sources excrete *Campylobacter spp.* (via fecal material) and thereby provide a continuous flux of *Campylobacter* into the environment. [Patterson (B-1910) P.10 L.3-4; Newell (B-1908) P.22 L.13-17]

157. Recent studies have implicated free-living birds and aquatic sources as the origin of near-universal colonization of commercial poultry flocks by *Campylobacter* (Piddock, et al., 2000). Wild birds have also been implicated in one *C. jejuni* waterborne community-wide disease outbreak (Sacks, et al. 1986) and one boarding school campylobacteriosis outbreak (Palmer, et al., 1983). In Japan, fluoroquinolone-resistant *C. jejuni* have been cultured from sparrows (Sorum and L'Abee-Lund, 2002). [Patterson (B-1910) P.10 L.7-12, citing to, *inter alia*, B-50, B-1774, B-1800]

158. Multiple antibiotic resistant (including fluoroquinolone-resistant) fecal bacteria have been isolated from wild deer and geese in suburban Morris County, NJ. All isolates were resistant to ciprofloxacin. [Patterson (B-1910) P.10 L.5-7]

159. Data indicate that free-ranging wild birds play a major role of in the dissemination of fluoroquinolone-resistant *Campylobacter*, including via fecal contamination of surface waters. [Patterson (B-1910) P.10 L.12-14, citing to, *inter alia*, B-50, B-1774, B-1800 and Sorum and L'Abee-Lund, 2002]

160. Biofilms are common and extensive in drinking water distribution systems. [Patterson (B-1910) P.19 L.13-14]

161. Biofilms in drinking water pipe distribution networks may harbor *Campylobacter*, from which the *Campylobacter* are then reintroduced into the distributed water. Biofilms may provide an organic substrate, a low dissolved oxygen environment, and protection from residual disinfectant. Residence within the biofilm was reported to approximately double the survival time of *Campylobacter jejuni* at 4°C. [Patterson (B-1910) P.19 L.9-13, Newell (B-1908) P.7 L.8-11]

162. Water has been established as a major source of campylobacteriosis in both outbreaks and in sporadic cases. [Patterson (B-1910) P.27 L.8-11]

163. Waterborne *Campylobacter* infections in the U.S. are indicated to exceed all other sources. [Patterson (B-1910) P.28 L.1-2]

164. There is evidence that the waterborne route is the predominant route for transmission of *Campylobacter* infections. [Patterson (B-1910) P.6 L.8-9]

165. There is evidence that the waterborne route is the predominant route for transmission of fluoroquinolone-resistant *Campylobacter* infections at least to the extent that such infections are environmentally derived (i.e., not related to direct

patient-to-patient transmission of fluoroquinolone-resistant *Campylobacter*). [Patterson (B-1910) P.6 L.9-11]

166. One epidemiological observation intriguing to epidemiologists searching for the sources of infection is the observed seasonal peak of human campylobacteriosis in all countries with longitudinal surveillance data. [Newell (G-1908) P.3 L.22-23, P.25 L.23 – P.26 L.1]

167. A strong seasonal pattern exists in the number of *Campylobacter* cases in the United States with cases peaking in June or July. [G-1452 Attachment 1 P.55; B-15; G-615; G-1679 P.23; Feldman (B-1902) P.11 L.6-7, citing B-215]

168. Fluoroquinolone resistance in *Campylobacter* peaks in the winter and declines in the summer. [Feldman (B-1902) P.11 L.9-10]

169. The seasonal pattern of *Campylobacter* infection is observed in all countries in the temperate climate zones, both in the northern and the southern hemisphere. The seasonal peak occurs in both humans and poultry. The mechanism behind this seasonal pattern remains obscure. [Wegener (G-1483) P.3 L.19-21; Newell (G-1908) P.26 L.1-11]

170. None of the poultry peaks obviously precede, or terminate before, the human peaks in each country, as would be expected if these were the sources of human infection. [Newell (G-1908) P.26 L.12-14]

171. The poultry and human seasonality peak data could be interpreted to suggest that the peak in the shedding of human *Campylobacters* into the environment could be the cause of the poultry flock peak. [Newell (G-1908) P.26 L.14-16]

172. There may be a common source of *Campylobacter* for both the humans and poultry flocks. [Newell (G-1908) P.26 L.20]

173. The fact that fluoroquinolone resistance in *Campylobacter* peaks in the winter and declines in the summer, indicates that there may be different sources in different seasons. [Feldman (B-1902) P.11 L.9-10]

174. Seasonality must be accounted for while examining many enteric pathogens because it is not unusual for the prevalence of the infecting organism and the incidence of the disease to vary over the course of a year in a cyclical pattern. If a case-control study examines too narrow of a window in time, the results may be skewed too high or too low. In either event, the prevalence and incidence found in a short-duration case-control study can not be properly used as a basis for annual prevalence or incidence rates. [Feldman (B-1902) P.30 L.13-18]

175. Naturally-occurring epidemiological experiments give no clear indication that poultry is a major source of human campylobacteriosis. [Newell (B-1908) P.24 L.17-19]

176. Data from the 1999 Belgian dioxin scare, which precipitated a sharp decrease in chicken consumption in Belgium show that no unusual drop in campylobacteriosis rates occurred in 1999 compared to the same months in other years. [Cox (B-1901) P.36]

177. Data from the 1999 Belgian dioxin scare, which precipitated a sharp decrease in chicken consumption in Belgium show a large change in chicken consumption was followed by no unusual changes in campylobacteriosis rates, suggesting that chicken consumption is not a detectable cause of campylobacteriosis. [Cox (B-1901) P.36; Newell (B-1908) P.23 L.18-21]

178. Data showing that poultry is not a major source of campylobacteriosis is consistent with evidence from England, where there was increased consumption of poultry meat during the foot and mouth disease (FMD) outbreak (and a reduction in the consumption of lamb, pork and beef) but no detectable increase in campylobacteriosis. [Cox (B-1901) P.37, citing Newell testimony (B-1908) P.24 L.10-14]

179. In Sweden the decrease in poultry flock infection from 50% to less than 10%, as a result of an active policy for enhanced biosecurity in poultry farms, has had no concomitant decrease in domestically-acquired campylobacteriosis. [Newell (B-1908) P.24 L.14-16]

180. *Campylobacter* present in poultry at the time of slaughter/processing do not multiply outside the chicken gut. From the moment the animal is slaughtered and the intestines are removed, the *Campylobacter* present on that carcass do not multiply further. [Wegener (G-1483) P.5 L.4-5]

181. Freezing and thawing of meat kills a proportion of the viable *Campylobacter* in the meat. [Wegener (G-1483) P.5 L.26-27; Joint Stipulation 24]

182. A chicken-product that has been frozen and thawed harbors less viable *Campylobacter* than the equivalent fresh product. [Wegener (G-1483) P.5 L.29-30]

183. Freezing of poultry reduces the number of live *Campylobacter* in the products. [Wegener (G-1483) P.5 L.31]

184. Under the normal conditions of food storage, freezing chicken products may reduce the population of *Campylobacter*. [Joint Stipulation 31]

185. Freezing chicken (and turkey) products may reduce the population of *Campylobacter*. [Joint Stipulation 24]

186. Red or white meat undergoing any heat treatment or freezing during processing will harbor less *Campylobacter* than meat produced without such treatment. [Wegener (G-1483) P.8 L.2-3]

187. Meat, which is dried, cured, salted, smoked, irradiated or exposed to other preservation methods, will harbor less *Campylobacter* compared to the unpreserved product. [Wegener (G-1483) P.5 L.4-6]

188. Like nearly all other bacteria *Campylobacter* is sensitive to cooking, and it is assumed that an adequately cooked chicken will harbour no viable *Campylobacter*. [Wegener (G-1483) P.9 L.21-23]

189. Unlike some other bacteria, *Campylobacter* does not tend to multiply in foods left out for many hours; indeed, it does not tolerate exposure to atmospheric oxygen or to drying. [Angulo (G-1452) P.9 L.29-31]

190. *Campylobacter* is sensitive to high temperatures and will be eliminated when poultry is properly cooked. [Jacobs-Reitsma (G-1459) P.5 L.26-28; Wegener (G-1483) P.9]

191. Food samples often contain only small numbers of *Campylobacter,* and the bacterial cells may also be seriously injured during processing such as freezing, cooling, heating, and sanitizing. [Meng (G-1466) P.2 L.2-4]

192. Many *Campylobacter* isolation and enrichment broths contain antimicrobial agents. [Joint Stipulation 33]

193. *Campylobacter* are not typically found in muscle tissue of poultry, but instead only on the surface of the birds. [B-196]

194. Double strength Bolton's Broth was used to enrich for *Campylobacters* in retail meat samples from the study reported in exhibit G-727. [G-727]

195. Food samples are enriched and cultured for *Campylobacter* so that detection of small numbers of sub-lethally damaged cells is promoted. [Meng (G-1466) P.2, L.4-7]

196. In a recent study Meng evaluated *in vitro* antimicrobial susceptibilities of 378 *Campylobacter jejuni* and *coli* isolates from 159 contaminated retail raw meats. Meng found 54% erythromycin resistance in those isolates. [Meng (G-1466) P.3 L.24-28]

197. From 1997-2001 the percentage of *Campylobacter* isolates resistant to erythromycin (MIC > 8) has decreased from 8% (17/217) in 1997 to 1% (5/324) in 2000. [G-749]

198. CVM does not have any facts or data demonstrating any increase in overall *Campylobacter* loads in live chickens or in live turkeys after fluoroquinolones were approved for use in chickens and turkeys. [CVM Response to Bayer's Interrogatory 23]

199. CVM does not have any facts or data demonstrating any increase in fluoroquinolone-resistant *Campylobacter* loads in live chickens or in live turkeys after fluoroquinolones were approved for use in chickens and turkeys. [CVM Response to Bayer's Interrogatory 25]

200. CVM does not have any facts or data demonstrating any increase in fluoroquinolone-resistant *Campylobacter* loads in retail chicken products or in retail turkey products after fluoroquinolones were approved for use in chickens and turkeys. [CVM Response to Bayer's Interrogatory 24]

201. CVM does not have any facts or data demonstrating any increase in fluoroquinolone-resistant *Campylobacter* in or on cooked chicken meat or cooked turkey meat ready for consumption after fluoroquinolones were approved for use in chickens and turkeys. [CVM Response to Bayer's Interrogatory 26]

202. Food handling practices and consumer knowledge of microbial food safety has markedly improved over the past decade, particularly from 1993, before enrofloxacin approval, to 2001. [Tompkin (A-204) P.9 L.29-30]

203. Four studies conducted by FDA to measure consumer knowledge and food handling practices demonstrate that progress is being made toward educating consumers and reducing risk from food borne microbiological pathogens. The FDA conducted a random digit-dial survey of a nationally representative sample of American consumers in 1988, 1993, 1998, and 2001 (Fein, Levy and Lando, 2002). The trends for both cross contamination measures and eating potentially risky foods were very similar. No improvement occurred between 1988 and 1993, and for one measure (washing hands after touching raw meat or chicken), the safety of the behavior became worse. Between 1993 and 1998, significant improvement on all of the measures of cross contamination was found, as was also the case on four of the six measures of eating potentially risky food. Then, between 1998 and 2001, most of the measures of cross contamination showed an additional but small improvement, which is an achievement after such a dramatic initial change. [Tompkin (A-204) P.10 L.4-18].

204. Large improvements in food safety practices were seen between 1993 and 1998 (as measured by cross contamination behaviors and consumption of risky foods). These gains were maintained between 1998 and 2001 for cross contamination and for consumption except for raw seafood. [Tompkin (A-204) P.10 L.9 – P.11 L.16]

205. In studies conducted by FDA, consumption of raw shellfish (3.2%) and undercooked hamburger (43%) were more common in Connecticut than the other four states. Raw milk consumption was more common among people who lived on a farm (8.6%) compared with people who lived in a city or urban area (1.1%).

Preference for undercooked hamburger was more common among men (35%), young adults (18 to 25 years, 33%), people with college education (38%), and among people with household income of more than $100,000/year (49%). African-Americans were less likely to prefer undercooked hamburger compared to other racial groups (10% versus 30%). Men washed their hands less often than women (89% versus 97%). Young adults compared to older adults were less likely to wash their hands after handling raw chicken (88% versus 95%). [Tompkin (A-204) P.12 L.4-13]

206. Unlike beef and seafood, poultry is not commonly consumed raw or rare on purpose. [Tompkin (A-204) P.12 L.19-20]

207. FoodNet is the Foodborne Diseases Active Surveillance Network. [Angulo (G-1452) P.2 L.15]

208. FoodNet is the principal foodborne disease component of the CDC's Emerging Infections Program. FoodNet is a collaborative project among the CDC, state health departments, the United States Department of Agriculture Food Safety and Inspection Service (FSIS), and the United States Food and Drug Administration (FDA). [Angulo (G-1452) P.2 L.15-16]

209. The objectives of FoodNet are to determine the frequency and severity of foodborne diseases in the US; determine the association of common foodborne diseases with eating specific foods; and describe the epidemiology of new and emerging bacterial, parasitic, and viral foodborne pathogens. [Angulo (G-1452) P.2 L.23-25, Kassenborg (G-1460) P.3 L.7-8]

210. FoodNet uses active surveillance and conducts related epidemiologic studies. [Angulo (G-1452) P.2 L.26-27]

211. FoodNet conducts population-based active surveillance for clinical laboratory isolations of *Campylobacter*, *Escherichia coli* (including *E. coli* 0157:H7), *Listeria*, and *Salmonella* infections in Connecticut, Georgia, Maryland, Minnesota, and Oregon, and selected counties in California, Colorado, New York, and Tennessee. [Angulo (G-1452) P.2 L.34-38]

212. FoodNet conducts active laboratory-based surveillance for culture-confirmed cases of *Campylobacter* and other foodborne pathogens. To identify cases, FoodNet personnel contact each clinical laboratory in their surveillance area either weekly or monthly depending on the size of the laboratory. Cases represent the first isolation of *Campylobacter* from a person by a clinical laboratory; most specimens are obtained for diagnostic purposes from ill persons. [Angulo (G-1452) P.4 L.37-41]

213. The FoodNet catchment area from which human origin specimens are drawn for NARMS surveillance has grown each year of the program. [Angulo (G-1452) P.4 L.43-48]

214. FoodNet surveillance began in 1996 in Minnesota and Oregon and selected counties in California, Connecticut, and Georgia. [Angulo (G-1452) P.4 L.43-44]

215. In 1998, the FoodNet surveillance area expanded; Connecticut surveillance became statewide and active surveillance began in selected counties in Maryland and New York. [Angulo (G-1452) P.5 L.46-47; DeGroot (A-200) P.16 L.7-8]

216. In 1999, the FoodNet surveillance area expanded; all remaining counties in Georgia were added and additional counties in New York were added. [Angulo (G-1452) P.5 L.47-48]

217. In 2000, the FoodNet surveillance area expanded to selected counties in Tennessee and additional counties in California were added. [Angulo (G-1452) P.5 L.48 – P.5 L.1]

218. In 2001, the FoodNet surveillance area included Connecticut, Georgia, Minnesota, and Oregon, and selected counties in California, Colorado, Maryland, New York, and Tennessee. [Angulo (G-1452) P.5 L.5-6]

219. The number of sites and population under surveillance by FoodNet nearly doubled between 1996 and 2001. Because of substantial variation in incidence of enteric among sites, adding new sites influences overall crude incidence. [Angulo (G-1452) P.5 L.15-17; see also DeGroot (A-200) P.15 L.17-23]

220. FoodNet activities are conducted within selected state health departments. [Angulo (G-1452) P.4 L.2]

221. Selection of state health departments to participate in FoodNet was based upon written responses to a Request for Proposals published in the Federal Register; state health departments were not chosen specifically to be representative of the United States population. [Angulo (G-1452) P.4 L.2-5]

222. Compared to the United States population, the population in the FoodNet surveillance area was slightly more likely to be Asian. [Angulo (G-1452) P.4 L.16-19]

223. Compared to the United States population, the population in the FoodNet surveillance area was less likely to be Black. [Angulo (G-1452) P.4 L.16-19]

224. Compared to the United States population, the population in the FoodNet surveillance area was less likely to be Hispanic. [Angulo (G-1452) P.4 L.16-19]

225. Compared to the United States population, the population in the FoodNet surveillance area was more likely to include urban residents. [Angulo (G-1452) P.4 L.16-19]

226. Compared to the United States population, the population in the FoodNet surveillance area was more likely to include residents in counties with lower population density. [Angulo (G-1452) P.4 L.16-19]

227. Compared to the United States population, the population in the FoodNet surveillance area was less likely to include persons living at or below poverty. [Angulo (G-1452) P.4 L.16-19]

228. Dr. Angulo acknowledges that there are slight demographic differences between the populations residing in the FoodNet surveillance area and the United States. [Angulo (G-1452) P.4 L.21-22]

229. Although FoodNet data provide detailed information regarding *Campylobacter* infections, the data do not reflect the entire US population." [Molbak (G-1468) P.5 L.17-21]

230. Data collected for the Human NARMS program do not represent the general United States population and the program contains no means to correct its estimates for inherent sampling biases to make them representative of the general population. [DeGroot (A-200) P.17 L.23-24 – P.18 L.1-2]

231. NARMS is the National Antimicrobial Resistance Monitoring System for enteric bacteria. [Angulo (G-1452) P.3 L.17]

232. Dr. Frederick Angulo of CDC, Dr. Paula Fedorka-Cray of USDA, and Dr. Linda Tollefson were the primary scientists involved in designing, developing and implementing the National Antimicrobial Resistance Monitoring System. That system became operational in January 1996 and is known as NARMS. [Tollefson (G-1478) P.4 L.41-47; DeGroot (A-200) P.3 L.2-5]

233. Linda Tollefson was the overall manager and director of NARMS since its inception in 1996 through the period covered by this Hearing. [Tollefson (G-1478) P.8 L.43-45]

234. The primary purpose of NARMS is to monitor antimicrobial resistance among foodborne enteric bacteria including *Campylobacter*, *Salmonella*, and *Escherichia coli* 0157:H7. [Angulo (G-1452) P.3 L.17-19, Tollefson (G-1478) P.8 L.2-4, Kassenborg (G-1460) P.3 L.21-23; DeGroot (A-200) P.3 L.2-5]

235. NARMS data are used to study emerging resistance and guide studies evaluating where and how people become infected with resistant foodborne bacteria. [Angulo (G-1452) P.3 L.19-21]

236. NARMS data are used by the CDC and state health departments to investigate outbreaks caused by particular bacteria, conduct other studies to better understand the circumstances under which resistant bacteria arise and spread, and guide efforts to mitigate antimicrobial resistance. [Angulo (G-1452) P.3 L.21-24]

237. NARMS is a collaborative project among the CDC, participating state health departments, the United States Food and Drug Administration, and the United States Department of Agriculture. [Angulo (G-1452) P.3 L.26-27]

238. NARMS consists of three testing sites, or arms, all using supplies purchased centrally and the same isolation, identification, and susceptibility testing procedures. [Tollefson (G-1478) P.6 L.18-20]

239. The NARMS testing sites are: 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). The third testing arm was established recently, in 2001. [Tollefson (G-1478) P.6 L.20-25]

240. 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 (G-1453) P.3 L.1-3]

241. In 1988 nalidixic acid susceptibility continued to be used as a diagnostic criterion. [Barrett (G-1453) P.3 L.11-12]

242. For NARMS testing in 1997, 1998, 1999 and 2000, confirmation of *Campylobacter jejuni/coli* was conducted as described in the FSIS Microbiology Laboratory Guidebook and included use of disk diffusion with susceptibility to nalidixic acid and resistance to cephalothin as identification of *Campylobacter jejuni/coli.* [Tollefson (G-1478) P.9 L.31-36]

243. Because only isolates that were nalidixic acid susceptible and cephalothin-resistant were considered *Campylobacter jejuni/coli*, 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 to fluoroquinolones. [Tollefson (G-1478) P.9 L.36-46]

244. The Center 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 (G-1478) P.19 L.22-27]

245. Any *Campylobacter* isolation, speciation and susceptibility testing protocol relying on nalidixic acid susceptibility as a criterion to identify *C. jejuni* or *C. coli* would have excluded all quinolone-resistant isolates from surveillance for these two species and therefore underreport resistance in *C. jejuni* and *C. coli* . [Barrett (G-1453) P.3 L.31-36]

246. Any *Campylobacter* isolation, speciation and susceptibility testing protocol that relied on susceptibility to nalidixic acid as one of the primary criteria to differentiate between the thermophilic *Campylobacters* (with *C. jejuni* and *C. coli* considered to be susceptible) would result in an underestimate of fluoroquinolone resistance, because some of the isolates discarded for not being *Campylobacter jejuni/coli* (because they were not susceptible to nalidixic acid) could in fact be resistant *Campylobacter jejuni/coli*, meaning they were resistant to both nalidixic acid and to fluoroquinolones. [Barrett (G-1453) P.3 L.1-3; Tollefson (G-1478) P.9 L.36-46]

247. There is no NCCLS or other generally accepted "standard" method of isolating *Campylobacters* from human, food or environmental sources. [Silley (B-1913) P.5 L.20-21]

248. Media for isolating *Campylobacters* from faeces, food and water have different attributes and, therefore are not optimal for recovering the representative diversity of *Campylobacter* species present in the original sample. There is no consensus concerning the best media and methods to isolate representative species of *Campylobacters* from the sample. [Silley (B-1913) P.5 L.22 – P.6 L.3]

249. Antimicrobials in selective media developed for *Campylobacters* have been chosen on the basis of those to which test strains are resistant and those most effective in inhibiting competitive flora. At least seventeen different single antimicrobials have been used (cephalothin, cephazolin, cefsulodin, cephalexin, cefoperazone, trimethoprim, polymyxin B, colistin, vancomycin, teicoplanin, rifampicin, novobiocin, bacitracin, cycloheximide, actidione, amphotericin, nystatin) either singly or more often in combination, including five different cephalosporins. [Silley (B-1913) P.6 L.8-14]

250. Incorporation of antimicrobials into selective media has the greatest significance with regard to introducing bias into the isolation procedure. [Silley (B-1913) P.6 L.15-17]

251. The choice of isolation medium will influence the relative distribution of *Campylobacter* spp. and phenotypes recovered. [Silley (B-1913) P.7 L.1-2]

252. Unlike *Salmonella* culture media, *Campylobacter* culture media have no indicator system to identify putative colonies. As a result, colonies are randomly chosen by lab technicians. [Silley (B-1913) P.35 ¶ 4 – P.36, ¶ 1, Attachment 1]

253. Studies have been reported where more than one strain of *Campylobacter* spp. has been found on 67% of infected carcasses and up to six strains on a single carcass. Studies such as these show that *Campylobacter* isolation techniques do not necessarily accurately isolate the species of *Campylobacter* present in a given sample, causing significant doubt on the inferences that may be drawn from such techniques, including inferences about the reservoir of the organism, and whether it has caused the disease. [Silley (B-1913) P.7 L.19 – P.8 L.2]

254. Filtration methods permit isolation without use of antimicrobial-containing media. [B-205 P.2]

255. The development of filtration techniques represents a significant advance over the use of selective media, and this method is now recommended for primary isolation of *Campylobacters*. [B-205 P.2]

256. Method of recovery can influence the subtypes of strains observed. Enrichment preferentially selects some strains. [G-457 P.4]

257. Culture methods for *Campylobacter* obtained from human stool samples are different than those used to isolate *Campylobacter* from retail food products. [Meng (G-1466) P.1 L.44 – P.2 L.9]

258. Bolton Broth contains antibiotics. Specifically, Bolton's Broth contains cefoperazone, vancomycin, trimethoprim, sulfamethoxazole and cyclohexamide. [Silley (B-1913) P.35 Attachment 1; ¶ 3]

259. Campy-Cefex Agar contains the following antibiotics; cefoperazone, nystatin, and rifampicin. [Silley (B-1913) P.35 Attachment #1; ¶ 3]

260. Human and animal fecal samples frequently contain more than one species of *Campylobacter* and/or more than one strain of the same species of *Campylobacter*. Culture methodologies employing antimicrobials are known to effect both the species, strain types, and antimicrobial susceptibilities of organisms isolated in such cases. [Silley (B-1913) Attachment 1 P.33; ¶ 3 – P.36 ¶ 1; Newell (B-1908) P.33 L.17-24]

261. Human and animal fecal samples frequently contain *Campylobacters* with varying antimicrobial susceptibilities. [Silley (B-1913) P.33 Attachment 1, ¶ 3]

262. The pre-enrichment step in carcass washes and retail product sampling can allow revived *Campylobacter* cells to multiply. Rapidly growing cells will have opportunity to overgrow slow growing cells, with resulting sample biasing. [Silley (B-1913) P.36 Attachment 1 ¶ 3; B-1062]

263. Enrichment culture in the presence of antimicrobials biases the recovery of *Campylobacters* originally present on the enriched sample. [Silley (B-1913) P.37, Attachment 1 ¶ 1]

264. The selection of single colonies suspected to be pure clones of *Campylobacter* from enrichment agar plates can be misleading because *Campylobacter* readily forms biofilms that cause strains of species and/or different species of *Campylobacter* to aggregate. This can result in both incorrect identification of species and incorrect interpretation of anti microbial susceptibility tests run on these isolates. This is estimated to occur roughly 10% of the time. [Silley (B-1913) P.37 Attachment 1 ¶ 1; B-1213]

265. An approved method for *Campylobacter* susceptibility testing for isolates of animal origin was not available until May 2002 when NCCLS published M31-A2. [Silley (B-1913) P.11; Joint Stipulation 29]

266. Antimicrobial susceptibility testing on isolates that are not pure cultures are invalid. [Silley (B-1913) P.37 Attachment 1 ¶1]

267. Sublethally damaged *Campylobacter* cells are often more sensitive to the selective antimicrobial agents used in traditional culture approaches than cells which are cultured from normal biological niche. [Silley (B-1913) P.38 Attachment 1 ¶ 2]

268. When submitting MIC data to FDA with regard to antimicrobial safety studies it is conditional that MIC's must be generated in isolates that have not been exposed to antimicrobials for at least three months prior to isolation. It is therefore difficult to place significant scientific weight on MIC data from isolates exposed to several antimicrobials during the isolation process. [Silley (B-1913) P.38 Attachment 1 ¶ 3]

269. *Campylobacter* isolates reported on in exhibits G-1484 (White WDT) and G-589 (Smith 1999) were exposed to several antimicrobials prior to susceptibility testing. [White (G-1484); G-589]

270. Studies have compared the same *Campylobacter* source samples by filtration isolation versus antimicrobial enrichment isolation and shown that the latter result in *Campylobacters* with reduced antimicrobial susceptibilities. This work also demonstrates genetic biasing via changes in RAPD profiles of the isolates. [Silley (B-1913) P.41 Attachment 1]

271. Disk diffusion studies with *Campylobacter* result in little or no inter-laboratory or intra-laboratory reproducibility. [Silley (B-1913) P.42 Attachment 1 ¶ 1]

272. The E-test is fundamentally a disk diffusion test. [Silley (B-1913) P.43 Attachment 1 ¶ 2]

273. Studies demonstrate that the E-test MIC's for the quinolones tended to be at least one dilution step higher (for resistant isolates) or lower (for susceptible isolates) than that from the agar dilution method. [Silley (B-1913) at P.43 Attachment 1 ¶ 1; Walker (G-1481) P.9]

274. MIC data generated using non-validated methodologies without standardized Quality Assurance procedures cannot be considered definitive. This applies to all NARMS generated antimicrobial susceptibility data as well as the studies of G-589, B-59 & B-22. [Silley (B-1913) P.45 Attachment 1 ¶ 8]

275. Efflux mechanisms are not necessarily specific to antibiotic class. [Joint Stipulation 34]

276. In *E. coli*, efflux pumps can be activated by a variety of compounds, including other antimicrobials, typically resulting in changes in fluoroquinolone MIC. [McDermott (G-1465) P.5]

277. White et al found that they could not culture *Campylobacter* from samples taken from their retail survey when the media was incubated at 35 degrees C (95 degrees F). [White (G-1352) Protocol Amendment, P.34]

278. White et al found that they had to incubate samples at 42 degrees C (107.6 degrees F) in order to recover *Campylobacter* from samples taken during their retail survey. [White (G-1352) Protocol Amendment, P.34]

279. Human isolate testing is conducted at the CDC's National Center for Infectious Diseases Foodborne Disease Laboratory in Atlanta, Georgia. [Tollefson (G-1478) P.6 L.35-37]

280. Clinical laboratories participating in FoodNet/NARMS isolate foodborne enteric bacteria usually from diagnostic specimens collected from ill persons and forward the isolates to state public health laboratories. [Angulo (G-1452) P.3 L.32-33]

281. Many NARMS activities are conducted within the framework of the CDC's Emerging Infections Program, including within FoodNet. Activities conducted at the CDC focus on foodborne enteric bacteria isolated from humans. [Angulo (G-1452) P.3 L.28-30]

282. NARMS susceptibility testing of human *Campylobacter* isolates is conducted exclusively in FoodNet sites. [Angulo (G-1452) P.3 L.46-47, Tollefson (G-1478) P.6 L.37-40]

283. NARMS testing of human *Salmonella* and *Escherichia coli* 0157:H7 isolates began in 1996. [Angulo (G-1452) P.3 L.35-36]

284. At its inception in 1996, NARMS began monitoring only *Salmonella* isolates of human and animal origin because of cost constraints. [Tollefson (G-1478) P.5 L.17-19]

285. NARMS testing of human *Campylobacter* isolates began in 1997. [Angulo (G-1452) P.3 L.36; DeGroot (A-200) P.3 L.8-10]

286. In NARMS, participating state health departments forward selected *Salmonella* and *E. coli* 0157:H7 isolates to the CDC for susceptibility testing. [Angulo (G-1452) P.3 L.36-38]

287. FoodNet conducts active laboratory-based surveillance for culture-confirmed cases of *Campylobacter* and other foodborne pathogens. [Angulo (G-1452) P.4 L.37-38]

288. Under the NARMS *Campylobacter* sampling protocol, each participating state health department in FoodNet sends the first selected *Campylobacter* isolate each

week to the CDC for susceptibility testing. [DeGroot (A-200) P.20 L.17-18; Angulo (G-1452) P.3 L.38-40; G-1679 P.28]

289. To identify *Campylobacter* cases to include in FoodNet NARMS sampling, FoodNet personnel contact each clinical laboratory in their surveillance area either weekly or monthly depending on the size of the laboratory. Cases represent the first isolation of *Campylobacter* from a person by a clinical laboratory. [Angulo (G-1452) P.3 L.38-41]

290. Diagnostic specimens collected by clinical laboratories participating in FoodNet/NARMS to isolate foodborne enteric bacteria are commonly stool specimens. [Angulo (G-1452) P.3 L.33-34]

291. Most FoodNet/NARMS *Campylobacter* specimens are obtained for diagnostic purposes from ill persons. [Angulo (G-1452) P.3 L.38-41; DeGroot (A-200) P.25 L.8-11]

292. The Human NARMS sample collection protocol calls for participating public health laboratories to submit only the first *Campylobacter* isolate received in each laboratory each week to CDC for susceptibility testing. [Tollefson (G-1478) P.7 L.32-34; Angulo (G-1452) P.7 L.26-30; G-1679 P.28]

293. Participating NARMS sites select every tenth non-typhi *Salmonella*, every tenth *Shigella*, and every fifth *E. coli* 0157 for susceptibility testing by CDC. [Tollefson (G-1478) P.7 L.23-34]

294. The National Antimicrobial Resistance Monitoring System (NARMS) began antimicrobial susceptibility testing of human *Campylobacter* isolates in 1997 when participating laboratories in California, Connecticut, Georgia, Minnesota, and Oregon selected and began forwarding *Campylobacter* isolates each week to the CDC. [Angulo (G-1452) P.7 L.26-29]

295. NARMS susceptibility testing of human *Campylobacter* isolates expanded in 1998 when participating laboratories were added in Maryland and New York. [Angulo (G-1452) P.7 L.29-30]

296. NARMS susceptibility testing of human *Campylobacter* isolates expanded in 1999 when participating laboratories were added in Tennessee. [Angulo (G-1452) P.7 L.29-30]

297. NARMS susceptibility testing of human *Campylobacter* isolates expanded in 2000 when participating laboratories were added in Colorado. [Angulo (G-1452) P.7 L.29-30]

298. There is substantial year-to-year and site-to-site variation in the rates of ciprofloxacin resistance among *Campylobacter* isolates. [Angulo (G-1452) P.8 L.16-18, L.26-27]

299. Rates of campylobacteriosis and fluoroquinolone-resistant *Campylobacter* are extremely variable among FoodNet sites. [Molbak (G-1468) P.4 L.38-44; P.6, Table 1; P.8 L.17-18; P.9, Table 3]

300. Human NARMS fails to distinguish isolates from patients with known factors for ciprofloxacin resistance such as foreign travel and prior fluoroquinolone use. [DeGroot (A-200) P.19 L.16-17]

301. Ciprofloxacin resistance rates in particular, are affected by such factors as prior antimicrobial use and foreign travel. [DeGroot (A-200) P.19 L.21-23, citing B-50 and G-589]

302. The Human NARMS program does not consistently characterize isolates with respect to either age or gender, important determinants of campylobacteriosis risk [http://www.cdc.gov/foodnet/annual/2000/2000_summary.htm] for which significant associations with resistance can also be reasonably hypothesized. [DeGroot (A-200) P.19 L.23 – P.20 L.1-2]

303. Ciprofloxacin resistance estimates generated from the Human NARMS *Campylobacter* sample selection protocol are erroneously elevated due to seasonal variation. [DeGroot (A-200) P.20 L.14-15]

304. Even though campylobacteriosis is the second most commonly identified bacterial cause of diarrhea in the U.S., the NARMS *Campylobacter* sampling protocol limits *Campylobacter* resistance submissions to 52 or 53 per participating site per year. [DeGroot (A-200) P.20 L.18-21]

305. Non- *Campylobacter* pathogens monitored by NARMS have a broader sampling protocol than that for *Campylobacter*, taking every 5th or 10th specimen, for example, rather than the first specimen of the week. [DeGroot (A-200) P.20 L.21-22. *See also* Tollefson (G-1478) P.7 L.23-34]

306. Participating state health departments submitted more *Shigella* and more *Escherichia coli* isolates to NARMS than *Campylobacter* isolates during 2000, even though these other diarrheal agents are not identified nearly as commonly as *Campylobacter*. [DeGroot (A-200) P.20 L.24 – P.21 L.1-2]

307. The Human NARMS protocol systematically collects a higher proportion of isolates available from winter, when campylobacteriosis incidence is lower, than from summer, when campylobacteriosis incidence is higher. [DeGroot (A-200) P.21 L.10-13]

308. Ciprofloxacin resistance among isolated *Campylobacter jejuni* is higher in the winter than it is in the summer. [DeGroot (A-200) P.21 L.13-14; Feldman (B-1902) P.11 L.9-10]

309. Yearly resistance estimates reported by the Human NARMS program are higher than the general level of ciprofloxacin resistance. [DeGroot (A-200) P.21 L.14-15]

310. In 2000 the Minnesota Department of Health website reported 11% overall incidence of *Campylobacter* resistance from all cases at www.health.state.mn.us/divs/dpc/ades/surveillance/table2000.pdf while at the same time Minnesota's year 2000 NARMS-submitted samples were 25% resistant. [DeGroot (A-200) P.16 L.21 – P.17 L.2; P.21 L.15 – P.25 L.6; P.45 L.8 – P.47 L.6 G-749, P.13]

311. Year-to-year comparisons of apparent prevalence of ciprofloxacin resistance among *Campylobacter* reported by Human NARMS are confounded with effects attributable to changes in the U.S. population and its exposure to factors known to increase risks of ciprofloxacin-resistant *Campylobacter* infection such as foreign travel or recent prior use of a fluoroquinolone. [DeGroot (A-200) P.25 L.18-22]

312. Foreign travel among residents of the FoodNet catchment area increased by 60% from 1998 to 2000. [DeGroot (A-200) P.25 L.16-17]

313. Antibiotic use among residents of the Food Net catchment area rose by 10% (12.0% (112/12755) to 13.2% (184/13113)) from 1998 to 2000. [DeGroot (A-200) P.25 L.17-18]

314. NARMS estimates not corrected for the confounders foreign travel and prior fluoroquinolone use cannot be meaningfully compared to the general population. [DeGroot (A-200) P.25 L.22-23]

315. Human NARMS Selection is biased by inconsistent diagnostic protocols employed by attending physicians. [DeGroot (A-200) P.27 L.5-24]

316. There is no statistical difference in the prevalence ratio estimate of fluoroquinolone resistance comparing 1997 NARMS data to 1998, 1999, 2000 and 2001 NARMS data when Connecticut data was removed from the analysis conducted by CDC. [Molbak (G-1468) P.9 Table 4]

317. There is no statistical difference in the prevalence ratio estimate of fluoroquinolone resistance comparing 1997 NARMS data to 1998 and 2000 NARMS when Connecticut data was left in the analysis conducted by CDC. [Molbak (G-1468) P.9 Table 4]

318. NARMS only tests a very small fraction of *Campylobacter* cases in the FoodNet catchment areas. [Burkhart (B-1900) P.44 L.2-3]

319. There is no statistical difference in the 13.6% resistance reported by NARMS in 1997 compared to the 17.6% reported in 1999. [DeGroot (A-200) P.50 L.12-14]

320. There is no statistical difference in the 13.3% resistance reported by NARMS in 1998 compared to the preliminary 2001 figure of 18%. [DeGroot (A-200) P.50 L.14-16]

321. No explanation of Human NARMS ciprofloxacin resistance estimates, used as part of the basis for the NOOH by CVM, is complete without a measure of sampling protocol compliance failure and data integrity violation. [DeGroot (A-200) P.52 L.10-12]

322. Both the preliminary and final Logistic Regression Model used to analyze the NARMS data include information on age categorization. [DeGroot (A-200) P.54 L.12-13]

323. A logistic regression model created by DeGroot, with the data made available to Bayer by CDC, clearly shows that reported yearly resistance varied not as the result of a generalized phenomena, but rather as the result of various effects operating within specific states in specific years. [DeGroot (A-200) P.54 L.2-4]

324. Resistance documented in Connecticut for the 1999 and 2001 NARMS collections were roughly twice as high as reported in the baseline year of 1997. [DeGroot (A-200) P.54 L.5-7]

325. The Logistic Regression Model used by CDC to analyze the NARMS data cannot be considered a true trend analysis. [DeGroot (A-200) P.54 L.17-20]

326. The Logistic Regression Model used by CDC to analyze the NARMS data compare yearly outcomes to a baseline year using sets of indicator variables. [DeGroot (A-200) P.54 L.20-21]

327. In conducting the Logistic Regression Model to analyze the NARMS data, CDC failed to explore how the independent variables and outcome measured vary with respect to passage of time; this analysis also obliterated the sequential relationship among temporal identifiers which precluded analysis of trends because each year was considered in isolation. [DeGroot (A-200) P.54 L.21 – P.55 L.4]

328. CDC fails to report on the ecological factors associated with varying ciprofloxacin resistance in different states and different years. [DeGroot (A-200) P.55 L.11-12]

329. The Human NARMS data provide no insight into national ciprofloxacin resistance trends among *Campylobacter* causing diarrhea in U.S. residents. [DeGroot (A-200) P.55 L.6-7]

330. CDC does not enforce the stated protocols for Human NARMS *Campylobacter* collection, resulting in haphazard specimen submission and potential data corruption. [DeGroot (A-200) P.30 L.1 – P.33 L.17]

331. At the 2002 NARMS Annual Scientific Meeting held November 19 - 22, in Hilton Head Island, SC, the human NARMS *Campylobacter* sampling methodology was described by Dr. Fred Angulo, Chief of the CDC NARMS Activity, as "artificial" and not population based as is the methodology for all of the other bacteria in the human NARMS program (i.e., the *Campylobacter* sampling methodology is

distinctly different than that for all of the other organisms in the NARMS programs). [Carnevale (A-199) P.11 L.14-19]

332. The consequences of CDC's "artificial" *Campylobacter* sampling methodology are significant, as it causes a marked over-representation of fluoroquinolone resistant *Campylobacter* isolates in the NARMS program. It has been shown that the incidence of human campylobacteriosis is highest during summer months while rates of resistance to fluoroquinolones are highest during the winter months. (For example, see A-71). Therefore, CDC's "artificial" program of selecting only the first isolate each week from the participating laboratories causes the level of fluoroquinolone resistance to be over-represented in the CDC program. [Carnevale (A-199) P.11 L.22 – P.12 L.2]

333. At the 2002 NARMS Annual Scientific Meeting held November 19 - 22, in Hilton Head Island, SC, Dr. Fred Angulo, Chief of the CDC NARMS Activity stated, "Now your question is to the extent that the prevalence we [CDC] identify is representative of the country, and I agree completely there are limitations in the generalization of our prevalence nationally." [Carnevale (A-199) P.13 L.12-15]

334. At the 2002 NARMS Annual Scientific Meeting held November 19 - 22, in Hilton Head Island, SC, Dr. Fred Angulo, Chief of the CDC NARMS Activity stated, " For *Campylobacter,* as you heard described, we [CDC] do not have a population based sampling methodology." [Carnevale (A-199) P.13 L.16-17]

335. At the 2002 NARMS Annual Scientific Meeting held November 19 - 22, in Hilton Head Island, SC, Dr. Fred Angulo, Chief of the CDC NARMS Activity stated, " We [CDC] agree completely, that there's a limitation in our sampling scheme of *Campylobacter.* That's why we're moving towards trying to develop a population based collection of *Campylobacter* isolates." [Carnevale (A-199) P.13 L.18-20]

336. At the 2002 NARMS Annual Scientific Meeting held November 19 - 22, in Hilton Head Island, SC, Dr. Fred Angulo, Chief of the CDC NARMS Activity stated, " So, and then *Campylobacter* is not population based, as was pointed out. So, I think that for all pathogens except *Campylobacter* we have a representative sample of culture confirmed cases at the state level." [Carnevale (A-199) P.13 L.21-24]

337. At the 2002 NARMS Annual Scientific Meeting held November 19 - 22, in Hilton Head Island, SC, Dr. Fred Angulo, Chief of the CDC NARMS Activity stated, "I agree. Its *[Campylobacter* resistance numbers] not a prevalence. It [*Campylobacter* resistance numbers] is not an estimate of the national prevalence because we [CDC] have artificially created this once a week sample." [Carnevale (A-199) P.13 L.24-28]

338. There was no statistical difference in the prevalence ratio estimate of flouroquinolne resistance comparing 1997 NARMS data to 1998, 1999, 2000 and 2001 NARMS data when Connecticut data was removed from the analysis conducted by CDC. [Molbak (G-1468) P. 9 Table 4]

339. There was no statistical difference in the prevalence ratio estimate of flouroquinolne resistance comparing 1997 NARMS data to 1998 and 2000 NARMS when Connecticut data was left in the analysis conducted by CDC. [Molbak (G-1468) P. 9 Table 4]

340. Bayer could not duplicate the Logistic Regression Model analysis since the data received contained numerous missing data on age. [Burkhart (B-1900) P. 43 L. 13-15]

341. NARMS only tests a very small fraction of *Campylobacter* cases in the FoodNet catchment areas. [Burkhart (B-1900) P. 44 L. 2-3]

342. There is no statistical difference in the 13.6% resistance reported by NARMS in 1997 compared to the 17.6% reported in 1999. [DeGroot (A-200) P. 50 L. 12-14]

343. There is no statistical difference in the 13.3 % resistance reported by NARMS in 1998 compared to the preliminary 2001 figure of 18%. [DeGroot (A-200) P. 50 L. 14-16]

344. No explanation of Human NARMS ciprofloxacin resistance estimates, used as part of the basis for the NOOH by CVM, is complete without a measure of sampling protocol compliance failure and data integrity violation. [DeGroot (A-200) P. 52 L. 10-12]

345. Both the preliminary and final Logistic Regression Model used to analyze the NARMS data include information on age categorization. [DeGroot (A-200) P. 54 L. 12-13]

346. A logistic regression model created by DeGroot, with the data made available to Bayer by CDC, clearly shows that reported yearly resistance varied not as the result of a generalized phenomena, but rather as the result of various effects operating within specific states in specific years. [DeGroot (A-200) P. 54 L. 2-4]

347. Resistance documented in Connecticut for the 1999 and 2001 NARMS collections were roughly twice as high as reported in the baseline year of 1997. [DeGroot (A-200) P. 54 L. 5-7]

348. The Logistic Regression Model used by CDC to analyze the NARMS data cannot be considered a true trend analysis. [DeGroot (A-200) P. 54 L. 17-20]

349. The Logistic Regression Model used by CDC to analyze the NARMS data compare yearly outcomes to a baseline year using sets of indicator variables. [DeGroot (A-200) P. 54 L. 20-21]

350. In conducting the Logistic Regression Model to analyze the NARMS data, CDC failed to explore how the independent variables and outcome measured vary with respect to passage of time; this analysis also obliterated the sequential relationship

among temporal identifiers which precluded analysis of trends because each year was considered in isolation. [DeGroot (A-200) P. 54 L. 21- P. 55 L. 4]

351. CDC fails to report on the ecological factors associated with varying ciprofloxacin resistance in different states and different years. [DeGroot (A-200) P. 55 L. 11-12]

352. The Human NARMS data provide no insight into national ciprofloxacin resistance trends among *Campylobacter* causing diarrhea in U.S. residents. [DeGroot (A-200) P. 55 L. 6-7]

353. Under the National Antimicrobial Resistance Monitoring program, there is not a population based sampling program for the collection of human *Campylobacter* isolates for antibiotic susceptibility testing. [Carnevale (A-199) P. 11 L. 2 – 22; P. 12 L. 17 – P. 13 L. 28; P. 85; P. 87; P. 88]

354. Under the National Antimicrobial Resistance Monitoring program, there is not a population based sampling program of human *Campylobacter* isolates for antibiotic susceptibility testing, as there is for all other bacteria monitored in the NARMS program. [Carnevale (A-199) P. 11 – L. 1 – 15; P. 13 L. 22 – 24]

355. Since there is not a population based sampling program for the collection of human *Campylobacter* isolates for antibiotic susceptibility testing under the NARMS program, the data generated by it for *Campylobacter* resistance cannot represent the rate of occurrence of *Campylobacter* resistant isolates in the United States or any representative subpopulation. [Carnevale (A-199) P. 12 L. 16 – P. 15 L. 15]

356. The antimicrobial susceptibility data for human *Campylobacter* isolates generated by the NARMS program do not represent the prevalence of fluoroquinolone resistant *Campylobacter*. [Carnevale (A-199) P. 12 L. 16 – P. 15 – 15; P. 88; P. 89]

357. Since a single year's NARMS data on human fluoroquinolone resistant *Campylobacter* is not population based and bears no relationship to the actual prevalence or rate of fluoroquinolone resistant *Campylobacter*, it is scientifically inappropriate and not meaningful to make year to year comparisons of the data. [Carnevale (A-199) P. 12 L. 16 – P. 15 – 15; P. 88; P. 89]

358. The NARMS data on human fluoroquinolone resistant *Campylobacter* has no bearing or relationship to the frequency of occurrence of fluoroquinolone resistant *Campylobacter* in any population. [Carnevale (A-199) P. 15 L. 2 – 15]

359. NARMS animal isolate testing is conducted at the USDA Agricultural Research Service (ARS), Russell Research Center in Athens, Georgia in Dr. Paula Fedorka-Cray's laboratory. [Tollefson (G-1478) P.7 L.45 – P.8 L.1-2]

360. The majority of the isolates included in the animal arm of NARMS are slaughter plant samples collected for the USDA Food Safety and Inspection Service (FSIS) compliance monitoring program that includes raw product from federally inspected

slaughter and processing establishments throughout the United States. [Tollefson (G-1478) P.8 L.7-12 P.9 L.15-16; DeGroot (A-200) P.3 L.12-13]

361. For NARMS FDA purposes, it is the slaughter plant isolates from the animal arm of NARMS that are analyzed. [Tollefson (G-1478) P.8 L.25-26; DeGroot (A-200) P.3 L.12-13]

362. Susceptibility testing of *Campylobacter* isolates from poultry were not added to the animal arm of NARMS until 1998. [Tollefson (G-1478) P.9 L.4-5; DeGroot (A-200) P.3 L.8-10, P.5 L.17-18]

363. The Poultry NARMS program suffers from methodological flaws and other problems that result in an inaccurate view of the overall prevalence of fluoroquinolone-resistant *Campylobacter* in production poultry. [DeGroot (A-200) P.4 L.1-3]

364. In order to utilize Poultry NARMS data to draw conclusions about the impact of any fluoroquinolone use in *poultry* on fluoroquinolone resistance levels in poultry *Campylobacter* isolates over time, one would need to know the level of pre-approval resistance. [DeGroot (A-200) P.5 L.5-8]

365. No credible pre-approval *Campylobacter* resistance data comparable to the NARMS poultry monitoring data are available that would serve as a baseline to allow for meaningful comparison to the 1998, 1999, 2000 and 2001 Poultry NARMS data. [DeGroot (A-200) P.5 L.18-21]

366. *Campylobacter* isolates stored and recovered later for testing can change susceptibility profiles. [DeGroot (A-200) P.5 L.23 – P.6 L.1]

367. As the Poultry NARMS program is designed, its data cannot show effects from fluoroquinolone use in poultry. No valid pre-1995 baseline of poultry *Campylobacter* resistance exists for comparison to post-1995 NARMS results. [DeGroot (A-200) P.6 L.3-5]

368. The yearly Poultry NARMS samples have been inconsistent with respect to poultry class and slaughter establishment type, season and geographic region across all years reported 1998 to 2001. [DeGroot (A-200) P.6 L.13-15]

369. The source of isolates provided by FSIS has not been consistent from year to year. [Tollefson (G-1478) P.9 L.15 – P.11 L.18; DeGroot (A-200) P.6 L.22-23]

370. For the period 1998 - 2000, all of the *Campylobacter* isolates from poultry in NARMS were obtained from raw product collected by the USDA/FSIS at federally inspected slaughter and processing establishments throughout the United States. [Tollefson (G-1478) P.9 L.5-9]

371. Isolates from the Animal division of NARMS underwent antimicrobial susceptibility testing in Dr. Paula Fedorka-Cray's laboratory. [Tollefson (G-1478) P.9 L.11-12]

372. FSIS personnel collected rinses from raw poultry carcasses from federally inspected slaughter establishments for NARMS testing. [Tollefson (G-1478) P.9 L.15-16]

373. In 1998, NARMS poultry carcass rinses (also called rinsates) were sent to one of three FSIS laboratories (Athens, Georgia; Alameda, California; St. Louis, Missouri) and analyzed for *Campylobacter jejuni/coli* according to the procedures described in the FSIS Microbiology Laboratory Guidebook using the most probable number method. [Tollefson (G-1478) P.9 L.18-24]

374. In 1998, Dr. Fedorka-Cray's laboratory only received isolates from the Athens, Georgia FSIS laboratory. [Tollefson (G-1478) P.9 L.25-27]

375. In February 1999, all three FSIS laboratories were directed to submit *Campylobacter* isolates to the ARS laboratory and in March 1999, ARS began receiving *Campylobacter* isolates from all three FSIS laboratories. [Tollefson (G-1478) P.9 L.27-31]

376. From approximately October 1998 until May 2000 FSIS conducted the Chicken Monitoring Program for *Campylobacter*. Rinses from all classes of raw chicken carcasses were analyzed for *Campylobacter jejuni/coli*. [Tollefson (G-1478) P.10 L.8-11; DeGroot (A-200) P.7 L.2-9]

377. From 1998-2000 NARMS methods used susceptibility to nalidixic acid as diagnostic of *Campylobacter jejuni/coli* identification. [Tollefson (G-1478) P.10 L.11-14, P.12 (chart)]

378. From November 1999 through November 2000 FSIS conducted the Nationwide Young Chicken (primarily broilers) Microbiological Baseline Data Collection Program to 1) estimate the prevalence of *Salmonella* and 2) to estimate the prevalence and levels of *Campylobacter jejuni/coli*. [Tollefson (G-1478) P.10 L.11-14, P.12 (chart); see, also DeGroot P.7 L.2-19]

379. From January 1999 through October 1999 FSIS conducted the Shakedown Nationwide Young Chicken Microbiological Baseline Data Collection Program for *Campylobacter* to estimate the national prevalence and levels of *Campylobacter jejuni/coli* in young chickens, primarily broilers. [Tollefson (G-1478) P.10 L.16-26; DeGroot (A-200) P.7 L.2-9]

380. If the NARMS data are to be used to measure the potential public health threat, and if multiple classes of birds are to be sampled, then the estimates produced must be adjusted to accurately reflect the different contribution each different class of bird makes to the overall campylobacteriosis risk. [DeGroot (A-200) P.7 L.16-19]

381. Poultry NARMS does not distinguish isolates by chicken type. Thus it is impossible to adjust estimates for the degree of risk posed to the consuming public by different classes of chickens processed at different slaughter facility types. [DeGroot (A-200) P.7 L.19-22]

382. NARMS poultry carcass rinse specimens from 1998 were collected only during the last quarter. [Tollefson (G-1478) P.10 L.22; DeGroot (A-200) P.8 L.7]

383. NARMS poultry carcass rinse specimens for 1999 were collected for the entire year. [Tollefson (G-1478) P.12 (chart); DeGroot (A-200) P.8 L.7-8]

384. NARMS poultry carcass rinse specimens for the year 2000 were only collected from January through October. [Tollefson (G-1478) P.10 L.38; DeGroot (A-200) P.8 L.8-9]

385. *Campylobacter* carriage in chickens varies with the season and resistance patterns of *Campylobacter* carried by chickens also vary by season. [DeGroot (A-200) P.8 L.9-11]

386. Because seasonality plays a role in *Campylobacter* carriage and resistance rates, yearly estimates presented by Poultry NARMS are confounded with seasonal variation. [DeGroot (A-200) P.8 L.11-13]

387. The most probable number method described in the FSIS Microbiology Guidebook and used in the FSIS microbiology laboratories for the period from October 1998 through October 2000 for identification of *Campylobacter jejuni/coli* used nalidixic acid susceptibility and cephalothin resistance as part of their identification protocol for identification of *Campylobacter jejuni/coli.* [Tollefson (G-1478) P.10 L.29-38]

388. In 2001, Dr. Paula Fedorka-Cray at ARS assumed responsibility for isolating *Campylobacter jejuni/coli* from the rinses. Culture methodology was changed to protocols routinely used in the ARS laboratory and use of nalidixic acid susceptibility and cephalothin resistance as a confirmatory test was discontinued. [Tollefson (G-1478) P.10 L.45 – P.11 L.1-3]

389. Beginning in January 2001, isolation of *Campylobacter* was conducted in Dr. Cray's ARS laboratory using spent FSIS *Salmonella* compliance broiler rinsates and using the ARS isolation and identification methods. That method did not use susceptibility to nalidixic acid and resistance to cephalothin for characterization of isolates. [Tollefson (G-1478) P.11 L.5-10]

390. Valid surveillance programs ensure that samples representative of the nation are taken if the data is to be used to extrapolate a national prevalence. [DeGroot (A-200) P.9 L.5-6]

391. Poultry NARMS sampling during all of 2001 and at least the first quarter of 2002 was not national in scope. [DeGroot (A-200) P.8 L.20-21]

392. For 2001, only rinsates from the Eastern FSIS laboratory (located in Athens, Georgia) were available for antimicrobial susceptibility testing in the animal arm of NARMS. [Tollefson (G-1478) P.11 L.10-13; DeGroot (A-200) P.8 L.21-23]

393. Poultry NARMS inappropriately tries to apply geographically limited data to the entire nation without a rational basis. [DeGroot (A-200) P.9 L.6-8]

394. A surveillance system must employ consistent laboratory methods in order to provide estimates that are validly comparable over time. [DeGroot (A-200) P.9 L.11-12]

395. Poultry NARMS employed non-standardized and varying microbiological isolation and testing methods over the reporting years from 1998 to 2001. [DeGroot (A-200) P.9 L.12-14]

396. Culture and isolation methods can affect subsequent antimicrobial susceptibility test results from the *Campylobacter* recovered. [DeGroot (A-200) P.10 L.4-5]

397. Resistance estimates resulting from different microbiological methods cannot be compared without first adjusting for the effects of the different methods. [DeGroot (A-200) P.10 L.5-6]

398. The very process of isolating *Campylobacters* for susceptibility testing can select for fluoroquinolone resistance. [DeGroot (A-200) P.10 L.13-14]

399. In 2001, two different methods (conventional and spin) for isolation of *Campylobacter* were being used when recovery of *Campylobacter* appeared to be lower for one of the methods. [Tollefson (G-1478) P.11 L.20-23]

400. A much lower recovery (approximately 11%) than previously reported by other laboratories, including reports by FSIS, was observed during 2001 with the conventional method. [Tollefson (G-1478) P.11 L.28-31]

401. Dr. Fedorka-Cray refers to the "spin method" as the "ARS Optimized method" for isolation of *Campylobacter*. [Tollefson (G-1478) P.11 L.36-38]

402. In 2002, isolation and testing of *Campylobacter* for the animal arm of NARMS is ongoing in the ARS lab using spent FSIS *Salmonella* compliance broiler rinsates and the ARS Optimized Method, which does not use susceptibility to nalidixic acid and resistance to cephalothin for identification of *Campylobacter jejuni/coli* isolates. [Tollefson (G-1478) P.11 L.40-45]

403. The National Antimicrobial Resistance Monitoring System ("NARMS") does not provide data that can be interpreted representing general patterns for the entire United States. [DeGroot (A-200) P.13 L.13-18, citing G-644]

404. NARMS is not designed to link emergent animal resistance and emergent human resistance. [DeGroot (A-200) P.13 L.13-18, citing G-644]

405. Annual NARMS data on *Campylobacter* antimicrobial resistance patterns cannot be meaningfully compared year to year because of differences in sampling patterns. [DeGroot (A-200) P.13 L.13-18, citing G-644]

406. Use of antimicrobials during culture can confound recovery. [DeGroot (A-200) P.12 L.7-8]

407. Further, mixed populations have been observed and aggregation of some strains not only affects speciation, but antimicrobial testing as well." [DeGroot (A-200) P.12 L.8-9]

408. NCCLS documents (specifically; NCCLS M31-A2, 2002, M7-A5, 2000) state that a specimen containing mixed growth of normal flora, in which the organisms bear little relationship to the infectious process being treated, susceptibility tests are often unnecessary, and the results may be misleading. [Silley (B-1913) Attachment 1 P.31 ¶ 2]

409. *C. upsaliensis* is a recently emerged pathogen in immunosuppressed patients as well as infants. [Silley (B-1913) P.34 ¶ 2 (see Goosens et.al.,1990)]

410. *C. upsaliensis* is found in high prevalence (27-55%) in dogs and is 11-19% prevalent in cats. [Silley (B-1913) Attachment 1 P.34 ¶ 2]

411. In a recent study report from Los Angeles, California *C. upsaliensis* was the second most frequently reported species of *Campylobacter* isolated from human patients. [Silley (B-1913) Attachment 1 P.35 ¶ 1]

412. Dogs and cats are a likely source of *C. upsaliensis* in people. [Silley (B-1913) Attachment 1 P.35 ¶ 1]

413. *C. upsaliensis* is easily missed on culture because it will be inhibited by antibiotics (specifically, cephalosporins) in most frequently used Campy enrichment media. [Silley (B-1913) Attachment 1 P.34 & 36.

414. The poultry NARMS programs has not been collected using a consistent sampling methodology year-to-year. [Carnevale (A-199) P.5 L.6-9; Tollefson (G-1478) P.9-10]

415. 1998 NARMS poultry data was collected: from May to September 1998, where the samples were taken for the purpose of isolating *Salmonella* under the FSIS HACCP program and were also used by FSIS to isolate *Campylobacter* for susceptibility testing in the NARMS program; and from October 1998 to December 1998, where FSIS *Campylobacter* cultures were obtained from a FSIS *Campylobacter* chicken monitoring program on various classes of young chickens, spent hens, etc., that originated primarily from the Eastern FSJS lab in Athens, GA. [Carnevale (A-199) P.5 L.11-19; Tollefson (G-1478) P.9-10]

416. 1999 NARMS poultry data was collected: from January 1999 to December 1999 from FSIS *Campylobacter* cultures obtained from a FSIS *Campylobacter* chicken monitoring program on various classes of young chickens, spent hens, etc., that originated primarily from the Eastern FSIS lab in Athens, GA; from January 1999 to October 1999, where *Campylobacter* isolates from the pilot program for a FSIS *Campylobacter* baseline study were utilized; and from November 1999 to December 1999, where *Campylobacter* isolates from a FSIS baseline study were utilized. [Carnevale (A-199) P.5 L.19-26; Tollefson (G-1478) P.9-10]

417. 2000 NARMS poultry data was collected: from January 2000 to May 2000 from FSIS *Campylobacter* cultures obtained from a FSIS *Campylobacter* chicken monitoring program on various classes of young chickens, spent hens, etc., that originated primarily from the Eastern FSIS lab in Athens, GA; and from January 2000 to October 2000, where *Campylobacter* isolates from a FSIS baseline study were utilized. [Carnevale (A-199) P.6 L.1-6; Tollefson (G-1478) P.9-10]

418. 2001 NARMS poultry data was collected from rinsates sent from HACCP testing in slaughter plants to the USDA Agriculture Research Service (ARS) after FSIS isolation of *Salmonella*. ARS then cultured these rinsates for *Campylobacter* and performed susceptibility testing on recovered isolates. [Carnevale (A-199) P.6 L.8-11; Tollefson (G-1478) P.10-11]

419. 2002 NARMS poultry data was collected from rinsates sent from HACCP testing in slaughter plants after the USDA Agriculture Research Service (ARS) after FSIS isolation of *Salmonella*. ARS then cultures these rinsates for *Campylobacter* and performs susceptibility testing on recovered isolates. [Carnevale (A-199) P.6 L.12-16; Tollefson (G-1478) P.11]

420. From the initiation of *Campylobacter* testing in 1998 through 2001, the sampling sources for *Campylobacter* isolates used in the animal NARMS program were very different. [Carnevale (A-199) P.6 L.17-18]

421. Because no defined, statistically sound, designed sampling source has been used for NARMS poultry, the fluoroquinolone susceptibility patterns determined by the analysis of the NARMS *Campylobacter* isolates neither represent the prevalence of fluoroquinolone-resistant *Campylobacter* present on chicken carcasses at the time of slaughter in the US nor such prevalence in live chickens. [Carnevale (A-199) P.6 L.21-26]

422. The culture techniques utilized to isolate *Campylobacter* in the animal NARMS program have not been consistent. For example, the methodology used to culture and isolate *Campylobacter* from HACCP samples by FSIS in 1998 and forwarded to ARS for susceptibility testing was significantly different than the methodology used by ARS in 2001 and 2002 to isolate *Campylobacter* from the rinsates received from FSIS after FSIS' use of the rinsates to isolate *Salmonella*. [Carnevale (A-199) P.7 L.2-8]

423. In 1998, 2001 and again in 2002, the animal NARMS program has used HACCP samples as the source of the *Campylobacter* tested. Analysis of *Campylobacter* isolated from HAACP samples will not allow the true prevalence rate of *Campylobacter* on chicken carcasses or their susceptibility patterns to be determined. [Carnevale (A-199) P.7 L.18-22]

424. HACCP samples are not a representative, random sample because the FSIS sampling program has a higher testing rate of poultry processing facilities with higher bacterial contamination. This biases the results of analysis of the samples toward higher levels of bacteria. [Carnevale (A-199) P.8 L.5-9]

425. The *Campylobacter* susceptibility patterns determined from analysis of the HACCP samples, initially collected by FSIS for *Salmonella* isolation and used for *Campylobacter* as an add-on, do not represent a national prevalence, and cannot be used for year to year comparison for trends. [Carnevale (A-199) P.8 L.16-19]

426. CVM contends that the causal relationship between fluoroquinolone use in poultry and increased cases of fluoroquinolone resistance is inferred because of a temporal relationship. [CVM Answer to Bayer Interrogatory 12]

427. There is no clear evidence that resistance to fluoroquinolones has increased over time, especially post licensing, in poultry *Campylobacters*. Moreover, data indicates that resistant poultry isolates were present even before the licensing of fluoroquinolones for use in poultry. [Newell (B-1908) P.14 L.17-20]

428. Evidence that veterinary use of fluoroquinolones results in the generation of fluoroquinolone resistance in *Campylobacter*, that such resistance is sustained over time, and that such strains can be transmitted to infect humans, is not convincing. [Newell (B-1908) P.39 L.6-8]

429. CVM's hazard identification and its whole risk assessment fail to assess any evidence for a causal relation between use of enrofloxacin in chickens and adverse human health consequences. [Cox (B-1901) P.26]

430. Any "temporal relation" between introduction of enrofloxacin and increase in fluoroquinolone-resistant *Campylobacter* rates only shows that some fluoroquinolone-resistant *Campylobacter* rates increased following introduction of enrofloxacin. This is not evidence of causation between the two. [Cox (B-1901) P.26]

431. CVM has not applied any generally accepted objective methods for identifying causal relations from the available data to discover whether causation is truly present and or to quantitatively estimate causal effects. [Cox (B-1901) P.27]

432. When generally accepted objective tests for identifying causal relations are performed on the available data, they indicate a complete absence of any significant positive causal relation between enrofloxacin use in chickens and fluoroquinolone-resistant campylobacteriosis rates in humans. [Cox (B-1901) P.27]

433. CVM's contention that the introduction of enrofloxacin in poultry in 1996 is the probable cause of the increase in fluoroquinolone-resistance in humans after 1996 is undermined by the fact that fluoroquinolone resistance in multiple bacteria in multiple countries has increased following the introduction and over-prescription of fluoroquinolones in human medicine, whether or not enrofloxacin has been used in food animals. [Cox (B-1901) P.27, citing B-119]

434. CVM's contention that the introduction of enrofloxacin in poultry in 1995 is the probable cause of the increase in fluoroquinolone-resistance in humans after 1995 is undermined by the fact that in no case has approval and use of enrofloxacin in other countries been demonstrated objectively to have influenced the existing trend of fluoroquinolone resistance in multiple bacteria in humans after the introduction and over-prescription of fluoroquinolones in human medicine. [Cox (B-1901) P.27]

435. CVM's contention that the introduction of enrofloxacin in poultry in 1996 is the probable cause of the increase in fluoroquinolone-resistance in humans after 1996 is undermined by the fact that in no case has approval and use of enrofloxacin in other countries been demonstrated objectively to have had any impact on the speed or magnitude of increase in fluoroquinolone-resistant campylobacteriosis in humans following the introduction of fluoroquinolones in human medicine. [Cox (B-1901) P.27]

436. CVM's contention that the introduction of enrofloxacin in poultry in 1995 is the probable cause of the increase in fluoroquinolone-resistance in humans after 1995 is undermined by the fact that the increase in fluoroquinolone-resistant campylobacteriosis over time has been comparable in countries with and without enrofloxacin use in broilers. [Cox (B-1901) P.27, citing B-29]

437. CVM's contention that the introduction of enrofloxacin in poultry in 1995 is the probable cause of the increase in fluoroquinolone-resistance in humans after 1995 is undermined by the fact that within each year, changes in *Campylobacter* carriage rates in humans tend to precede changes in *Campylobacter* carriage rates in chickens. This makes it unlikely that the human CP rates are caused primarily by the chicken rates and also provides some evidence for reverse causation (i.e., human *Campylobacter* may help cause chicken *Campylobacter*, perhaps through contaminated water, or perhaps a common environmental source contributes to both). [Cox (B-1901) P.27-28; *See also* Newell (G-1908) P.26 L.12-20)]

438. A study in Sweden published in 1981, long before fluoroquinolones had been introduced, showed that 39% of *C. jejuni* isolates from chickens were then already resistant to nalidixic acid, as were 11% of human isolates. [Gonder, (A-201 P.14 L.9-11; citing B-1851 (Svedhem 1981)]

439. Fluoroquinolones were not in use in chickens in Sweden in 1981. [Gonder (A-201) P.14 L.11-12; Joint Stipulation 64]

440. There exist temporal patterns that refute the hypothesis that human fluoroquinolone-resistant *Campylobacter* rates are caused by enrofloxacin use in poultry. [Cox (B-1901) P.29]

441. Analyzing airsacculitis condemnation data (as a proxy for enrofloxacin use) in Minnesota in relation to human fluoroquinolone-resistant *Campylobacter* rates in Minnesota from 1996-1999 shows that within each year, human fluoroquinolone-resistant *Campylobacter* rates are negatively correlated with recent airsacculitis condemnation rates in chickens. [Cox (B-1901) P.29]

442. Analyzing airsacculitis condemnation data (as a proxy for enrofloxacin use) in Minnesota in relation to human fluoroquinolone-resistant *Campylobacter* rates in Minnesota from 1996-1999 shows that within each year, human fluoroquinolone-resistant *Campylobacter* rates are significantly positively correlated with future airsacculitis condemnation rates in chickens, suggesting that use of enrofloxacin in chicken does not cause the human fluoroquinolone-resistant *Campylobacter*. [Cox (B-1901) P.29]

443. Nonparametric nonlinear regression analysis of the 1996-1999 Minnesota data suggests that there was an increase in the slope of the human fluoroquinolone-resistant *Campylobacter* rate (a change point) in early 1998, years after the introduction of fluoroquinolones in chickens. [Cox (B-1901) P.29]

444. Change points occurring at any time in the interval between 1995 and 2001, such as the one identified in 1998 by a nonparametric nonlinear regression analysis of the 1996-1999 Minnesota data can explain the types of "temporal relations" and "trends" that CVM and its witnesses refer to (e.g., Molbak testimony, G-1468, P.8, paragraph 24). [Cox (B-1901) P.29]

445. The increase in the slope of the human fluoroquinolone-resistant *Campylobacter* rate (a change point) in early 1998 is not related to anything that happened in 1995 or 1996, including enrofloxacin introduction. [Cox (B-1901) P.29]

446. Interpreting a statistically non-significant increase in prevalence ratio between 1997 and 2001 as evidence for an effect caused by a product introduced in 1995 is not scientifically valid. [Cox (B-1901) P.29, referring to Molbak (G-1468) P.8 L.29-32]

447. Fluoroquinolone-resistant campylobacteriosis trends have increased in countries without substantial enrofloxacin use. [Cox (B-1901) P.42]

448. CVM's contention that the introduction of enrofloxacin in poultry in 1995 is the probable cause of the increase in fluoroquinolone-resistance in humans after 1995 is undermined by the fact fluoroquinolone-resistant campylobacteriosis trends have also increased in countries without substantial enrofloxacin use. [Cox (B-1901) P.42]

449. Fluoroquinolone-resistant campylobacteriosis trends have also increased in species that are *not* prescribed enrofloxacin. [Cox (B-1901) P.42]

450. CVM's contention that the introduction of enrofloxacin in poultry in 1995 is the probable cause of the increase in fluoroquinolone-resistance in humans after 1995 is undermined by the fact fluoroquinolone-resistant *Campylobacter* trends have also increased in species that are *not* prescribed enrofloxacin. [Cox (B-1901) P.42]

451. CVM has never presented, nor is there evidence, any analysis showing that introducing fluoroquinolones into animal use has had any impact whatsoever on trends or the time series of human fluoroquinolone-resistant campylobacteriosis rates. [Cox (B-1901) P.42]

452. Without an analysis showing that introducing fluoroquinolones into animal use has had an impact on trends or the time series of human fluoroquinolone-resistant campylobacteriosis rates, there is no sound basis in time series analysis or statistical methodology for inferring that fluoroquinolone use in poultry is a cause of observed fluoroquinolone resistance in human campylobacteriosis cases. [Cox (B-1901) P.42]

453. Data on human fluoroquinolone-resistant campylobacteriosis rates from before the introduction of enrofloxacin for poultry use (such as the data reported in of Smith et al., 1999 and in Nachamkin et al., 2002) refute the hypothesis of a change in the time series of human fluoroquinolone-resistant campylobacteriosis rates in 1995 or 1996. [Cox (B-1901) P.42, referring to G-589 (Smith 1999) and G-1517 (Nachamkin 2002); *See also* DeGroot (A-200) P.55 L.20 – P.59 L.13]

454. Based on data on human fluoroquinolone-resistant campylobacteriosis rates from before the introduction of enrofloxacin for poultry use (such as the data reported in of Smith et al., 1999 and in Nachamkin et al., 2002), it appears that the trend of increasing human fluoroquinolone-resistant campylobacteriosis rates occurred before the introduction of enrofloxacin and continued without change when enrofloxacin was introduced. [Cox (B-1901) P.42, referring to G-589 (Smith 1999) and G-1517 (Nachamkin 2002)]

455. In the Nachamkin et al. data, human fluoroquinolone-resistant campylobacteriosis rates were lower in 1996 and 1997 than in 1995, and a significant (but unexplained) increase did not occur until 2000. [Cox (B-1901) P.42, referring to G-1517 (Nachamkin 2002); DeGroot (A-200) P.58 L.5-17; Newell (B-1908) P.42 L.1-5]

456. Human NARMS data for fluoroquinolone-resistant *Campylobacter* show a large degree of heterogeneity within states. [Cox (B-1901) P.43]

457. Human NARMS data for fluoroquinolone-resistant *Campylobacter* show that there is not an increasing trend of fluoroquinolone-resistant *Campylobacter* in some states. [Cox (B-1901) P.43; DeGroot (A-200) P.50 L.6 – P.54 L.10]

458. Human NARMS data for fluoroquinolone-resistant *Campylobacter* show that while the "trend" is upward in three states (CA, MN, OR), it is downward in one (NY) and uneven in 3 others (CT, GA, MD with only 2 submissions in 2000). Comparing 2000 to 1999, there were 5 increases and 3 decreases in

fluoroquinolone-resistant *Campylobacter* rates among 8 states sampled in both years. [Cox (B-1901) P.43]

459. Although the debate over fluoroquinolone-resistant *Campylobacter* has been shaped largely by data from MN, this data is not representative of the general US population, nor of other states such as NY. [Cox (B-1901) P.43, referring to G-589 (Smith 1999)]

460. Using trend data from Europe, one could find a "temporal relationship" (by CVM's criteria) between *bans* on animal antimicrobials in Europe and subsequent trends of *increasing* campylobacteriosis and salmonellosis rates in Europe. [Cox (B-1901) P.44]

461. CVM's finding of a "temporal relationship" to support its contention that use of fluoroquinolones in poultry causes increased fluoroquinolone-resistant campylobacteriosis in humans is an instance of a well-known logical fallacy (the ex post or false cause fallacy) and is not supported by more detailed data analysis. [Cox (B-1901) P.44]

462. CVM has not cited any facts or data indicating that fluoroquinolone use in chickens explains any part of the observed trends or levels of fluoroquinolone-resistant campylobacteriosis in humans in the US. [Cox (B-1901) P.54]

463. From 1995 to present, per capita chicken consumption in the United States has increased every year compared to the prior year, and over all has increased 12%. [Cox (B-1901) P.28]

464. Fluoroquinolone-resistant *Campylobacter* were identified in humans before 1995, before fluoroquinolones were approved for use in any food animal, including poultry. [DeGroot (A-200) P.59 L.14 – P.60 L.11]

465. In 1988 Barrett found 5% quinolone resistance in *Campylobacter jejuni* isolated from humans, before fluoroquinolones were approved for use in any food animal, including poultry. [Barrett (G-1453) P.3 L.3-10; G-1609; Cox (B-1901) P.71]

466. Kiehlbach found 12% quinolone resistance in *Campylobacter* isolated from humans from August 1992 to April 1995, before fluoroquinolones were approved for use in any food animal, including poultry. [DeGroot (A-200) P.59 L.18-20, citing B-39]

467. In 1992 Smith found 1.3 % fluoroquinolone resistance in *Campylobacter* isolated from humans, and 6% resistance in isolates from 1995, both before fluoroquinolones were approved for use in any food animal, including poultry. [Cox (B-1901) P.34; *See also* G-589]

468. Williams found 3.3% quinolone resistance in *Campylobacter* isolated from humans in 1993, before fluoroquinolones were approved for use in any food animal, including poultry. [DeGroot (A-200) P.59 L.22 – P.60 L.4, citing B-67]

469. Nachamkin found over 20 % fluoroquinolone resistance in *Campylobacter* isolated from humans in 1995, before enrofloxacin was approved and sarafloxacin was actively marketed for use in any food animal, including poultry. [Cox (B-1901) P.34; *See also* G-1517]

470. CVM has not used any scientifically accepted methodologies for drawing causal inferences from time series, trend data, and pre-approval/post-approval comparisons to support its contention that use of fluoroquinolones in poultry causes increased fluoroquinolone-resistant campylobacteriosis in humans. [Cox (B-1901) P.44]

471. Applying scientifically accepted methodologies for drawing causal inferences from time series, trend data, and pre-approval/post-approval comparisons to examine CVM's contention that use of fluoroquinolones in poultry causes increased fluoroquinolone-resistant campylobacteriosis in humans demonstrates that a causal inference is not justified. [Cox (B-1901) P.44]

472. CVM has not performed any formal statistical tests of the causal hypothesis that fluoroquinolone use in chickens causes increases in fluoroquinolone-resistant *Campylobacter* infections in humans. [CVM's Answer to Bayer's Interrogatory 13]

473. CVM has not performed any formal statistical tests of the causal hypothesis that fluoroquinolone use in chickens causes decreases in fluoroquinolone-resistant *Campylobacter* infections in humans. [CVM's Answer to Bayer's Interrogatory 14]

474. CVM has not performed any conditional independence tests for possible causality in any sets of data that involve fluoroquinolone use in chickens and fluoroquinolone-resistant *Campylobacter* infections in humans, including data sets from the CDC 1998 - 1999 *Campylobacter* Case-Control study (G-1644), Smith et al. (G-589), and Effler et al. (G-185). [CVM's Answer to Bayer's Interrogatory 16]

475. A causal analysis (conditional independence tests for causality (e.g., Shipley, 2000)) on the data sets from the CDC 1998 - 1999 *Campylobacter* Case-Control study (Friedman et al., 2000), Smith et al. (1999), and Effler et al. (2001), indicate that there is no detectable causal relation between chicken consumption and fluoroquinolone-resistant campylobacteriosis rates in people. [Cox (B-1901) P.45, referring to G-1644 (Friedman 2000), G-589 (Smith 1999) and G-185 (Effler 2001)]

476. CVM has not developed any causal graph models or path analysis models from data that involve fluoroquinolone use in chickens and fluoroquinolone-resistant *Campylobacter* infections in humans, including data sets from the CDC 1998 - 1999 *Campylobacter* Case-Control study (G-1644), Smith et al. (G-589), and Effler et al. (G-185). [CVM's Answer to Bayer's Interrogatory 17]

477. Causal graph modeling for the CDC 1998 - 1999 *Campylobacter* Case-Control study data set (G-1644) and the Smith et al. (G-589) case-control data set, refutes the hypothesis that chicken consumption is a likely cause of campylobacteriosis cases or fluoroquinolone-resistant campylobacteriosis cases in humans. [Cox (B-1901) P.47, citing B-1020 and B-1252]

478. CVM has not performed any formal statistical tests for omitted explanatory variables and/or confounders in analyzing possible statistical associations between fluoroquinolone use in chickens and fluoroquinolone-resistant *Campylobacter* infections in humans. [CVM's Answer to Bayer's Interrogatory 18]

479. Application of formal statistical tests for omitted explanatory variables and/or confounders in analyzing possible statistical associations between fluoroquinolone use in chickens and fluoroquinolone-resistant *Campylobacter* infections in humans explains away 100% of the campylobacteriosis and fluoroquinolone-resistant campylobacteriosis risks that CVM attributes to chicken. [Cox (B-1901) P.47]

480. If any confounder or combination of confounders fully explains away an observed positive relation between chicken consumption and campylobacteriosis risk or fluoroquinolone-resistant campylobacteriosis risk, then banning enrofloxacin could reduce the prevalence of fluoroquinolone-resistant *Campylobacter* in chicken without affecting risk to humans at all. [Cox (B-1901) P.49]

481. The effects of confounders (non-causal statistical associations) must be removed in order to isolate the true causal relation (probably negative) between fluoroquinolone use in chickens and risk of campylobacteriosis and fluoroquinolone-resistant campylobacteriosis illness-days in people. Until corrected for confounders, data sets from the CDC 1998 - 1999 *Campylobacter* Case-Control study (G-1644), Smith et al. (G-589), and Effler et al. (G-185) cannot be interpreted or used to predict what human health effects will be caused by actions such as withdrawing the NADA for enrofloxacin. [Cox (B-1901) P.49]

482. When data sets from the CDC 1998 - 1999 *Campylobacter* Case-Control study (G-1644), Smith et al. (G-589), and Effler et al. (G-185) are corrected for confounders, no association between chicken consumption and increased campylobacteriosis risk or fluoroquinolone-resistant campylobacteriosis risk remains. [Cox (B-1901) P.49, citing B-1252]

483. Attributable fraction calculations do not in general identify, adjust for, or remove the effects of confounders or other risk factors. [Cox (B-1901) P.49, P.62]

484. Causal graph modeling allows the effects of confounders to be modeled and the direct causal contribution of chicken consumption to campylobacteriosis risk to be isolated. Applying this technique shows that removing the effects of confounding removes the entire association between chicken consumption and human campylobacteriosis risk. [Cox (B-1901) P.49, citing B-1020 and B-1252]

485. CVM's estimation of a non-zero risk between chicken consumption and human campylobacteriosis is based entirely on failure to properly correct for confounders. [Cox (B-1901) P.49]

486. CVM has not applied any generally accepted methods of causal inference for interrupted time series and/or quasi-experimental designs to demonstrate a probable causal relation between fluoroquinolone use in chickens and fluoroquinolone-resistant *Campylobacter* infections in humans. [CVM Answer to Bayer Interrogatory 40]

487. In interpreting historical trends and data on associations between fluoroquinolone use in chickens and fluoroquinolone-resistant *Campylobacter* infections in humans, CVM did not control for the possibility of spurious regression. [CVM Answer to Bayer Interrogatory 41]

488. Spurious regression provides another potential non-causal explanation (in addition to confounding) for associations between variables that are tracked over time. [Cox (B-1901) P.53]

489. In the absence of controls for spurious time series associations and threats to validity, CVM's inference of a causal relation from the claimed "temporal relationship" between enrofloxacin introduction and increasing fluoroquinolone-resistant campylobacteriosis rates in humans is unwarranted. [Cox (B-1901) P.53]

490. The most discriminating molecular subtyping methods do not independently provide "causal" evidence. They can however provide information supportive of epidemiologic findings. [Besser (G-1455) P.6 L.43 – P.7 L.3] [Tenover (G-1476) P.4 L.10-12; L.34-36]

491. Smith et al conducted a retail survey of chicken products from September 8 to November 3, 1997, in the Minneapolis-St. Paul metropolitan area. These samples were cultured for *Campylobacter*. [Smith (G-589) P.2]

492. Smith et al conducted their retail survey of chicken carcasses during the time of the year that has historically proven to give the highest probability of finding *Campylobacter jejuni* positive carcasses, as pointed by Willis in G-701. [Smith (G-589), Harris (B-387) P.3-4, Willis (G-701) P.3]

493. Domestically acquired fluoroquinolone-resistant *C. jejuni* isolates from MN residents for the calendar year of 1997 having the same fla-A PCR/RFLP types as fluoroquinolone-resistant *C. jejuni* from chicken products isolated in the Minneapolis/St. Paul area in September, October, and November of 1997 were not shown to be "clonal" in the study referred to be Smith as exhibit G-589. [Smith (G-1473); G-589]

494. Only "clonal" isolates of *C. jejuni* can support epidemiologic evidence for "causality" in studies where both types of work are performed. [Newell (B-1908) P.31 L.5 – P.32 L.4]

495. Clones are genetically related isolates that are indistinguishable from each other by a variety of genetic typing tests or are so similar that they are presumed to be derived from a common parent. [Besser (G-1455) P.3 L.6-8]

496. Fla-A PCR/RFLP subtyping is a weakly discriminatory subtyping test, (roughly equivalent to serotyping) as compared to PFGE, MLST, or AFLP subtyping methods. PFGE, MLST, and AFLP are considered to be more discriminatory and better able to establish clonality than fla-A PCR/RFLP subtyping. [Nachamkin (G-1470) P.8 L.29; Barrett (G-1453) P.5 L.27,28; Besser (G-1455) P.9 L.5-8; Endtz (G-1457) P.5; L.8-12 & L.12-15; G-1752; G-176; Newell (B-1908) P.34 L.19 - P.35 L.21]

497. The most discriminating molecular subtyping methods such as PFGE, MLST, AFLP, are useful in assessing clonal similarities and "genetic overlap" between animal, human and environmentally sourced organisms including *Campylobacter* sps. [Newell (B-1908) P.34 L.19 – P.35 L.21, P.36 L.5-24; G-1785; G-1629; G-1630]

498. Molecular subtyping methods may be useful in supporting or undermining epidemiological findings. [Besser (G-1455) P.6 L.43 – P.7 L.3]

499. All molecular subtyping methods have their greatest utility in supporting or undermining epidemiological findings when isolates obtained are closely linked in time and space with the epidemiological findings. [Tenover (G-1476) P.4 L.27,28 & P.7 L.9-23]

500. Molecular subtyping methods are more supportive in "outbreak" investigations than in cases of sporadic Campylobacteriosis because the temporal and geographical relationship of isolated organisms to epidemiologic findings are more easily established. [Tenover (G-1476) P.4 L.32-34] [Besser (G-1455) P.9 L.5-8 & P.6 L.43 – P.7 L.3]

501. Molecular subtyping methods are less useful in assessing clonal relationships in isolates disparate in time and space due to genetic drift of the organisms. [Tenover (G-1476) P.8 L.18,19] [Newell (B-1908) P.30 L.6-14]

502. *Campylobacter* sps of human and animal origins have been shown to be genetically unstable. B-33. [Newell (B-1908) P.29 L.23 – P.30 L.14]

503. Inter and intragenomic recombination has been shown to occur within the fla A&B loci of *Campylobacter jejuni*. [B-33]

504. The flagellin locus in *C. jejuni* is considered to be unstable. [B-33]

505. Control strains of *C. jejuni* are not reported to have been used in the fla-A subtyping study reported in exhibit [G-589]

506. Low discriminating molecular subtyping methods are generally less useful for interpretive purposes than high discrimination molecular subtyping methods. [Newell (B-1908) P.35 L.1-12]

507. The only reference placed in evidence on genetic overlap in the U.S. involving human and poultry isolates in a similar region, over a similar time frame is that of Avery Dickins et. al. [G-1785]

508. The M. Avery Dickins et. al. study (G-1785) estimates human/poultry clonal overlap of *Campylobacters* to be 6-8 %. [G-1785]

509. *Campylobacter jejuni* isolates from different sources may share identical fla –A banding patterns. This could result in erroneously concluding that two non-clonal isolates were the same. [Tenover (G-1476) P.4 L.18-20] [Smith (G-1473) P.14 L.20,21]

510. By chance, epidemiologically unrelated isolates can have similar or indistinguishable genotypes. [Tenover (G-1476) P.4 L.18-20] [Smith (G-1473) P.14 L.20,21]

511. Fla – A subtyping is considered to be of low to moderate discrimination value and cannot establish clonal relationships from isolates disparate in time and space. [Newell (B-1908) P.30 L.15-18]

512. PFGE is a superior (more discriminatory) subtyping method to fla-A subtyping. [Nachamkin (G-1470) P.8 L.29]

513. Because fluoroquinolone-resistant *Campylobacters* are different from wild type *Campylobacters* by only a single base pair change, they can be considered a smaller subset of the fluoroquinolone-susceptible *Campylobacter* population. [Meng (G-1466) P.4 L.10-14]

514. Clonally shared populations of *Campylobacter* in humans and poultry are most likely to be identified in the populations, all things being equal, since these represent the largest fraction in each group. [Newell (B-1908) P.31-36]

515. Clonal overlap studies in the U.S.(G-1785) describe smaller clonal overlap populations in human and poultry *Campylobacters* than do studies from Canada (B-553) and other European countries [B-380]

516. The "concordance" argument of Besser (G-1455; P.11) between the alignment of fluoroquinolone-resistant fla-A types in human cases and poultry products is not biologically plausible because fluoroquinolone-susceptible strains are far more prevalent in MN resident cases and in MSP purchased chicken products than are fluoroquinolone-resistant strains. All things being equal, it would be far more likely to see concordance between the types in higher prevalence than between the types of low prevalence. [Newell (B-1908) P.31-36]

517. There is no evidence that fla-A PCR/RFLP typing was "blinded" in the subtyping analysis performed in the report of exhibit G-589. [Tenover (G-1476) P.4 L.31,32]

518. PFGE is validated for *Campylobacter*. [Tenover (G-1476) P.10 L.8]

519. Discriminatory power is an important attribute of any typing system. [Tenover (G-1476) P.10 L.1-2]

520. Common source routes of infection cannot be ruled out for populations that have overlapping *Campylobacter* genotypes. [Newell (B-1908) P.38 L.17-20] [Smith (G-1473) P.14 L.20-25]

521. The epidemiologic findings of G-589 are negative for poultry as a source of *Campylobacter* or fluoroquinolone-resistant *Campylobacter*. [G-589]

522. Consumption of poultry meat is not a risk factor for infection with fluoroquinolone-resistant *Campylobacter* in domestically acquired *Campylobacter* cases in the UK. [Newell (B-1908) P.40 L.16-22]

523. Data showing a genetic overlap between *Campylobacter* isolated from chicken and *Campylobacter* isolated from humans are consistent with the hypotheses of reverse causation (human effluents contaminate chicken flocks, perhaps via intermediate vectors) and common third causes (both humans and chickens are contaminated by some other environmental source). [Cox (B-1901) P.28, citing Hanninen, G-1458, P.7 ¶ 11)]

524. Evidence that chickens share *Campylobacter* subtypes with lambs and other animals (presumably not because one species eats the other) indicates that the common third cause interpretation may be the most plausible hypothesis. [Cox (B-1901) P.28]

525. CVM's hazard identification step of the CVM/Vose Risk Assessment incorrectly identifies chicken as the predominant source of campylobacteriosis and fluoroquinolone-resistant campylobacteriosis in humans. [Cox (B-1901) P.14]

526. Correct causal analysis of CDC and other data shows that chicken consumption per se is not a predominant cause of human campylobacteriosis, [Cox (B-1901) P.15, citing Exhibit G-1681); *See also* G-1488 (Friedman 2003) and G-1489 (Nelson 2003)]

527. Chicken handled or prepared at home is associated with a statistically significant *reduction* in risk of campylobacteriosis. [Cox (B-1901) P.15, citing G-1644 (Friedman 2000), G-185 (Effler 2001) and B-1252 (Cox 2002); *See also* G-1488 (Friedman 2003) and G-1489 (Nelson 2003)]

528. The finding that chicken handled or prepared at home is associated with a statistically significant *reduction* in risk of campylobacteriosis is not considered in the CVM/Vose Risk Assessment model. [Cox (B-1901) P.15]

529. Not accounting for the finding that chicken handled or prepared at home is associated with a statistically significant *reduction* in risk of campylobacteriosis in the CVM/Vose Risk Assessment model results in the chicken-attributable fractions and other quantities in the CVM/Vose Risk Assessment model incorrectly describing the chicken-campylobacteriosis relation in the current general US population. [Cox (B-1901) P.15, P.57-64]]

530. Analyzing the recent large case-control data set provided by CDC (The 1998 - 1999 FoodNet *Campylobacter* case-control study data set) shows that, with high statistical confidence, there is no detectable association between chicken consumption and risk of fluoroquinolone-resistant campylobacteriosis in humans. [Cox (B-1901) P.15; citing B-1252]

531. The finding that there is no detectable association between chicken consumption and risk of fluoroquinolone-resistant campylobacteriosis in humans has also been confirmed by analyzing the Smith et al. 1999 data set. [Cox (B-1901) P.15; citing B-1252]

532. A traditional definition of exposure assessment is "the qualitative and/or quantitative evaluation of the degree of intake likely to occur". The traditional risk assessment framework considers that the amount of contamination ingested by individuals (e.g., expressed as a population frequency distribution of CFUs, or colony-forming units, of *Campylobacter* ingested in meals) is crucial for quantifying risk. This reflects the fundamental principle that "the dose makes the poison". [Cox (B-1901) P.16]

533. FDA has recognized the key concept of exposure assessment in its own previous definitions for other microbial risk assessments, e.g., in defining exposure assessment as "A component of a risk assessment that characterizes the source and *magnitude* of human exposure to the pathogen", while equating magnitude of human exposure (i.e., "dose") to "The *amount* or *number* of a pathogen that is ingested or interacts with an organism (host)". [Cox (B-1901) P.16]

534. The CVM/Vose Risk Assessment model does not quantify or characterize the amount of exposure of humans to *Campylobacter* or fluoroquinolone-resistant *Campylobacter*. [Cox (B-1901) P.16]

535. The CVM/Vose Risk Assessment model does not follow the established concepts of exposure assessment. Instead, it seeks to quantify the "total *prevalence* of *Campylobacter* [and of fluoroquinolone-resistant *Campylobacter*] among broiler carcasses" (G-953, P.4-2 emphasis added). That is, it examines only the proportion of carcasses with some *Campylobacter* contamination present, but does not quantify how much contamination is present. [Cox (B-1901) P.16; citing G-953]

536. Rosenquist et al. (G-1788) demonstrated the need for quantitative detection methods; "The minor effect [less than 10% reduction] on the number of [*Campylobacter*-] positive carcasses at the end of slaughter [of] ... a relatively

large reduction in the number of *Campylobacter* on the chickens, for example, a reduction of 3 $\log_{10}$ CFU/chicken... demonstrates the need for quantitative detection methods. ... The incidence of campylobacteriosis related to consumption of chicken was reduced significantly by reducing the number of *Campylobacter* on the carcasses, even though such a reduction had almost no influence on the fraction of positive chickens." [Cox (B-1901) P.16-17; citing G-1788 (Rosenquist 2002)]

537. Despite the demonstrated need for quantitative detection methods as delineated in the scientific literature, the CVM/Vose Risk Assessment model does not incorporate quantitative assessment of microbial load, instead using the fraction of *Campylobacter* positive chickens. [Cox (B-1901) P.17]

538. As demonstrated by Rosenquist et al., the mere the fraction of *Campylobacter* positive chickens is insensitive to changes in microbial loads that greatly affect human health. Hence, in general, human health risks are *not* proportional to the fraction of *Campylobacter*-positive ("contaminated") chickens, in contrast to the CVM/Vose Risk Assessment model's major assumptions. [Cox (B-1901) P.17; citing G-1788 (Rosenquist 2002)]

539. The linear CVM/Vose Risk Assessment model assumed by CVM should not be expected to produce realistic or accurate answers about the effects of risk management interventions because the change in human health risk is *not* directly proportional to the prevalence of contamination. For example a reduction of less than 10% in the fraction of positive chickens leaving the slaughterhouse can correspond to more than a 30-fold reduction in human campylobacteriosis cases. The change in human health risk (30-fold) is *not* directly proportional to the prevalence of contamination (1.1-fold). [Cox (B-1901) P.17; citing G-1788 (Rosenquist 2002)]

540. The fraction of *Campylobacter*-positive chicken (which the term "a qualitative method" refers to) is *not* an adequate exposure metric from which to predict human health risk. [Cox (B-1901) P.17; citing G-1788 (Rosenquist 2002)]

541. The CVM/Vose Risk Assessment model ignores all dose-response information. Instead, it misapplies attributable fraction calculations to assign blame for most human campylobacteriosis cases to chicken, even though most human campylobacteriosis are actually caused by other factors. [Cox (B-1901) P.18, citing B-1252 (Cox 2002); P.64-70]

542. For other microbial risk assessments, FDA has previously defined risk assessment as a process that "consists of the following steps: hazard identification, exposure assessment, hazard characterization (dose-response), and risk characterization". It defined dose-response assessment as "The determination of the relationship between the magnitude of exposure and the magnitude and/or frequency of adverse effects." Similarly, the Codex Alimentarius Commission states that "For biological or physical agents, a dose-response assessment should be performed if the data are

obtainable." [Cox (B-1901) P.18; citing *Draft Assessment of the Relative Risk to Public Health from Foodborne Listeria monocytogenes Among Selected Categories of Ready-to-Eat Foods*, http://www.foodsafety.gov/~dms/lmriskgl.html]

543. Dose-response data for *Campylobacter* in human volunteers are readily obtainable and have been used to create several published dose-response models. [Cox (B-1901) P.18, citing B-517/G-411 (Medema 1996), B-748/G-629 (Teunis 1999), G-628 (Teunis and Havelaar 2000) and G-1788 (Rosenquist 2002)]

544. Despite dose-response data for *Campylobacter* in human volunteers being readily obtainable and the existence of several published dose-response models, the CVM/Vose Risk Assessment model did not perform any dose-response assessment. [Cox (B-1901) P.18]

545. Other *Campylobacter* risk assessment models such as B-1260 (Cox and Popken 2002) and G-1788 (Rosenquist 2002) incorporate relevant dose-response information for *Campylobacter*. [Cox (B-1901) P.18]

546. The CVM/Vose Risk Assessment model does not incorporate relevant *Campylobacter* dose-response information. [Cox (B-1901) P.18]

547. Rather than incorporate relevant *Campylobacter* dose-response information, the CVM/Vose Risk Assessment model relies on the assumptions that probability of campylobacteriosis in a person is directly proportional to the quantity of chicken consumed, and that the chicken-attributable risk of fluoroquinolone-resistant campylobacteriosis is proportional to the quantity of *Campylobacter*-contaminated chicken consumed, regardless of the amount of the contamination. [Cox (B-1901) P.18, P.64-70]

548. The assumption that the probability of campylobacteriosis in a person is directly proportional to the quantity of chicken consumed is incorrect. [Cox (B-1901) P.18]

549. The assumption that the chicken-attributable risk of fluoroquinolone-resistant campylobacteriosis is proportional to the quantity of *Campylobacter*-contaminated chicken consumed, regardless of the amount of the contamination, is incorrect. [Cox (B-1901) P.18]

550. Despite CVM's assertion, the parameter K_{res} does not "establish[ ] an exposure-response relationship between the quantity of chicken contaminated with fluoroquinolone-resistant *Campylobacter* and the number of human cases with fluoroquinolone-resistant *Campylobacter*". [Cox (B-1901) P.54, citing CVM Answer to Bayer Interrogatory 49]

551. The CVM/Vose Risk Assessment model's "K" ratio cannot correctly be interpreted as a dose-response relation, since neither a quantitative dose metric nor response probability as a function of dose has been quantified. [Cox (B-1901) P.19, P.64-70]

552. The CVM/Vose Risk Assessment model's "K" ratio is a ratio of two positive numbers such that "K" can never be a negative number. [Cox (B-1901) P.19]

553. The CVM/Vose Risk Assessment model seeks to quantify the average exposure of the "average consumer". But this quantity cannot be used to accurately predict risk, either for individuals or for populations. The average exposure level for the average consumer is irrelevant for predicting risk of Campylobacteriosis (fluoroquinolone-resistant or not) since, as experimental data indicate, risks are caused primarily by concentrations of CFUs much higher than average in ingested foods. [Cox (B-1901) P.67]

554. The overall causal relation between chicken consumption and risk of *Campylobacter* infections can be negative if consuming chicken is preventative/protective of getting a *Campylobacter* infection. [Cox (B-1901) P.19; *See also* Endtz (G-1457) P.4 L.23-24]

555. In traditional risk assessment frameworks, risk characterization is supposed to integrate hazard identification, exposure assessment, and dose-response information to determine the probable frequency and severity of adverse health effects that exposure to a hazard causes in a population. [Cox (B-1901) P.20]

556. In the CVM/Vose Risk Assessment framework, neither hazard identification, nor exposure assessment, nor hazard characterization (i.e., dose-response modeling) has been carried out according to generally accepted risk assessment standards and principles. [Cox (B-1901) P.20]

557. The case-control data of Friedman et al. (2000) show that the correct proportion of human *Campylobacter* illnesses attributable to chicken consumption must be much smaller than 60%. [Cox (B-1901) P.20, citing The 1998-1999 FoodNet *Campylobacter* Case-Control Study data set (e.g. G-1644) and B-1020]

558. A recent prospective case-control study from Quebec identifies poultry as the "principal suspected source of infection" in only about 10% of cases, comparable to drinking tap water at home (9%). [Cox (B-1901) P.20, citing G-1681 (Michaud 2002)]

559. Genetic data suggest that only about 20% of human CP isolates (5 of 24) were genetically related to genotypes found in chickens. [Cox (B-1901) P.20, citing G-1684 (Nadeau 2002)]

560. Lamb and chicken share a significant proportion of *Campylobacter jejuni* subtypes with humans, suggesting the possibility of a common environmental source and indicating that shared subtypes need not arise from consumption of one species by another. [Cox (B-1901) P.20, citing G-1670 (Kramer 2000)]

561. Despite multiple data sources to the contrary, the CVM/Vose Risk Assessment Model uses 60% as the fraction of human campylobacteriosis cases attributable to chicken consumption. [Cox (B-1901) P.20]

562. The CVM/Vose risk Assessment Model uses attributable risk numbers that do not control for known confounders and risk factors for campylobacteriosis (e.g., male sex, contact with puppies and dogs, income and insurance coverage, dining out in restaurants, etc.). [Cox (B-1901) P.21; *See also* Feldman (B-1902) P.29 L.9-P.30 L.5]

563. The risk of campylobacteriosis that CVM attributes to chicken is in reality primarily due to other, non-chicken sources. [Cox (B-1901) P.21]

564. The CDC's 1998 - 1999 *Campylobacter* Case-Control study provide data that can be used to estimate chicken-attributable fractions directly for fluoroquinolone-resistant campylobacteriosis cases from data on chicken consumption and fluoroquinolone-resistant *Campylobacter*. [Cox (B-1901) P.22, P.57-64]

565. Instead of the nearly 60% chicken-attributable fraction used in the CVM/Vose Risk Assessment, a more realistic value of the chicken-attributable fraction for fluoroquinolone-resistant campylobacteriosis, based on the CDC's 1998 - 1999 *Campylobacter* Case-Control study data, is between –11.6% (protective effect) and 0.72%, (not statistically significantly different from zero) depending on how missing data values are treated. [Cox (B-1901) P.22, P.57-64]

566. The fraction of nearly 60% chicken-attributable fraction used in the CVM/Vose Risk Assessment is nearly 80-fold too high compared to the 0.72% fraction for fluoroquinolone-resistant campylobacteriosis (or even higher, if the true chicken-attributable risk is zero, consistent with the data). [Cox (B-1901) P.22, P.57-64]

567. By assuming that its model form (i.e., excess risk = K*exposure) is correct despite overwhelming evidence to the contrary (e.g., risk actually decreases with consumption of chicken and increases disproportionately with microbial loads above 500 CFU), CVM under-states uncertainty in its results and produces artificially narrow confidence bands. [Cox (B-1901) P.23]

568. CVM's risk assessment does not address *interindividual variability in susceptibility,* even though dose-response data show that only about 20% of people experimentally exposed to *Campylobacter* became sick even at the highest concentrations. [Cox (B-1901) P.23, citing B-748/G-629 and G-628 (Teunis 1999 and Teunis 2000)]

569. By assuming that one constant, K, essentially plays the role of a dose-response, the CVM/Vose Risk Assessment model fails to address the fact that only a minority of those exposed may be susceptible – and that the factors affecting susceptibility may have nothing to do with chicken consumption. Thus, neither uncertainty nor variability has been correctly characterized in the CVM/Vose Risk Assessment model. [Cox (B-1901) P.23]

570. Analysis of CDC's 1998 - 1999 *Campylobacter* Case-Control study data demonstrates that chicken consumption at home has a significant association with reduced risk of becoming ill with campylobacteriosis. [Cox (B-1901) P.24]

571. Analysis of CDC's 1998 - 1999 *Campylobacter* Case-Control study data demonstrates that chicken consumption as a whole is not associated with increased risk of becoming ill with campylobacteriosis. [Cox (B-1901) P.24]

572. Analysis of CDC's 1998 - 1999 *Campylobacter* Case-Control study data demonstrates that consumption of both home-cooked chicken and restaurant-prepared chicken are non-significantly negatively associated with becoming ill with a fluoroquinolone-resistant case of campylobacteriosis. [Cox (B-1901) P.24]

573. Cases of campylobacteriosis associated with recent chicken consumption are less virulent (fewer average illness-days) than fluoroquinolone-resistant campylobacteriosis associated with other (non-poultry) sources. [Cox (B-1901) P.24]

574. If attention is restricted to patients who report recently eating chicken, then fluoroquinolone resistance is associated with decreased days of illness. [Cox (B-1901) P.24]

575. People who eat chicken now have significantly lower risk of acquiring fluoroquinolone-resistant campylobacteriosis than people who do not. [B-1252 Figure 4, P. 3832, citing data of Smith et al., 1999).

576. CVM's hazard identification step of the CVM/Vose Risk Assessment incorrectly identifies domestic chicken-borne fluoroquinolone-resistant *Campylobacter* as the predominant cause of adverse health effects (e.g., campylobacteriosis followed by treatment failure and excess days of diarrhea) when in fact these effects are demonstrably caused by other factors including foreign travel and restaurant dining. [Cox (B-1901) P.15, citing B-1020 (Cox 2001), B-1252 (Cox 2002) and G-1711 (Rodrigues 2001)]

577. Applying conditional independence tests for causality to the CDC 1998 - 1999 *Campylobacter* Case-Control data set reveals that after correcting for confounders (i.e., variables that are associated with both chicken consumption and fluoroquinolone-resistant campylobacteriosis cases), overall consumption of chicken is not a risk factor for campylobacteriosis. [Cox (B-1901) P.29, citing G-1644 (Friedman 2000); Burkhart (B-1900) P.9, L.39-41]

578. Applying conditional independence tests for causality to Effler data set (funded and supported by CDC under cooperative agreement #U50/912395-03) reveals that after correcting for confounders (i.e., variables that are associated with both chicken consumption and fluoroquinolone-resistant campylobacteriosis cases), overall consumption of chicken is not a risk factor for campylobacteriosis. [Cox (B-1901) P.29, citing G-185 (Effler 2001)]

579. Preparation and consumption of chicken at home and buying or handling raw chicken are statistically significantly protective against campylobacteriosis. [Cox (B-1901) P.29, citing G-1644 (Friedman 2000); Burkhart (B-1900) P.9, L.39-41]

580. The finding that preparation and consumption of chicken at home and buying or handling raw chicken are statistically significantly protective against campylobacteriosis is consistent with conclusions from several studies including Rodrigues et al., 2001 and Effler et al., 2001. [Cox (B-1901) P.29-30, citing G-185 (Effler 2001) and G-1711 (Rodrigues 2001)]

581. The CDC 1998 - 1999 *Campylobacter* Case-Control data set shows that exposure to chicken juice and raw chicken are not risk factors for getting campylobacteriosis but instead tend to reduce the risk of being a campylobacteriosis case. [Cox (B-1901) P.29, citing G-1644 (Friedman 2000)]

582. Because exposure to chicken juice and raw chicken are not risk factors for getting campylobacteriosis but instead tend to reduce the risk of being a campylobacteriosis case, it is not plausible that chicken per se is usually well cooked and safe, but still causes excess risk of campylobacteriosis via cross-contamination in the kitchen. [Cox (B-1901) P.29]

583. The CDC 1998 - 1999 *Campylobacter* Case-Control data set shows that restaurant dining/consumption of commercially prepared food, including chicken, is a risk factor for campylobacteriosis – but not significantly more so for chicken than for other meats. [Cox (B-1901) P.29, citing G-1644 (Friedman 2000)]

584. The CDC 1998 - 1999 *Campylobacter* Case-Control data set and other studies show that restaurant dining, rather than chicken consumption per se, appears to be the major human health threat for getting campylobacteriosis. [Cox (B-1901) P.29, citing G-1644 (Friedman 2000), G-185 (Effler 2001), G-1711 (Rodrigues 2001) and other international studies G-10 (Adak 1995), G-182 (Eberhart-Phillips 1997) and Kassenborg testimony G-1460 P.8); Newell (B-1908) P.25 L.15-18]

585. The human health risk of campylobacteriosis (cases per capita-year) has steadily decreased in the US since Baytril was introduced [Endtz (G-1457 P.2, Para. 3; Molbak (G-1468) P.4, L.18); CVM Response to Bayer's Interrogatory 28]

586. Chicken consumption per capita has steadily increased since Baytril was introduced. [Cox (B-1901) P.36]

587. Since Baytril was introduced, chicken consumption (pounds per capita-year) has steadily increased while human health risk of campylobacteriosis per pound of chicken consumption has steadily and significantly decreased. [Cox (B-1901) P.36; Endtz (G-1457) P.2 ¶ 3; Molbak (G-1468 P.4 L.18; CVM's Response to Bayer's Interrogatory 28]

588. The fact that campylobacteriosis incidence rate has steadily decreased while chicken consumption has steadily increased argues against CVM's contention that chicken is the predominant source of human campylobacteriosis and that campylobacteriosis rates (as well as fluoroquinolone-resistant campylobacteriosis rates) in the US population are directly proportional to chicken consumed. [Cox (B-1901) P.36]

589. There are substantial differences in the incidence of *Campylobacter* case rates among the nine FoodNet sites (more than a four-fold difference in 2001 between high and low rates and more than a 15-fold difference for infants less than 1 year. [Cox (B-1901) P.36; citing Molbak (G-1468) P.4, Para.17]

590. The substantial differences in the incidence of *Campylobacter* case rates among the nine FoodNet sites are not explained by differences in chicken consumption per capita (CDC case-control data) and thus suggest the importance of other, non-chicken factors in causing the observed rates of campylobacteriosis. [Cox (B-1901) P.36]

591. Epidemiological data from a recent prospective case-control study from Quebec identifies poultry as the "principal suspected source of infection" in only about 10% of cases – far from the "predominant cause" suggested by CVM. [Cox (B-1901) P.36 citing G-1681 (Michaud 2002)]

592. Outbreak data clearly indicate that chicken consumption is not a predominant source of campylobacteriosis outbreaks in humans, but that drinking water is. [Cox (B-1901) P.36]

593. The data that the CVM/Vose Risk Assessment model relies on to calculate attributable fractions came from two studies from the 1980s: Harris et al., and Deming et al. [Bartholomew (G-1454) P.8 L.13-15; Cox (B-1901) P.38, referring to G-953 (the CVM/Vose Risk Assessment), G-268 (Harris 1986) and G-162 (Deming 1987); [Feldman (B-1902) P.17 L.20-23]

594. Despite CVM's assertion that "most of the data used [in the CVM/Vose Risk Assessment model (G-953)] were from large national collections." (CVM's Answer to Bayer Interrogatory 21), the most important part of the risk assessment calculation, the estimation of the chicken-attributable fraction, was based on Harris (G-268) and Deming (G-162), two relatively small, outdated studies that were not from large national collections. [Cox (B-1901) P.50; Feldman (B-1902) P.34 L.12-21]

595. Relying on Harris (G-268) and Deming (G-162), the CVM/Vose Risk Assessment attributed a high proportion (nearly 60%) of campylobacteriosis risk to chicken consumption. [Cox (B-1901) P.38, referring to G-953 (the CVM/Vose Risk Assessment), G-268 (Harris 1986) and G-162 (Deming 1987)]

596. The populations in the Harris (G-268) and Deming (G-162) studies were not representative of the current US population in terms of age, income, travel habits, dietary habits, and other relevant risk factors. [Cox (B-1901) P.38, P.57-64]

597. The attributable fractions calculated in the Harris (G-268) and Deming (G-162) studies cannot correctly be applied to US population case rates. [Cox (B-1901) P.38, P.57-64]

598. The Harris (G-268) and Deming (G-162) studies cannot be used to support a correct calculation of the chicken-attributable fraction for fluoroquinolone-resistant campylobacteriosis, since neither contains any data on fluoroquinolone-resistant campylobacteriosis. [Cox (B-1901) P.39, P.57-64]

599. Neither the Harris (G-268) study nor the Deming (G-162) study isolated the portion of campylobacteriosis risk associated with chicken consumption that is actually caused by chicken-borne *Campylobacter*, as opposed to being caused by other things (e.g., restaurant dining, income, male sex) that are correlated with patterns of chicken consumption. [Cox (B-1901) P.38-39, P.57-64]

600. The CVM/Vose Risk Assessment model misidentifies chicken as the predominant cause of campylobacteriosis. Chicken accounts for 50%-70% of human campylobacteriosis cases in the CVM/Vose Risk Assessment model, but accounts for an undetectably small fraction of cases in reality, based on relatively large data sets such as the CDC 1998 - 1999 *Campylobacter* Case-Control data set, for which univariate logistic regression yields a negative population attributable risk (PAR). [Cox (B-1901) P.38, P.57-64]

601. A more accurate estimate of the population-attributable risk (PAR) for chicken consumption as a whole, based on the CDC 1998 - 1999 *Campylobacter* Case-Control data set, is negative (protective effect) while that for restaurant chicken is 3.1% (calculated via the standard PAR formula with a = 665, b=341, c=1439, d=976.) These are univariate PARs. A multivariate PAR that removes the effects of confounders would be closer to zero. Thus, an attributable fraction of 0 to 3.1% is more realistic than CVM's 57%. [Cox (B-1901) P.56, P.57-64]

602. CVM's interpretation of PAR (as set out in Angulo (G-1452) P.10 L.38 and Kassenborg (G-1460) P.8 Para.16) as referring to cases that are caused by or "due to" a factor or cases that would be reduced if a factor was eliminated is not correct. For example, PAR fractions for different factors can easily sum to several hundred percent, and it is incorrect to interpret the PAR for a factor as the fraction of cases that it causes or that would disappear if the factor were removed. [Cox (B-1901) P.57]

603. The CDC 1998 - 1999 *Campylobacter* Case-Control data (G-1644), which is from a large national collection, was not used in the estimation of the chicken-attributable fraction in the CVM/Vose Risk Assessment model (G-953). [Cox (B-1901) P.50, 56]

604. The CVM/Vose Risk Assessment attributes none of the risk for domestically acquired, non-treatment-related campylobacteriosis cases to well-known risk factors identified in other studies, such as drinking raw milk or water, restaurant dining and eating non-poultry meat prepared outside the home, sex of the victim, contact with puppies, or income. [Cox (B-1901) P.38, citing G-1711 (Rodrigues 2001), G-589/G-1723 (Smith 2001), G-185 (Effler 2002), G-1644 (Friedman 2000)]

605. The fraction of campylobacteriosis cases that CVM attributes to chicken is inflated by the exclusion of well-known risk factors that are known to be important in the general population, such as drinking raw milk or water, restaurant dining and eating non-poultry meat prepared outside the home, sex of the victim, contact with puppies, or income. [Cox (B-1901) P.38, referring to risk factors identified in G-1711 (Rodrigues 2001), G-589/G-1723 (Smith 2001), G-185 (Effler 2002), G-1644 (Friedman 2000)]

606. There is no logically necessary connection between attributable fractions for campylobacteriosis cases in general and attributable fractions specifically for fluoroquinolone-resistant campylobacteriosis cases. [Cox (B-1901) P.39]

607. The CVM/Vose Risk Assessment calculates the product $p_{ca}*p_{rh}$ = "probability that a *Campylobacter* case is attributable to chicken" * "probability that a *Campylobacter* case from chicken is fluoroquinolone-resistant", but this is mathematically not the same as the probability that a fluoroquinolone-resistant *Campylobacter* case is due to chicken. [Cox (B-1901) P.39]

608. Results from the large pilot case-control study of sporadic cases of *Campylobacter* infection conducted by the Foodborne Diseases Active Surveillance Network (FoodNet) in 1996-1997 found numerous factors to be associated with increased risk of *Campylobacter* infection, including eating poultry meat in restaurants, eating non-poultry meat in restaurants, eating raw seafood, international travel, contact with puppies and farm animals, and male gender. [G-1452 Attachment 1 P.46]

609. Results from the large pilot case-control study of sporadic cases of *Campylobacter* infection conducted by the Foodborne Diseases Active Surveillance Network (FoodNet) in 1996-1997 found a number of different exposures to be independent protective factors, including eating poultry meat at home, eating non-poultry meat at home, and eating at fast-food restaurants. [Angulo (G-1452) Attachment 1 P.46; Burkhart (B-1900) P.9, L.39-41]

610. The largest case-control study of sporadic *Campylobacter* infections was conducted in the United States in the FoodNet sites in 1998 and 1999. ("The 1998-1999 FoodNet *Campylobacter* case-control study.") [Angulo (G-1452) P.9 L.46-47]

611. Data from the 1998-1999 FoodNet *Campylobacter* case-control study have been used in three analyses: (a) comparison of *Campylobacter* cases and well community controls to determine the risk factors for becoming infected with *Campylobacter*; (b) comparison of ciprofloxacin-resistant *Campylobacter* cases and well community controls to determine the risk factors for becoming infected with ciprofloxacin-resistant *Campylobacter*; and (c) comparison of the medical consequences of ciprofloxacin-resistant and ciprofloxacin-susceptible *Campylobacter* cases. [Angulo (G-1452) P.10 L.7-12]

612. Cindy R. Friedman et. al performed the comparison of *Campylobacter* cases and well community controls to determine the risk factors for becoming infected with *Campylobacter*, the results of which are reported in G-1644 (Friedman 2000) and Attachment 3 to G-1452. [G-1644; Angulo (G-1452) Attachment 3]

613. Heidi Kassenborg et. al performed the comparison of ciprofloxacin-resistant *Campylobacter* cases and well community controls to determine the risk factors for becoming infected with ciprofloxacin-resistant *Campylobacter*, the results of which are reported in G-337 (Kassenborg 2000) and Kassenborg's testimony (G-1460). [G-337; 1460]

614. Jennifer Nelson (nee McClellan) et al performed the comparison of the medical consequences of ciprofloxacin-resistant and ciprofloxacin-susceptible *Campylobacter* cases, the results of which are reported in G-1679 (McClellan 2000), G-780 (McClellan 2002), G-1367 and Attachment 4 to G-1452. [G-780; G-1367; G-1432 Attachment 2; G-1699]

615. G-1679 is a thesis that Jennifer Nelson (nee McClellan) published in 2000 and submitted to the Rollins School of Public Health Thesis Committee of Emory University in partial fulfillment of the degree of Master of Public Health. [McClellan (G-1679) P.1]

616. Dr. Fred Angulo was a Field Advisor for the McClellan Thesis G-1679 and approved it on December 14, 2000. [G-1679 McClellan (2000) P.2]

617. The "Null Hypothesis" set forth in G-1679 was: "There is no difference in severity (i.e., duration of diarrhea) between individuals with fluoroquinolone-resistant *Campylobacter* infections and individuals with fluoroquinolones-susceptible *Campylobacter* infections in the 1998-1999 FoodNet *Campylobacter* study. [McClellan (G-1679) P.38]

618. At the suggestion of Dr. Angulo, thesis advisor for the G-1679 McClellan Thesis, McClellan conducted a "survival analysis" of the CDC *Campylobacter* case-control data. The results of the "survival analysis" are the basis for the findings presented in the thesis. [G-1679 McClellan (2000) P.4 &5]

619. In a preliminary analysis, the full model used to analyze data in G-1679 examined the relationship between ciprofloxacin resistance and duration of diarrhea while adjusting only for age, race, gender, residence, FoodNet site, education, and household income. Reduced models were generated from this preliminary model to further explore the data. [McClellan (G-1679) P.45-46]

620. The final, full Cox proportional hazards model, utilized by McClellan in G1679 examined the association between ciprofloxacin resistance and duration of diarrhea controlling for age, sex, residence, FoodNet site, education, and household income, stratified by race. [McClellan (G-1679) Pg. 54]

621. McClellan posits in G-1679 that a bias could have been introduced into her study. She states that a disproportionate number of cultures could have been collected from individuals with fluoroquinolone-resistant infections and that this could have biased the final sample population. [G-1679 McClellan (2000) P.60]

622. Selection bias can significantly alter the findings of a study. Inferences may be drawn to the study population but not to the general population. [Feldman (B-1902) P.30 L.6-7]

623. Analysis of the 1998-1999 *Campylobacter* Case-Control study by Nelson revealed that, when not adjusting for antimicrobial or antidiarrheal use, there was no statistical difference in mean duration of diarrhea between patients with a ciprofloxacin-resistant infection (8 days) compared to patients with a ciprofloxacin-susceptible infection (7 days), (p value = 0.1) [Angulo (G-1452), Attachment #4, P.116; P.117]; [Nelson (G-1489) P.10]

624. Analysis of the 1998-1999 *Campylobacter* Case-Control study by Nelson revealed that adjusting for fluoroquinolone and antidiarrheal use, there was no statistical difference in mean duration of diarrhea between patients with a ciprofloxacin-resistant infection who took a fluoroquinolone and no antidiarrheal agent (8 days) compared to patients with a ciprofloxacin-susceptible infection who took a fluoroquinolone and no antidiarrheal agent (6 days), (p value=0.08). [Angulo (G-1452), Attachment 4, P.118]; [Nelson (G-1489) P.11]

625. Nina Marano et. al also performed a comparison of the medical consequences of ciprofloxacin-resistant and ciprofloxacin-susceptible *Campylobacter* cases, the results of which are reported in G-394 (Marano 2000). [G-394]

626. G-1644 Friedman (2000), G-337 Kassenborg (2000), G-1679 McClellan (2000), G-394 (Marano 2000) and G-780 (Nelson 2002) all analyze and report on data from the 1998-1999 FoodNet *Campylobacter* case-control study. [Angulo (G-1452) P.10 L.7-12]

627. According to Angulo (G-1452), data from the 1998-1999 FoodNet *Campylobacter* case-control study show that among non-travelers, 24 percent of sporadic cases of campylobacteriosis in the United States are due to eating chicken in a restaurant. [Angulo (G-1452) P.10 L.41-43]

628. Data from the 1998-1999 FoodNet *Campylobacter* case-control study show that the largest population attributable fractions were for eating chicken in a restaurant and eating non-poultry meat in a restaurant. [Angulo (G-1452) P.10 L.36-37]

629. Data from the 1998-1999 FoodNet *Campylobacter* case-control study show that 21 percent of sporadic campylobacteriosis cases in the U.S. are due to eating non-poultry meat in a restaurant. [Angulo (G-1452) P.10 L.43]

630. Data from the 1998-1999 FoodNet *Campylobacter* case-control study show that 4 percent of sporadic campylobacteriosis cases in the U.S. are due to eating turkey in

a restaurant in the seven days prior to illness onset. [Angulo (G-1452) P.10 L.43-44]

631. Data from the large, recent, national case-control study of sporadic *Campylobacter* infections conducted in the FoodNet sites 1998 and 1999 (The 1998-1999 FoodNet *Campylobacter* case-control study), demonstrate that sporadic *Campylobacter* infections have multiple sources. [Angulo (G-1452) P.10 L.46 – P.11 L.2]

632. The 1998-1999 FoodNet *Campylobacter* case-control study found independent risk factors for acquiring a *Campylobacter* infection included drinking untreated water from a lake, river, or stream, drinking raw milk, eating undercooked poultry, eating raw seafood, having a pet puppy, having contact with farm animals, and having contact with animal stool. [Angulo (G-1452) Attachment 3 P.88]

633. The 1998-1999 FoodNet *Campylobacter* case-control study found that consumption of poultry prepared at a restaurant and consumption of non-poultry meat prepared at a restaurant accounted for nearly one half of all *Campylobacter* illnesses. [G-1452 Attachment 3 P.88]

634. The 1998-1999 FoodNet *Campylobacter* case-control study found that consumption of poultry prepared at home and consumption of non-poultry meat prepared at home was associated with a reduced risk of *Campylobacter* disease. [G-1452 Attachment 3 P.88; Burkhart (B-1900) P.9, L.39-41]

635. Of the 64 patients in the Kassenborg case-control study (G-337) with fluoroquinolone-resistant *Campylobacter* infection who were interviewed, 2 were from California, 27 from Connecticut, 4 from Georgia, 3 from Maryland, 17 from Minnesota, 10 from New York, and one from Oregon. [Kassenborg (G-1460) P.6 L.11-13]

636. In the Kassenborg case-control study (G-337), 84.4% of the patients interviewed in the 1998 - 1999 *Campylobacter* case-control study with fluoroquinolone-resistant infections were from Connecticut, Minnesota, and New York. [Kassenborg (G-1460) P.6 L.11-13]

637. McClellen found that persons with fluoroquinolone-resistant infections more frequently lived in Connecticut. [McClellan (G-1679) P.49-50]

638. There was a high prevalence of ciprofloxacin-resistant *Campylobacter* found in Connecticut in 1999. [Angulo (G-1452 P.8 L.30-31; Molbak (G-1468) P.8 L.28-29 & L.21-23]

639. In the Kassenburg case-control study (G-337) Connecticut and New York FoodNet sites had the highest percentages of fluoroquinolone-resistant *Campylobacter* isolates. [Kassenborg (G-337) P.7]

640. In the Kassenborg case-control study (G-337), potential subjects were interviewed with 21 days of their stool sample collection date; potential controls were

interviewed within 7 days after the case subject's interview. All subjects were asked about food and water consumption, child daycare, travel, animal exposures, and food-handling practices during the 7-day period before the case subject's onset date. Thus controls were questioned about foods consumed as long as 35 days previously. [Angulo (G-1452), Attachment 3, P.83] [Friedman (G-1488) P.5] [Kassenborg (G-1460) P.4 L.21-23] [Feldman (B-1902) P.28 L.7-17]

641. In the Kassenborg case-control study (G-337), patients with fluoroquinolone-resistant *Campylobacter* infections were 7.6 times more likely to report having traveled outside the United States during the 7 days prior to illness onset than were those with fluoroquinolone-sensitive infections. [Kassenborg (G-1460) P.7 L.5-7]

642. In the Kassenborg case-control study (G-337), foreign travel was a risk factor for fluoroquinolone-resistant *Campylobacter* infection when the interviewed fluoroquinolone-resistant *Campylobacter* cases were compared to their age-matched well controls. [Kassenborg (G-1460) P.7 L.9-11]

643. The Kassenborg case-control study (G-337) found that 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, eating in a non-fast food restaurant during the 7 days before illness onset, and using an antacid during the 4 weeks before illness onset. [Kassenborg (G-1460) P.8 L.2-6]

644. In the Kassenborg case-control study (G-337) 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. [Kassenborg (G-1460) P.8 L.18-20]

645. The Kassenborg case-control study (G-337) did not evaluate whether travel-associated cases may also be a consequence of fluoroquinolone use in food-producing animals. [Kassenborg (G-1460) P.10 L.3-5]

646. The association between eating poultry outside the home and the risk for *Campylobacter* infection has been reported in other case-control studies of general *Campylobacter* infections. [Kassenborg (G-1460) P.10 L.8-10]

647. Since risky food handling practices (i.e., not washing hands after handling raw poultry) increase the risk of acquiring a *Campylobacter* infection, reducing the frequency of risky food handling practices will reduce the incidence of *Campylobacter* infections. [Angulo (G-1452) P.13 L.24-26]

648. In the United States, the incidence of *Campylobacter* infections as measured through the Foodborne Disease Active Surveillance Network (FoodNet) decreased by 27% between 1996 and 2001. [G-1452 Attachment 3 P.82; CVM Response to Bayer's Interrogatory 28]

649. An estimated 1.4 million persons in the U.S. in 1999 were infected with *Campylobacter*. [Angulo (G-1452) P.7 L.13-14, P.9 L.16-18, P.17 L.10]

650. The human health impact of interest is campylobacteriosis caused by *Campylobacter jejuni* and/or *Campylobacter coli* that are resistant to fluoroquinolones, a class of antimicrobials including molecules such as ciprofloxacin, lomefloxacin and norfloxacin for use in human medicine and enrofloxacin and sarafloxacin developed for use in veterinary medicine. [JS 41; (B-549) P. 6]

651. There is a strong overlap in susceptibility of *Campylobacter* to these agents, so called "cross resistance", i.e. resistance to one fluoroquinolone implies resistance to all fluoroquinolones. [Newell (B-1908) P.18 L. 3-4]

652. Campylobacteriosis caused by *Campylobacter* jejuni or *Campylobacter* coli is a type of human intestinal infection caused by the oral ingestion of the bacteria. [(G-70) P. 2]

653. Campylobacteriosis infections of relevance to this hearing are restricted to:

- infections from *Campylobacter* jejuni and/or *Campylobacter* coli which are,
- fluoroquinolone resistant due to use of Baytril in poultry in the United States, to the extent that they:
- result in less effective treatment in people treated with a fluoroquinolone,
- result in more protracted illness because the *Campylobacter* are resistant, and/or
- result in increased hospitalization.

[CVM Answers to Interrogatories 51-60; 67 FR 7700, 7701 (February 20, 2002); Tollefson (G-1478) P.15, L.34-39]

654. As of 1999, the total number of persons in the United States who contract campylobacteriosis annually, while in the United States or abroad due to foreign travel, has been estimated at about 1.4 million, or about 0.5% of the US population. [Angulo (G-1452) P. 17 L.10]

655. The total number of persons in the United States who contract campylobacteriosis annually, while in the United States or abroad, is estimated to have decreased by about 27% from 1996 to 2001. [Angulo (G-1452) P. 5 L.21-23]

656. A number of *Campylobacter* must be ingested to cause a human infection with clinical symptoms. [(G-70) P.3; (G-441) P.3; Nachamkin (G-1470) P. 4 L. 43 - 46, P. 5 L. 1-8]

657. Based on experimental data, the minimum number of *Campylobacter* capable of causing campylobacterioris has been estimated to be about 500 - 800 organisms (minimum infectious dose). [(G-70) P.3; (G-441) P.3; Nachamkin (G-1470) P. 4 L. 43 - 46, P. 5 L. 1-8]

658. Thus, the risk that a given meal will lead to campylobacteriosis depends at least in part on the number of *Campylobacter* ingested. [JS 27]

659. The capability of *Campylobacter* to cause illness (its "pathogenicity") is dependent in part on the susceptibility of the potential host, in addition to the inoculum size, or minimum infectious dose. [(B-205) P.3; (G-70) P. 3; (G-707) P. 9]

660. Thus, many persons with campylobacteriosis - perhaps as many as 25% of all persons infected - do not exhibit clinical symptoms and are therefore "asymptomatic". [Pasternack (B-1909) P. 3 L. 23, P. 4 L. 1-3; (G-70) P.3]

661. *Campylobacter* are thought to cause human disease by colonizing the mucous overlying the intestinal lining, however the mechanisms by which *Campylobacter* cause diarrhea remain unknown. [Newell (B-1908) P. 44 L.20-21, P. 45 L. 5-6; (G-441) P.3]

662. Nearly all *Campylobacter* infections remain localized in the small and large intestines, where they cause inflammation i.e., *Campylobacter* enteritis ("enteritis" means inflammation of the intestines, which can be caused by microbes including bacteria and other agents). [Ohl (G-1485) P.6 L.20-21; Kist (B-1906) P.4 L.17-18]

663. In approximately 1% or less of reported campylobacteriosis cases, the bacteria penetrate the intestinal lining and enter the blood stream, a condition known as bacteremia. [Tauxe (G-1475) P. 18 L. 28-30; (G-511) P. 1, 4; (B-742) P. 1]

664. Since most strains of *Campylobacter* jejuni and *Campylobacter* coli are susceptible to the bactericidal (killing) activity of blood-serum, bacteremia is usually self-limiting and often remains untreated. [Kist (B-1906) P.5 L.7-9]

665. In very rare cases, campylobacteriosis can cause systemic illness once in the blood stream (sepsis) and in extremely rare cases, infections can become present in extra-intestinal organs. (Blaser 1992) [Ohl (G-1485) P.7 L.1-3; (G-580) P.7, 8]

666. Bacteremia is a prerequisite for spread of the pathogen to extra intestinal tissues ("focal" infections), thus focal infections are extremely rare and are mostly presented in the literature as single cases (Skirrow and Blaser 1995). [Kist (B-1906) P. 5 L. 7-9]

667. The clinical symptoms of *Campylobacter* enteritis include some or all of the following:

- about 24 to 72 hours of early, flu-like symptoms of fever, headache, muscle aches and abdominal pain (known as the "prodrome");

- following the prodrome, in some cases diarrhea typically lasting 3 to 10 days, about 5-7 days in most cases;
- in some cases, vomiting;
- in some cases, blood and/or white blood cells in the stool; and,
- in some cases, elevated white blood cell count.

[Pasternack (B-1909) P. 4 L. 5; (G-70) P.4; Endtz (G-1457) P.2 L. 31-36]

668. These clinical symptoms are indicative of campylobacteriosis but are not definitive, since they are similar to the symptoms of other forms of bacterial enteritis, such as those caused by Shigella, E. coli and Salmonella. [(G-191) P. 7; (G-1789) P. 3; (B-205) P.5]

669. *Campylobacter* enteritis resolves itself without treatment in the vast majority of cases (e.g., is "self-limiting") whether fluoroquinolone susceptible or fluoroquinolone resistant. [Pasternack (B-1909) P. 3 L. 16-17; (G-240) P. 1; (G-530) P. 1; (G-622) P. 1]

670. Only a very small fraction of persons with campylobacteriosis seek treatment and are evaluated by a physician; e.g., based on 1996 - 1997 FoodNet data, it was estimated that only 1 in 18 persons with campylobacteriosis seek treatment. [(G-615) P. 3; Pasternack (B-1909) P. 4 L. 4-6]

671. The most common potential complication of *Campylobacter* enteritis is dehydration. [(G-261) P. 9, 10]

672. Administration of fluids, orally or intravenously, to correct or prevent dehydration is the most common form of treatment. [Kist ((B-1906) P.9 L. 17-20]

673. Only a small number of individuals with moderate to severe symptoms may require antibiotics as part of their therapy. [Ohl (G-1485) P. 9 L. 46, P. 10 L. 1-7; Pasternack (B-1909) P. 7 L. 17-22, P. 18 L. 15-18; (G-70) P. 6; Iannini (B-1905) P. 5 L. 9-12; Molbak (G-1468) P.3 L.21]

674. For this small percentage of people, the antibiotic of choice for treatment of campylobacteriosis is a macrolide such as erythromycin or azithromycin or the new rifaximin. [Iannini (B-1905) P.4 L. 8-11; Pasternack (B-1909) P. 14 L. 1-16; Endtz (G-1457) P. 6 L. 44-45; Thielman (G-1477) P.2 Para. 4; Morris (G-1469) P.5 L. 3-5; (G-557) P. 3; (B-816) P. 2]

675. Ciproflaxacin is an additional antibiotic frequently used for treatment of campylobacteriosis. [Iannini (B-1905) P.4 L. 3-11; Ohl (G-1485) P.13 L. 20-38; (G-191) P. 6]

676. In the context of this proceeding, "empiric treatment" means the treatment of enteritis with an "antimicrobial" (antimicrobial is a term used to describe agents that treat infections with microbes; antibiotics and fluoroquinolone are antimicrobials) based on the individual patient's clinical symptoms and medical history, without first obtaining the results of a culture or other test in order to ascertain what micro-organism(s), or other agent is actually causing the clinical symptoms. [Ohl (G-1485) P.11 L. 31-38]

677. If the treating physician does not have the results of a culture, and must decide on empiric treatment, the 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. [JS 42.]

678. Routine empiric antimicrobial treatment is generally not recommended for diarrheal illness. [Iannini (B-1905) P. 3 L. 15-18; Ohl (G-1485) P.9 L.36-46, P.10 L.1-7]

679. Ciproflaxacin and azithromycin, i.e. fluoroquinolones and macrolides, are used or can be used for the empiric treatment of enteritis, including campylobacteriosis. [Iannini (B-1905) P.4 L. 3-21; Pasternack (B-1909) P.4 L.10-17, P.13 L.11-12; Ohl (G-1485) P.11 L.44-46, P.13 L.1]

680. Even without correcting for foreign travel and prior fluoroquinolone use, it has been estimated that the potential number of treatment failures in the US due to fluoroquinolone-resistant *Campylobacter* infections would be less than 150, or less than 0.00005% of the US population, which is over two orders of magnitude less (< 1 in a million) than the 1 in 10,000 risk level that FDA accepted as safe in its bottled water standard for microbial infections. [Kist (B-1906) P. 11 L. 15-22 - P. 12 L. 1-11; Bayer Response to NOOH P. 16 and authorities cited]

681. The need for empiric treatment of campylobacteriosis by fluoroquinolones has been diminished by the recent introduction of a new test which allows *Campylobacter* infections to be identified within two hours [(B-1143) P. 1-3]; and by the emergence of azithromycin as an effective, broad-spectrum antibiotic that is well tolerated and to which resistance is low, and a soon to be approved antimicrobial rifaximin. [Pasternack (B-1909) P. 13 L.11-21, P.14 L.1-16; Iannini (B-1905) P.4 L.9-16, P. 6 L.1-5; Ohl (G-1485) P. 13 L.31-33]

682. Ciprofloxacin is not approved for the treatment of enteritis or campylobacteriosis in pediatric patients and adolescents (less than 18 years of age), pregnant women, and lactating women. [JS 25; Pasternack (B-1909) P. 4 L. 19; (G-529) P.3; (B-121) P. 2]

683. The general use of empiric antimicrobial treatment in the management of acute or severe bacterial enteritis was derived from the efficacy of empiric therapy for the treatment of Traveler's Diarrhea, where the majority of illnesses were originally attributable to another class of bacteria, enterotoxigenic E. coli. [Pasternack (B-1909) P. 18 L.5-8]

684. "Traveler's Diarrhea" is a term used to describe enteritis acquired in the course of travel to less developed countries or areas. [Ohl (G-1485) P.7 L.20-22]

685. Traveler's Diarrhea is the most common travel-related medical problem, afflicting 20-50% of travelers visiting the developing world, including 7 million US residents. [Ohl (G-1485) P. 7 L. 22-24]

686. Traveler's Diarrhea is most commonly caused by E. coli, but other causes include *Campylobacter*, Salmonella, Shigella and the parasites Giardia and Entamebae, as well as viral agents. [Ohl (G-1485) P. 7 L.24-29]

687. The main risk factors for Traveler's Diarrhea are point of origin, destination, host factors and exposure to contaminated food and water. [(B-121) P. 1]

688. Traveler's Diarrhea usually develops during the first week after arrival in a high risk region, but it may also appear at any time during or after short-term trips, and it may occur more than once during the same trip. [(B-121) P. 1]

689. Some travelers who develop diarrhea secondary to viruses (10-12% of Traveler's Diarrhea cases with an identified cause) or parasites (2-4%), will not improve with empiric antimicrobial therapy, and may develop persistent diarrhea lasting longer than 14 days, which is reported in approximately 3% of travelers with acute diarrhea. [(B-121) P. 2]

690. Fluoroquinolone resistant *Campylobacter* jejuni is highly associated with Traveler's Diarrhea: e.g., in the Smith Study, 75% of the 130 persons with fluoroquinolone resistant *Campylobacter* jejuni likely acquired their infections in a foreign country [(G-589) P. 4-6]

691. Based on the CDC 1998-1999 *Campylobacter* case-control study, Traveler's Diarrhea may account for 13% of the campylobacteriosis cases for which persons seek treatment in the United States each year. [Angulo (B-1452) P. 81, Attachment 3]

692. In addition to being acquired outside the United States from sources outside the United States, Traveler's Diarrhea is also distinctive in that it has a longer mean duration of diarrheal symptoms than campylobacteriosis acquired in the United States, regardless of whether the disease is due to *Campylobacter* which are susceptible or resistant to fluoroquinolones. [Burkhart (B-1900) P. 36 Table 8]

693. This longer duration may be caused by some other risk factor for which foreign travel is a marker, such as exposure to more heavily contaminated foods or water,

or to novel strains of *Campylobacter* to which the traveler has no immunity. [(G-1711) P. 5, 6; Feldman (B-1902) P.37 L.6-8]

694. Approximately 5% to 9% of the persons who seek treatment for campylobacteriosis in the United States each year have taken a fluoroquinolone antibiotic within a month before seeking treatment for their campylobacteriosis. [Smith (G-1473) P.9 L.44-45, P.10 1-2; Kassenborg (G-1460) P.7 L.1-4]

695. Human use of a fluoroquinolone, including use for treatment of campylobacteriosis, can lead to the emergence of fluoroquinolone-resistant *Campylobacter* in the treated individual. [JS 8; (B-127 P. 1; (G-589 P. 4, 6; (G-707) P. 11]

696. Empiric treatment of enteritis with a fluoroquinolone has been shown to select for fluoroquinolone resistant *Campylobacter* during treatment in 25% of the cases, constituting a further reason not to routinely treat adult patients empirically. [(B-816) P. 2; (B-857) P. 1; (G-250) P. 4; (G-529) P. 2; (G-589) P. 4; (B-127) P. 2-4]

697. When performing quantitative assessments of domestically acquired campylobacteriosis, it is necessary to remove cases of Traveler's Diarrhea as it consistently appears as a confounder in the population group or subgroup under consideration. [Burkhart (B-1900) P.4 L.16-18, P.13 L.20-46, P.14 L.1-22; Cox (B-1901) P.5 L.14-21, P.31, Attachment 1; Feldman (B-1902) P.16 L.3-14, P.36 L.13-21, P.37 L.1-8, P.38 L. 20-22, P.39 L.1-7, P.42 L.5-14]

698. Traveler's Diarrhea is a confounder (it is significantly positively associated with both fluoroquinolone resistance and days of illness) and is significantly different from domestically acquired diarrhea. [Cox (B-1901) P.22]

699. When performing quantitative assessments of domestically acquired, fluoroquinolone-resistant campylobacteriosis, it is necessary to remove cases of Traveler's Diarrhea and cases previously treated with fluoroquinolone from the population group or subgroup under consideration. [Burkhart (B-1900) P.4]

700. When cases of Traveler's Diarrhea and previous fluoroquinolone treatment are removed from the "CDC 1998-1999 *Campylobacter* case-control study" and Smith et al. study populations, there is no statistical difference between the mean durations of diarrhea for fluoroquinolone-resistant and fluoroquinolone-susceptible *Campylobacter* cases. [Burkhart (B-1900) P. 35 L. 4-6; P. 36 L. 4-5]

701. CVM does not have any facts or data demonstrating any increase in the rate or extent of complications (including but not limited to Guillain-Barre Syndrome) from infections caused by fluoroquinolone-resistant *Campylobacter* as compared to infections caused by fluoroquinolone-susceptible (non-resistant) *Campylobacter*. [CVM Interrogatory Answer 60]

702. Clinical and bacterial relapses are seen in untreated patients as well as in fluoroquinolone treated patients with *Campylobacter* initially susceptible to

fluoroquinolones. [Kist (B-1906) P.13 L.15-21; (G-1616) P.3; (G-422) P.3; (B-127) P.2; (G-497) P. 2-4]

703. There are no data associating either complications or increased mortality with fluoroquinolone resistant *Campylobacter* infections as compared to infections with susceptible *Campylobacter*. [Kist (B-1906) P.16 L.6-7, P.18 L.6-7, 12-13; Newell (B-1908) P.47 L.23-24, P.48 L.1-2]

704. A fatal outcome of campylobacteriosis is rare and is usually confined to very young or elderly patients, almost always with an underlying serious disease. [Kist (B-1906) P.3 L.19-20; (B-44) P. 1; (G-580) P. 4; (G-1644) P. 4]

705. Guillain-Barre Syndrome ("GBS") cases have been associated with campylobacteriosis but are not affected by prior antibiotic treatment. [Kist (B-1906) P.14 L. 18-19; Pasternack (B-1909) P. 19 L.6-8; (G-1661) P. 4]

706. Reactive arthritis is characterized by pain and swelling of joints, typically 1 to 2 weeks after onset of enteritis, caused by agents such as *Campylobacter*. [Kist (B-1906) P. 7 L.3-4]

707. *Campylobacter* associated reactive arthritis is usually self-limiting. [Kist (B-1906) P. 7 L.5-6]

708. *Campylobacter* associated reactive arthritis is rare (0-1.7% of reactive arthritis cases) and is not affected by prior antibiotic treatment. [Kist (B-1906) P. 7 L.11-13; Pasternack (B-1909) P. 19 L.6-8]

709. In deciding whether to treat a patient with enteritis empirically with antimicrobials such as a fluoroquinolone or a macrolide, the physician must make a prudent judgement whether the potential benefits of treatment of that patient outweigh the potential risks. [Ohl (G-1485) P.9 L.36-46, P.10 L.1-7; Pasternack (B-1909) P.18 L.12-22, P.19 L. 1-22, P.20 L.1-2; (G-261) P. 11; (G-250) P.1]

710. The potential benefits of empiric treatment of campylobacteriosis with a fluoroquinolone are uncertain because the data from the relevant studies are conflicting regarding whether the duration of diarrhea will be shortened and whether treatment requires "early" treatment, i.e., before the elapse of the time it takes to obtain the results of a stool culture or other test. [Pasternack (B-1909) P. 11 L.19-22, P.12 L.1-22, P.13 L.1-8; (B-44) P. 7; (B-127) P. 4; (G-705) P. 1]

711. Some of the relevant studies show no statistically significant benefit, in the form of reduction of the mean duration of diarrhea, from treatment of susceptible *Campylobacter* with fluoroquinolones. [(B-816) P. 2-3; (G-188) P. 1, 3, 4, 5; (G-172) P. 3]

712. While some studies claim that "early" treatment of campylobacteriosis is required to obtain a reduction in the mean duration of diarrhea, the study that shows the greatest benefit from such treatment (Dryden et al.) concerned patients who had

received treatment, on average, 4 or more days after the onset of their diarrhea. [(B-1127) P.1; (G-172) P. 3; Pasternack (B-1909) P. 12 L. 14-20]

713. On the other hand, empiric treatment of enteritis with fluoroquinolones entails the risk that, if the disease is caused in whole or in part by Salmonella bacteria, carriage of the bacteria will be prolonged and an acute infection may be turned into a chronic one. [Pasternack (B-1909) P.5 L.18-20, P.8 L.17-18]

714. Further, empiric treatment of enteritis with antimicrobials such as fluoroquinolones also presents a risk of a life-threatening complication of hemorragic E. coli infection known as the hemolytic-uremic syndrome, whose risk is thought to be increased significantly following antibiotic treatment of hemorrhagic E. coli enteritis. This is less common in adults than in children, but is present nonetheless. [Iannini (B-1905) P.3 L. 19-21, P.4 L.1-2; Pasternack (B-1909) P.5 L.8-17, P.8 L.18-21; (B-1559) P.1, 3, 4, 6]

715. Empiric use of antimicrobials, including fluoroquinolones, for the treatment of enteritis is undergoing reexamination, and more recent treatment guidelines are more cautious about recommending the use of such therapy. [Pasternack (B-1909) P. 4 L.10-21, P.5 L.1-20, P.11 L.1-18, P.18 L. 21-22, P.19 L.1-22, P.20 L.1-2; Iannini (B-1905) P. 3 L.15-18; (B-857) P.2; (G-253) P.5; (G-707) P.9]

716. As a consequence of the emerging and improved knowledge of the relative risks and benefits of empiric treatment of enteritis with fluoroquinolones, such empiric treatment of enteritis which is not Traveler's Diarrhea is properly, and increasingly, limited to adult patients with severe abdominal pain, frequent episodes of diarrhea, fever, blood or white blood cells in the stool, who seek medical attention relatively early, and those individuals who weakened as a result of any of a variety of medical illnesses or conditions due, e.g., to age, pregnancy, etc. [(B-127) P.1; (G-172) P.5,6; (G-292) P.1]

717. Immunocompromised patients (patients whose immune systems have been compromised, e.g., chronically ill patients, HIV-infected patients, organ transplant patients) fall into a special category since they are prone to suffer more severe disease, a potentially higher risk of bloodstream infection, and are at risk for relapsing and chronic infections. In these patients, antibiotic therapy is necessary. [Iannini (B-1905) P. 5 L.18-19; Kist (B-1906) P. 17 L.11-21; Pasternack (B-1909) P.5 L.21-22, P.6 L.1-22, P.7 L.1-13; (B-742) P. 1; (G-1616) P. 3; (G-442) P. 3]

718. Since clinical and microbiological failures have been documented following the administration of a single antibiotic agent to immunocompromised individuals known to have *Campylobacter* enteritis due to an antibiotic-susceptible strain, the administration of a combination antibiotic therapy is the prudent treatment. [Iannini (B-1905) P. 5 L.18-20; (B-742) P. 5]

719. In the rare instances of bacteremia and extraintestinal infections requiring antibiotic treatment, particularly among those patients with underlying immunodeficiency

states, parenteral (intramuscular or intravenous, not oral, treatment) combination therapy with imipenem and gentamicin is the recommended treatment. [Pasternack (B-1909) P.8 L.21-22, P.9 L.1-3; Iannini (B-1905) P. 5 L. 6-8; (B-273) P.7; (B-742) P.5]

720. Prolonged and relapsing *Campylobacter* enteritis in immunocompromised patients, as well as complications and death in such patients, has no relation to whether the *Campylobacter* was initially fluoroquinolone resistant or susceptible. [(B-742) P.3-5]

721. The prognosis of campylobacteriosis in HIV-infected individuals depends on the severity of immunodeficiency rather than on issues of initial antibiotic susceptibility: there are well-documented cases of fluoroquinolone-susceptible *Campylobacter* enteritis and bacteremia who nevertheless failed therapy. [Pasternack (B-1909) P. 6 L.11-12, P. 7 L.8-12]

722. In the most thoroughly reported case study, the deaths of 3 HIV-infected patients were attributed to *Campylobacter* jejuni bacteremia infections, however, fluoroquinolone resistance was not a factor in causing the deaths. [(B-742) P. 3-5; Pasternack (B-1909) P. 6 L.17-22, P.7 L.1-13]

723. Resistance of domestically acquired *Campylobacter* to fluoroquinolones in patients not recently treated with fluoroquinolones does not appear to be a very significant clinical concern in the United States: the most recent, broad-based studies in the United States "CDC 1998-1999 *Campylobacter* case-control study" and Smith et al. do not show any difference in the mean durations of diarrhea for susceptible and resistant cases when appropriate adjustments are made to exclude foreign travel and prior treatment. [Burkhart (B-1900) P. 36 (Table 8); (B-50) P. 2]

724. Even outside the United States, where infections may be more serious, recent studies of patients receiving fluoroquinolone for enteritis in settings where fluoroquinolone resistance among *Campylobacter* isolates was almost universal show that treatment failures are limited (approximately 2.6-25%), even among populations with very high in vitro resistance rates (in vitro means outside the host, e.g. laboratory tests). [(B-1920) P.4; (B-50) P.2]

725. Treatment failures among patients with susceptible *Campylobacter* isolates receiving fluoroquinolones are in a similar range to such treatment failures among patients with resistant isolates:

- In the Piddock study, the frequency of treatment failures for fluoroquinolone resistant *Campylobacter* was approximately 2.6% (1/39), and in the Sanders study the treatment failure rate was approximately 25%, producing a fluoroquinolone-resistant treatment failure range of approximately 2.6% to 25%.

- In the Kuschner study, the frequency of treatment failures for fluoroquinolone-susceptible *Campylobacter* was approximately 4.2% (1/24), and in a clinical trial of empiric ciprofloxacin treatment for acute diarrhea, the treatment failure rate for fluoroquinolone-susceptible *Campylobacter* was approximately 20% (2/10), producing a fluoroquinolone susceptible range of approximately 4.2% to 20%. [(B-20) P.2; (B-1920) P.4; (G-354) P.3; Pasternack (B-1909) P.12 L.20-22, P.13 L.1]

726. It is likely that the apparent efficacy of fluoroquinolone in "resistant" cases can be attributed to the very high concentrations of fluoroquinolone achieved in vivo in the human intestine and found in stool samples. The achievable stool concentration of fluoroquinolone typically exceeds the in vitro benchmarks for "resistance" (which are based on blood concentrations for other bacteria) for fluoroquinolone in *Campylobacter* by an order of magnitude or more. [(G-172) P. 1]

727. There is both conceptual evidence as well as clinical experience to suggest that the current definition of in vitro *Campylobacter* jejuni resistance to ciprofloxacin is overly stringent and not relevant to the clinical management of *Campylobacter* jejuni enteritis in almost all cases. [Pasternack (B-1909) P. 17 L. 4-6]

728. Epidemiological data support the conclusion that treatment of fluoroquinolone-resistant *Campylobacter* illness patients with ciprofloxacin is usually effective, and as effective as treatment of patients with fluoroquinolone-susceptible *Campylobacter* illness. [Cox (B-1901) P. 78]

729. Treatment results, in combination with pharmacokinetic data, support the conclusion that the breakpoints for fluoroquinolone resistance in *Campylobacter* set based on extrapolations from in vitro testing are too low to have clinical significance. [Silley (B-1913 P. 17 L. 15-23, P. 18 L. 1-15; Pasternack (B-1909) P. 14 L. 19-22 – P. 15 L. 1-16]

730. There is no clinically established threshold or official breakpoint for resistance to ciprofloxacin among *Campylobacter* isolates in any country. [Kassenborg (G-1460) P.4 L.3-4; (B-44) P. 6; (G-1789) P. 11; (G-191) P. 4;(G-624) P. 1; Silley (B-1913); citing Piddock et. al., 2000, Attachment 1 P.46 ¶ 2]

731. There are no standardized methods for the measurement of fluoroquinolone resistance in Campylobacters. Both the methods used and the breakpoints adopted by different studies vary so the comparison of studies between countries within laboratories in the same country, is difficult. [Newell (B-1908) P.13 L.17-20]

732. In general, CDC uses a breakpoint of 4 ug/ml because the National Committee for Clinical Laboratory Standards (NCCLS) uses an MIC of 4 ug/ml for ciprofloxacin resistance to Enterobacteriaceae. [Kassenborg (G-1460) P.4 L.4-6]

733. The in vivo clinical importance of *Campylobacter* deemed to be "resistant" by in vitro testing remains unknown. [Newell (B-1908) P.14 L.1-2; Burkhart (B-1900) P.4 L.22-24, P.10 L.1-2]

734. For fluoroquinolones, the best clinical outcomes are associated with peak/MIC ratios >/= 10. [Silley (B-1913) attachment # 1 P.50 ¶ 2]

735. If a high enough peak to MIC ratio can be achieved then not only will the parent organism be killed but also the "resistant" mutant. [Silley (B-1913) attachment # 1 P.51 ¶ 1]

736. Peak to MIC ratios can easily exceed 10 in the gastrointestinal tract of patients with Campylobacters that have an MIC of 32 when patients are treated with 500mg ciprofloxacin BID. [Silley (B-1913) attachment # 1 P.51 ¶ 1, 2]

737. *Campylobacter* infection occurs in the gastrointestinal tract. [(G-444)]

738. Given the high levels of ciprofloxacin reported in the gastro-intestinal tract it is not surprising that clinical cure can be demonstrated for organisms with an MIC of 32 ug/ml. [Silley (B-1913) attachment # 1 P.52 ¶ 1]

739. A proportion of the isolates tested in the NARMS program have been shown to be impure cultures, this will lead to a degree of misinterpretation of the data. [Silley (B-1913) attachment # 1 P.55 ¶ 4]

740. It is highly inappropriate to consider that *Campylobacter* spp. With an MIC of 4 ug/ml will be clinically resistant to ciprofloxacin. [Silley (B-1913) attachment # 1 P.55 ¶ 6]

741. Available data supports a breakpoint of 64 ug/ml. Such a breakpoint would need to be substantiated in accordance with NCCLS guidelines. [Silley (B-1913) attachment # 1 P.56 ¶ 2]

742. The NCCLS breakpoint for two different bacteria to the same antimicrobial may be very different. [Walker (G-1481) P.5 ¶ 10]

743. Testing methods not endorsed by NCCLS and interpretive criteria that are not set by NCCLS may be of questionable value. [Walker (G-1481) P.9 ¶ 13]

744. Epidemiological data also suggest that treatment of fluoroquinolone-resistant *Campylobacter* illness patients with ciprofloxacin is usually effective. [Cox (B-1901) P.78, citing G-394 (Marano 2000 data)]

745. CVM's risk assessment does not show that harm to human health has occurred, can occur, or is likely to occur as a result of continued use of Baytril. It does not provide valid evidence that harm to human health is likely or plausible, nor does it quantify harm to human health caused by enrofloxacin use (or by a ban). [Cox (B-1901) P.7 L.4-7]

746. CVM's hazard identification step of the CVM/Vose Risk Assessment does not identify any adverse human health effects specifically caused by fluoroquinolone-resistant campylobacteriosis in humans. [Cox (B-1901) P.14]

747. CVM's hazard identification step of the CVM/Vose Risk Assessment does not identify by objective causal analysis of data any adverse human health effects caused (or probably caused) specifically by fluoroquinolone-resistance in *Campylobacter*. [Cox (B-1901) P.15]

748. Analyzing the recent large case-control data set provided by "CDC 1998-1999 *Campylobacter* case-control study" shows that, with high statistical confidence, there is no detectable causal relation between fluoroquinolone-resistant campylobacteriosis in humans and adverse human health consequences (excess days of diarrhea). [Cox (B-1901) P.15]

749. Analyzing the recent large case-control data set provided by "CDC 1998-1999 *Campylobacter* case-control study" shows that, with high statistical confidence, there is not a significant statistical association between fluoroquinolone-resistant campylobacteriosis in humans and days of diarrhea, after correcting for confounders. [Cox (B-1901) P.15; Burkhart (B-1900) P.49 L.12-14]

750. The excess days of diarrhea attributed by CVM to fluoroquinolone-resistant campylobacteriosis are completely explained away by foreign travel (Cox and Popken, 2002) – a key confounder not correctly controlled for in the statistical analyses that CVM has relied on. [Cox (B-1901) P.15; Burkhart (B-1900) P.49 L.12-14]

751. If the statistical analyses that CVM relies on to demonstrate that fluoroquinolone-resistant campylobacteriosis leads to excess days of diarrhea are completely explained away by foreign travel as a confounder, then no hazard to human health from chicken-borne fluoroquinolone-resistant *Campylobacter* has been demonstrated. [Cox (B-1901) P.15; Burkhart (B-1900) P.49 L.12-14]

752. CVM's risk assessment has not identified or quantified any adverse human health effects specifically caused fluoroquinolone-resistant *Campylobacter*. Although CVM has discussed various adverse health effects of campylobacteriosis in connection with its risk assessment, including excess days of diarrhea, Guillian-Barre Syndrome, and death, the risk assessment model itself identifies no specific adverse human health effect(s) that are caused by chicken-borne fluoroquinolone-resistant *Campylobacter*, as opposed to *Campylobacter* in general. [Cox (B-1901) P.25]

753. Analysis of "CDC 1998-1999 *Campylobacter* case-control study" data demonstrates that fluoroquinolone-resistant campylobacteriosis is not associated with longer illness duration. [Cox (B-1901) P.24]

754. After correcting for confounding due to foreign travel, there is no significant association between fluoroquinolone-resistant campylobacteriosis and duration of

diarrhea in the "CDC 1998-1999 *Campylobacter* case-control study" data set. [Cox (B-1901) P.30-31; Newell (B-1908) P.46 L.10-13; Burkhart (B-1900) P.49 L.12-14]

755. After correcting for confounding due to foreign travel, there is no significant association between fluoroquinolone-resistant campylobacteriosis and duration of diarrhea in the Smith et al. data set. [Cox (B-1901) P.30-31; Newell (B-1908) P.46 L.10-13; Burkhart (B-1900) P.49 L.12-14]

756. CDC researcher Jennifer Nelson (a/k/a Jennifer McClellan) anticipated that after correcting for confounding due to foreign travel, there might be no significant association between fluoroquinolone-resistant campylobacteriosis and duration of diarrhea in the "CDC 1998-1999 *Campylobacter* case-control study" set, stating in her thesis; "An alternative explanation of why people with fluoroquinolone-resistant *Campylobacter* infections have an increased hazard of having longer diarrhea could be unmeasured confounders. For example, if people were more likely to acquire fluoroquinolone-resistant bacteria during international travel..." [Cox (B-1901) P.31, citing (G-1679) (McClellan 2000)]

757. McClellan found that "The hazard ratio for the association between ciprofloxacin resistance and duration of diarrhea, adjusting for age, sex, residence, FoodNet site, education, and household income, stratified by race, was 1.2 (95% CI: 0.92, 1.57)." [Cox (B-1901) P.31, citing (G-1679) (McClellan 2000)]

758. McClellan found no statistically significant relation between ciprofloxacin resistance and duration of diarrhea, even without adjusting directly for international travel. [Cox (B-1901) P.31, citing G-1679 (McClellan 2000)]

759. Only by improperly ignoring confounders can an apparent positive association between fluoroquinolone-resistant campylobacteriosis and duration of diarrhea in the "CDC 1998-1999 *Campylobacter* case-control study" data set be created. [Cox (B-1901) P.31]

760. The finding of no association between fluoroquinolone-resistant campylobacteriosis and excess days of illness in the "CDC 1998-1999 *Campylobacter* case-control study" data set is consistent with data from a recent analysis of 9089 cases of campylobacteriosis, investigated in the Sentinel Surveillance Study of England and Wales (G-1772), in which there was no significant difference in the mean duration of illness associated with a fluoroquinolone-resistant organism (7.9 days) compared to infection with a sensitive organism (7.7 days) (p=0.4). [Cox (B-1901) citing Newell (B-1908); Newell (B-1908) P.46 L.13-22]

761. Correction for confounding is a crucial part of objective causal analysis of epidemiological data. [Cox (B-1901) P.39-40; Feldman (B-1902) P.8 L.25-29]

762. After correctly accounting for confounding (or "explaining away" of the association) by foreign travel there is no evidence of any excess days of illness

caused by a fluoroquinolone-resistant *Campylobacter* infection compared to a susceptible (non-fluoroquinolone-resistant *Campylobacter* infection. [Cox (B-1901) P.39]

763. The "CDC 1998-1999 *Campylobacter* case-control study" shows no differentiation between hospitalizations related to fluoroquinolone-resistant *Campylobacter* infections and those related to fluoroquinolone-susceptible ones. [Friedman (G-1488) P. 87]

764. The "CDC 1998-1999 *Campylobacter* case-control study" showed that fluoroquinolone-resistant *Campylobacter* cases had fewer days of hospitalization (a mean of 2 days) than did fluoroquinolone-susceptible *Campylobacter* cases (a mean of 3 days). [Nelson (G-1452) P. 117, 118, 128]

765. When adjusted to remove Travelers Diarrhea and prior treatment, the "CDC 1998-1999 *Campylobacter* case-control study" showed that campylobacteriosis patients with fluoroquinolone-resistant *Campylobacter* were hospitalized less frequently than *Campylobacter* patients with fluoroquinolone-susceptible *Campylobacter* (9.3% vs. 10.5%) and for fewer days (median of 1 day vs. median of 3 days). [Burkhart (B-1900) P. 38]

766. There were no differences in length of illness or admission to hospital between patients with a ciproflaxacin-resistant infections and patients with susceptible infections as reported in a recent large case control study conducted in the United Kingdom, which was stratified by foreign travel. [Molbak (G-1468) P. 19 L. 37-40]

767. Different conclusions, regarding significance of duration of diarrhea, were drawn by Marano compared to McClellan following the evaluation of the same "CDC 1998-1999 *Campylobacter* case-control study". [Molbak (G-1468) P. 19 L. 25-26 & L. 35]

768. The Danish registry data set utilized for (G-1799) and Molbak (G-1468) did not contain adequate information to study the effects of antimicrobial drug resistance or treatment with antimicrobial drugs. [(G-1799) P. 4; Molbak (G-1468) P. 20 L. 11-13]

769. There was no statistically significant difference between time elevated risks of complications from fluoroquinolone-resistant and complications from fluoroquinolone-susceptible infections in the Danish registry data set utilized for (G-1799) and (G-1468). [Molbak (G-1468) P. 21 L. 11-15]

770. There was no statistically significant difference in mortality between patients infected with fluoroquinolone resistant and sensitive strains in the Danish registry data set utilized for (G-1799) and (G-1468). [Molbak (G-1468) P. 22 L. 1-2]

771. Among the patients studied in (G-1799) and (G-1468), the diagnosis of a gastrointestinal infection such as *Campylobacter* may be a marker of excess mortality rather than a contributing cause. [(G-1799 P. 4]

772. While a fluoroquinolone may be used for the early empirical treatment of campylobacteriosis, erythromycin is often used following the diagnosis of *Campylobacter* species, and erythromycin resistance to *Campylobacter* is low, so treatment failures resulting in severe outcomes such as death or persistent blood stream infection are more difficult to find. [Molbak (G-1468) P. 19 L. 1-6]

773. The relevance to this proceeding of the *Campylobacter*-related mortality inferences drawn in (G-1799) and Molbak (G-1468) is unknown and in doubt, since they provide no information regarding the species of *Campylobacter* involved in the Danish register data set or in their analyses of it, and c. fetus, which is not generally thermophilic or relevant to this proceeding, is well-known to cause or to be associated with many of the life-threatening conditions identified in those data. [G-444 P. 323-324]

774. In a study in Denmark, Neimann, Molbak et al. found no statistically significant difference between the mean duration of illness from fluoroquinolone-resistant *Campylobacter* and the mean duration of illness from fluoroquinolone-suspectible *Campylobacter* ($p = 0.109$). [(G-455) P. 1]

775. Neimann, Molbak et al. also found that the median duration of disease was similar between fluoroquinolone-resistant *Campylobacter* cases and fluoroquinolone-susceptible cases among patients who did not receive antibiotic treatment. [(G-455) P. 1]

776. Neimann, Molbak et al. also found that fluoroquinolone resistance in *Campylobacter* isolates was highly associated with foreign travel (75.6% for resistant isolates vs. 22.3% for susceptible isolates). [(G-455) P. 1]

777. The hypothesis that *Campylobacter* which are intrinsically resistant to fluoroquinolones are capable of producing illness (virulence) that is more severe than *Campylobacter* which are intrinsically susceptible to fluoroquinolones, is based on a paucity of data which have methodological limitations, and does not apply to domestically acquired fluoroquinolone-resistant *Campylobacter*, since domestically acquired fluoroquinolone-resistant *Campylobacter* do not produce more prolonged or severe illness. [Pasternack (B-1909) P.20 L.12-15; Burkhart (B-1900) P.40 L.3-7]

778. There is no evidence in the epidemiological experience available to date that there is an increase in virulence associated with FQ resistant *Campylobacter*. [Burkhart (B-1900) P.3 L. 17-18]

779. There are no robust data that existed prior to the approval of FQs for use in US poultry that provide an estimate of the pre-approval background (baseline) rate of domestic FQ resistance. [Burkhart (B-1900) P.3 L. 35-37]

780. The Minnesota 1992-1998 database, cited by Smith, (G-589) does not adjust for foreign travel or prior FQ use. Therefore these data can not be used to determine

whether domestic cases without FQ use have increased in incidence. [Burkhart (B-1900) P.3 L. 39-41]

781. There is no public health basis to conclude that the approval of NADA 140-828 should be withdrawn. [Burkhart (B-1900) P.3 L.5-6]

782. Temporal reporting data from other countries cited by the FDA as affirming that FQ use in US poultry is significant cause of resistant *Campylobacter* in the US, cannot be used to reach such a conclusion. [Burkhart (B-1900) P.3 L.27-29]

783. The FoodNet case control study has severe limitations in its questionnaire design that limit its overall value for both resistant and non-resistant *Campylobacter*. [Burkhart (B-1900) P.4 L. 12-14]

784. Both the Smith study and the CDC *Campylobacter* case-control study have too few domestic FQ resistant cases without prior FQ use to be of value in studying the cause of FQ resistance. [Burkhart (B-1900) P.4 L. 14-15]

785. It is scientifically reasonable to consider the possibility that human benefits could be attributable to the treatment of poultry with FQs. [Burkhart (B-1900) P.7 L. 18-19]

786. The FDA was concerned about the emergence of resistant bacteria during their deliberations about whether to approve a FQ for use in poultry, and the agency knew that FQ treatment selects for resistant *Campylobacter* in humans and animals. [Burkhart (B-1900) P.8 L. 11-14]

787. A complexity of interpreting data from foreign sources is determining the similarities of use of FQ in food production in those countries. [Burkhart (B-1900) P.8 L. 34-36]

788. Cases of FQ resistant *Campylobacter* caused by either foreign travel or prior FQ use before culture are not germane to an individual country's concern that FQ us in farm animals in that country could be causing domestically acquired FQ resistant cases. [Burkhart (B-1900) P.8 L. 44 – P.9 L. 1]

789. The FDA also believes that temporal correlations observed in other countries provide supporting evidence that FQ use in US poultry production accounts for a significant degree of the FQ resistance that has been observed in the US. Interpretation of the foreign data hinges on the same problem that exists in the US data in that there are limited historical resistance data for comparison. [Burkhart (B-1900) P.8 L.30-34]

790. It is not so clear how foreign data generalizes to the use of FQs to treat specific microbial infections in the US. [Burkhart (B-1900) P.8 L.37-38]

791. There are fairly high resistance rates that have been reported in several countries that do not even have FQs approved for use in farm animals. [Burkhart (B-1900) P.8 L.38-40]

792. Both human use of FQs and the rate of international travel have increased dramatically in the 1990's. [Burkhart (B-1900) P.9 L.1-2]

793. One cannot interpret trends from data when missing information on foreign travel and prior FQ use given that both human use of FQs and the rate of international travel have increased dramatically in the 1990's and that a significant proportion of FQ resistant cases are attributable to these two factors. [Burkhart (B-1900) P.9 L. 1-4]

794. The belief that foreign travelers who develop FQ resistant *Campylobacter* because of FQ use in farm animals in another country is not evidence that can be used to evaluate the cause of resistance in the country of interest. [Burkhart (B-1900) P.9 L. 4-7]

795. Based upon the Smith and Kassenborg data, a significant proportion of resistant cases that are observed in US residents are unlikely to be linked to enrofloxacin use in poultry production. [Burkhart (B-1900) P.13 L. 13-15]

796. Since it is well established that prior FQ use quickly selects for *Campylobacter* that are resistant in vitro, it is epistemologically difficult to assign causality for such resistance to another factor, given the prior FQ use. [Burkhart (B-1900) P.13 L. 22-25]

797. *Campylobacter* caused by some exposure associated with foreign travel can not address the question of whether FQ use in US poultry production causes a significant degree of resistant disease in US residents. [Burkhart (B-1900) P.13 L. 35-37]

798. Controlling for foreign travel in case control studies requires that sufficient numbers of controls with foreign travel be included in the study. [Burkhart (B-1900) P.13 L. 43-44]

799. Foreign travelers must be excluded from primary analysis when sufficient numbers of controls with foreign travel are not included in a case control study. [Burkhart (B-1900) P.13 L. 44-46]

800. International travelers may delay seeking medical treatment until returning to the US, thereby self selecting for longer courses of illness. [Burkhart (B-1900) P.14 L. 5-6]

801. NARMS/ FoodNet sites do not capture data on foreign travel or prior FQ use on a routine basis. [Burkhart (B-1900) P.17 L. 6-8]

802. The Smith study (G-589) failed to identify any risk factor for the 20% of resistant cased that were acquired domestically and did not have prior FQ use before culture. [Burkhart (B-1900) P.18 L. 27-29]

803. The Smith analysis (G-589) did not consider the possibility that foreign travel could confound an evaluation of illness duration. [Burkhart (B-1900) P.18 L. 34-35]

804. In the Smith data set (see, e.g., G-589), foreign travel is associated with longer duration of illness irrespective of in vitro FQ resistance. [Burkhart (B-1900) P.19 L. 37-38]

805. In the Smith data set (see, e.g., G-589), the most complete data is for the year 1997. [Burkhart (B-1900) P.22 L.7-25]

806. In the Smith data set (see, e.g., G-589), patients from 1997 without foreign travel, had a mean duration of illness of 9.2 in the resistant cases and 9.0 in the 144 non-resistant controls. Likewise, in patients with foreign travel, the mean duration was 11.1 in the 52 patients with resistant *Campylobacter* compared to 12.2 in the 32 patients with non-resistant *Campylobacter*. [Burkhart (B-1900) P.19 L. 43 – P.20 L.4]

807. Smith's finding of a longer duration of illness in patients with resistant *Campylobacter* must have been because of his failure to control for foreign travel. [Burkhart (B-1900) P.20 L. 21-23]

808. Burkhart, after analyzing Smith's data, found that chicken and turkey exposure was less likely in resistant cases. [Burkhart (B-1900) P.20 L. 38]

809. There is no evidence of any difference in risk factors between resistant and non-resistant cases in the Smith data. If anything, the Smith data suggest that resistant cases are less likely to be associated with consumption of chicken/turkey compared to non-resistant cases." [Burkhart (B-1900) P.22 L. 40-43 referring to G-589 (Smith 1999)]

810. McClellan and Marano reanalyzed the Kassenborg dataset and reported their findings in G-1679 and G-394. [Burkhart (B-1900) P.25 L. 4-7]

811. The questionnaire used in the 1998 - 1999 CDC *Campylobacter* case-control study, was different for collection data on chicken/turkey consumption than that for other foods and meats. [Burkhart (B-1900) P.25 L. 27-28]

812. The 1998 - 1999 CDC *Campylobacter* case-control study questionnaire asked about the cumulative consumption for each poultry product, but skipped collecting such data for other foods. [Burkhart (B-1900) P.25 L. 31-33]

813. After analyzing data from the 1998 - 1999 CDC *Campylobacter* case-control study, Burkhart finds no evidence of increased morbidity with resistant *Campylobacter*

when controlling for foreign travel and prior FQ use. [Burkhart (B-1900) P.33 L. 2-4]

814. After analyzing data from the 1998 - 1999 CDC *Campylobacter* case-control study, Burkhart found patients who claimed foreign travel had a longer duration of diarrhea than patients who did not travel (7.8 days vs 6.9 days). [Burkhart (B-1900) P.34 Table 5]

815. After analyzing data from the 1998 - 1999 CDC *Campylobacter* case-control study, Burkhart found that patients with resistant infections had less days of illness than those with susceptible infections when they reported no foreign travel, had no prior FQ use, and were treated with a FQ following culture. (6.0 days vs 6.9 days) [Burkhart (B-1900) P.36. Table 8]

816. After analyzing data from the 1998 - 1999 CDC *Campylobacter* case-control study, Burkhart found that resistant cases who use an antidiarrheal agent tended to have 1-2 days less diarrhea. [Burkhart (B-1900) P.37 L.7]

817. Burkhart found, after analyzing data from the 1998 - 1999 CDC *Campylobacter* case-control study, that foreign travel must be controlled for when analyzing illness length. [Burkhart (B-1900) P.40 L. 3-4]

818. The NARMS dataset does not have information on foreign travel and prior FQ use. [Burkhart (B-1900) P.44 L. 42-43]

819. NARMS has no value in estimating the incidence or in determining if there has been a temporal decrease or increase in resistance after enrofloxacin approval. [Burkhart (B-1900) P.50 L. 8-10]

820. Approximately 99 % of the *Campylobacter* infections in the United States are caused by C. jejuni. [McClellan (G-1679) P.12]

821. The United States poultry industry is a fully integrated system of animal agriculture. Each poultry company has control over all fiscal and bird husbandry aspects of production, from the day-old parent breeders to the marketing and distribution of the final products to the retailer. [Hofacre (A-202) P.2 L.15-17]

822. A typical poultry complex in the US is a stand-alone, fully integrated operation that provides all the needed inputs to progress from the baby parent chick to the finished product for the consumer. The entire process is centrally planned and coordinated for maximum efficiency and control. Other than the use of contract growing facilities, the birds, feed, and other inputs are owned and controlled by the integrated company, and production is accomplished by company employees. In the case of contract facilities, the farm owner provides the facility, which must meet company specifications, and supplies the labor. The company supplies the birds and feed, and usually supplies, or at the very least strictly specifies any other inputs such as pesticides, rodenticides, and medications. Each company typically has a complete growing program, which includes all aspects of husbandry, such as

temperatures, air quality, water quality, lighting programs, feeding programs, vaccination, and so forth. Companies have agents who administer these plans, provide growers with advice and assistance, and monitor compliance. There are various arrangements for provision of certain inputs such as bedding (litter), heat, and power, but the company either provides these or specifies and monitors their provision. [Smith (B-1914) P.7 L.1-14]

823. The vertically integrated arrangement in the poultry industry is extremely efficient and allows a high degree of quality control over the entire process. Implicit in this degree of control is the ability to effectively prevent disease on a population basis, control microbial contamination, and control chemical residues, including pesticides, antibiotics, and other drug residues. [Smith (B-1914) P.7 L.14-18]

824. The poultry industry is highly integrated. The typical commercial company is divided into operational units, usually referred to as "complexes." The complex starts with a baby breeder chick, and accomplishes all phases of production through and including the production of the finished and packaged poultry product, ready for shipment to the market. The complex typically is headed by a complex manager, who reports to the President/CEO level. Most complexes consist of a live production division and a processing division, with managers who report to the complex manager. The live production division is responsible for producing the live poultry and delivering them to the processing plant. The processing division is responsible for slaughter, dressing, packaging, and distribution. The live production division is where antibiotic usage occurs. [Smith (B-1914) P.3 L.18 through P.4 L.8]

825. Most poultry companies maintain extensive records of all phases of the operation, from pullet weights to hen performance (egg production and hatchability), broiler performance (livability, feed conversion, condemnation), live haul performance (efficiency, DOAs), and plant efficiency. The amount of data collected and analyzed is staggering in its detail. In terms of health monitoring of broilers, some of the more important parameters are morbidity and mortality. [Smith (B-1914) P.14 L.20 through P.19-23]

826. The typical US broiler chicken farm typically has on the order of 100,000 chickens, divided equally into four houses. As in a city of 100,000 people, disease prevention becomes imperative for the poultry industry. Poultry veterinarians practice preventive medicine, utilizing two primary tools, biosecurity and vaccination. [Hofacre (A-202) P.3 L.15-18]

827. Broiler chicks hatch in 21 days (28 days for turkeys). Day-old chicks are vaccinated in the hatchery to help prevent two respiratory diseases, Newcastle disease and infectious bronchitis. [Hofacre (A-202) P.5 L.17-19]

828. Day old 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 (A-202) P.5 L.20-22]

829. In the United States, a broiler grow-out house typically contains on the order of 20,000 to 25,000 broilers. [Joint Stipulation 37]

830. In the United States, a turkey grow-out house typically contains on the order of 10,000 to 20,000 turkeys. [Joint Stipulation 38]

831. Broilers are typically reared to 42 - 58 days of age. [Russell (B-1912) P.3 L.15-16]

832. Automatic nipple (closed) water systems are found in virtually all broiler houses in the United States. Water is provided by a series of four or more pipes that run the length of the house, suspended from the ceiling by a centrally adjustable cable system. The pipe is equipped approximately every foot with a nipple that dispenses water when pecked by the chicken. The suspension system allows for easy height adjustment as the birds grow. This closed system provides a constant supply of clean water, while minimizing spillage and wet litter. The industry converted to these closed drinking systems in the early 1990's. An automatic nipple water system throughout the house maintains a constant supply of drinking water to the birds. Fresh water from a municipal system or a potable water well flows into the house and can only exit the system when a bird pecks or touches the nipple thus allowing water to go only into their mouth. [Hofacre (A-202) P.6 L.2-8; Smith (B-1914) P.10 L.13-18]

833. Modern poultry housing facilities afford the birds with unlimited access to feed and water with a very comfortable climactic environment in which to live. They are free to roam about the grow-out house. [Hofacre (A-202) P.28 L.12-15]

834. Dr. Aaestrup's is not an expert in poultry husbandry in the U.S. [Hofacre (A-202) P.28 L.17-18]

835. A broiler farm typically has only one age of birds growing at one time. When the birds reach slaughter age (average is 49 days old in U.S.) all birds on the farm are caught and loaded onto trucks and delivered to the processing plant. [Hofacre (A-202) P.7 L.5-7]

836. Most live poultry presented for slaughter are healthy and have not been fluoroquinolone treated. (Prucha (A-203) P.14 L.1-2)

837. Air sacculitis is an infection of the air sacs by *Escherichia coli*. [Russell (B-1912) P.18 L.5]

838. *E. coli* is a normal inhabitant of all poultry intestines and therefore will always be found in all broiler and turkey farms. Many normal inhabitant *E. coli* possess virulence factors; however, they do not normally cause disease by themselves. In most instances, *E. coli* is a secondary infection following a primary viral or environmental insult (see B-1412). *E. coli* plays a key role in the severity of the reaction to vaccination of chickens with Newcastle disease and infectious bronchitis vaccines. It has also been shown to play a role as a secondary invader following *Mycoplasma* sp. and *Bordetella avium* infections in both turkeys and

chickens. In turkeys, colibacillosis is a frequent infection following hemorrhagic enteritis. Environmental insults, such as, ammonia, CO_2, and dust, which damage the upper respiratory tract's natural defense system of cilia and mucus secretion, leads to greater susceptibility to *E. coli* infections. Even in localized infections, such as cellulitis, *E. coli* is a secondary invader following trauma to the skin from scratches. [Hofacre (A-202) P.7 L.20 – P.8 L.11]

839. *E. coli* infection is the most common malady in broiler production, and is the target condition for enrofloxacin. [A-54; Smith (B-1914) P.18 L.8-9]

840. *E. coli* is rarely a primary infection. Rather, some other insult, typically a viral agent, damages either the non-specific and/or the specific defense mechanisms, allowing *E. coli* to penetrate beyond those barriers and set up infection. [B-1412; Smith (B-1914) P.18 L.9-11; Hofacre (A-202) P.8 L.12-17]

841. While pathogenic *E. coli* tend to possess certain virulence characteristics that distinguish them from nonpathogens, the pathogens appear to be almost ubiquitous in broiler production. [Smith (B-1914) P.18 L 11-13]

842. There is currently no vaccine for *E. coli* in poultry, and no effective means to eliminate it from the broiler environment. A sufficient primary insult almost guarantees a secondary *E. coli* infection. [Smith (B-1914) P.18 L.13-15]

843. Respiratory colibacillosis is the most common manifestation of *E. coli* infectious disease in broilers. In this case, the damage is done to the non-specific pulmonary defense mechanisms. [Smith (B-1914) P.18 L.16-18]

844. The virus that most often causes *E. coli* infections in broiler chickens is IBV. IBV is a coronavirus that easily undergoes genomic reassortment and homologous recombination in the bird. Over time, new genotypes of the virus arise and large populations of birds may be susceptible. This necessitates development and production of a new vaccine for billions of birds throughout the U.S. Since it takes from 3-5 years to produce a USDA-approved live virus vaccine, the only ways to control losses in the interim are to prevent introduction of the virus and to treat the secondary *E. coli* infections with antibiotics when prevention fails. [Hofacre (A-202) P.10 L.17 through P.11 L.2].

845. The initial signs of an impending outbreak of *E. coli* are those of the primary, inciting agent. Depending on the nature of that agent, they can be obvious or subtle. Signs can appear subtle to the novice, but experienced growers, supervisors, and clinicians can spot them with practice. Postmortem examination at this stage will reveal conjunctivitis, mild to moderate rhinitis and tracheitis, and mild foamy exudates in the abdominal air sacs. As the disease progresses and secondary *E. coli* infection enters the picture, these signs intensify to the point that casual observers may be able to detect them. Birds with grossly swollen heads due to subcutaneous cellulitis may appear, and the lesions in the air sacs become purulent (air sacculitis), and may progress to fibrinous exudates on the heart, lungs,

liver, and all abdominal organs. Lame birds with purulent arthritis and others with lesions of "IP" may appear. (Smith (B-1914) P.21 L.2-18; Hofacre (A-202) P.8 L.12-17]

846. Determination that a house has *E. coli* is relatively easy. *E. coli* is by far the most common secondary invader in respiratory disease in chickens. [B-1412; Smith (B-1914) P.24 L.15-16]

847. Historically a new genotype of IBV occurs about every 5 to 10 years that causes an industry-wide problem and results in a period of poor flock health and elevated condemnations for airsacculitis and septicemia in a particular region of the country. [Hofacre (A-202) P.11 L.3 -10].

848. As in people with influenza (flu), there is a seasonality to the viral infections, and most often this occurs in the winter. Also like flu in people, these viruses tend to be a regional problem that may or may not spread throughout the entire country. [Hofacre (A-202) P.10 L.15-17].

849. Poultry veterinarians know that the poultry industry is presently (2002 - 2003) in one of the periods where the IBV virus has not shifted its genome enough to elude available vaccines, however, it is inevitable that this will occur. When that happens, the need for Baytril will again be high to treat *E. coli* infections in broiler chickens. [A-202 P.12 L.12 through P.13 L.2]

850. The welfare and care guidelines of most poultry companies require a careful inspection of each poultry house a minimum of twice daily (more is preferable). These guidelines generally require that the lights be bright enough, and that the observer pass close enough to all birds and areas of the house to verify the health and welfare of the flock and the proper functioning of all equipment (fans, vents, heaters, drinkers, feeders). Growers are instructed to notify the flock supervisor as soon as possible if unusual signs of morbidity are noted. Most experienced growers are quite adept at this, and are more than willing to comply. The signs that are cause for concern are well known to growers, and may include a variety of conditions such as abnormal respiratory noises, nasal or ocular discharges, diarrhea, wet litter, ruffled feathers, depression, and changes in feed or water consumption (which are also monitored by the growers in most companies). [Smith (B-1914) P.15 L.2-11]

851. Most companies require that growers (whether contract or company employees) pick up and record mortalities on a regular schedule, typically at least twice a day. Both spontaneous deaths and birds culled for morbidity or other abnormalities are recorded. The expected rate after 7 days of age is 0.1% per day or (preferably) less. Growers are generally instructed to contact the flock supervisor immediately if daily mortality exceeds a specified level, often as low as 0.15 or 0.2% per day. Most growers willingly comply, as they are paid by the pound produced, and mortality cuts into profits. Flock supervisors contact management or the veterinarian if the cause and solution is not obvious. [Smith (B-1914) P.15 L.13-20]

852. Most companies and growers prefer to prevent disease as opposed to treating it. In the U. S., it is generally attempted to exclude exotic diseases from the country as a whole, and vaccinate against endemic diseases. Nevertheless, there are sporadic and unforeseen diseases that must be constantly guarded against. Biosecurity practices vary from region to region and within a region, based on perceived risks. [Smith (B-1914) P.15 L.22 - P.16 L.3]

853. Recent advancements in poultry medicine and husbandry, such as the enclosed drinking and feeding systems, efficient ventilation and cooling systems, litter management practices and amendments, precise feed formulation, and modern vaccine programs described, have all led to unprecedented levels of bird health. Birds are now grown larger, faster, and more efficiently than ever, with better health and lower condemnation levels than ever. However, in spite of all efforts, disease events still occur. Disease agents naturally mutate, and selection naturally favors those that can escape control measures, such as vaccines. Development and application of a new management procedure, or development and licensing of a new vaccine to combat a new or altered disease challenge is a slow, laborious, and expensive process. Nevertheless, those methods have been and will remain the best options for long-term disease control. In the meantime, access to effective therapeutic agents remains critical in the modern management systems. Judicious and strategic use of an effective therapeutic agent reduces spread of disease, losses from morbidity and mortality, and animal suffering. Effective control of disease likely improves product quality and wholesomeness. [Smith (B-1914) P.17 L.16 through P.18 L.6]

854. Broiler companies employ one or more veterinarians who specialize in poultry medicine. These veterinarians are full time employees of the company. About 75% of the broilers produced in the US receive veterinary care from in-house veterinarians. The other 25% receive veterinary care and advice from state and university diagnostic laboratory or extension veterinarians and technical service veterinarians with the poultry biological and pharmaceutical companies. The veterinarian will typically be involved in all phases, including pullets, breeders, hatcheries, and even live haul and processing. The veterinarian will either design or advise on the complete vaccination program, from the day old pullet all the way to the processed broiler. The veterinarian designs and monitors medication and other health programs, and is a partner in the management team on all issues affecting bird health, productivity, and food safety. [Smith (B-1914) P.7 L.20 through P.8 L.11]

855. Mortality begins to increase dramatically as *E. coli* infection spreads. In a house of 20,000 birds, in which normal daily mortality should be less than 20 per day (and frequently 5 to 10 per day), mortality may double daily, peaking at 100, 150, or more per day. In other cases, the premonitory signs can be much more subtle, even when severe disease results. [Smith (B-1914) P.21 L.18-21]

856. In a broiler house with *E. coli* infection, all birds are in a stage of morbidity and all need treatment. Although the signs may be obvious to the practiced observer, it is

difficult to stage the disease in an individual, short of a post mortem examination. The disease progresses extremely rapidly in both the individual and in the flock. The presence of the inciting factors almost guarantees the occurrence of *E. coli*, especially if the presence of pathogenic strains is established and they are currently circulating in sick birds in the house. [Smith (B-1914) P.22 L.9-14]

857. Some diseases occasionally resolve spontaneously. *E. coli* infections do not. Disease may eventually peak or plateau, but once a flock has reached mortality levels in the range of 0.4% per day, spontaneous resolution is extremely uncommon. The disease will continue to circulate in the flock, and elevated morbidity and mortality will continue until the birds are slaughtered unless effective treatment is administered. [Smith (B-1914) P.23 L.2-6]

858. Once an outbreak has been well characterized, early detection and accurate prediction of the outcome becomes much easier. [Smith (B-1914) P.24 L.13-14]

859. If a grower has birds that become sick or an abnormal number die (>1 bird per day per 1000, i.e. >25 per day in a 25,000 bird house) then they immediately contact their broiler serviceperson (available 24 hours/day). These broiler servicepersons are trained by veterinarians to perform necropsies (analogous to autopsies in humans) or they may deliver diseased or dead birds to a diagnostic laboratory veterinarian. [Hofacre (A-202) P.6 L.9-19]

860. If a farm has a history of *E. coli* respiratory disease that is refractory to the tetracycline class of antibiotics or the mortality becomes very high, the serviceperson will normally contact the diagnostic laboratory or company veterinarian for assistance. These servicepersons are most often trained to be the lay technician for the veterinarian for most poultry companies. If they or the veterinarian perform a necropsy or if the birds are taken to a diagnostic laboratory and the clinical signs of an *E. coli* infection are observed, a swab for culture confirmation and antibiotic sensitivity will frequently be performed. The veterinarian will most often recommend beginning the therapy while waiting for the culture and sensitivity results. [Hofacre (A-202) P.20 L.7-14]

861. The US average level of death loss (mortality) in the typical 100,000-bird broiler farm is 4-5%. There is also loss of approximately 0.5-1.5% of the birds in the processing plant, when birds are removed by the United States Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) inspectors, primarily for visible signs of respiratory disease, most often due to an *Escherichia coli* infection. [Hofacre (A-202) P.3 L.18-22]

862. The American Veterinary Medical Association, with the support of the FDA, published the 15 general principles for judicious use of antimicrobials for poultry veterinarians. (G-113). These principles are: 1) Preventive strategies, such as appropriate husbandry and hygiene, routine health monitoring, and immunization, should be emphasized; 2)Other therapeutic options should be considered prior to antimicrobial therapy; 3) Judicious use of antimicrobials, when under the direction

of a veterinarian, should meet all the requirements of a valid veterinarian-client-patient relationship; 4) Prescription, veterinary feed directive, and extralabel use of antimicrobials must meet all the requirements of a valid veterinarian-client-patient relationship; 5) Extralabel antimicrobial therapy must be prescribed only in accordance with the Animal Medicinal Drug Use Clarification Act amendments to the Food, Drug, and Cosmetic Act and its regulations; 6) Veterinarians should work with those responsible for the care of animals to use antimicrobials judiciously regardless of the distribution system through which the antimicrobial was obtained; 7) Regimens for therapeutic antimicrobial use should be optimized using current pharmacological information and principles; 8) Antimicrobials considered important in treating refractory infections in humans or veterinary medicine should be used in animals only after careful review and reasonable justification. Consider using other antimicrobials for initial therapy; 9) Use narrow spectrum antimicrobials whenever appropriate; 10) Utilize culture and susceptibility results to aid in the selection of antimicrobials when clinically relevant; 11) Therapeutic antimicrobial use should be confined to appropriate clinical indications. Inappropriate uses, such as for uncomplicated viral infections, should be avoided; 12) Therapeutic exposure to antimicrobials should be minimized by treating only for as long as need for the desired clinical response; 13) Limit therapeutic antimicrobial treatment to ill or at risk animals, treating the fewest animals indicated; 14) Minimize environmental contamination with antimicrobials whenever possible; 15)Accurate records of treatment and outcome should be used to evaluate therapeutic regimens. [G-113; Hofacre (A-202) P.18 L.1 through P.19 L.13)

863. Poultry veterarians must treat the entire house of birds whenever viral-induced *E. coli* is diagnosed. By the time the grower notices sickness, dying, or dead birds in a particular house, there are already a tremendous number of animals who have been exposed and are incubating the virus and exposing more birds. The grower may even have inadvertently carried the virus on his/her shoes or clothing into the next house, etc. This is the reason poultry veteranrians must treat the entire house of birds as sick, because the vet cannot tell which birds are incubating the disease once it is introduced into the house. [Hofacre (A-202) P.13 L.15-22; Carey (G-1456) P.4 L.34-37; TerHune (B-1915) P.4-5]

864. Most clinicians will routinely culture the initial cases in an outbreak, and at least selected cases periodically to confirm the diagnosis and the sensitivity pattern. Once the outbreak is established, and several isolations of *E. coli* have been made, a presumptive diagnosis can be made on the appearance of lesions. The main reason to pursue further cultures is to continue to monitor the sensitivity pattern. Most staff veterinarians are intimately familiar with the typical sensitivity patterns in their complexes. Because of the rapidity with which the disease moves, the diagnosis is made on signs and lesions, swabs taken, treatment begun, and results are received later to confirm the diagnosis and give guidance in the event of failure of the initial therapeutic choice, and in subsequent cases in the complex. [Smith (B-1914) P.24 L.23 - P.25 L.6]

865. Sick birds typically stop eating but continue to drink water. The only way to quickly stop the progression of a disease is to provide the antibiotic in the birds' drinking water. This precludes the use of any antibiotic with an in-feed only label. [Hofacre (A-202) P.22 L.14-17; Glisson (B-1903) P.4 L.6-10]

866. The broiler chicken growers' pay is based on the pounds of broilers delivered to the processing plant utilizing the least amount of feed for growth. Any birds that are condemned by the USDA as unwholesome for human consumption are deducted from this weight. Therefore, there is a strong incentive to follow company husbandry guidelines. Also, many poultry company contracts require the cost of any medication used to be deducted from the growers pay. Growers therefore also have an incentive to avoid treating a flock unless certain that the flock is sick. [Hofacre (A-202) P.6 L.20 - P.7 L.4]

867. Because of the high drug cost, the decision to write a prescription for enrofloxacin will be based on how high the mortality has gone or if the farm has a history of previous treatment failures with the tetracyclines. Only a house with elevated mortality will be treated. [Hofacre (A-202) P.20 L.14-17]

868. The decision to use enrofloxacin is not taken lightly. Veternarians will not use an intervention unless they expect to at least break even on the cost of treatment. The return on treating *E. coli* is based primarily on prevention of mortality, condemnation, and interruptions in the processing plant. There are likely also benefits from reduction in morbidity (in terms of growth rate and feed conversion, by getting sick birds back on feed), but these are hard to quantitate and are usually not considered. The bottom line is that enrofloxacin is used only in those cases where extreme mortality or condemnation is expected. The drug is used only by prescription. [Smith (B-1914) P.25 L.20 through P.26 L.4].

869. Under U. S. conditions (and in most of the world), it is not feasible to treat broilers except by feed or water medication. [Smith (B-1914) P.26 L.19-20; Carey (G-1456) P.4 L.31-32; TerHune (B-1915) P.4 L.15 – P.5 L.2; B-926, B-1117]

870. Some therapeutic antibiotics are also approved for inclusion into feed (not enrofloxacin), but this is generally a poor delivery method and not often used, particularly in broiler chickens. [Glisson (B-1903) P.4 L.5-6]

871. The only opportunity to treat broilers individually (such as by injection) is in the hatchery, on the day of egg transfer or hatch. The broiler industry categorically and voluntarily rejected the use of the only approved injectable fluoroquinolone (sarafloxacin) for mass, prophylactic use in the hatchery. [Smith (B-1914) P.20-23]

872. The only class of antimicrobials for water treatment that are not over-the-counter are the fluoroquinolones. [Hofacre (A-202) P.20 L.3-4]

873. Baytril is administered by water. [A-54; Smith (B-1914) P.26 L.19]

874. Water delivery has long been accepted by the industry. FDA has long accepted drinking water delivery as a safe and effective means to administer therapeutic animal drugs, including antibiotics, to commercially grown broiler chickens and turkeys." [Joint Stipulation 18]

875. 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. [A-54; Hofacre (A-202) P.21 L.21-22].

876. Most veterinarians will determine the dose and duration of the enrofloxacin treatment based on their clinical judgment when observing the flock for depressed birds and the severity of the mortality. If it is a severe case of *E. coli* infection, the prescription may be for 50 ppm on day 1, 25 ppm for days 2, 3, or 4. If the house is severe enough to justify enrofloxacin but not extreme, then the prescription may be written for 25 ppm for 3 days. Rarely is it necessary to treat broiler chickens with enrofloxacin longer than 3 days or higher than the 25 ppm (except the first day/loading dose). [Hofacre (A-202) P.20 L.17 – P.21 L.2]

877. Baytril is used according to the label instructions. Since the label instructions allow a dosage of 25 ppm – 50 ppm for 3-7 days (B-1011, G-822), the veterinarian has some choice in the prescribed treatment. This choice is influenced by the veterinarian's assessment of the severity of the disease and the relative value of the affected flock. In general, because of the proven high efficacy in broilers of a 25-ppm, 3-day regimen, as well as for economic considerations, the lower dosage and shorter duration of treatment is commonly used. [Glisson (B-1903) P.5 L.7-12; TerHune (B-1915) P.6 L.6-11; A-54]

878. The use of enrofloxacin in the United States poultry industry is well-controlled. It is only used under veterinary prescription and supervision and is generally used as the treatment of last resort. It is not being used for growth promotion, but only for therapeutic uses. It is delivered through the drinking water in a manner that insures proper dosing, minimizes development of resistance, and minimal contamination of the environment. [Glisson (B-1903) P.11 L.20 – P.21 L.2; TerHune (B-1915) P.6 L.11-14; A-54; Wages (B-1917) P.21 L.8-11]

879. At the time enrofloxacin was approved in 1996, CVM determined that use of enrofloxacin to treat broiler chickens and turkeys was safe under the approved labeled conditions of use. [Joint Stipulation 26]

880. 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. [Joint Stipulation 36; Gonder (A-201) P.28 L.6-7]

881. Even if one could isolate and treat individual birds, or even sections of a poultry house (which one cannot in the broiler industry), such a course would not be indicated, and in fact would be guaranteed to fail with the dynamics of the disease. The assertion by some that the poultry industry routinely treats the entire house

when a few birds show signs is patently false. Once the presence of the disease is established, and professional opinion indicates the likelihood of progression, then treatment of the entire flock is the only medically valid course of action. [Smith (B-1914) P.22 L.19 – P.23 L.2; Carey (G-1456) P.4 L.34-37]

882. A recent study by Glisson et al. demonstrates that Baytril (enrofloxacin) is effective for controlling air sacculitis and other commonly used medications are not. Thus, Baytril is the most effective medication for controlling air sacculitis. [Russell (B-1912) P.26 L.15-17; Glisson (B-1903) P.14-25]

883. Baytril is the only practical efficacious antimicrobial available for poultry veterinarians to use to treat *E. coli*. It can be administered in the drinking water which is the ideal method for treating sick birds. Also, the label dose range and duration of treatment allows the veterinarian to use his/her professional judgment when writing the prescription that should result in a successful treatment outcome using the least amount of drug. This should maintain the useful life of the drug by limiting the level of resistance development not only in the target *E. coli* but also poultry commensal bacteria. There is really no practical alternative therapy to Baytril for systemic *E. coli* infections in poultry. [Hofacre (A-202) P.30 L.13 - P.31 L.31; Smith (B-1914) P.32 L.8]

884. Enrofloxacin is the most efficacious antibiotic available in the United States for treatment of *E. coli* infections in broiler chickens and *E. coli* and *Pasteurella multocida* infections in turkeys. The pharmacokinetics of the compound are such that high levels of enrofloxacin are reached in the respiratory tissues of treated birds, which is the desired site for effective treatment of both *E. coli* and *P. multocida* infections. This characteristic, coupled with the typical low Minimum Inhibitory Concentration (MIC) values of enrofloxacin against avian *E. coli* and *P. multocida*, (G-59, G-256) insures that levels reached at the site of infection are far higher than the MIC required for an effective outcome. This also minimizes the potential for resistance development in the target organism. [Glisson (B-1903) P.5 L.21 - P.6 L.7; B-1914 P.32 L.4]

885. Enrofloxacin is needed by poultry producers to treat *E. coli* respiratory infections. *E. coli* strains that infect poultry are highly resistant to sulfa drugs and tetracyclines. [TerHune (B-1915) P.8 L.13-14)

886. Bayer selected the prescribed dose range based on the pharmacokinetics of the drug, the characteristics of the commercial delivery systems, the resulting serum levels that could be obtained under commercial conditions, and known sensitivity patterns of broiler *E. coli* isolates. [B-1914 P.28 L.6-8]

887. Enrofloxacin almost uniformly produces a dramatic, measurable clinical response, and controls morbidity, mortality, and condemnation in the manner expected of an effective antimicrobial. [Smith (B-1914) P.L.15-17]

888. Enrofloxacin is the product of choice to treat broiler flocks infected with air sacculitis. [Robach (B-1911) P.15 L. 18]

889. The safety and efficacy for enrofloxacin were established using data obtained from studies where groups of birds (houses or pens) were the experimental unit. [B-926; B-1117; TerHune (B-1915) P.5 L. 7-8]

890. The safety and efficacy data for enrofloxacin demonstrate that diseased birds drank the medicated water in sufficient quantities to treat disease. In one study, turkeys were challenged with *Pasteurella multocida* (Fowl Cholera) and 98% of the nonmedicated control birds died compared to 8%, 0% and 0% of the enrofloxacin treated birds, treated at 12.5, 25 and 50 ppm respectively. [B-1117; TerHune (B-1915) P.5 L. 14-16; Smith (B-1914) P.28 L.2-5]

891. The current label dose for enrofloxacin is 25 to 50 ppm for broiler chickens and turkeys. The safety and efficacy studies demonstrate that when medicating the drinking water with enrofloxacin, individual birds are dosed adequately, even at half the lowest recommended dose. This study also indicates that a superior result could not have been obtained with the use of an individually dosed injectable product. [B-1117; TerHune (B-1915) P.5 L. 16 through P.17 L. 5)

892. The pharmacokinetic data were provided on the Baytril label along with Minimum Inhibitory Concentration (MIC) data for label pathogens allow for the selection of peak serum concentrations (Cmax) to MIC ratios that minimize the selection of resistant organisms. [B-1117; TerHune (B-1915) P.7 L. 12-15]

893. Testimony presented by Patrick F. McDermott (Exhibit G-1465) expresses concern for birds receiving an adequate dose of a medication when it is administered in the drinking water, referencing Exhibit G-52 because it describes how water consumption is variable in poultry and dependent on numerous variables, such as bird weight, maturity (age) and ambient temperature. [TerHune (B-1915) P.6 L. 15-19; G-1465]

894. Exhibit G-52 is the Baytril® product information document from Bayer that describes the variables associated with poultry water consumption, and the appropriate levels of enrofloxacin to use in different circumstances. The labeling of enrofloxacin for poultry explicitly addresses the variables associated with poultry water intake and allows the veterinarian to administer the product in a safe and efficacious manner. [TerHune (B-1915) P.6 L. 19 - P.7 L. 1]

895. Dr. McDermott's concern, stated in his testimony (G-1465) that birds do not or may not birds receive an adequate dose of a medication when it is administered in the drinking water, conflicts with the efficacy data submitted to CVM in support of the NADA and published data which clearly demonstrate that adequate quantities of enrofloxacin are consumed. [TerHune (B-1915) P.6 L. 15 - 7 L. 3]

896. Dr. McDermott's testimony about how sub-optimally dosed birds lead to an increase in the probability for selecting for resistant *E. coli* in healthy and diseased

birds is not supported by available data that clearly demonstrate that poultry receive an adequate dose. [TerHune (B-1915) P.7 L. 1-7]

897. The use of enrofloxacin to treat respiratory *E. coli* infections such as air sacculitis in broilers results in healthier birds during grow-out and entering the processing plants. [Robach (B-1911) P.15 L.23 through P.16 L.2]

898. When water-soluble medications such as enrofloxacin are used they do not expose a greater numbers of animals than just the few with clinical signs of disease, contrary to CVM's publicly concern stated at 65 Fed. Reg. 64957. [Terhune (B-1915) P.3 L.21-23, P.4 L.1-4; B-1117]

899. Water-soluble medication of enrofloxacin for poultry is not non-discriminating for the target (clinically sick animals) as compared to injectable products, and does not raise the possibility of development of resistant organisms in greater numbers than if the drugs were to be administered in an individual injectable dosage form contrary to CVM's publicly concern stated at 65 Fed. Reg. 64957. [Terhune (B-1915) P.5 L.3-10, 12; B-1117]

900. If a ranch or farm has multiple houses then only the house(s) with diseased birds will be medicated, not all houses. [B-1117; Terhune (B-1915) P.4 L.3-4]

901. CVM is incorrect if it has concluded if one bird is sick then the entire flock (every house on the ranch) will be treated, because the medication is water-soluble and administered through the water. [Terhune (B-1915) P.4 L.10-12]

902. Poultry houses are designed to provide nutrients and medications through the house water supply, therefore, houses are equipped to be medicated individually and not on a ranch or "flock" basis. [Terhune (B-1915) P.4 L.12-14]

903. As a house (diseased unit) of birds develops clinical signs associated with disease, the animals with observable clinical signs are at a different progression point of the disease, but the whole house (diseased unit) is exposed and at risk. When a house of birds is medicated, it is the same as systemically treating a sick calf (diseased unit), potentially exposing other portions of the house where bacteria live to drugs in order to save the unit. [Terhune (B-1915) P.4 L.15 through P.5 L.2; B-926; B-1117]

904. The overall exposure of poultry and their environment to the fluoroquinolone is the same whether the poultry house is treated through the drinking water, or if theoretically one were able to individually inject every bird, and an increased rate of resistance of *Campylobacter* to fluoroquinolones is not associated with the method of drug delivery. [Terhune (B-1915) P.5 L.3-6]

905. CVM's statement that "wide spread contamination by water leakage and animal waste that occurs when large numbers of animals are treated, which result in untreated animals being exposed to the drug" was another concern with water-soluble medications [(65 Fed. Reg. 64957)] is not correct since the whole house is

the diseased unit and the treatment target; every bird in the house is a treatment target because every bird is at some stage of disease, from exposure to clinical disease. [Terhune (B-1915) P.7 L.16 through P.8 L.2]

906. Routine water leakage associated with birds watering at troughs, bells or cups has been eliminated in commercial broiler houses because of the standard use of nipple waterers. Nipple waterers are designed specifically to eliminate water leakage or water spillage when birds drink. In addition, the cost of the medication prohibits the poultry integrator from indiscriminant regard to water leakage. [Terhune (B-1915) P.8 L.3-7]

907. There are no viable alternatives to enrofloxacin in the United States poultry industry for treating *E. coli* infections in broiler chickens and turkeys. [Glisson (B-1903) P.12 L. 3-4]

908. Dr. TerHume's studies in the early 1990s demonstrated the superiority of fluoroquinolones over tetracyclines to treat *E. coli* airsacculitis due to tetracycline resistance. [B-1579; B-1376; TerHune (B-1915) P.8 L. 14-16).

909. For layer chickens, the choice of antibiotics available to poultry veterinarians for the treatment of bacterial infections is limited (see Table 1 below). Until the fluoroquinolones were approved for poultry, the tetracyclines were the primary antibiotic class available for treating *E. coli* infections. [Hofacre (A-202) P.15 L. 15 – P.16 L.2]

910. Poultry veterinarians have very few antibiotics available for treatment of *E. coli* infections. The available options are to use those for which there is a specific label indication for *E. coli* and those for which AMDUCA allows the veterinarian to use his discretion for extra label use. [Hofacre (A-202) P.24 L. 2-5]

911. While AMDUCA does allow veterinarians to use some drugs in an extralabel manner, the pharmacokinetics and practicality of administration of these drugs must be taken into account. 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. (Joint Stipulation 36). The drug ceftiofur (a cephalosporin class of antibiotic) is not effective when administered orally in either the drinking water or feed; therefore it must be administered by injection to each bird individually. Water and feed are the only practical manner to treat poultry. This means ceftiofur cannot be used by poultry veterinarians to treat a flock of 20,000 birds with an *E. coli* infection. For gentamicin (aminoglycoside class), it can only be given by injection. Also, the legal withdrawal time (safety of no drug residue in the meat) for gentacicin is 35 days and since most broilers are slaughtered at 42-49 days of age, this makes it even more impractical to treat with gentamicin. The same is true for spectinomycin (aminocyclitol similar to the aminoglycosides) and sulfomyxin that must be injected, these cannot be practically administered to a commercial flock of birds. This leaves only chlortetracycline, oxytetracycline, and

the fluoroquinolones available in Dr. Tollefson's table to treat *E. coli.* [Hofacre (A-202) P.28 L.19 – P.29 L.19]

912. In general, the alternatives to enrofloxacin for therapeutic use in poultry are the tetracyclines and the sulfa drugs. If the NADA for enrofloxacin is withdrawn, the only available drugs specifically approved to treat *E. coli* infections in chickens older than three days of age and *E. coli* and *Pasteurella multocida* infections in turkeys older than three days of age are: sulfa drugs (such as sulfamethazine, sulfaquinoxaline, sulfadimethoxine) and tetracyclines (such as tetracycline, oxytetracycline, chlortetracycline). [Glisson (B-1903) P.7 L.5-10; Hofacre (A-202) P.24 L.5-9; Wages (B-1917) P.19 L.6-9]

913. Tetracycline usage for treatment of *E. coli* infections in poultry is usually ineffective or poorly effective because of widespread resistance to tetracyclines among avian *E. coli* isolates. High tetracycline resistance is seen in other surveillance systems as well. Tetracyclines have been used for decades in the United States poultry industry, without veterinary prescription requirements, to treat *E. coli* infections. (B-1377). Resistance to this antibiotic class has become the norm, not the exception. [B-1376; B-1379; B-1377; B-Glisson (B-1903) P.7 L. 11-17; Wages (B-1917) P.19 L.1-2]

914. Sulfa drugs have also been available for decades to the United States poultry industry for therapeutic use. Usage of sulfas has been very limited in recent years because of serious concerns for sulfa residues in poultry meat and poultry products. Sulfa drugs typically have long withdrawal periods. Since respiratory disease in broilers usually occurs in the late stages of the production cycle, it is difficult to use a sulfa drug for treatment in broilers without risking product residues. [Glisson (B-1903) P.8 L.9-14]

915. The potential problem of having sulfa drug residues is potentially enhanced in areas of the country that have acidic drinking water. Sulfa drugs are typically less soluble in acidic water and can precipitate in water lines. Sulfa residues in water lines can potentially cause residues in poultry tissues even when the treatment is withdrawn at the proper time. Since poultry companies are focused on product quality, the potential for sulfa residues in poultry products is considered to be too high of a risk and consequently many companies voluntarily abstain from the use of sulfa drugs. [Glisson (B-1903) P.8 L.14 – P.9 L.2]

916. There are only two practical alternatives for treatment of a systemic *E. coli* infection – in poultry, tetracyclines or enrofloxacin. Since nearly 90% of the *E. coli* isolates are resistant to the tetracyclines (Bass, 1999), loss of Baytril would leave poultry veterinarians with no real alternatives. [Hofacre (A-202) P.27 L. 1-4; B-1903; Smith (B-1914) P.32 L. 9].

917. For commercially grown broiler chickens and turkeys in the U.S., it is neither feasible nor practical to administer antibiotics on an individual bird basis. (Joint Stipulation 36). This limits the extra-label alternatives. For example use of the

aminoglycosides and cephalosporins are eliminated as an option due to their very poor oral activity. Although there is a label for streptomycin for water administration for *E. coli* therapy, clinical experience indicates it is not very efficacious. [Hofacre (A-202) P.24 L. 9-14]

918. Since sick birds continue to drink, in a disease situation, as a practical matter, the treating veterinarian will want to choose an antibiotic labeled for use in the drinking water. The veterinarian's choices of antibiotics available for water therapy of chickens are: bacitracin, chlortetracycline, oxytetracycline, tetracycline, erythromycin, enrofloxacin, lincomycin, neomycin, streptomycin, and sulfadimethoxine. However, each of these choices has limitations as follows:

- Bacitracin is a polypeptide antibiotic which is poorly absorbed from the intestine and are primarily effective against gram positive bacteria (*E. coli* is a gram negative bacteria).

- Tetracycline class (chlortetracycline, oxytetracycline, tetracycline) – these are broad spectrum antibiotics that were very effective against gram positive and negative bacteria when first introduced into the market decades ago. They are very safe but no longer very effective for treatment of *E. coli* infections. They are bacteriostatic, meaning they stop growth of the bacteria and the birds' immune system must kill the bacteria, thus any reduction in immune function will result in poor efficacy. However, as seen in figure 4, nearly 90% of the clinical *E. coli* isolates have become resistant to this class of antibiotics since these have been the only reasonably effective drugs for *E. coli* infections for 30 years.

- Erythromycin – this is a macrolide antibiotic that is most effective against gram positive bacteria. It has been tried for use against *E. coli* airsacculitis but has not been effective.

- Enrofloxacin – a fluoroquinolone antimicrobial that has a broad spectrum of activity, readily absorbed from the intestine and very safe and effective.

- Lincomycin is a lincosamide antibiotic that is similar in function to the macrolides. It is poorly absorbed when administered orally, therefore it is used primarily to treat gram positive bacterial enteritis, such as, *Clostridium perfringens*. This drug is not effective against *E. coli,* which is a gram negative bacteria.

- Aminoglycosides – neomycin and streptomycin are both labeled for drinking water treatment. It is estimated that less than 25% of this class of antibiotics is absorbed when administered orally. Therefore, it would be prohibitively expensive and impractical to administer enough of these drugs to get an adequate drug concentration to kill the bacteria in the respiratory tract.

- Penicillin – penicillin is a beta-lactam antibiotic that inhibits primarily gram positive bacteria. It has little or no effect on *E. coli.*

- Sulfadimethoxine – the sulfonamide antibiotics or "sulfas" have very good activity against gram negative bacteria, like *E. coli.* Also, they are readily absorbed from the intestines into the blood stream. However, the sulfa class has a very narrow margin of safety. This means that birds can become quickly overdosed and die if they drink more water than is anticipated (weather gets too warm). Also, the sulfonamides become protein bound and have long half lives so the withdrawal prior to slaughter becomes a concern. The U.S.D.A.-FSIS has historically had the greatest violations of drug residues due to sulfonamide therapy, so few poultry companies use the sulfas to avoid any residue violation. Also, in some areas of the country, depending on the pH of the water supply, sulfa drugs precipitate out in the water lines. This leaves an available concentration of the sulfa drug to which the birds may be exposed even in the withdrawal period and can impact tissue residues.

  [Hofacre (A-202) P.24 L.15 – P.26 L.22)

919. Regardless of whether or not, in Denmark: (1) there is always another antibiotic in Denmark, other than enrofloxacin, available to treat bacteria in poultry; (2) enrofloxacin is very easy to use in the absence of a proper diagnosis or accurate identification of the infectious agent; (3) a total ban on all usage of fluoroquinolones would not cause major problems in the food animal production, if any; and, (4) fluoroquinolones are convenient drugs to use in veterinary medicine, but they are rarely important and never essential, none of these statements are true with respect to the U.S. poultry industry. [Hofacre (A-202) P.27 L.6 – P.28 L.11]

920. In the U.S., drugs such as ampicillin, colistin, tiamulin are not available to use in poultry. [Hofacre (A-202) P.28 L.8-9]

921. Drs. Glisson and Greg Mathis (Southern Poultry Research, Inc.) designed and conducted an experiment to compare the efficacy of enrofloxacin, oxytetracycline, and sulfadimethoxine for the treatment of *E. coli* infections in broiler chickens. [Glisson (B-1903) P.9 L.6-8, P.14-25]

922. In Dr. Glisson's and Mathis's study:

- 1600 one-day-old broiler chicks were randomly distributed into 80 floor pens. The 80 floor pens were randomly assigned to one of four treatments. One group was to remain untreated and three were to be treated. The treatments were: 1) enrofloxacin (25 ppm) administered in the drinking water for three consecutive days, 2) oxytetracycline (400 mg/gal) administered in the drinking water for six consecutive days, 3) sulfadimethoxine (1875 mg/gal) administered in the drinking water for six consecutive days. All treatments were consistent with industry practices and, where applicable, label indications.

- The birds were reared for 20 days in normal conditions. On day 21, all birds in all pens were sprayed with live Newcastle disease vaccine virus and live infectious bronchitis vaccine virus. Subsequent to the vaccine application, environmental ammonia levels were allowed to elevate above normal levels. These events created an environment conducive to the natural occurrence of respiratory *E. coli* infection in the broilers.

- An outbreak of colibacillosis was confirmed when at least 0.5% of the birds died from colibacillosis in a 72 hour period. At that point, treatment was begun.

- The experiment ended at 42 days of age. The parameters measured were weight gain, feed conversion, mortality, and air sac lesion scores.

- The study data confirmed the greater efficacy of enrofloxacin when compared to the other treatments. All parameters measured favored enrofloxacin treatment, but the two important factors, mortality and air sac lesions, provided the most striking evidence of the efficacy of enrofloxacin. Enrofloxacin treatment prevented all further *E. coli* associated mortality and reduced air sac lesion scores very significantly. Oxytetracycline and sulfadimethoxine provided marginal mortality reductions when compared to the nonmedicated treatment and had essentially no effect on air sac lesion scores.

  [Glisson (B-1903) P.9 L. 9 - P.10 L. 9; P.14-25]

923. Drs. Glisson and Mathis's study reproduced very closely the effect seen when:

- enrofloxacin is used in the field to treat *E. coli* infections in broilers--typically a dramatic reduction in mortality and a dramatic reduction in the lesions in the respiratory tract at slaughter;

- oxytetracycline or sulfadimethoxine treatment is used in the filed to treat *E. coli* infections in broilers-- typically the reduction in mortality was entirely unacceptable in a commercial setting and those treatments had no real effect on internal lesions of the respiratory tract.

[Glisson (B-1903) P.10 L.10-16]

924. Drs. Glisson and Mathis's study confirms the results of a previous study that using a similar design and protocol, enrofloxacin treatment provided a significant difference in feed conversion and air sac lesion scores when compared to oxytetracycline treatment. [Glisson (B-1903) P.10 L.20 – P.11 L.2]

925. 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. [Joint Stipulation 47]

926. 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. [Joint Stipulation 48]

927. Any stipulation relating to the registration date of Bayer's ciprofloxacin products or Bayer's enrofloxacin products contains no representation regarding the approval date or date of first use of non-Bayer ciprofloxacin products, enrofloxacin products or fluoroquinolones. [Joint Stipulation 49]

928. Any stipulation relating to the registration date of Bayer's ciprofloxacin products or Bayer's enrofloxacin products for poultry contains no representation regarding the dates of sale or use, if any, of Bayer's ciprofloxacin products or Bayer's enrofloxacin products for poultry in any country, or whether such registrations are currently in effect. [Joint Stipulation 50]

929. 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. [Joint Stipulation 62]

930. 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. [Joint Stipulation 63]

931. One of the most effective means of controlling microbial contamination on poultry products is to understand that the health of the incoming poultry is of key importance. [Russell (B-1912) P.12 L.18 –20; Robach (B-1911) P.13 L.18-21]

932. The scientific community and USDA agree that preventing carcass contamination with fecal mater is an essential element in reducing the prevalence of *Campylobacter* and *Salmonella* on raw poultry. [Tompkin (A-204) P.58 L.3-5]

933. In the federal register notice on Pathogen Reduction, Hazard Analysis and Critical Control Point (HAACP) Systems; Final rule on July 25, 1996, the FSIS stated that "...microbial pathogens associated with fecal contamination are the single most likely source of potential food safety hazard in slaughter establishments, preventing and removing fecal contamination and associated bacteria are vital responsibilities of slaughter establishments." (9CFR). [Hofacre (A-202) P.9 L.5-9]

934. Healthier poultry generally are more resistant to colonization by *Salmonella* and *Campylobacter* and are less likely to be subjected to processing errors due to gut tears or cuts or lack of flock uniformity. [Robach (B-1911) P.16 L.2-4]

935. Studies by Arakawa et al. (B-1821), Baba et al. (B-1822), and Fukata et al. (1987) (B-1823) demonstrate that poultry with a disease condition, such as coccidiosis, were colonized more effectively by *Salmonella* compared to poultry that were coccidia free. Thus, there is a relationship between the health of poultry and the ability of intestinal pathogens, such as *Salmonella* and *Campylobacter* to colonize the chickens. [Russell (B-1912) P.12 L.21 through P.13 L.3]

936. Researchers have demonstrated a link between *E. coli* infection and low body weight in flocks of turkeys. In a study by Marrett *et al.* (2000), a group of turkey poults exposed to naturally occurring populations of *E. coli* in litter were treated using an antibiotic and another group remained untreated. These researchers found that the antibiotic treated poults had higher body weight after only 15 days than the untreated groups (Marrett *et al.*, 2000). Sell *et al.* (1997) reported that weight gain and feed efficiency were markedly impaired by *E. coli* infection of turkeys after only 7 days of exposure. These studies suggest that *E. coli* infections impact body weight, and factors that lead to non-uniform or underweight birds should be controlled to prevent fecal contamination during processing. [Russell (B-1912) P.38 L.7-15]

937. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF-1997) reported that because processing of raw broilers does not involve a lethal heat process, such as pasteurization, delivering live chickens to the processing plant with as few pathogens as possible is necessary to control contamination of carcasses with *Salmonella* and *Campylobacter*. Morishita *et al.* (1997) stated that reducing *C. jejuni* colonization in live chickens may potentially reduce the incidence of *C. jejuni* infections in humans. Thus, controlling factors that contribute to colonization during growout should significantly impact pathogenic bacterial contamination in the processing plant. [Russell (B-1912) P.37 L.9-16]

938. Morishita *et al.* (1997) observed that intestinal colonization of *Campylobacter jejuni* within a flock plays a major role in carcass contamination during slaughter. [Russell (B-1912) P.39 L.1-3]

939. A common trait of both broilers and turkeys is that when they get sick, they "go off feed," i.e., stop eating. (B-1117, at p.25). Chickens respond to illness similarly to people in that an infection results in fever. The fever causes the animal to decrease feed consumption. [Russell (B-1912) P.16 L.13-15; Wages (B-1917) P.11 L.21-22]

940. Chickens that are sick with air sacculitis and are not effectively treated will continue to drink but usually stop eating, especially when they become feverish. They will sit down on the floor and eat any spilled feed from the automatic feeder they can reach. Therefore, they will consume large quantities of bacteria, viruses, and coccidia from the bedding material (litter). [Hofacre (A-202) P.14 L.1-4; Smith (B-1914) P.24 L.5-8; Robach (B-1911) P.13 L.28-29; Glisson (B-1903) P.4. L.6-7]

941. Turkeys that are sick with pasteurella or *E. coli* and are not effectively treated stop eating. [Gonder (A-201) P.21 L.12-13 and P.22 L.18-19 and P.26 L.23; Wages (B-1917) P.11 L.21-22]

942. Studies by Bilgili and Hess (B-1829), Savage (B-1836) and Bilgili (B-1830) have demonstrated the link between decreased feed consumption and poor intestinal tensile strength in chickens and turkeys. [Russell (B-1912) P.16 L.15-17 and P.17

typical indicator organism for enteric pathogens in poultry. [Robach (B-1911) P.12 L.7-13; Tompkin (A-204) P.7 L.14-16]

956. Failure to control *E. coli* and *Salmonella* increases the likelihood of a higher prevalence and concentration of *Campylobacter* on raw poultry. [Tompkin (A-204) P.58 L.13-14]

957. Chill water and the chilling process can be a source of pathogen contamination contributing to cross contamination between carcasses during chilling. [Logue (G-1464) P.2 L.18-20]

958. A small number of contaminated carcasses may have an impact in spreading contamination. [Logue (G-1464) P.2 L.21-22]

959. Procedures used during processing such as handling (during carcass orientation and hanging), defeathering, and evisceration also contribute to cross contamination between carcasses. [Logue (G-1464) P.2 L.22-24]

960. In the study described in the Written Direct Testimony of Catherine Logue (G-1464) the chill water in Plant B was hyperchlorinated to a concentration of 20 ppm. [Logue (G-1464) P.7 L.5-7, L.16-17]

961. In the study described in the Written Direct Testimony of Catherine Logue (G-1464) the chill water in Plant A was unchlorinated well water. [Logue (G-1464) P.7 L.2-3]

962. In the study described in the Written Direct Testimony of Catherine Logue (G-1464) more *Campylobacter* isolates from Plant B showed a higher degree of resistance and displayed resistance to more antibiotics compared to isolates from Plant A. [Logue (G-1464) P.8 L.12-20; P.20,21]

963. Leakage of intestinal contents during the slaughtering process almost inevitably contaminates poultry carcasses with *Campylobacter*. [Meng (G-1466) P.1 L.39-40]

964. Because chicken broilers are usually more uniform in size than turkeys, chicken processing plants tend to be much more automated than turkey processing plants. [Minnich (G-1467) P.2 L.17-18]

965. Live chickens arrive at slaughter plants in crates which are stacked on top of each other on the back of tractor trailers. [Minnich (G-1467) P.2 L.32-33]

966. Chickens are unloaded from the crates onto conveyor belts that transport them inside of the plant. [Minnich (G-1467) P.2 L.38-39]

967. Chickens are slaughtered by cutting their necks (by hand or through the use of a mechanical blade). Next, chickens enter a scalding system. The scalder is a tank that is between 60-120 feet or more long and contains over 2,000 gallons of water that is 130 degrees F or greater. The scalder's heating function allows the chicken's

feathers to be more easily removed in subsequent steps. Fresh water is added to the scalder constantly to both maintain the temperature and provide make-up water lost as birds exit the unit. [Minnich (G-1467) P.2 L.41-46]

968. With the addition of fresh water, it takes approximately 1 –3 hours for the water in the scalder to completely exchange. There are 300 or more chickens in the scalder at any given time and they remain there for 1 - 3 minutes. [Minnich (G-1467) P.3 L.1-3]

969. Upon exiting the scalder, the chickens enter one or more mechanical picking machines which remove the feathers. Picking machines are metal cabinets with rubber projections that vibrate. Chickens pass through these projections (called fingers) that beat the feathers loose from the carcass. [Minnich (G-1467) P.3 L.5-8]

970. Once the birds are placed on the evisceration line, their body cavities are opened mechanically through the use of a circular blade that surrounds the vent (or anal) area and cuts it free from the surrounding skin. While doing this it also pulls the vent upward and extracts the lower portion of the rectum outward. This extraction helps to prevent fecal contents from leaking into the interior of the bird during the remainder of the opening and evisceration process. The remainder of the opening process is accomplished by the use of a mechanical blade. The viscera (internal organs) are extracted by means' of a piece of equipment (called the eviscerator) with a spoon-shaped protuberance. The spoon-shaped object enters the. interior of the chicken through the underbelly and scoops out the viscera positioning them just outside of the body cavity opening. [Minnich (G-1467) P.3 L.21-30]

971. Chickens move in front of at least one plant employee who ensures proper opening and positioning of the extracted viscera, then the chickens pass in front of USDA inspection personnel who determine adulteration and wholesomeness of the chickens. [Minnich (G-1467) P.3 L.32-34]

972. Chickens and turkeys with visible internal digestive tract contamination are either sent to an off-line reprocessing area by the USDA inspector or are later reprocessed on the slaughter line. Off-line reprocessing occurs after a USDA inspector determines that the chicken has visible contamination and consists of removing the chicken from the processing line and manually washing the interior and exterior of the bird with water that has at least 20 PPM of chlorine added to it. The carcass is washed until it is visibly clean of digestive tract contents. In many plants on-line reprocessing is a standard, part of the slaughter process. During on-line reprocessing each chicken carcass is mechanically washed at the end of the slaughter 'line with plain water and then with an antimicrobial solution (such as TriSodium Phosphate). In plants that utilize an on-line reprocess step, the USDA inspector will not single out individual carcasses due to visible contamination, since all of the carcasses are washed at the end of the line. Even with these extra washes, bacterial contamination is not completely eliminated from the carcasses. [Minnich (G-1467) P.3 L.36 – P.4 L.1-2; Russell (B-1912) P.5 L.8-16]

973. The majority of the intestinal tract is removed from the bird by a mechanical blade at the level of the jejunum (mid-portion of the intestines). The remaining viscera, including the stomach, gizzard, and upper intestines are removed by mechanical means. The viscera puller (otherwise called the "pac-man") has a claw-like protuberance that enters the body cavity of the bird and grasps the GI tract just before the proventriculus. It pulls out the proventriculus, gizzard, and upper intestines. [Minnich (G-1467) P.4 L.13-18]

974. Chilling tanks are filled with either pre-chilled water or ice and water. Chicken chilling tanks are metal structures that are 125 - 140 feet or more in length. They hold over 20,000 gallons of water when full. Chickens remain in the chiller for approximately 1 - 2 hours in order to decrease their body temperature from approximately 90 degrees F to 40 degrees F or less depending on the size of the bird and chill tank. Water is continually added to the chiller units to both maintain the cold temperature and make-up the lost water from the exiting carcasses. This water allows for an exchange of volume to occur every 3 - 5 hours on average. [Minnich (G-1467) P.5 L.4-11; Russell (B-1912) P.6 L.3-9]

975. Each chicken slaughter plant may slaughter a unique size and weight of bird; however, the size and weight is usually consistent within that facility. [Minnich (G-1467) P.6 L.14-16]

976. Chicken and turkey processing facilities are dependent for efficient operation on processing chicken and turkeys of uniform weight and size. [Carey (G-1456) P.3 L.27; Hofacre (A-202) P.2 L.16-21]

977. Poultry processing is highly automated. Variable size of poultry is problematic because processing equipment is set for the average size of a uniform flock. [Hofacre (A-202) P.9 L.16-21]

978. The variation in turkey carcass size makes it more practical to manually process turkeys rather than trying to fit and adjust equipment to a variety of bird sizes. [Minnich (G-1467) P.6 L.18-19]

979. It is possible for chickens and turkeys that were free from *Campylobacter* at the farm to become contaminated with it during the transportation and slaughter process. Throughout the process, there are numerous places where cross-contamination may occur between animals or carcasses with *Campylobacter*, and if present, fluoroquinolone-resistant *Campylobacter*, and those carcasses without *Campylobacter*. [Minnich (G-1467) P.7 L.23-28]

980. Cross-contamination between carcasses with bacteria may occur. [Minnich (G-1467) P.7 L.31]

981. 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. Because of the crowding of animals and the design of these crates (openings on the top, bottom and sides), it is very easy for feces that

may contain bacteria such as *Campylobacter* to spread or drop from one animal to another. [Minnich (G-1467) P.8 L.12-16]

982. When chickens and turkeys are taken out of crates and placed on conveyor belts to transport them into the plant, the conveyor belt can become contaminated with chicken or turkey feces and contaminate the exterior of the animals with bacteria. [Minnich (G-1467) P.8 L.18-20]

983. When chickens and turkeys are slaughtered, the mechanical blade or hand held knife could spread contamination. If there were feces on a chicken's or turkey's neck when it passed through the hand-held knife or mechanical blade, not only could it contaminate the deeper tissues of that carcass, but also it could pass that contamination on to subsequent chickens or turkeys slaughtered with the same knife or blade. [Minnich (G-1467) P.8 L.22-27]

984. Chickens and turkeys are not washed prior to entering the scalder; therefore, any external debris or contamination frequently comes off into the scalding tank. Turnover of the scalder with fresh tap water takes about 1-3 hours on average at a chicken processing plant. Even with these efforts, scalder water at chicken plants, which stays murky brown throughout each day from the dirt and feces in it, represents a potential for bacterial cross-contamination between chickens. [Minnich (G-1467) P.8 L.29-34]

985. The picking machine equipment is not washed between carcasses. Any external contamination on the carcasses could be passed on to the picking fingers and to subsequent carcasses entering the picking machines. [Minnich (G-1467) P.8 L.36-38]

986. The head puller bars used to remove the chickens' heads are not washed in between each carcass. Equipment or utensils used to remove turkeys' heads are likewise sources of cross-contamination. Any external contamination on the upper neck or lower head area could be spread between carcasses by the equipment. [Minnich (G-1467) P.8 L.40-43]

987. The machinery used to detach the feet from the chickens and turkeys present a vehicle for cross-contamination. If there is contamination on the foot or hock joint of the chicken or turkey, that contamination can be spread to subsequent carcasses. [Minnich (G-1467) P.9 L.1-3]

988. Mechanical equipment used to transfer chickens from the kill line to the evisceration line may present a point of cross-contamination. [Minnich (G-1467) P.9 L.5-6]

989. 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 the plant employees or their equipment. The intestinal contents (and pathogens such as *Campylobacter* contained within the intestines) could then be spread between animals. In my experience, most chicken plants have a visible

contamination rate following venting, opening, and evisceration of 5% or more. [Minnich (G-1467) P.9 L.8-13]

990. 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 (G-1467) P.9 L.15-17]

991. The chilling tanks for chilling chicken and turkey giblets may be a point of cross-contamination of giblets with bacteria. [Minnich (G-1467) P.9 L.19-20]

992. During the removal of the intestinal tract from the bird, fecal material could spill out onto the birds' wings and necks, located below (the birds are hung upside down during this process). [Minnich (G-1467) P.9 L.22-24]

993. The final trimming station in chicken, and turkey processing represents another point of cross-contamination. Bacteria from the knife and from employee's hands can be a source of such contamination. [Minnich (G-1467) P.9 L.26-28]

994. When the oil glands are removed from the chickens or turkeys, if there were contamination on the exterior of the tail area, that contamination could be spread between carcasses by the mechanical or hand held blade. [Minnich (G-1467) P.9 L.30-32]

995. Chill tanks represent a point for cross-contamination for chickens and turkeys mainly due to the number of carcasses within it at any given time (i.e. thousands) and the water media which facilitates spread of bacteria. [Minnich (G-1467) P.9 L.34-36; Russell (B-1912) P.16 L.6-9]

996. Poultry companies have identified a critical control point (CCP) just prior to the chiller. At this point, they evaluate carcasses for the presence of fecal material. However, at line speeds of 70 broilers/minute or higher, it is impossible to detect all contaminated carcasses. [Russell (B-1912) P.15 L.15-17]

997. Packing and further processing (cut-up and deboning) areas represent another point of cross-contamination. Not only do the tables or conveyors present risks of cross contamination, the carcasses commonly contact many other carcasses during this process (for example, one conveyor or table could easily hold 25 or more chickens at one time). [Minnich (G-1467) P.9 L.38-42]

998. HACCP requires plants to identify and prevent problems during the processing of meat and poultry items that could cause harm to consumers. It requires plants to establish controls for the cleaning of equipment, utensils, & facilities; hygiene of employees; the control of certain pathogenic bacteria (e.g. *Salmonella*); and the prevention of other food hazards (such as foreign material including metal and glass contamination). [Minnich (G-1467) P.10 L.14-19; Robach (B-1911) P.9 L.17-22]

999. Reducing the prevalence rate of *Campylobacter* and *Salmonella* on raw poultry requires a farm-to-table approach that incorporates the principles of HACCP and the use of GMPs. [Tompkin (A-204) P.58 L.1-2]

1000. FSIS's food safety goal is to reduce the risk of foodborne illness associated with the consumption of meat and poultry products. [Minnich (G-1467) P.10 L.32-33]

1001. The Hazard Analysis Critical Control Point (HACCP) portion provided a science-based process control system for food safety that included provisions for the prevention and control of biological, chemical and physical hazards in food. The Pathogen Reduction portion included plant criteria for *E. coli* levels on slaughtered carcasses and *Salmonella* performance standards for slaughtered carcasses and raw ground products. HACCP requires plants to look for hazards in foods starting with the receipt of raw materials and continuing through the shipping of the finished product. [Minnich (G-1467) P.10 L.42 – P.11 L.1]

1002. Since the implementation of the Pathogen Reduction/HACCP final rule, FSIS testing has shown that the percentage of raw carcasses testing positive for *Salmonella* has decreased. [Minnich (G-1467) P.11 L.4-6]

1003. There are no current USDA reduction standards in place for *Campylobacter*. [Minnich (G-1467) P.11 L.6]

1004. The potential cross-contamination risk posed by the transportation and processing of poultry increases the likelihood that carcasses leaving the processing plants are contaminated with *Campylobacter*. [Minnich (G-1467) P.11 L.14-17]

1005. Surveys of chicken, turkeys, ducks and geese, indicate they are all reservoirs of *Campylobacter*. There are large variations in the proportions of flocks that are infected. The large variation depends on the type of production system, the geographical location and on the time of year. [Wegener (G-1483) P.3 L.9-11]

1006. Flocks of birds reared with access to open areas, such as organic and other types of free ranging production, are more frequently infected than birds reared under strict biosecurity, probably because of exposure to wild bird's droppings and water contaminated by wild animal's faeces. [Wegener (G-1483) P.3 L.12-15]

1007. *Campylobacter* does not usually cause disease in the food animals. *Campylobacter* bacteria colonize the intestines together with hundreds of other species of harmless bacteria. *Campylobacter* grow and multiply in the animal intestine, and can be isolated from the faeces of the animals in numbers ranging from thousands to hundreds of millions per gram of faeces (usually 1 05- 1 O8 bacterial cells per gram faeces). [Wegener (G-1483) P.4 L.2-6]

1008. Broiler chicken and other poultry, where there is no contact between the parent bird and the progeny, acquire the infection from the environment. [Wegener (G-1483) P.4 L.17-20]

1009. The animal gastrointestinal tract is probably the only significant place where *Campylobacter* grow and multiply in the farm to fork chain. [Wegener (G-1483) P.4 L.18-20]

1010. Because *Campylobacter* and other enteric bacteria is present in animal faeces, *Campylobacter* and other enteric bacteria can be found in all places where faeces contamination occurs. This includes the surface of the animals and the farm environment. Most importantly however, faeces and enteric bacteria can, and will, contaminate the carcass during slaughter, and consequently *Campylobacter* and other enteric bacteria is smeared onto the surface of the meat during processing of the fresh meat products. [Wegener (G-1483) P.4 L.22-27]

1011. 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 matter how stringent hygiene measures are applied. [Wegener (G-1483) P.5 L.1-3]

1012. A risk/benefit analysis on the withdrawal of the NADA for enrofloxacin should include an analysis of the total effect on human health risks from the withdrawal of the NADA for enrofloxacin, including whether the human health benefits of using the drug outweigh the human health risks from use of the drug. [Cox (B-1901) P.12; ALJ Davidson's March 3, 2003 Order (OR31), P.1]

1013. Evaluating the total effect on human health risks from the withdrawal of the NADA for enrofloxacin includes not only the effect on fluoroquinolone-resistant campylobacteriosis, but also the effect on fluoroquinolone-susceptible campylobacteriosis (i.e., illness from susceptible strains) and on illnesses due to other chicken-borne pathogens, such as *Salmonella*. [Cox (B-1901) P.12]

1014. CVM did not consider any human health risks and benefits of enrofloxacin use in chickens or turkeys in making the decision to propose to withdraw the NADA for enrofloxacin. [CVM Response to Bayer's Interrogatory 83; Burkhart (B-1900) P.2 L.44-45]

1015. The CVM/Vose Risk Assessment has not fully assessed the human health effects of withdrawing the NADA for enrofloxacin because it focuses on only one organism (*Campylobacter*) and one main issue (fluoroquinolone-resistance) without evaluating the withdrawal's probable total effects on human health risks. [Cox (B-1901) P.12]

1016. The CVM/Vose Risk Assessment model does not identify or quantify any specific adverse human health effects, nor does it show how the frequency or severity of such health effects (e.g., illness-days) would change depending on continued use of enrofloxacin or the withdrawal of the NADA for enrofloxacin. [Cox (B-1901) P.55, P.83-87]

1017. The CVM/Vose Risk Assessment does not model how enrofloxacin reduces human exposures to *Campylobacter* and other pathogens by changing the distribution of

microbial loads reaching people via chickens. Thus, the model does not and cannot provide accurate or useful estimates of human health risks from use of enrofloxacin in chickens. [Cox (B-1901) P.55, P.83-87]

1018. Banning Baytril will greatly increase human health risks from campylobacteriosis and salmonellosis. A ban is expected to cause more than 25 additional days of campylobacteriosis and over 90 days of salmonellosis for each hypothetical day of fluoroquinolone-resistant *Campylobacter* (Fluoroquinolone-resistant CP) illness prevented. [Cox (B-1901) P.7 L.15-18, P.25, P.83-87]

1019. Withdrawing the NADA for enrofloxacin will prevent far fewer days of illness than CVM has estimated. Withdrawing the NADA for enrofloxacin may have no human health benefits. [Cox (B-1901) P.7 L.13-14, P.77-79, 82]

1020. The CVM/Vose Risk Assessment does not meet the minimal standards of technical competence and correctness necessary for acceptance in peer-reviewed journals because of its failures to correctly characterize risk, scope the enrofloxacin risk management problem, incorporate available relevant data on causality, exposure and dose-response, or alert decision-makers to the potential adverse human health consequences of an enrofloxacin ban. [Cox (B-1901) P.25]

1021. Enrofloxacin is used to treat respiratory problems in chickens, often manifested as airsacculitis. [Cox (B-1901) P.84]

1022. Airsacculitis flocks have higher initial levels of pathogens such as *Campylobacter*, *E. coli*, and *Salmonella*. [Cox (B-1901) P.84]

1023. A consequence of withdrawing the NADA for enrofloxacin (and probably other therapeutics and growth promoters such as those banned in Europe in 1999) is to increase the variance in the sizes and weights of broilers arriving at processing plants. [Cox (B-1901) P.83, citing B-1912, Attachment _ (Russell, 2002)]

1024. During processing, airsacculitis significantly increases the levels and incidence of *Campylobacter*, *E. coli*, and *Salmonella*. [Cox (B-1901) P.84]

1025. Airsacculitis-positive flocks have greater variability in carcass sizes and weakened digestive tracts, which in turn increase processing errors such as tears and cuts in digestive organs. [Cox (B-1901) P.84]

1026. Increased variance in the sizes and weights of broilers arriving at processing plants leads to more cuts and fecal contamination during processing, as more birds fall outside the tolerance of the evisceration equipment and process. [Cox (B-1901) P.83]

1027. Increased processing errors such as tears and cuts in digestive organs increase fecal contamination levels. [Cox (B-1901) P.84]

1038. Choice of an antibiotic is guided by the availability of antibiotics approved in a given country for a given animal species and the ease of use. [Aarestrup (G-1451) P.3 L.6-7]

1039. Fluoroquinolones such as enrofloxacin have activity against a wide range of different organisms and have very good distribution in the body. [Aarestrup (G-1451) P.3 L.9-10]

1040. Dr. Russell examined the impact on processed broilers if Baytril is not available because the health of the incoming bird is important to the pathogen load of the finished product. [Russell (B-1912) P.16 L.11-12]

1041. Eliminating the use of Baytril within the poultry industry will dramatically increase the number of human *Campylobacter* infections. [Russell (B-1912) P.26 L.15-22]

1042. Only about one-half of the cases of food borne illness caused by the major bacterial pathogens (*Campylobacter*, *Salmonella*, *E. coli* 0157:H7, and *Listeria*) are attributable to meat and poultry sources. [Prucha (A-203) P.4 L.3-5]

1043. Dr. Russell's research confirms what HACCP managers have long known, that birds that are not treated for diseases like air sacculitis infections will have: (1) greater intra-flock variability in weight at the time of slaughter leading to increased processing errors and increased fecal contamination; (2) higher pathogen contamination due to increased fecal contamination; and (3) increased numbers of infectious processes within carcasses at time of slaughter. This means that birds with untreated air sacculitis are more likely to carry pathogens, cross-contaminate other carcasses during processing, and are more likely to contain pathogens leaving the processing plant than are birds whose disease is treated. [Prucha (A-203) P.11 L.13-21]

1044. The enrofloxacin delivery method is effective. Baytril is administered in the drinking water of the infected house. Sick birds drink even if they do not eat. FDA has long accepted drinking water delivery as a safe and effective means to administer therapeutic animal drugs, including antibiotics, to commercially grown broiler chickens and turkeys. (Joint Stipulation 18). [Wages (B-1917) P.18 L.1-6]

1045. Recent data shows that carcasses with high levels of pathogens are more likely to cause disease than product with low levels of these pathogens. [Robach (B-1911) P.15 L.21-23]

1046. The removal of enrofloxacin from the broiler producer's arsenal of weapons would be a major step backwards in our multiple- threshold strategy to reduce the incidence of enteric pathogens in fresh poultry. [Robach (B-1911) P.16 L.4-6]

1047. It is of utmost importance that the poultry industry continue to have access to disease-control agents such as Baytril, in order to implement the multiple control-point strategy so necessary to the continuous improvement of the microbiological

quality of our products and the public health. [Robach (B-1911) P.16 L.23 through P.17 L.3]

1048. The implementation of HACCP has brought sound science, risk assessment and hazard analysis to the decision making process. [Robach (B-1911) P.17 L.9-10]

1049. Processing healthier birds will result in improved microbiological quality both at plant arrival and in the chilled carcass. [Robach (B-1911) P.17 L.10-11]

1050. In the absence of an effective disease treatment for *E. coli* respiratory infections it is possible that these diseased and weakened birds will be more susceptible to colonization by enteric pathogens of human health significance. Without an effective treatment for respiratory disease (*E. coli*) in broilers, our industry loses a valuable weapon in our arsenal against foodborne illness. Losing this weapon puts enormous additional pressures on other parts of the process. [Robach (B-1911) P.17 L.11-16]

1051. In the United States, enrofloxacin is approved for use only by prescription and only under veterinary supervision. (Joint Stipulation 15). [Wages (B-1917) P.18 L.7-8; Gonder (A-201) P.27 L.22-23]

1052. In the United States, enrofloxacin is approved for therapeutic use only and is not approved for growth promotion. (Joint Stipulation 16). [Wages (B-1917) P.18 L.8-9; Gonder (A-201) P.27 L.23 – P.28 L.1]

1053. In the United States, extra-label use of enrofloxacin is prohibited by law for food producing animals. (Joint Stipulation 17 & 46). [Wages (B-1917) P.18 L.9-11; Gonder (A-201) P.28 L.1-3]

1054. Proper and judicious use of enrofloxacin has been publicized in the poultry industry (G-113, B-1263). [Wages (B-1917) P.19 L.3-4]

1055. The OIE approach for risk assessment consists of four stages: release assessment, exposure assessment, consequence assessment and risk estimation. Release assessment describes the pathways for entry of an adverse agent into the environment. Exposure assessment quantifies the likelihood and extent of such introduction to occur, and the resulting impact (in terms of dose) to the (human) population affected. Consequence assessment relates the exposure to the probability and nature of the adverse human health outcomes. Risk estimation integrates the results of the prior stages into a measure of the adverse outcome predicted to occur. [Haas (B-1904) P.6 L.19 through P.7 L.2]

1056. As noted in the OIE approach for risk assessment, there are distinctive parallels between the OIE approach and the NAS (1983) approach. The principal differences are that in the OIE approach, hazard identification is considered to be part of a (preliminary) risk analysis phase, and (the NAS) exposure assessment is subdivided (in the OIE framework) into phases of exposure assessment and consequence assessment. However it is clear that the principal differences are in

terminology rather than in substantive activity that must be taken to produce an estimate of adverse consequence from a policy or event. [Haas (B-1904) P.7 L.3-9]

1057. The FDA Draft Guidance ("Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to their Microbiological Effects on Bacteria of Human Health Concern," September 6, 2002) adopts the OIE approach, and sets forth specific tasks to be undertaken in a *qualitative* risk assessment of new drug approvals. It is noteworthy that, while the draft guidance does not forbid quantitative risk assessment, it only discusses qualitative risk assessment. In particular, in the approach outlined, the results of each of the steps of release assessment, exposure assessment, and consequence assessment are captured in descriptive terms (high, medium or low). The final risk estimate is obtained from this lexical descriptor and used as a basis for regulatory decision making. [Haas (B-1904) P.7 L.11-19]

1058. The "risk analysis" prepared by CVM in connection with the proposal to withdraw approval of use of fluoroquinolones (FQ) in chickens has a number of major flaws, errors and omissions. On this basis, it cannot be considered as a reliable basis to estimate the impact of fluoroquinolone use in chickens on occurrence of fluoroquinolone-resistant *Campylobacter* in humans. In particular these flaws, errors and omissions are likely to have resulted in a substantial overestimate of the risk to humans. [Haas (B-1904) P.7 L.21 through P.8 L.4 relying on B-1904]

1059. The CVM/Vose Model cannot be considered a "Risk Assessment." [Haas (B-1904) P.8 L.5 through P.10 L.18, excluding P.9 L.3-6, and P.9 L.10 through P.10 L.2]

1060. The concept of a risk assessment derives from the 1983 NAS paradigm including steps of hazard characterization, dose-response analysis, exposure analysis, and risk characterization. The recent OIE paradigm bears great similarity to the NAS paradigm. Additionally, a recent consensus document developed under the auspices of the International Life Sciences Institute (ILSI) -- including participation by scientists from USDA, FDA and USEPA -- contains analytical phases that are similar to both the NAS and OIE frameworks. The FDA Center for Food Safety and Applied Nutrition has published a framework for conducting major risk assessments, in which it adopts the elements of the NAS paradigm (both in terminology and in substance). A similar breakdown, specifically for the area of food risk assessment has been adopted by *Codex Alimentarius*. [Haas (B-1904) P.8 L.6-15]

1061. While there has been an evolution in the practice of risk analysis, particularly with respect to the integration of communication and stakeholder input at all steps of the process, it is clear that the key technical aspects of risk assessment remain consistent from the 1983 NAS paradigm until today (although terminology may differ with the particular application). In particular, assertions that the 1994 NRC report ("Blue Book") or the 1996 NRC report ("Orange Book") have supplanted the 1983 paradigm are factually erroneous. [Haas (B-1904) P.8 L.15 through P.9 L.2]

1062. It is also noteworthy that an assessment of the impact of fluoroquinolone-resistant *Campylobacter jejuni* derived from beef cattle has been conducted by the Georgetown University Center for Food AND Nutrition Policy using the NRC/*Codex* paradigm. (B-147). In fact, a co-author (Crawford) of this study is now Deputy Commissioner of FDA. [Haas (B-1904) P.9 L.6-9]

1063. It is difficult to associate the steps required for a quantitative risk assessment with the actual tasks performed in the CVM/Vose model (G-953). For example, exposure is only portrayed with respect to pounds of chicken consumed, and pounds consumed containing fluoroquinolone (FQ) resistant *Campylobacter*. In other words, the risk of consuming a portion of chicken with 1 *Campylobacter* is assumed to be equal to the risk of consuming a portion with 1000 organisms. There is no specific quantification of the number (either of fluoroquinolone sensitive or fluoroquinolone-resistant) of organisms per portion being consumed in the exposed population. There is no specific construction or utilization of a dose-response relationship, despite the availability of a relationship (B-517, B-748), and despite the fact that other risk assessors (B-147) have used that model. [Haas (B-1904) P.10 L.3-12]

1064. The alternative approaches used by CVM (G-953) are therefore at variance with the steps that have become to be generally regarded as key to the validity and usefulness of quantitative microbial risk assessment. [Haas (B-1904) P.10 L.13-15]

1065. The CVM/Vose Model (G-953) fails to meet the NAS criteria for risk assessments. [Haas (B-1904) P.10 L.16-18]

1066. The CVM/Vose Model underestimates the probability that a person with campylobacteriosis will seek care (p_{mn}). [Haas (B-1901) P.10 L. 16 – P. 12 L.5]

1067. The CVM/Vose Model overestimates the attributable risk from chicken. [Haas (B-1904) P.12 L.10 – P.15 L.7, excluding P.12 L.15 through 18 and P.13 Figure 1]

1068. The CVM/Vose Model shows an inconsistency between "K" values for total and fluoroquinolone-resistant *Campylobacter*. [Haas (B-1904) P.15 L.11 – P.16 L.2]

1069. The K" values in the CVM/Vose risk assessment cannot properly be interpreted as dose-response factors since the K's are ratios between the aggregate case burden and the aggregate consumption. [Haas (B-1904) P.15 footnote 5]

1070. The CVM/Vose Model dismisses the distributional importance of pathogen load. [Haas (B1904) P.16 L.3-18]

1071. Due to the lack of adherence to standard practices in microbial risk assessment, lack of consideration of significant variables, and use of outdated information, the CVM/Vose analysis is not useful in understanding or quantifying the risks posed by the use of fluoroquinolone in treating chickens. Due to the lack of grounding in conventional risk assessment practices, it does not appear possible to quantify the

degree of error that may have been made. [Haas (B-1904) P.16 L.19 through P.17 L.2]

1072. The CVM/Vose Model (G-953) does not meet the SDWA Criteria As Required by OMB Regulations in the following among other aspects. Based on his analysis, Dr. Hass concluded that there are significant deficiencies in the CVM/Vose analysis with respect to the OMB guidelines such that it is not an adequate risk assessment under OMB requirements. In particular, the CVM/Vose analysis fails to meet data quality requirements (peer review, objectivity) mandated in the OMB guidelines. [Haas (B-1904) P.17 L.3-10]

1073. Although the epidemiological studies used by Vose to estimate the poultry related fraction of campylobacteriosis were peer reviewed in refereed journals, the fact that they are old studies and contain methodological flaws with respect to present practice would lead to questioning with respect to data quality. It would have been possible for these to have been subject to the additional peer review contemplated by OMB, but this has apparently not occurred. The NARMS data used, to my knowledge, has not been subject to external peer review. The OMB guidelines on objectivity specify that peer review provides a rebuttably presumptive test of data and analytic results. The guidelines themselves appear to be silent with respect to analytic methods. Vose's approach represents a new analytical method, which to my knowledge has not been subject to peer review or other tests of objectivity. [Haas (B-1904) P.17 L.11-20]

1074. It is likely that any risk assessment that used an experimental method (e.g., for determination of concentration of a toxic material) which had not been subject to the objectivity test of OMB (e.g., peer review), would be held up to question. It is therefore reasonable to consider that a method for handling of data (calculation) that had not been subject to the objectivity test would be equally suspect. I am not aware of any peer-reviewed manuscript that delineates the K factor approach. Whether the CVM/Vose analysis went through an alternative independent and open peer review process is not clear, but I have seen nothing to suggest it has. [Haas (B-1904) P.19 L.12 through P.20 L.6]

1075. The OMB and HHS/FDA guidelines indicate that documents should include "Additional studies not used to produce the risk estimate that support or fail to support the findings of the assessment, and the rationale of why they were not used." This delineation and critique of alternative studies does not appear to be part of the CVM/Vose model. [Haas (B-1904) P.20 L.7-10]

1076. The OIE Risk Assessment Framework does not meet the regulatory requirements of the OMB regulations for risk assessment. There is no intrinsic inconsistency between the OIE risk framework and the OMB guidelines. However, the OMB, in incorporating the Safe Drinking Water Act requirements for health risk assessment, which refer to central tendency estimates and upper and lower bound estimates for risk, would appear to strongly argue for the use of *quantitative* rather than *qualitative* risk assessment.

1077. The CVM/Vose Model (G-953) does not meet the OIE Risk Assessment Framework. The steps of the OIE framework for risk assessment consist of release assessment, exposure assessment, consequence assessment and risk estimation. The OIE definition of Consequence Assessment (in typical US applications, this would be termed Dose Response Assessment) is inVose *et al.* at page 815, expanded upon at page 816. It is clear that the CVM/Vose model is an attempt to develop a quantitative risk assessment. The CVM/Vose analysis, however, does not meet the OIE framework for quantitative risk assessments, because it does not adequately consider or model the dose at the moment of exposure. The metric for dose used in the CVM/Vose analysis is whether or not a particular amount of chicken does or does not contain fluoroquinolone-resistant *Campylobacter*. It does not consider the amount of fluoroquinolone-resistant *Campylobacter* that might be present. The principle of "The dose makes the poison" is applicable to quantitative risk assessment, and by neglecting the amount of bacteria ingested, the CVM/Vose analysis appears to overlook one of the key principles contemplated in the OIE framework. [Haas (B-1904) P.20 L.20 through P.21 L.23]

1078. The CVM/Vose Model (G-953) does not meet the recently proposed CVM Guidance for Industry: Evaluating the Safety of Antimicrobial New Animal Drugs With Regard to Their Microbiological Effects on Bacteria of Human Health Concern. The CVM Guidance expounds a *qualitative* risk assessment framework as being appropriate for assessing safety of new animal drugs. Under the CVM Guidance, the evaluation of each of the stages of release assessment, exposure assessment, and consequence assessment are to be conducted using a final descriptor of "high," "medium" or "low." The CVM/Vose analysis does not go through readily separable phases of release assessment, exposure assessment and consequence assessment, nor does it describe in a formal sense any of these aspects using the lexical descriptors designated under the CVM Guidance. Hence, the CVM/Vose model does not meet the CVM Guidance. [Haas (B-1904) P.21 L.24 through P.22 L.9]

1079. The CVM/Vose Model (G-953) is not a reliable basis to estimate the impact of fluoroquinolone use in chickens on occurrence of fluoroquinolone-resistant *Campylobacter* infections in humans. In the absence of meeting the NAS criteria, or any other recognized criteria (e.g., OIE), the burden is on the risk assessor to establish scientific credibility to demonstrate that the assessment is correct. This is typically done through peer review or validation with existing data. [Haas (B-1904) P.22 L.10-18]

1080. The methodologies used in the CVM/Vose analysis (G-953) have not been subject to peer review. There are some inputs (e.g., NARMS data) that have not apparently been subject to external peer review, and there are other inputs (epidemiological studies) where, despite being published in peer review journals, would require additional peer review to validate their utility. The December 1999 Workshop does not constitute peer review under generally accepted definitions. [Haas (B-1904) P.22 L.18-23]

1081. The CVM/Vose analysis methodology (G-953) is defective in a number of key aspects., including among other defects: (1) There are a number of assumptions that are made which have not be in explicitly grounded in data. For example, at the public meeting in January 2001, Dr. Kimberly Thompson of Harvard questioned whether there is support for a linear assumption between disease burden and frequency of consumption of positive portions. Dr. Condon, as noted above, questioned the appropriateness of analyzing risk without explicitly considering the number of organisms ingested. Therefore, it is reasonable to believe that the methodology as employed *would not be capable* of meeting the normal tests of adequacy inherent in the peer review process for scientific journals. [Haas (B-1904) P.23 L.1-9]

1082. Based on Dr. Haas analysis, the CVM /Vose analysis (G-953) should not be considered as a reliable basis on which to make a decision. [Haas (B-1904) P.23 L.10-11]

1083. It is clear both from the Cox study, as well as those of other workers, that a more traditional risk assessment could have been performed on *Campylobacter* in poultry. [Haas (B-1904) P.23 L.11-12]

1084. It is in Dr. Haas' opinion clear from the Cox study, as well as those of other workers, that a more traditional risk assessment should be performed prior to making a decision by CVM to withdraw approval of enrofloxacin in poultry. [Haas (B-1904) P.23 L.11-13]

1085. The CVM/Vose Analysis (G-953) does not consider potential microbial benefits from the use of fluoroquinolone in chicken production. [Haas (B-1904) P.23 L.14-15]

1086. The literature indicates that the prevalence of air sacculitis in chickens with respiratory diseases increases in flocks that are not administered efficacious antibiotics. Recent work also indicates that chickens that have air sacculitis have greater levels of enteric pathogens such as *Campylobacter* and Salmonella on their carcasses.(B-1912, Attachment 1). Inasmuch as greater carcass bacterial loadings will lead to greater loadings of microorganisms on the food product as prepared or consumed, even considering HACCP, providing that handling and cooking processes do not differ, the diminished use of efficacious antibiotics, all other factors remaining constant, would increase the exposure of people to pathogens and therefore increase the risk of human disease. [Haas (B-1904) P.23 L.16 – P.24 L.2]

1087. The CVM/Vose Analysis (G-953) explicitly considers only chicken and not turkey. [Haas (B-1904) P.24 L.7]

1088. Even if the CVM/Vose analysis was to be regarded as providing a rational basis for making a regulatory judgement with respect to the issue of use of fluoroquinolone in chickens, it could not be regarded as providing a rational basis with respect to turkeys. There is no consideration of distinctiveness in microbial prevalence,

exposure (food consumption), case rate or strain differences between turkeys and chickens. These latter factors make the two problems (although perhaps naively similar) different enough to merit distinct and separate analysis. Hence the scientific basis for reaching a regulatory decision in the case of fluoroquinolone use in turkeys is even less than may exist for chickens. [Haas (B-1904) P.24 L.8-15]

1089. The CVM/Vose model (G-953) cannot be considered a risk assessment under the NAS 1983 paradigm. [Haas (B-1904) P.25 L.9-10]

1090. The OIE paradigm has substantive similarities to the NAS 1983 paradigm, and therefore the CVM/Vose model (G-953) does not appear to contain the necessary elements contemplated under the OIE framework. [Haas (B-1904) P.25 L.11-13]

1091. The CVM/Vose analysis (G-953) makes a number of assumptions that are not substantiated by peer reviewed information. [Haas (B-1904) P.25 L.14-15]

1092. Some data used in the CVM/Vose analysis (G-953) is from dated literature no longer likely to reflect actual exposure patterns in the general population. [Haas (B-1904) P.25 L.16-17]

1093. The CVM/Vose analysis (G-953) fails to consider distributional (of organism) and dose-response issues that are essential for an adequate quantitative microbial risk assessment. [Haas (B-1904) P.25 L.18-19]

1094. The overall analytical framework used in the CVM/Vose analysis (G-953) has not been subject to a peer review as required in the OMB guidelines on data quality. [Haas (B-1904) P.25 L.20-21]

1095. The CVM/Vose analysis (G-953) does not provide a basis to perform a qualitative risk assessment according to the CVM Guidelines. [Haas (B-1904) P.26 L.1-2]

1096. The CVM/Vose analysis (G-953) provides an inappropriate basis on which to make a risk-based regulatory decision. [Haas (B-1904) P.26 L.3-4]

1097. The flaws in the CVM/Vose analysis (G-953) rise to the level such that they do not permit the use of the CVM/Vose analysis to infer a risk from the use of FQ in chickens. [Haas (B-1904) P.26 L.5-6]

1098. The CVM/Vose analysis (G-953) neglects potential benefits with respect to human exposure to pathogens resulting from the use of fluoroquinolone in chicken rearing. [Haas (B-1904) P.26 L.7-8]

1099. The CVM/Vose analysis (G-953) neglects the additional risk (in the form of increased carcinogenic water disinfection byproducts) that would result from the withdrawal of use of fluoroquinolone in chicken rearing. [Haas (B-1904) P.26 L. 9-11]

1100. The CVM/Vose analysis (G-953) explicitly considers only risks posed with respect to fluoroquinolone-resistant *Campylobacter* in chicken, and does not provide explicit consideration with respect to turkey. [Haas (B-1904) P.26 L.12-14]

1101. The CVM/Vose Risk Analysis does not objectively address the question of whether data suggest that enrofloxacin use in chickens causes increased risk of harm to humans, such as treatment failures or morbidity, although it is driven by many untested assumptions and opinions on this point. [Cox (B-1901) P.5 L.8-11]

1102. Objective tests for potential causality, though readily available, have not been used by CVM in their Risk Analysis. [Cox (B-1901) P.5 L.11-12]

1103. When objective tests for potential causality are used, they refute the CVM/Vose Risk Assessment's main assumptions and predictions. [Cox (B-1901) P.5 L.11-13]

1104. CVM's risk assessment does not meet widely accepted standards for risk assessment. It lacks generally accepted intellectual foundations for drawing valid conclusions about risk, and, indeed, the conclusions that it draws are not valid. [Cox (B-1901) P.7 L.1-3]

1105. CVM's risk assessment does not use the best available data and methods. [Cox (B-1901) P.7 L.8]

1106. CVM's risk assessment model does not and cannot provide accurate or useful estimates of human health risks from use of Baytril in chickens. [Cox (B-1901) P.7 L.9-10]

1107. CVM's risk model cannot be used to support rational or useful risk-management decision-making. [Cox (B-1901) P.7 L.11-12]

1108. Since the 1970s, human health risk assessment has become a relatively well-established discipline, featuring both well-structured methodological approaches for assessing health risks from known or suspected hazards and also a substantial body of technical content and methods supporting the methodological structure. [Cox (B-1901) P.9-10]

1109. The traditional logical structure of risk assessment includes the following steps: 1) Scoping the analysis to support decisions by estimating the causal relation between decisions, exposures, and their probable total human health consequences (see e.g. Vose testimony, G-1480, P.3, paragraph 7, citing SRA and NRC). To guide rational regulatory decision-making, traditional quantitative risk analysis seeks to quantify the causal relation between regulatory actions that might be taken and their total probable human health consequences. This step is often not listed explicitly, but it is a crucial part of risk analysis frameworks (e.g., EPA's multipathway risk assessment framework) designed to support effective and rational health risk management decision-making; 2) Hazard identification, which means to use data to provide and assess evidence of a causal relation between exposures (e.g., to *Campylobacter*-contaminated chicken) and adverse human health response (e.g.,

illness-days per capita per year); 3) Exposure assessment, which means presenting data-based estimates of the population frequency distribution of individual exposures (e.g., frequencies and magnitudes of ingested microbial loads of *Campylobacter*) in a human population (e.g., the population of chicken-eaters in the US). Exposure modeling also addresses how human exposures would change if different risk management actions (e.g., a ban on enrofloxacin) were undertaken. 4) Dose-response modeling or exposure-response modeling, which quantifies the causal relation between levels of exposure and probabilities of specified adverse human health consequences for individuals with various characteristics or risk factors; 5) Risk characterization, which integrates information from the exposure assessment and exposure-response models and presents their implications for the frequency and magnitude of exposure-related adverse health effects in the exposed population; 6) Uncertainty characterization, which describes uncertainty, variability, and sensitivities in the estimated exposure-response relation for the exposed population. It should characterize both model uncertainties and data uncertainties. Variability analysis should describe the extent of inter-individual heterogeneity in risks, e.g., due to differences in other risk factors and covariates among individuals. [Cox (B-1901) P.10]

1110. FDA's Center for Food Safety and Applied Nutrition has offered a definition of risk assessment that is: "The scientific evaluation of known or potential adverse health effects resulting from human exposure to hazards. The process consists of the following steps: hazard identification, exposure assessment, hazard characterization (dose-response), and risk characterization". The Center for Food Safety and Applied Nutrition has offered a definition risk as "The likelihood of the occurrence and the magnitude of the consequences of exposure to a hazard on human health. [Cox (B-1901) P.11]

1111. The CVM/Vose Risk Assessment for enrofloxacin use in poultry does not follow the content or methods of the traditional risk assessment approach. [Cox (B-1901) P.11]

1112. As defined by the Codex Alimentarius Commission, one of the necessary steps of Hazard Characterization as a part of risk assessment is a dose-response assessment should be performed if the data are obtainable. [Joint Institute for Food Safety and Applied Nutrition (A-30)]

1113. For risk assessments the term "dose-response assessment" means the deterimination of the relationship between the magnitude of exposure (dose) to a chemical, biological or physical agent and the severity and/or frequency of associated adverse health effects (response). [Joint Institute for Food Safety and Applied Nutrition (A-31) P. 1]

1114. The General Principles of Microbiological Risk Assessment as posted on the foodriskclearinghouse.umd.edu, website for the Joint Institute for Food Safety and Applied Nutrition, are: 1. Microbiological Risk Assessment must be soundly based upon science; 2. There should be a functional separation between Risk Assessment

and Risk Management; 3. Microbiological Risk Assessment should be conducted according to a structured approach that includes Hazard Identification, Hazard Characterization, Exposure Assessment, and Risk Characterization; 4. A Microbiological Risk Assessment should clearly state the purpose of the exercise, including the form of Risk Estimate that will be the output; 5. A Microbiological Risk Assessment should be transparent. This requires: full and systematic documentation, statement of assumptions and value judgments and rationale, and a formal record; 6. Any constraints that impact on the Risk Assessment such as cost, resources or time, should be identified and their possible consequences described; 7. Data should be such that uncertainty in the Risk Estimate can be determined; data and data collection systems should, as far as possible, be of sufficient quality and precision that uncertainty in the Risk Estimate is minimized; 8. The Risk Estimate should contain a description of uncertainty and where the uncertainty arose during the Risk Assessment process; 9. A Microbiological Risk Assessment should explicitly consider the dynamics of microbiological growth, survival, and death in foods and the complexity of the interaction (including sequelae) between human and agent following consumption as well as the potential for further spread; 10. Wherever possible, Risk Estimates should be reassessed over time by comparison with independent human health data; 11. A Microbiological Risk Assessment may need reevaluation as new relevant information becomes available. [Joint Institute for Food Safety and Applied Nutrition (A-31)]

1115. As posted on the foodriskclearinghouse.umd.edu, website for the Joint Institute for Food Safety and Applied Nutrition, the risk assessment step of Exposure Assessment includes an assessment of the extent of actual or anticipated human exposure. [Joint Institute for Food Safety and Applied Nutrition (A-31) P. 2]

1116. As posted on the foodriskclearinghouse.umd.edu, website for the Joint Institute for Food Safety and Applied Nutrition, factors that must be considered for Exposure Assessment include the frequency of contamination of foods by the pathogenic agent and its level in those foods over time. These factors are influenced by the characteristics of the pathogenic agent, the microbiological ecology of the food, the initial contamination of the raw material, the level of sanitation and process controls, the methods of processing, packaging, distribution and storage of the foods, as well as any preparation steps such as cooking. Another factor that must be considered in the assessment is patterns of consumption. This relates to socio-economic and cultural backgrounds, ethnicity, seasonality, age differences (population demographics), regional differences, and consumer preferences and behaviour. [Joint Institute for Food Safety and Applied Nutrition (A-31) P. 3]

1117. As posted on the foodriskclearinghouse.umd.edu, website for the Joint Institute for Food Safety and Applied Nutrition, the risk assessment step of Exposure Assessment should describe the pathway from production to consumption. [Joint Institute for Food Safety and Applied Nutrition (A-31) P. 3]

1118. As posted on the foodriskclearinghouse.umd.edu, website for the Joint Institute for Food Safety and Applied Nutrition, the risk assessment step of Exposure

Assessment estimates the level, within various levels of uncertainty, of microbiological pathogens or microbiological toxins, and the likelihood of their occurrence in foods at the time of consumption. [Joint Institute for Food Safety and Applied Nutrition (A-31) P. 3]

1119. Two human feeding studies of *Campylobacter jejuni* are referenced on the foodriskclearinghouse.umd.edu website for the Joint Institute for Food Safety and Applied Nutrition. [Joint Institute for Food Safety and Applied Nutrition (A-32) P. 1]

1120. As posted on the foodriskclearinghouse.umd.edu, website for the Joint Institute for Food Safety and Applied Nutrition, the risk assessment step of Hazard Characterization dose-response data is utilized when available. [Joint Institute for Food Safety and Applied Nutrition (A-33)]

1121. Human feeding studies to assess dose-response relationships for various pathogens are referenced on the foodriskclearinghouse.umd.edu, website for the Joint Institute for Food Safety and Applied Nutrition. [Joint Institute for Food Safety and Applied Nutrition (A-33)]

1122. In a joint document, the FDA's Center for Food Safety and Applied Nutrition and the USDA's Food Safety and Inspection Service indicate that the generally accepted framework for microbial risk assessments divides the risk assessment into four distinct components: (1) hazard identification, (2) exposure assessment, (3) hazard characterization, and (4) risk characterization. [Interpretive Summary: Draft Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods (A-34) P. 5]

1123. In a joint document, the FDA's Center for Food Safety and Applied Nutrition and the USDA's Food Safety and Inspection Service indicate that under the generally accepted framework for microbial risk assessments the steps of exposure assessment, hazard characterization, and risk characterization involve the use available data and, where necessary, science-based assumptions, to develop mathematical models that estimate how often consumers eat food contaminated with the organism, the number of the bacteria likely to be in that food, and the risk of serious illness or death to the age-based groups when they are exposed to the hazard. [Interpretive Summary: Draft Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods (A-34) P. 5 - 6]

1124. The FDA's Center for Food Safety and Applied Nutrition's Foodborne Pathogenic Microorganisms and Natural Toxins Handbook recognizes that human feeding studies suggest that about 400 – 500 *Campylobacter jejuni* may cause illness in some individuals, while in others greater numbers are required. [Foodborne Pathogenic Microorganisms and Natural Toxins Handbook (A-104) P.1]

1125. The CVM/Vose Risk Assessment's parameter p_{ca} (probability a *Campylobacter* case is attributable to chicken) is too high. [Cox (B-1901) P.77]

1126. The CVM/Vose Risk Assessment's parameter p_{rh} (probability a *Campylobacter* case from chicken is fluoroquinolone-resistant) is too high. [Cox (B-1901) P.78, citing B-1260]

1127. The CVM/Vose Risk Assessment's chicken-attributable fraction specifically for fluoroquinolone-resistant campylobacteriosis cases is too high; a value based on the data from the CDC 1998 - 1999 *Campylobacter* Case-Control data set is between – 11.6% and 0.72% (depending on how missing data are treated) and is not statistically different from zero. [Cox (B-1901) P.78]

1128. The CVM/Vose Risk Assessment estimates the total number of cases in which ciprofloxacin is administered to a patient with at least one CFU of fluoroquinolone-resistant *Campylobacter*, but not every case of fluoroquinolone-resistant *Campylobacter* illness treated with ciprofloxacin will experience treatment failure or diminished effectiveness. [Cox (B-1901) P.78, citing B-50 (Piddock 1999)]

1129. Not all domestically acquired, non-medication-related fluoroquinolone-resistant campylobacteriosis cases come from chickens. [Cox (B-1901) P.79]

1130. By assuming that fluoroquinolone use is the only source of fluoroquinolone-resistant *Campylobacter* chickens, the CVM risk assessment model over-estimates the true fraction of fluoroquinolone-resistant *Campylobacter* in chickens that come from fluoroquinolone use. [Cox (B-1901) P.79]

1131. A *Campylobacter* risk assessment should take into account on farm strategies and their impact on the level of *Campylobacter* on or in poultry entering the processing plant. [Tompkin (A-204) P.40 L.8-9]

1132. Existing data from FoodNet fail to show a significant epidemiologic link between the consumption of turkey meat and campylobacteriosis and salmonellosis. [Tompkin (A-204) P.58 L.15-16]

1133. Most turkey production systems (over 90%) are completely integrated and provide direct management control over turkey breeders, hatcheries, feed mills, producers and processors. [Gonder (A-201) P.3 L.16-18; Wages (B-1917) P.3 L.1-4]

1134. Female turkeys may be marketed at 10-22 weeks of age and weigh 10-26 pounds; males may be marketed from 16-24 weeks and weigh 25-45 pounds. [Gonder (A-201) P.3 L.10-11]

1135. Two ages of birds may be present on the farm at the same time. [Gonder (A-201) P.4 L.15-16]

1136. Having birds of multiple ages on one farm means having birds with different exposure histories and different immunities in close proximity to each other. This

increases the need for closely monitoring bird health and heightens the need for early and effective treatment interventions. [Gonder (A-201) P.4 L.22 through P.5 L. 2]

1137. Within the last eight years, weights and ages of marketed turkeys have increased in response to market demands. [Gonder (A-201) P.5 L.3-4]

1138. Most turkey farms have 2 to 4 houses while the national range is 2 to 10 houses. [Gonder (A-201) P.6 L.1-2]

1139. Generally, brooder houses will contain 10,000-20,000 poults, although some very large houses used in a few locations may contain 70,000 poults. Finishing or grow-out houses usually contain one-third to one-half as many birds as brooder houses due to the need to provide additional space for the birds to grow. [Gonder (A-201) P.6 L.9-14; Wages (B-1917) P.4 L.7-9]

1140. Microbial contamination from incoming feed ingredients is kept to a minimum by heat treatment during processing. It is provided to the birds in automatic feeders filled from storage tanks outside the house. When the feed level in a control pan becomes low, this triggers an auger system that fills the pans from the tanks. Supplemental, small hand-filled feeders are usually used while the poults are very young and in the brooder rings. [Gonder (A-201) P.6 L.18-22; Wages (B-1917) P.4 L.17-18]

1141. Water is provided either by one of several different systems of nipple drinkers (a closed system which the bird must activate by picking a "nipple" in the brooder houses), or plastic bell (Israeli, Plasson) drinkers where the water flow is controlled by a valve that responds to the weight of the water in the drinker – less water in the circular channel at the base of the hanging drinker triggers additional water flow. Waterers are generally washed with iodine every 1-2 days to control microbial contamination. [Gonder (A-201) P.7 L.2-7; Wages (B-1917) P.5 L.1-4]

1142. All water systems in poultry houses must be managed to avoid spillage and keep the litter as dry as possible to reduce foot and leg problems, and gastrointestinal disease. This is particularly true in turkeys compared to chickens due to their heavier market weights, older ages, and relative intolerance of environmental ammonia (commonly produced by wet litter). [Gonder (A-201) P.7 L.12-15]

1143. Virtually all producers require the observation of all flocks at least once per day. Additionally, company service technicians visit each farm at least once per week. Those observing flocks look for changes in bird behavior, equipment performance, mortality, morbidity, and biosecurity. [Gonder (A-201) P.8 L.4-8)

1144. Mortality on turkey ranches is recorded daily. Generally, if mortality exceeds 1 per thousand per day without an anticipated cause, the service technician must be contacted; compliance is generally not an issue. [Gonder (A-201) P.8 L.10-13; Wages (B-1917) P.6 L.1-4]

1145. Those working daily with turkeys are quite cognizant of the birds' activity level and normal behavior. Changes in these parameters are usually reported on the day they occur and are investigated. Checking for morbidity is more subjective than checking mortality, so a higher level of husbandry experience is generally required. [Gonder (A-201) P.8 L.15-18]

1146. During the service technician's visit, each house is usually completely "walked" down its length, observing the flock's activity, eating and drinking behavior, droppings (feces) appearance, respiratory activity, evidence of lameness, feed and water availability and quality, litter condition, air quality, and temperature and humidity conditions. Any necessary changes are recommended or made immediately. If a disease is suspected, arrangements are made to obtain a diagnosis. Either a company veterinarian is consulted or birds are taken to a State or company laboratory. Various samples to aid diagnosis can be taken. Flocks in many companies are monitored regularly by laboratory examination for diseases that may occur in the area or that are of interest. [Gonder (A-201) P.9 L.19 through P.10 L.5]

1147. Biosecurity procedures vary somewhat usually depending on the degree of vertical integration of the birds' owner. In general, almost every company requires a change in footwear and a change in outer clothing when going from farm to farm. Companies with separate brooder farms, or breeders, generally require personnel to shower and wear farm-specific clothing when moving between farms. Equipment that is moved from farm to farm is cleaned and disinfected. Within farms, personnel usually will wear different footwear for each house, or step in a disinfectant bath before entering each house. Younger birds are generally visited first, then older birds, since older birds have lived longer and had more opportunity to be exposed to disease. Houses that are not healthy are visited after those that are. Breeder farms and larger meat turkey farms may have wash stations to clean and disinfect vehicles and equipment before entering the farm, and when leaving. [Gonder (A-201) P.9 L.7-19]

1148. Brooder houses are usually completely cleaned out between flocks. All litter is removed, and the house and all equipment are washed and disinfected prior to the arrival of the next flock. [Gonder (A-201) P.10 L.15-16]

1149. Turkeys and chickens are different species. Chickens are not just big turkeys. Factors that influence the diagnosis, prevalence and treatment of disease in one are not the same as in the other. [Gonder (A-201) P.11 L.10-12]

1150. Because of differences between how turkeys and chickens are colonized by microflora, how they are raised and how they are processed, the risk of transmitting *Campylobacter jejuni* infection to humans are inherently different between turkeys and chickens. [Gonder (A-201) P.11 L.12-14]

1151. Turkey is a highly unlikely source of infection for human campylobacteriosis [Gonder (A-201) P.13 L.6-7 relying on A-201 generally]

1152. There are relevant grow-out and processing differences between turkeys and chickens that contribute to reduced bacterial loads on turkeys. Turkeys have more frequent house clean-out and their live-haul equipment is more routinely sanitized between processed flocks. [Gonder (A-201) P.11 L.14-17]

1153. More than one age of turkey is often present on the farm and the birds are raised to a greater age, making disease control somewhat more complex and more critical. [Gonder (A-201) P.11 L.17-19]

1154. Turkey processing uses higher scalding temperatures than broilers, which can kill more *Campylobacter*. Wempe (Appl Env Micro 45:355-359. 1983) reports *C. jejuni* prevalence in scalding tank overflow water at California chicken plants to be 13.3, 20, and 20% for scalding temperature of 60, 53, and 49^{0}C (140, 127, and 121^0 F). Yusufu (J Food Prot 46:868-872. 1983) reports California turkey plants had a prevalence of 5.7 and 5.6% at 60 and 57C (140 and 134^{0}F) - rather a large difference. [Gonder (A-201) P.11 L.20 through P.12 L.2]

1155. Turkeys undergo manual evisceration and cropping so the risk of enteric pathogen contamination is greatly reduced as compared to automated processing in the broiler industry. Turkeys undergo extended chilling to reduce carcass temperature due to larger body mass. This uses more chlorinated water (at 3-5 ppm active chlorine) and chilling capacity than chickens so that bacterial loads should be further reduced since both chlorination (Luechtefeld, J Clin Micro 13:266-268, 1981; Genigeorgis, Proceeding of the Western Poultry Disease Conference, 1986; (B-1857) Blaser, Appl Env Micro 51:307-311, 1986)(B-1855)) and washing (Izat, Poultry Sci 67: 1568-1572. 1988) (B-1853) reduce *Campylobacter* levels during processing. [Gonder (A-201) P.12 L.4-11]

1156. Turkeys are sold at a more physiologically and immunologically mature age than chickens; therefore it seems reasonable that their intestinal microflora may be more similar to that of adult chickens. Indeed, the microflora from adults has been shown to have protective effects against campylobacteria infections in young chicks (Soerjade-Liem, Avian Dis 28:139-146, 1984). (B-1868) [Gonder (A-201) P.12 L.11-15]

1157. Recent studies corroborate an additional major difference between chickens and turkeys. These studies show that the predominant *Campylobacter* recovered from turkeys is *C. coli* not *C. jejuni.* These studies include, Zhao et. al. (G-727), who recently reported on *Campylobacter* prevalences from retail meats in the greater Washington, D.C. area. They report a 14% prevalence on turkey products versus 71% prevalence on chicken products. The major organism recovered was *Campylobacter coli* which was found in 86 of 112 isolates (77%) while *C. jejuni* was only identified 16 of 112 isolates (14%). The remaining isolates were other *Campylobacter* spp. Other studies include 165 ground turkey samples were cultured for *Campylobacter* – 14 (8.5%) were positive for *Campylobacter jejuni/coli*, while 73 of 162 (45%) of ground chicken samples were positive (Food Safety & Inspection Service, USDA, Nationwide Raw Ground Chicken

Microbiological Survey and Nationwide Raw Ground Turkey Microbiological Survey, 1994). [Gonder (A-210) P.12 L.16 through P.13 L.3]

1158. Hollinger (G-945, pg.4 & table #2; pg. 9) has reported that turkey carcasses have a 20 fold lower organism contamination rate with *Campylobacter* spp. than chicken. Other studies show that *C. jejuni* could not be isolated from turkeys or turkey products (Genigeorgis, Rosef, Acta Vet Scand 23: 128-134, 1982 (B-1857); Baker, Poultry Sci 66: 1766-1770, 1987 (B-1858)). [Gonder (A-201) P.13 L.3-9]

1159. CDC reports that 95 – 99% of human *Campylobacter* infections are caused by *C. jejuni.* [Gonder (A-201) P.13 L.5-6]

1160. Broilers are generally marketed at 6-9 weeks of age. Turkeys are marketed at 13-21 weeks of age in most situations. [Gonder (A-201) P.14 L.14-15]

1161. The turkey is more immunologically and physiologically mature at processing, the intestinal microflora should be much more stable. This maturity should result in an intestinal microflora more closely resembling that of a competitive exclusive culture, which should reduce the number of enteric pathogens, including *Campylobacter*, that are present (Snoeyenbos, Avian Dis 22:273-278. 1978) [Gonder (A-201) P.14 L.15-19]

1162. As compared to broiler chickens, far more turkey meat is produced for further processed sales. Such further processing usually includes cooking which kills bacteria, including *Campylobacter*, that may otherwise be present on the raw carcass (Genigeorgis (B-1857)). [Gonder (A-201) P.14 L.19-22]

1163. Modern consumers buy very little raw turkey, even at Thanksgiving. [Gonder (A-201) P.14 L.22-23]

1164. As the consumption patterns changed, it became obvious to producers that plant-cooked turkey is what the individual and food-service consumer desired. [Gonder (A-201) P.14 L.23 through P.15 L.2]

1165. Several major producers now cook all their turkey product in-plant and do not provide turkey to the non-processed market. [Gonder (A-201) P.15 L.3-4]

1166. Goldsboro Milling produces about 10 million market turkeys. In 1986, virtually all its product was sold as fresh or frozen turkey. Goldsboro cooks over 70% of product before it leaves the plant. Goldsboro is not unique in the industry. [Gonder (A-201) P.15 L.5-9]

1167. There are marked differences in the diseases that affect turkeys compared to chickens. Turkeys do not become ill with infectious bronchitis, infectious laryngotracheitis, chicken infectious anemia, Marek's disease, lymphoid leucosis, infectious coryza, viral arthritis, EDS-76, infectious bursal disease, and the chicken species of coccidia (Eimeria acervulina, necatrix, tenella, brunetti), and are markedly less susceptible to viscerotropic velogenic Newcastle disease, necrotic

enteritis, and avian encephalomyelitis than chickens. Chickens do not become significantly ill with bordetella, turkey viral hepatitis, turkey coronaviral enteritis, turkey species of coccidia (*E. adenoeides, meleagrimitis, and gallopavonis*), paramyxovirus-3, *Mycoplasmas iowae* and *meleagridis*, turkey rhinotracheitis, *Salmonella arizona*, and *Ornithobacterium rhinotracheale*. [Gonder (A-201) P.16 L.1-11]

1168. There are many differences between turkeys and chickens including differences in *Campylobacter* prevalence between the two species that have been known for years, and apparently still exist. [Gonder (A-201) P.16 L.15-17]

1169. Two of the most important turkey diseases are colibacillosis (also known as *E. coli*, *coli*, colisepticemia) and fowl cholera (also known as pasteurellosis, *Pasteurella multocida* infection). Both are caused by bacteria. [Gonder (A-201) P.16 L.19-21]

1170. Colibacillosis has two primary manifestations: respiratory and enteric. Respiratory *E. coli* includes infection of lungs (pneumonia) and airsacs (airsacculitis). Enteric *E. coli* includes infection of the gastrointestinal tract. Both forms usually follow infection of the target tissue by a virus or other invader. [Gonder (A-201) P.16 L.22 through P.21 L.2]

1171. The bacteria *E. coli* is a normal inhabitant of the intestines of all animals, but when a tissue is damaged, it may multiply to unusual numbers, move to unusual places in the body, or produce unusual byproducts (toxins). These unusual events result in disease that can be more severe and damaging than the virus infection or other precipitating event. [Gonder (A-201) P.17 L.2-6]

1172. Different strains of *E. coli* can be more damaging than others, or can work by different mechanisms (toxin production vs. cellular invasion, etc.). This tends to make the infections in turkeys quite unpredictable in terms of morbidity and mortality. [Gonder (A-201) P.17 L.6-9]

1173. Colibacillosis does generally occur at the ages when viral or protozoal infections are most common – within the first 2-3 weeks of age when intestinal viruses frequently occur, and again about 1-2 weeks after moving to the finishing house and exposure to a new environment, possibly with old litter, when respiratory infection may be more common. Occurrences at other times are episodic, depending on the diseases prevalent in the geographic area. [Gonder (A-201) P.17 L.9-14]

1174. Some of the most common inciting agents for colibacillosis in turkeys are Newcastle disease, bordetellosis, hemorrhagic enteritis, turkey coronaviral enteritis, coccidiosis, or high levels of atmospheric ammonia. (B-1412). [Gonder (A-201) P.17 L.14-16.

1175. *E. coli* disease can be difficult to treat in turkeys due to the persistence of the underlying cause, and the poor blood supply to the respiratory airsacs – one of the organs principally affected. [Gonder (A-201) P.17 L.16-18]

1176. Successful treatment of *E. coli* disease in turkeys depends on early intervention with an antibiotic with good tissue distribution to affected tissues, or efficacy at low tissue levels. [Gonder (A-201) P.17 L.18-19]

1177. *Pasteurella multocia* is not a normal inhabitant of the turkey's body. [Gonder (A-201) P.17 L.20-21]

1178. *Pasteurella multocia* can cause primary disease in turkeys, usually a consolidating pneumonia or septicemia. [Gonder (A-201) P.17 L.21-22]

1179. Different strains of *Pasteurella multocia* vary in disease severity, but virtually all can cause some disease and some are quite deadly. [Gonder (A-201) P.17 L.22-23]

1180. It is not unusual for mortality from pasteurellosis in turkeys to double every day without successful treatment. Uncontrolled breaks may result in over 50% mortality. [Gonder (A-201) P.17 L.23 through P.18 L.1]

1181. *Pasteurella multocia* is carried by rodents and other mammals. [Gonder (A-201) P.18 L.1-2]

1182. Exposure to *Pasteurella multocia* is most common in finishing houses in older birds. [Gonder (A-201) P.18 L.2-3]

1183. Early diagnosis and fast, effective treatment of pasteurellosis in turkeys is critical. [Gonder (A-201) P.18 L.1-2]

1184. Successful treatment of pasteurellosis in turkeys depends on rapid treatment with an antibiotic that works quickly – most strains will kill affected birds within 1-3 days. [Gonder (A-201) P.18 L.4-5]

1185. Outbreaks of colibacillosis and fowl cholera are not predictable in turkeys and, therefore, having effective, fast acting antibiotics as a back-up to our disease control efforts is imperative. [Gonder (A-201) P.20 L.4-8]

1186. It is not correct that enrofloxacin is not a valuable drug because it is used so sparingly and national average turkey mortality is low and controlled. National average mortality rate is not relevant to this discussion. While the national average mortality rate may decline, there will still be those flocks that may suffer many times that national rate of mortality. Those are the flocks in which enrofloxacin makes a difference – to reduce that damagingly high rate and relieve animal suffering in the individual flock. This is one of the ways in which poultry veterinarians make progress in the national rates of mortality and condemnation. [Gonder (A-201) P.20 L.9-17]

1187. Diseases in a region can wipe out a flock in a region/devastate a region's turkey production. Fowl cholera, in an individual flock, can have startlingly high mortality. [Gonder (A-201) P.20 L.18-21]

1188. In unsuccessfully treated outbreaks or slow-moving outbreaks of infectious diseases, chronically affected turkeys will be noticeably smaller than unaffected birds. [Gonder (A-201) P.21 L.7-12]

1189. In unsuccessfully treated outbreaks or slow-moving outbreaks of infectious diseases in turkeys feed consumption drops as the number of infected birds increases. (B-1117, at 25). [Gonder (A-201) P.21 L.7-13]

1190. In unsuccessfully treated outbreaks or slow-moving outbreaks of infectious diseases in turkeys water consumption usually increases initially since both diseases can cause a fever. (B-1117, at P.25). [Gonder (A-201) P.21 L.7-14]

1191. In unsuccessfully treated outbreaks or slow-moving outbreaks of infectious diseases in turkeys diarrhea usually begins as feeding activity decreases and the intestinal microflora changes as dietary starches and sugars are no longer available. Depending on age and exposure, coccidiosis may occur as feed consumption drops to levels that do not provide enough coccidiostat to prevent this particular disease. [Gonder (A-201) P.21 L.7-21]

1192. In unsuccessfully treated outbreaks or slow-moving outbreaks of infectious diseases in turkeys, depending on the virulence (disease-causing ability) of the particular strains of disease involved, death can be rapid (1-2 days), or prolonged (1-2 weeks), or result in the bird becoming so small or crippled that it must be killed. [Gonder (A-201) P.21 L.7-21]

1193. If there are 10,000 birds in a house, a colibacillosis outbreak, or mild fowl cholera outbreak, frequently shows a per day mortality daily pattern of 4-6-8-7-10-16-25-45. A veterinarian would hope to intervene on the day mortality reached 10, especially if the flock was showing other signs such as depression, sneezing or coughing and would certainly intervene on the day mortality reached 16. [Gonder (A-201) P.21 L.23 through P.22 L.6]

1194. A severe fowl cholera outbreak is more rapid than a colibacillosis outbreak and would show a per day mortality pattern more like 4-6-8-7-18-45-150-275 in a turkey house of 10,000 birds. Waiting "a day or two to see if it went back down" is not acceptable in this situation. [Gonder (A-201) P.22 L.8-10]

1195. In a turkey house with *E. coli* or *Pasteurella multocida* infection, all birds will be exposed to the pathogen and are in danger of disease. They all require treatment. They drink from the same waterers (glasses), and eat from the same feeders (plates). They live in contact with each other, and each other's secretions. Disease will predictably spread from organism to organism when they are in close proximity. [Gonder (A-201) P.22 L.11-16]

1196. A common trait of both broilers and turkeys is that when they get sick, they "go off feed," i.e., stop eating. (B-1117, at P.25). As a result, their intestines become fragile. (Russell, 2002) (B-1912). This can be due to increased water consumption, actual intestinal disease resulting in edema of the wall of the intestine (coccidiosis,

E. coli), or changes in the bacterial population of the intestines due to altered eating patterns (necrotic enteritis), leading to diarrhea, gas, and actual damage to the intestinal lining. (Russell, 2002). [Gonder (A-201) P.22 L.18-23; Wages (B-1917) P.11 L.21-22]

1197. Clinically ill turkeys frequently have diarrhea and interrupted eating patterns. (B-322). Both of these conditions increase intestinal fragility and the difficulty of removing intestines intact at the processing plant. Interrupted eating leads to uneven loading in the intestinal tract, making both mechanical and manual evisceration more difficult. This increases the chance that the birds' flesh will be contaminated with intestinal contents and intestinal bacteria at the processing plant. It is an undesirable situation, and an unsafe one if the processor or consumer does not safely handle and cook the meat prior to consumption. [Gonder (A-201) P.23 L.1-7; Wages (B-1917) P.12 L.1-4]

1198. When turkeys "go off feed," they are also more susceptible to enteric problems, including parasites such as coccidiosis and the overgrowth of pathogenic bacteria. Some of the problems arise because feed consumption in sick birds may be inadequate to provide enough coccidiostat (anti-coccidial medication) to prevent coccidiosis, or growth-promoting antibiotic to stabilize the intestinal bacterial population and prevent clostridial overgrowth (necrotic enteritis). This is a food safety issue in humans. [Gonder (A-201) P.23 L.8-12; Wages (B-1917) P.12 L.5-8]

1199. Turkeys that "go off feed" are more likely to be populated with *Campylobacter, Salmonella,* and *Clostridia* as the nutrient mix and pH of the intestinal contents changes to conditions more suitable to the growth of these bacteria (low volatile fatty acids in the ceca, higher intestinal pH, decreased starch and sugar levels, etc.). [Gonder (A-201) P.23 L.13-16]

1200. In turkeys with intestinal disease and some septic diseases, the intestinal wall actually becomes thinner. This decreases tensile strength and increases intestinal breaking at processing. The intestines may also become swollen with fluid and gas as diarrhea develops. These swollen intestines are difficult to remove from the bird without breaking them at processing, resulting in fecal contamination. [Gonder (A-201) P.23 L.17-21; Wages (B-1917) P.12 L.9-13]

1201. In deciding whether to treat a sick turkey flock a veternarian's oath requires that the public health be served, including as part of human health the production of adequate wholesome food for the human population with a minimum of environmental damage. [Gonder (A-201) P.24 L.14-17]

1202. In order to determine if a turkey house has *E. coli* or *Pasteurella multocida* (fowl cholera), one conducts a necropsy (post-mortem examination), supplemented by bacteriological cultures and sensitivities and microscopic examination of stained smears from affected tissues. Frequently, a preliminary diagnosis is possible from necropsy alone since fowl cholera frequently has specific pathological changes, but this preliminary diagnosis should be confirmed with bacterial cultures, since some

fowl cholera and colibacillosis cases can have similar appearances. [Gonder (A-201) P.25 L.1-7]

1203. The decision to treat a turkey house or not depends on the results of the necropsy, how quickly the mortality appears to be increasing, what the extent and manifestations of morbidity are in that house, and whether it's fowl cholera or colibacillosis. If it's colibacillosis, the attending veterinarian must try to determine the inciting cause, how quickly it can be remedied or may resolve, whether similar cases have been seen recently, how those cases behaved, whether there have been similar occurrences on previous flocks on the same farm, and if so, how they have responded to treatment. [Gonder (A-201) P.25 L.8-14; Wages (B-1917) P.6 L.4-8]

1204. Selecting what to treat a turkey house with is based on various factors, including the legal and logistical availability of the medication, how expensive the medication will be in the age of bird involved, whether the market will permit recovery of cost of the treatment, whether the treatment has worked in the past in similar situations, whether the withdrawal period would interfere with timely flock sale, whether repeat treatment may be required, and whether the flock is old enough to be sent to market rather than treated if treatment would be expensive or the mortality is increasing rapidly, and whether the medication would have any potential for interaction with other agents or age or environment-related toxicity. [Gonder (A-201) P.25 L.15-22]

1205. Enrofloxacin is prescribed for turkeys only in cases of colibacillosis or fowl cholera with severe disease potential, where the mortality may become high quickly. (Joint Stipulation 15). [Gonder (A-201) P.26 L.4-5]

1206. Most veterinarians must see birds from the flock, obtain cultures, determine the amount of medication required, note it on the case report, and call the company warehouse to authorize disbursement of enrofloxacin, and most follow the Judicious Use Guidelines for Use of Antimicrobials in Poultry. [Gonder (A-201) P.26 L.12-19]

1207. Sick turkeys generally consume water more reliably than they consume feed. This is one of the main reasons that feed medication works better to prevent diseases such as coccidiosis rather than for treatment. As soon as birds become ill, they usually quit eating. [Gonder (A-201) P.26 L.21-23]

1208. Delivery of enrofloxacin through drinking water systems is appropriate and effective. FDA has long accepted drinking water delivery as a safe and effective means to administer therapeutic animal drugs, including antibiotics, to commercially grown broiler chickens and turkeys. (Joint Stipulation 18). [Gonder (A-201) P.27 L.1-5]

1209. Label dosage for enrofloxacin is 25 ppm - 50 ppm. (A-54). Dr. Gonder typically uses Baytril at 25 ppm. [Gonder (A-201) P.27 L.6]

1210. Enrofloxacin is the most expensive medication per unit in the history of poultry medicine – waste is not tolerated. [Gonder (A-201) P.27 L.12-13]

1211. Turkey veterinarians and companies use enrofloxacin prudently since, among other reasons, and unlike most humans, turkey flocks don't have insurance companies paying for the medication. Therefore, it makes no sense to spend money wildly on antibiotics. [Gonder (A-201) P.29 L.11-18]

1212. Enrofloxacin is extremely effective when used prudently and can result in no clinical failures for the treatment of colibacillosis and pasteurellosis (fowl cholera) in turkeys in over one hundred cases. [Gonder (A-201) P.29 L.20-23;Wages (B-1917) P.18 L.19]

1213. There are many historical examples of Baytril's efficacy. [Gonder (A-201) P.30 L.4]

1214. In general, there are no good alternatives to enrofloxacin for turkey flocks with fowl cholera or colibacillosis. [Gonder (A-201) P.30 L.20]

1215. The fluoroquinolones represent the first new class of antibiotics for drinking water medication in turkeys in over 20 years. [Gonder (A-201) P.30 L.21-22]

1216. Poultry veterinarians have vast experience in trying to coax efficacy form severely limited armamentarium available prior to fluoroquinolone introduction. [Gonder (A-201) P.30 L.22 – P.31 L.1]

1217. Fluoroquinolones are the sole antibiotic effective against enteric-origin systemic colibacillosis at 2-3 weeks of age in Dr. Gonder's company, and produces, against historic standards of treatment for severe cases of fowl cholera and respiratory colibacillosis, truly spectacular, rapid reductions in mortality. [Gonder (A-201) P.31 L.1-4]

1218. Vaccine technology lags behind disease introduction – antibiotic use fills the gaps until vaccines or management strategies can be developed. [Gonder (A-201) P.31 L.22 – P.32 L.1]

1219. The withdrawal of the approval of enrofloxacin would adversely impact turkey health, primarily in the area of colibacillosis. [Gonder (A-201) P.32 L.8-10]

1220. If increased amounts of colibacillosis-caused airsacculitis, osteomyelitis, and fecal contamination can affect the quality of USDA-inspected turkey product, then FDA withdrawal of enrofloxacin from the market would harm that quality. [Gonder (A-201) P.32 L.10-13]

1221. Fecal contamination is usually increased in flocks marketed with active disease. If that translates into a potential degradation in human health due to increased amounts of enteric pathogens of whatever type entering the food chain, then FDA

withdrawal of enrofloxacin wouldn't be good for human health either. [Gonder (A-201) P.32 L.13-16]

1222. Due to their bacteriostatic nature, the tetracyclines tend to work rather slowly. This is not a good situation for diseases with rapidly increasing mordidity/mortality rates, like fowl cholera or a severe colibacillosis outbreak. The control achieved with tetracycline products in treatment situtations is generally incomplete; repeat treatment is often necessary due to failure to completely control the situation. At least *in vitro*, many *E. coli* isolates are resistant to tetracyclines (B-700); this is frequently associated with lack of efficacy in the field. [Gonder (A-201) P.34 L.6-10]

1223. Tetracyclines have been in use in poultry for over 35 years. [Gonder (A-201) P.34 L.12]

1224. Tetracyclines are fairly inexpensive, generally arguing for use as a preventive medication, such as to avoid a fowl cholera outbreak after a dog attack, or reduce the occurrence of osteomyelitis associated with susceptible bacterial infections. [Gonder (A-201) P.34 L.12-15]

1225. Sulfa medications typically work more quickly on susceptible bacteria than tetracyclines, but not as fast as fluoroquinolones. They are quite useful in cases of coccidiosis, fowl cholera, or colibacillosis in younger turkeys. However, resistance is not unusual, since again, sulfas have been in use for over 30 years. [Gonder (A-201) P.34 L.16-19]

1226. Sulfa medications are generally limited to use in water medication in turkeys since their residue potential greatly complicates controlling their use in a high-volume feed mill. Sulfas use may also be limited by palatability and solubility problems with particular water supplies, and toxicity problems if birds are dehydrated or adequate water consumption cannot be maintained due to lameness or changes in drinking equipment due to scheduled movements. [Gonder (A-201) P.34 L.19 through P.35 L.1]

1227. The lengthy withdrawal period (10-14 day withdrawal period in-house, depending on the particular formulation available) for use of sulfa medications in turkeys also precludes their use in older birds close to slaughter age. The residue limit for sulfa compounds in poultry is ≤ 0.1 ppm, depending on the compound (21 CFR 556.6xx depending on compound) in edible tissue. Sulfas tend to persist in feed and water systems, tending to aggravate the residue potential. [Gonder (A-201) P.35 L.1-5]

1228. Exceeding the residue limit triggers an investigation by CVM. Frequently, flocks sold subsequently from the affected farm must be tested prior to slaughter until a pattern of compliance is established. If several flocks from different farms within the same company are involved, all flocks from the affected company may be tested for a period of time. The test generally requires the submission of 30 birds per flock to the USDA FSIS veterinarian at the slaughter facility within 1-2 weeks

of the regular processing date. Capturing, transporting, and processing these birds is difficult and expensive, especially since the birds are usually condemned as offal since they would otherwise be required to be stored under separate seal until testing results are received. Many plants cannot store small numbers of birds for a short period of time in a cost-effective manner. Violations may also be published in the *FDA Veterinarian*, which is bad publicity. [Gonder (A-201) P.35 L.8-18]

1229. *E. coli* and *Pasteurella multocida* infections in turkeys are serious diseases. These infections cause severe animal suffering in turkeys that should be treated to alleviate the suffering. [Gonder (A-201) P.35 L.20-22]

1230. Infections are cyclical and regional. Just because flocks may be in general good health at present and enrofloxacin is not being used extensively, it is only a matter of time before a fowl cholera outbreak hits, or a virus hits that will cause secondary *E. coli* infections. Then enrofloxacin will be needed in that region because an outbreak can devastate a region's production. [Gonder (A-201) P.36 L.1-5]

1231. Baytril is the only efficacious drug for treating *E. coli* or *Pasteurella multocida* infections in turkeys. [Gonder (A-201) P.36 L. 5-6]

1232. Due to high resistance to tetracyclines and sulfa drugs, and the residue concerns with the sulfa drugs, there are no practical alternatives to Baytril on the market. [Gonder (A-201) P.36 L.6-7]

1233. Water medication to the entire exposed turkey house is appropriate because all are exposed and at some stage of the disease process. Administration by water is safe and effective. [Gonder (A-201) P.36 L.8-10]

1234. The watering systems in turkey houses are such that Baytril is administered in a manner to minimize spillage and environmental contamination. [Gonder (A-201) P.36 L.10-11]

1235. There was no information presented in the Notice of Opportunity for Hearing demonstrating that turkeys should be included. Turkeys have been arbitrarily lumped in with broiler chickens under the heading of "poultry". This is not correct in this instance. [Gonder (A-203) P.36 L.13-15]

1236. No information has been presented in the Notice of Opportunity for Hearing showing that the *Campylobacter* risk is similar between turkeys and chickens, and ample information exists showing that it is not. [Gonder (A-201) P.36 L.15-17]

1237. The turkey industry markets approximately 270 – 280 million birds annually. [Wages (B-1917) P.3 L.4]

1238. Over 90 % of the turkey industry is integrated. [Wages (B-1917) P.3 L.12]

1239. The integrator almost always contracts to growers to raise flocks. Some companies that are cooperatives or family owned and operated may have significant company

farm production. Following the integrator company's specifications, the grower typically builds and owns the houses, furnishes the watering devices, feeders and brooding equipment, and provides labor to grow the turkeys to market weight until processing. [Wages (B-1917) P.3 L.14-19]

1240. All turkey feed in the US industry is pelleted. Feed for the first 3 weeks of age is pelleted and then crumbled for easier consumption. Pelleting is a time/temperature/moisture process that, among other things, kills bacteria that can be present in feed. It is typically a corn/soybean-based diet supplemented with vitamins and minerals. [Wages (B-1917) P.4 L.11-14]

1241. The feeding system consists of automatic pan feeders that deliver feed to the pan via a closed auger system. As birds eat feed from the pans, the pans are automatically re-filled. [Wages (B-1917) P.4 L.17-18]

1242. The majority of turkey farms are on well water systems. Some farms have access to municipal systems but that would be the exception rather than the rule. [Wages (B-1917) P.4 L.19-20]

1243. Most turkey farms chlorinate their water during the grow-out period through a proportioner or through shock treating the well water with chlorine at regular intervals. [Wages (B-1917) P.4 L.21-22]

1244. The integrator company provides "best management practices" guidelines to the grower covering how birds are to be raised by the grower. Often the grower will observe overall flock health multiple times per day. When the flock health supervisor observes the flock during his/her visit overall health and welfare issues are addressed. [Wages (B-1917) P.5 L.13-17]

1245. The grower and flock health supervisor routinely assess morbidity and mortality in the flock. This is usually communicated to the company veterinarian to determine if the veterinarian needs to assess the flock for treatment or perform diagnostic work ups. Morbidity of greater than ½% of the flock usually requires a veterinarian assessment. [Wages (B-1917) P.5 L.18-21]

1246. Mortality is assessed daily and logged onto a mortality sheet. Mortality is picked up in the house when the grower observes the birds usually 3-5 times daily. Mortality is usually reported to the integrator company weekly; in cases of increased mortality, mortality may be reported daily. [Wages (B-1917) P.6 L.1-4]

1247. A flock health supervisor will see the flock usually twice per week and more often if morbidity or mortality dictates. He communicates any potential disease with the integrator company veterinarian to determine if the veterinarian should assess the flock for potential treatment. When mortality reaches 1 bird per 1000 turkeys, a diagnostic work-up is usually performed. [Wages (B-1917) P.6 L.4-8]

1248. The diagnostic work up will typically involve bacterial cultures, serology and possibly histological samples. These samples are taken to determine the appropriate course of action. [Wages (B-1917) P.6 L.8-10]

1249. Preventative vaccination is performed in flocks. Hemorrhagic enteritis vaccine is administered via water at 4 weeks of age. Other disease agents, such as Newcastle disease and fowl cholera vaccinations are used on a case-by-case basis depending on exposure to these agents on certain farms. [Wages (B-1917) P.6 L.21 through P.7 L.2)

1250. If litter is not scheduled for replacement between turkey placements, any moist or caked litter is removed. The litter is then tilled to provide adequate bedding for the flock entering. If needed, new litter, usually wood shavings, is added. Acidifying litter treatment products may be used between house placements in the grow-out houses to reduce residual bacterial contamination and to control ammonia in the litter. [Wages (B-1917) P.7 L.8-12]

1251. Acidification treatment is bactericidal as bacteria do not survive acidic environments. Such treatment is known to control *Campylobacter*. (B-1850). [Wages (B-1917) P.7 L.12-14]

1252. Feeder height and feed depth in the feed pan as well as drinker or waterer height and water depth in the waterer are carefully managed on a daily basis as both of these factors are crucial for good litter quality management. If water management is not performed properly, the litter can become wet which results in increased ammonia production. Ammonia affects the cilia of the trachea which is a natural disease defense mechanism that prevents the introduction of bacteria down into the lower respiratory tract. [Wages (B-1917) P.7 L.15-20]

1253. 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. (Joint Stipulation 39). [Wages (B-1917) P.8 L.6-8]

1254. It is important to understand *E. coli* and *Pasteurella multocida* diseases in turkeys to fully understand the need for enrofloxacin and the consequences of withdrawing the NADA for enrofloxacin. [Wages (B-1917) P.8 L.8-10]

1255. *Pasteurella multocida*, also known as fowl cholera or "cholera," is an acute primary infectious disease in turkeys. (B-1190, B-1262, B-1264). It strikes turkeys as young as 6-8 weeks of age but usually strikes when they are greater than 10-12 weeks of age. [Wages (B-1917) P.8 L.11-13].

1256. Clinically, fowl cholera can occur as an acute septicemia or a subacute to chronic respiratory disease causing a fibrinous airsacculitis and pneumonia. I have personally observed this disease produce very high (>40%) mortality. [Wages (B-1917) P.8 L.15-17]

1257. Once a house comes down with fowl cholera observable to the veterinarian, all birds in the house are in some stage of exposure to the disease and need to be treated. [Wages (B-1917) P.8 L.18-20]

1258. *E. coli* infections are secondary infections in turkeys. (B-1412). The most common is the respiratory form causing a fibrinous pneumonia, air sacculitis, pericarditis and perihepatitis. It occasionally causes an acute septicemia. *E. coli* infections are usually secondary bacterial infections that strike when the birds immune system is weak from fighting primary viral infections, such as Newcastle disease, turkey rhinotracheitis virus (pneumovirus infection), turkey coronavirus, arboviruses, influenza viruses and hemorrhagic enteritis or other bacterial infections such as bordetellosis, mycoplasmosis, or *Ornithobacterium rhinotracheale* infection. When the aforementioned primary infectious agents occur in a flock, secondary *E. coli* infection (colibacillosis) usually occurs. (B-1412). [Wages (B-1917) P.8 L.21 through P.9 L.7]

1259. Morbidity in a turkey flock from *E. coli* infections can be greater than 60% with mortality as high as 20%. [Wages (B-1917) P.9 L.7-8]

1260. Once a house comes down with *E. coli* infections observable to the veterinarian, all birds in the house are in some stage of exposure or disease and need to be treated. [Wages (B-1917) P.9 L.8-9]

1261. *E. coli* or *Pasteurella multocida* infection are characterized by 5 stages: 1) exposure; 2) incubation period; 3) clinical disease; 4) recovery period; 5) carrier period. All birds in a house do not receive their initial exposure to the disease at the same time. [Wages (B-1917) P.9 L.11-14]

1262. A turkey grow-out house that is hit with a bacterial disease has an incubation period of 7-10 days and a clinical disease period of 5-7 days. On any given day after observing clinical disease you will have some turkeys that were just exposed, some turkeys that are incubating the disease and some showing clinical disease. The rates of clinical disease and mortality grow geometrically. Effective treatment can occur in both the incubation period (prevention/control) and clinical disease period (treatment). In large populations of animals, the entire population is at some stage of the disease exposure, so treating the disease for the benefit of the population for mortality prevention is extremely important. [Wages (B-1917) P.9 L.14-22]

1263. Observations of increased morbidity include visual observations such as depression, anorexia (not eating), huddling, increased or decreased activity and mortality. There are also audible signs such as respiratory noise, snicking, sneezing, coughing and altered vocalization. Increased mortality is easy to track by numbers of dead birds. Mortality can range from 1 bird per thousand to 50 birds per thousand per day. [Wages (B-1917) P.10 L.1-6]

1264. In a turkey house with *E. coli* or *Pasteurella multocida* infection, all birds are in various stages of morbidity. When you have 5,000-10,000 birds in a house, it is impossible to actually assess every bird. There is not enough time. Birds will die before you can initiate treatment if the veterinarian would have to individually assess each bird. A veterinarian has to practice population medicine and assess the entire flock, do diagnostic work-ups and evaluate if the disease is acute or chronic. Decisions are made based on all of the flock information. [Wages (B-1917) P.10 L.6-11]

1265. Outbreaks of *E. coli* and *Pasteurella multocida* infections tend to be regional and can be cyclical. Like all infectious diseases, some are endemic in areas and occur naturally while others remain dormant for seasons before occurring in the commercial turkey population. Disease vectors such as insects and rodents may play active roles in increasing infectious pressures in certain areas. (B-1190). [Wages (B-1917) P.10 L.13-17]

1266. A geographical area may experience periods of good flock health and poor flock health. The season of the year, environmental temperature and humidity play important roles in predisposing areas or regions to disease. (B-108). A grower may go for quite a few grow-out cycles before experiencing disease. It is many times simply unpredictable. [Wages (B-1917) P.10 L.18-21]

1267. Many specific diseases occur only in certain regions for a variety of reasons known and unknown. Poultry density or the number of flocks in an area can significantly impact the incidence of disease. Infectious agents that are vector driven may occur in the southern states whereas agents that replicate much better in colder moist areas may occur more in the northern states. Even within a State there may be variations. [Wages (B-1917) P.11 L.1-11]

1268. Merely looking at annual national average disease mortality to assess the need or value of enrofloxacin is misleading. [Wages (B-1917) P.11 L.12-13]

1269. Diseases in a particular region can devastate that region's turkey production. For example turkey production in Georgia has been wiped out because of turkey coronavirus. A major turkey company in North Carolina no longer produces turkeys because of turkey coronavirus. Other integrators have vacated production areas because of repeated disease occurrences. [Wages (B-1917) P.11 L.15-19]

1270. Veterinarians have a duty to treat sick animals. [Wages (B-1917) P.12 L.15]

1271. The AAAP Guidelines on Judicious Therapeutic Antimicrobial Use in Poultry provide a guide for veterinarians in treating fowl cholera (pasteurellosis) and *E. coli* infections (colibacillosis) in turkeys. The guidelines address diagnostics, non-antimicrobial intervention, antimicrobial intervention, treatment duration, treatment assessment, other treatment considerations, and prevention. [Wages (B-1917) P.13 L.8 – P.17 L. 21]

1272. The enrofloxacin delivery method is safe and effective. Baytril is administered in the drinking water of the infected house. Sick birds drink even if they do not eat. FDA has long accepted drinking water delivery as a safe and effective means to administer therapeutic animal drugs, including antibiotics, to commercially grown broiler chickens and turkeys. (Joint Stipulation 18). [Wages (B-1917) P.18 L.2-6]

1273. Turkeys and broilers get a sufficient dose of enrofloxacin. [Wages (B-1917) P.18 L.19]

1274. The enrofloxacin dose in turkey's is designed to minimize resistance in the target pathogen. [Wages (B-1917) P.18 L.19-20]

1275. Mortality has been observed to go from 100 birds per day to less than 10 in 36 hours after enrofloxacin administration in turkeys. [Wages (B-1917) P.18 L.21 through P.19 L.1]

1276. Enrofloxacin is an outstanding therapeutic tool, especially when considering the high level of resistance to other therapeutic agents. [Wages (B-1917) P.19 L.1-2]

1277. Proper and judicious use of enrofloxacin has been publicized in the poultry industry. (G-113, B-1263). [Wages (B-1917) P.19 L.3-4]

1278. If the NADA for enrofloxacin is withdrawn, the only available drugs specifically approved to treat *E. coli* infections in chickens older than three days of age and *E. coli* and *Pasteurella multocida* infections in turkeys older than three days of age are: sulfa drugs and tetracyclines (Tetracycline, Oxytetracycline, Chlortetracycline). [Wages (B-1917) P.19 L.6-9; Gonder (A-201) P.34 L.1-5]

1279. The main problem with using tetracyclines and sulfa to treat turkeys is a high level of clinical resistance drugs. [Wages (B-1917) P.19 L.10-11]

1280. Sulfa drugs do still have limited value in some cases of cholera but are not effective in treating colibacillosis in turkeys. [Wages (B-1917) P.19 L.11-12]

1281. In reality, there are no alternatives to enrofloxacin use in turkeys. [Wages (B-1917) P.19 L.13-16]

1282. Based on enrofloxacin's price, if other antimicrobial agents work, the turkey veterinarians will use them. [Wages (B-1917) P.19 L.17-18]

1283. Turkeys and chickens are biologically different and there are differences in how the two are reared and raised. Turkeys are not just big chickens. Many of the biological and rearing differences are significant to the issues in this hearing. [Wages (B-1917) P.19 L.20-22]

1284. Although *Campylobacter* colonizes both turkeys and chickens, the predominant *Campylobacter* that colonizes turkeys is *C. coli* not *C. jejuni*. In chickens, the predominant species is *C. jejuni*. This means that any risk assessment that takes

into account the impact of human illness caused by *Campylobacter jejuni* from chickens is not relevant or applicable to turkeys. [Wages (B-1917) P.20 L.1-5]

1285. Studies have shown that the overall prevalence of *Campylobacter* is different in chickens and turkeys. [Wages (B-1917) P.20 L.6-7]

1286. Any risk assessment that takes into account the prevalence of *Campylobacter* in chickens is not relevant or applicable to turkeys. [Wages (B-1917) P.20 L.7-9]

1287. Turkeys are raised to an older age than broiler chickens. This means that turkeys have a greater opportunity to become ill with respiratory infections. [Wages (B-1917) P.20 L.10-11]

1288. *E. coli* and *Pasteurella multocida* infections in turkeys are serious diseases. [Wages (B-1917) P.21 L.2]

1289. Baytril is the only consistently efficacious drug for treating *E. coli* or *Pasteurella multocida* infections in turkeys. [Wages (B-1917) P.21 L.6-7]

1290. Baytril is administered in a manner to maximize efficacy. [Wages (B-1917) P.21 L.8]

1291. Baytril is administered in a manner to minimize resistance. [Wages (B-19179) P.21 L.9]

1292. There are no practical alternatives to Baytril on the market. [Wages (B-1917) P.21 L.11]

1293. The steps used in chicken and turkey processing in the United States are very similar; however, because chicken broilers are usually more uniform in size than turkeys, chicken processing plants tend to be much more automated than turkey processing plants. [Minnich (G-1467) P.2 L.16-18]

1294. The transportation and slaughter process for turkeys is similar to that for chickens, with the differences mainly to accommodate larger and heavier animals. Although all turkey slaughter plants have some mechanical equipment, it is less than that seen in chicken slaughter plants. This is because of the great variation in size of birds slaughtered within most turkey plants. [Minnich (G-1467) P.6 L.10-14]

1295. Each chicken slaughter plant may slaughter a unique size and weight of bird; however, the size and weight is usually consistent within that facility. [Minnich (G-1467) P.6 L.14-16]

1296. An individual turkey slaughter plant would very likely slaughter small (approximately 10 pound birds) to very large (40 pound or larger birds) often within the same day. The variation in carcass size makes it more practical to manually process turkeys rather than trying to fit and adjust equipment to a variety

of bird sizes. The main differences between chicken processing and turkey processing include: [Minnich (G-1467) P.6 L.16-20]

1297. Live turkeys are transported to the slaughter plant in crates approximately 18 cubic feet in size, containing 8-24 turkeys each. An average transport truck will hold 32 or more crates, and a plant may slaughter up to 20-30 trucks of turkeys in a shift. The average turkey slaughter plant operates 1-2 shifts per day, 5 days per week. (About 23,000 - 60,000 turkeys or more a day for all shifts combined). [Minnich (G-1467) P.6 L.22-26]

1298. The scalder tank at turkey processing plants is larger than those at chicken processing plants. The scalder is about 40-65 feet long, and contains over 7,000 gallons of water. With the addition of the fresh water it takes approximately 3 or more hours for the water in the scalder to completely exchange. There are over 100 turkeys in the scalder at any given time and they remain there for 1-3 minutes. [Minnich (G-1467) P.6 L.28-32]

1299. Turkeys are usually manually transferred onto evisceration shackles, as opposed to mechanically hung. [Minnich (G-1467) P.6 L.34-35]

1300. In turkey, evisceration is usually accomplished manually rather than mechanically. [Minnich (G-1467) P.6 L.37]

1301. Turkey heads are usually removed after USDA inspection. This allows turkeys to be hung on the evisceration line by a "3 point" suspension and facilitates the inspection of the internal body cavity by USDA personnel. Head removal may be done manually or mechanically. [Minnich (G-1467) P.6 L.39-42]

1302. Lungs are removed manually by a plant employee with a metal cylindrical suctioning device (otherwise known as a lung gun). Necks are either manually removed at this point or they may be removed after exiting the chiller. [Minnich (G-1467) P.6 L.44-46]

1303. Turkey chill tanks are over 160 feet in total length. They hold over 120,000 gallons of water when full. Turkeys remain in the chiller for approximately 3-6 hours in order to decrease their body temperature from over 90 degrees F to 65 degrees F or less depending on the size of the bird and chill tank. Water is continually added to the chiller units to both maintain the cold temperature and make-up the lost water from the exiting carcasses. Due to the large volume of a turkey chilling system, under normal circumstances, the water in the turkey chill tank would be overturned approximately once per shift. [Minnich (G-1467) P.7 L.2-9]

1304. The most common USDA inspection technique for postmortem dispositions of turkeys is the New Turkey Inspection System (NTIS). It is the oldest of the high line speed methods for inspection and therefore the most common. This system utilizes a method of inspection where the viscera remains attached to the carcass until after passing the USDA inspection area. The average number of slaughter lines in a turkey plant is 2. There are 2 USDA inspectors per slaughter line.

Maximum line speeds for NTIS systems ranges from 5 1 turkeys per minute for young turkeys weighing less than 16 pounds, 41 turkeys per minute for young turkeys weighing over 16 pounds, to approximately 30 turkeys per minute for larger (40 pounds or more) breeder turkeys. This equates to 25 %, 20 %, and 15 turkeys per inspector per minute respectively. [Minnich (G-1467) P.7 L.11-20]

1305. Epidemiologic data in the US made available through FoodNet, the most sensitive means employed by the public health community to document the extent of diarrheal disease in the US, do not support turkey meat as a significant source of human campylobacteriosis or salmonellosis. [Tompkin (A-204) P.15 L.11-15]

1306. Existing data from FoodNet fail to show a significant epidemiologic link between the consumption of turkey meat and campylobacteriosis and salmonellosis. [Tompkin (A-204) P.58 L.15-16]

1307. 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. However, there is evidence for differences in the live birds in the pathological consequences of infection (Lam *et al.*, 1992) (Glunder, 1989) (Wallace *et al.*, 1998), on-set and rate of dissemination of colonization (Wallace, *et al.*, 1998), chronicity of infection and shedding (Glunder, 1989) and diversity of infective strains (Wallace, *et al.*, 1998) (Rogol & Sechter, 1987). [Newell (B-1908 P.4 L.1-7]

1308. There is also some suggestion that turkeys may be preferentially colonized by *C.coli* rather than *C.jejuni* (Zhao *et al.*, 2001) (Nielsen & Nielsen, 1999). Overall these observations suggest that *Campylobacter* colonization in broilers and turkeys may have significant host-specific differences. [Newell (B-1908) P.4 L.7-12]

1309. Whilst there are many reports on *Campylobacter* colonization in chickens very little is known about this infection in turkeys. Few studies have been undertaken on the live birds. [Newell (B-1908) P.10 L.8-9]

1310. In some studies turkey poults appear to become infected earlier (at 1-7 days) than chicks (Wallace, *et al.*, 1998). However, some flocks may remain negative for longer (Luechtefeld and Wang, 1981). [Newell (B-1908) P.10 L.9-12]

1311. Dissemination of infection throughout a turkey flock may take a longer time than in a broiler flock. [Newell (B-1908) P.10 L.12-13]

1312. Each poultry company has control over all fiscal and bird husbrandry aspects of production, from the day-old parent breeders to the marketing and distribution of the final products to the retailer. [Hofacre (A-202) P.2 L.16-17]

1313. The 'poultry industry' is actually three different industries, commercial layers, broilers, and turkeys. [Hofacre (A-202) P.2 L.18-19]

1314. Enrofloxacin is not approved for use in "laying hens producing eggs for human consumption" (as stated on label). [Hofacre (A-202) P.3 L.1-2]

1315. The size of US turkey industry is at 272 million, whereas the size of the broiler chicken industry is at 8.5 billion. [Hofacre (A-202) P.3 L. 4-5]

1316. Typically, the integrated broiler chicken or commercial turkey 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 (A-202) P.3 L.5-14]

1317. *E. coli* is a normal inhabitant of all poultry intestines and therefore will always be found in all broiler and turkey farms (Drasar, 1985). Many normal inhabitant *E. coli* possess virulence factors; however, they do not normally cause disease by themselves. [Hofacre (A-202) P.7 L.20 through P.8 L.1]

1318. In most instances, *E. coli* is a secondary infection following a primary viral or environmental insult (Glisson, 1998) (see also B-1412). *E. coli* plays a key role in the severity of the reaction to vaccination of chickens with Newcastle disease and infectious bronchitis vaccines (Smits, 1976; Cook, 1986). It has also been shown to play a role as a secondary invader following *Mycoplasma* sp. and *Bordetella avium* infections in both turkeys and chickens (Goren, 1991; Pierson, 1996). [Hofacre (A-202) P.8 L.6]

1319. In turkeys, colibacillosis is a frequent infection following hemorrhagic enteritis (Sponenberg, 1985). Environmental insults, such as, ammonia, CO_2, and dust, which damage the upper respiratory tract's natural defense system of cilia and mucus secretion, will lead to greater susceptibility to *E. coli* infections (Nagaraja, 1984; Goren, 1991). Even in localized infections, such as cellulitis, *E. coli* is a secondary invader following trauma to the skin from scratches (Peighambari, 1995). [Hofacre (A-202) P.8 L.6-11]

1320. Sick birds typically stop eating but continue to drink water. The only way to quickly stop the progression of a disease is to provide the antibiotic in the birds' drinking water. This precludes the use of any antibiotic with an in-feed only label. (Indicated in Table 1.) [Hofacre (A-202) P.22 L.14-16]

1321. 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. (A-54). The drug cannot be used in laying chickens laying eggs for human consumption. (A-54). [Hofacre (A-202) P.22 L.21 through P.23 L.1]

1322. There is also a 48 hour period of time that the birds must have fresh water (no Baytril) prior to slaughter to avoid any residue of the drug remaining in edible tissues. In the U.S., Baytril can only be given to birds by prescription and only under the supervision of a veterinarian. (Joint Stipulation 15). It is approved for therapy only; it has not nor will it ever be used as a growth promotant. (Joint Stipulation 16). [Hofacre (A-202) P.23 L.1-6]

1323. In one study, turkeys were challenged with *Pasteurella multocida* (Fowl Cholera) and 98% of the nonmedicated control birds died compared to 8%, 0% and 0% of the enrofloxacin treated birds, treated at 12.5, 25 and 50 ppm respectively. [Terhune (B-1915) P.5 L.14-16]

1324. The current label dose for enrofloxacin is 25 to 50 ppm for broiler chickens and turkeys. The safety and efficacy studies demonstrated that when medicating the drinking water with enrofloxacin, individual birds are dosed adequately, even at half the lowest recommended dose. This study also indicates that a superior result could not have been obtained with the use of an individually dosed injectable product. [Terhune (B-1915) P.5 L.16 through P.6 L.5]

1325. Enrofloxacin is the most efficacious antibiotic available in the United States for treatment of *E. coli* infections in broiler chickens and *E. coli* and *Pasteurella multocida* infections in turkeys. The pharmacokinetics of the compound are such that high levels of enrofloxacin are reached in the respiratory tissues of treated birds, which is the desired site for effective treatment of both *E. coli* and *P. multocida* infections. This characteristic, coupled with the typical low Minimum Inhibitory Concentration (MIC) values of enrofloxacin against avian *E. coli* and *P. multocida*, (G-59, G-256) insures that levels reached at the site of infection are far higher than the MIC required for an effective outcome. This also minimizes the potential for resistance development in the target organism. [Glisson (B-1903) P.5 L.21 – P.6 L.7]

1326. We have no viable alternatives to enrofloxacin in the United States poultry industry for treating *E. coli* infections in broiler chickens and turkeys. [Glisson (B-1903) P.12 L.3-4]

1327. The CVM/Vose analysis explicitly considers only risks posed with respect to fluoroquinolone-resistant *Campylobacter* in chicken, and does not provide explicit consideration with respect to turkey. [Haas (B-1904) P.26 L.12-14]

1328. There is limited data with regard to the prevalence of food borne pathogens (*Salmonella* and *Campylobacter*) among turkey carcasses at slaughter. [Logue (G-1464) P.2 L.26-27]

1329. In one study of two full-time turkey processing plants, the plants were each visited at monthly intervals for a period of one year. During each visit, surface swabs were obtained from turkey carcasses at two selected points on the production line, one point pre-immersion chilling and the other points being post-chill. Although samples of the chill water from the chill tanks were examined, it was not possible to sample the same carcass at both sampling sites (pre and post chill) because of logistics and time constraints. [Logue (G-1464) P.3 L.4-25]

1330. All carcass sampling was carried out using a non-invasive, non-destructive procedure originally devised by Lasta *et al.* (1992) for beef. [Logue (G-1464) P.4 L.4-5]

1331. The isolation and detection of *Campylobacter spp.* from the carcass swabs and the chill water was carried out using modified methods that were lab-specific. [Logue (G-1464 P.4 L.18-20]

1332. Current work on examining the antimicrobial resistance profiles of isolated *Campylobacter* is ongoing. A total of 94 *Campylobacter* isolates recovered from the birds processed at the processing plants have been evaluated. [Logue (G-1464) P.4 L.31 through P.5 L.5]

1333. Results from the study show differences in the overall incidence of the pathogen detected between the two processing plants. [Logue (G-1464) P.5 L.27-29]

1334. Also, in some cases, it was not possible to exclusively identify a single *Campylobacter* isolate beyond the genus level as the identification software could only discriminate based on differing biochemical reactions. [Logue (G-1464) P.6 L.3-5]

1335. Differences were observed with regards to prevalence of *Campylobacter* species recovered at each individual plant. [Logue (G-1464) P.6 L.7-8]

1336. Factors such as the individual processing plant examined, the time of the year, and farms that were providing animals may be contributory factors to the findings of this study. [Logue (G-1464) P.6 L.30-32]

1337. The plants also followed different plant processing practices, which can affect results. For example, plant A indicated that they did not use chlorinated water (the source was a well) in their chilling regime and relied on the quality of water supplied from the city which was used in the chill ice and "top up" water in the tanks. In contrast, Plant B stated that they hyperchlorinated the chill water to a concentration of 20 ppm. In both cases, the chlorine concentrations of the water in the chill immersion tanks was not established at the time of the study. [Logue (G-1464) P.7 L.1-7]

1338. The incidence of turkey carcass samples that tested positive for *Campylobacter* species was higher at plant A than plant B. Again, such noted differences may be related to the processing conditions used. Plant A used a batch chill process and

did not add any additional chorine to their chill tanks, aside from city water which was used to make ice chips. In contrast, plant B hyperchlorinated their water supply to a level of 20 ppm and used a continuous chill process. [Logue (-1464) P.7 L.9-17]

1339. Wang *et al.* (1983) reported that a chlorine concentration of 1.25 mg/L as sodium hypochlorite caused significant reductions in *Campylobacter* during disinfectant trials. This did not, however, take account of the presence of organic material which can reduce the effectiveness of chlorine compounds (El-Assaad *et al.* 1998). [Logue (G-1464) P.7 L.17-21]

1340. The rate of production is likely of importance in understanding *Campylobacter* contamination rates of turkey carcasses. [Logue (G-1464) P.7 L.23-24]

1341. Dr. Logue's data supports those of Acuff *et al.* (1986) who indicated that size and processing line speed were factors influencing overall carcass contamination rates and that the ultimate source of the *Campylobacter* contamination was the birds themselves. [Logue (G-1464) P.7 L.31 through P.8 L.2]

1342. According to Dr. Angulo, multivariate logistic regression model was used to determine risk factors for acquiring a *Campylobacter* infection among persons who did not travel outside the United States. In the final multivariate model, cases were 2.2 times more likely to have eaten chicken in a restaurant in the seven days prior to illness onset than controls (95 percent confidence interval, 1.7 to 2.9); 44 percent of cases ate chicken in a restaurant compared with 26 percent of controls. Cases were 2.5 times more likely to have eaten turkey in a restaurant in the seven days prior to illness onset than controls (95 percent confidence interval, 1.3 to 4.7); 6 percent of cases ate turkey in a restaurant compared with 3 percent of controls. [Angulo (G-1452) P.10 L.22-29]

1343. According to Dr. Angulo, the largest population attributable fractions were for eating chicken in a restaurant and eating non-poultry meat in a restaurant. The population attributable fraction for eating chicken in a restaurant was 24 percent (95 percent confidence interval, 17 to 30 percent), and for eating non-poultry meat in a restaurant was 21 percent (95 percent confidence interval, 13 to 30 percent). The population attributable fraction suggests that, among non-travelers, 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 3 restaurant in the seven days prior to illness onset. [Angulo (G-1452) P.10 L.36-44]

1344. Young chickens that are produced for meat are called broilers. The number of broilers raised per house varies considerably depending on climatic conditions and body size. There can be a number of houses per farm. Broilers are typically raised at 0.75 square feet per bird. Market age and weight for broilers ranges from 4.2 pounds for 38 day old birds to greater than 6 pounds for 50 day old birds. [Carey (G-1456) P.2 L.4-8]

1345. Male turkeys are called Toms and female turkeys are called Hens. Turkeys are housed at 7.5-8.0 pounds of market weight per square foot of floor space. Hens are marketed at weights of 14-23 pounds and Toms are marketed at 22-35 pounds. [Carey (G-1456) P.2 L.10-12]

1346. Broilers and turkeys are raised in larger enclosures. Typical broiler houses are 40-50 feet wide and 400-600 feet long, so this size house would be 16,000 to 30,000 square feet, and would contain 21,000 to 40,000 broilers, depending on their size. There is considerable variation in turkey house size, however, widths of 50 feet and lengths of 500-600 feet are common. Thus, the area of the house would be 25,000 to 30,000 square feet, and would contain 8,000 to 17,000 hens per house; or 5,000 to 10,000 toms per house. Most broiler and turkey facilities are one story with the floor constructed of concrete or packed soil. Broilers are housed within these structures throughout their lifetime. [Carey (G-1456) P.2 L.24-32]

1347. Turkey flocks are often raised to 5-6 weeks in brooder facilities then moved to grower facilities at the same or nearby farms. Most turkeys remain in these facilities throughout their lifetime, however, some turkeys are provided with access to an outdoor area for part of the day. [Carey (G-1456) P.2 L.34-37]

1348. Ventilation can range from very simple open-sided naturally ventilated to totally enclosed mechanically ventilated systems. Typical modern turkey brooder and broiler facilities have some type of heating system to maintain house temperature for very young birds despite cold outdoor temperatures. Turkey grow-out facilities typically do not have heating systems. [Carey (G-1456) P.3 L.23-27]

1349. *Campylobacter* colonization of broilers is mainly found in the caecum, as well as in other parts of the intestinal tract. There are approximately 10 million to 1 billion *Campylobacter* colony forming units (CFUs) per gram of caecal contents in a colonized broiler (Jacobs-Reitsma, 2000; Corry and Atabay, 2001). [Jacobs-Reitsma (G-1459) P.2 L.48 through P.3 L.2]

1350. Recently, our research team examined 35 caecal samples of 16 week-old turkeys. The average concentration of campylobacters (per gram of caecal content) was estimated at 1.6×10^6 CFUs {Nico Bolder, personal communication}. Our findings are in line with results of other studies, which found 1.2×10^4 to 15×10^7 (median: 2.7×10^5) CFUs of C. fetus subspecies *jejuni* (*Campylobacter jejuni*) per gram of caecal content {Luechtefeld et al., 1981). [Jacobs-Reitsma (G-1459) P.3 L.4-9]

1351. Data from Denmark indicate a seasonal variation in the presence of *Campylobacter* in turkey flocks at slaughter. [Jacobs-Reitsma (G-1459) P.5 L.39-41]

1352. From June 1999 to July 2000, Meng's laboratory conducted a survey of retail fresh meats for *Campylobacter, E. coli,* and *Salmonella* in the Washington, D.C. area. Among 825 meat samples randomly collected from retail stores of the four supermarket chains, 184 chicken carcasses and 172 turkey breasts were examined for the presence of *Campylobacter*. The prevalence of *Campylobacter* in retail

chicken and turkey was 70.7% and 14.5%, respectively (Zhao et al. 2001). The report of our study can be found on this Docket as Exhibit G-727. [Meng (G-1466) P.2 L.26-35]

1353. In Meng's study (G-727), 722 *Campylobacter* isolates (595 from chicken, 112 from turkey, 11 from pork and 4 from beef) were studied. Approximately half (53.6%) of the isolates were identified as *C. jejuni,* 41 -3% as *C. coli,* and 5.1% as other species. Both *C. jejuni* and *C. coli* were isolated more frequently from retail chicken than from turkey, pork, or beef. Interestingly, *C. coli* was more often recovered from retail turkey samples than *C. jejuni.* Nineteen retail poultry meats (18 chicken, and 1 turkey) contained more than one *Campylobacter* species. [Meng (G-1466) P.3 L.13-19]

1354. Antimicrobial resistance phenotypes of *Campylobacter* differ according to species of the organism and source of isolation. [Meng (G-1466) P.3 L.29-31]

1355. A recent investigation of sporadic *Campylobacter* infection in Hawaii had similar findings (Effler 2001). This study was based on cases occurring in the summer of 1998. In this study, eating chicken from a restaurant was associated with illness, and eating turkey or ham was of borderline statistical significance. [Tauxe (G-1475) P.9 L.5-9]

1356. The CVM Iowa State retail meat study shows significant differences between retail chicken and turkey in terms of ciprofloxacin resistant *Campylobacter.* [White (G-1484) P.11 Table 1]

1357. The CVM "Iowa Meat Study" (G-1484 P.10; figure #1) shows that only 9 of 151 turkey products was positive for *Campylobacter.* (G-1484)

1358. A considerable amount of information on *Campylobacters* and the infections they cause was available to the scientific community prior to 1996. [Newell (G-1908) P.19 L.16-17]

1359. Much of the information given on the colonization of *Campylobacters* by poultry was based on studies undertaken in the early 1990s and therefore was available before late 1996. [Newell (B-1908) P.4 L.16-18]

1360. As early as 1981 the development of resistance to antimicrobials of therapeutic importance, including erthyromycin and nalidixic acid in both human and animal strains, was being reported (Goldstein *et al.*, 1982) (Vanhoof *et al.*, 1982). [Newell (B-1908) P.11 L.4-7]

1361. Because of the controversy that surrounded the use of fluoroquinolones in food-producing animals even before they were approved in the United States, FDA held a joint Veterinary Medicine and Anti-Infective Drugs Advisory Committee (Joint Advisory Committee) meeting in May 1994 to address the specific issue of approval of fluoroquinolones for use in poultry. [Tollefson (G-1478) P.4 L.18-23]

1362. The Joint 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. Extra label use means any use other than that specifically stated on the label, i.e., the approved use. [Tollefson (G-1478) P.4 L.26-38]

1363. In late 1993 or early 1994 CVM became aware of foreign studies asserting that fluoroquinolone use in chickens or in turkeys can act as a selection pressure and result in the emergence and dissemination of fluoroquinolone-resistant *Campylobacter*. FDA was concerned that the slaughter, inspection and packaging process for poultry in the United States was such that if the chickens or turkeys are contaminated with the resistant pathogens at the time of slaughter, food products could transmit the resistant organisms to humans. If the resistant *Campylobacter* cause an illness in a consumer who needs treatment, medical therapy may be compromised. [Tollefson (G-1478) P.13 L.22-33]

1364. Prior to approval of enrofloxacin, CVM concluded that the public health risks arising from the use of fluoroquinolones in poultry could be managed such that there would be minimal impact on human health. [Tollefson (G-1478) P.14 L.1-4]

1365. The importance of thermophilic *Campylobacters* as a cause of acute human bacterial enteritis was first widely recognized in 1977 with the development of simple techniques for culture and diagnosis from human diahorreic stools (Skirrow, 1977). By 1981 it had become clear that there were two major bacterial species involved; *C. jejuni* and *C. coli*. [Newell (B-1908) P.19 L.4-7; Tompkin (A-204) P.14 L.9-10]

1366. The first International *Campylobacter* workshop was organized in England 1981 and the proceedings were published (Newell, 1982). Information on the epidemiology, clinical consequences, pathogenesis, antimicrobial resistance etc. of these organisms have been presented at subsequent *Campylobacter* international workshops biannually since that date (Pearson et al., 1983, 1985) (Kaijser & Falsen,1988). (Anon, 1993) (Newell et al., 1996) (Lastovica et al., 1998). Thus a considerable amount of information on *Campylobacters* and the infections they cause was available to the scientific community prior to 1996. [Newell (B-1908) P.19 L.11-17]

1367. Prior to 1996, it was known that *Campylobacter jejuni* and *C. coli* colonize the intestinal mucous of a range of hosts, including humans, live stock, wild animals, companion animals, wild birds and poultry. Asymptomatic excretion is common in animals and birds. However, in susceptible humans, particularly in the industrialized world, colonization is associated with disease (Skirrow, 1994). [Newell (B-1908) P.19 L.18-22]

1368. Prior to 1996, resistance mechanisms in bacteria for all known antimicrobial agents were identified. (Jacoby and Archer 1991, Murray 1991, Skolnick 1991). This includes both natural and synthetic compounds. [Aarestrep (G-1451) P.1 L.47–P.2 L.1]

1369. Laboratory isolation of fluoroquinolone-resistant *Campylobacter* isolates of human or animal origin was first noted in 1985 (Taylor et al., 1985) (B-739). [van den Bogaard (B-1916) P.3 L. 16-18]

1370. In the late eighties and early nineties several *in vitro* studies were published which demonstrated the rapid selection of quinolone-resistant *Campylobacters* (Taylor & Courvalin, 1988 (B-1123); Segreti et al., 1990; Gootz & Martin, 1991(B-367). This is because *Campylobacter* spp. are intrinsically less susceptible to fluoroquinolones than other Gram-negative bacteria and in this genus, one-step mutations in the *gyrA* subunit of DNA gyrase (substitution of Thr-86) causes clinical resistance (Gootz & Martin, 1991 (B-367); Wang et al., 1993 (B-826); Gibreel et al., 1998 (B-354); Ruiz et al., 1998 (G-544)). [van den Bogaard (B-1916) P.3 L. 18 - P.4 L. 2]

1371. Prior to 1996, it was known that fluoroquinolones inhibit the activity of a bacterial enzyme called DNA gyrase. In most bacterial species, resistance is largely due to mutations in this gene. This is the case in *Campylobacter,* where fluoroquinolone resistance appears mainly to be due to mutations in the *gyrA* gene encoding part of the A subunits of DNA gyrase. Cloning and sequencing of the C. *jejuni gyrA* gene have demonstrated that mutations in gyrA at certain positions (Thr-86, Asp-90 and Ala-70) are responsible for resistance (Wang et al. 1993 (B-826)). The most common mechanism of resistance among wild type isolates is a mutation at position threonine-76. This mutation (ACT 4 ATT) causes an amino acid change to isoleucine (Wang et al. 1993 (B-826)), resulting in poor binding of the drug to the mutated target protein. [Aarestrep (G-1451) P.8 L.8-21]

1372. Prior to 1995, it was shown for *Campylobacter jejuni* that additional factors such as increased efflux and decreased uptake resulting in diminished accumulation of fluoroquinolones may also influence quinolone resistance (Segreti et al., 1992 (B-667); Charvalos et al., 1995; Lin et al., 2002 (B-1209)). [van den Bogaard (B-1916) P.4 L.2-5]

1373. It was reported in 1991 that only a single genetic event, which occurs in approximately 1 to 5 in 100 million cells (i.e., 1-5 in 10^8 cells), will result in high-level fluoroquinolone resistance in *Campylobacter.* [McDermott (G-1465) P.5 L. 8-10]

1374. In 1993 it was reported that mutations in the gyrA gene have been linked to fluoroquinolone resistance in *Campylobacter* and that only a single gyrA mutation is necessary to confer fluoroquinolone resistance in *Campylobacter.* [McDermott (G-1465) P.2 L.18 - 19, P.4. L.8-9]

1375. In 1992 and 1993, Jacobs-Reitsma tested 617 isolates from 187 broiler flocks from 160 Dutch different farms. 29.3% of the isolates were found to be cross-resistant to the quinolones tested (naladixic acid, flumequine, enrofloxacin and ciprofloxacin). [Jacobs-Reitsma (G-1459) P.6 L.43-50; G-319]

1376. In early 1990s Jacobs-Reitsma et al., did a longitudinal survey of two Dutch broiler farms, including weekly sampling of fresh caecal droppings of all flocks during eight consecutive production cycles. Overall, the prevalence of *Campylobacter* at the two farms was 56% of 32 flocks and 91% of 22 flocks, respectively, and *Campylobacter* was generally isolated for the first time in broilers between 3 and 4 weeks of age (Jacobs-Reitsma et al., 1995). [Jacobs-Reitsma (G-1459) P.3 L.20-40]

1377. A 1990 study showed that once the water supply within a house, especially the drinkers, has become contaminated by any *Campylobacter*-positive birds then this becomes a major contributing factor for the rapid dissemination of infection within the flock (Shanker et al., 1990). [Newell (B-1908) P.7 L.20-23]

1378. In 1995 it was reported that 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 et al., 1995). [Jacobs-Reitsma (G-1459) P.4 L.13-17]

1379. Prior to 1996 it had been estimated that *Campylobacter* are present in the chicken gut at approximately 10^5-10^9 organisms per gram of fecal material. [McDermott (G-1465) P.5 L.19-20]

1380. In late 1993 or early 1994, before fluoroquinolones were approved for use in chickens and turkeys, CVM management understood and accepted that fluoroquinolone use in chickens and in turkeys could act as a selection pressure resulting in the emergence and dissemination of fluoroquinolone-resistant *Campylobacter* spp. in chickens and in turkeys. [Joint Stipulation 2]

1381. Based on the *in vitro* findings in the late eighties and early nineties (Taylor & Courvalin, 1988 (B-1123); Segreti et al., 1990; Gootz & Martin, 1991(B-367)), the development of resistance to fluoroquinolones in *Campylobacter* spp. in vivo was clearly predictable and understandable. [van den Bogaard (B-1916) P.5 L. 26-29]

1382. The emergence of fluoroquinolone-resistance of *Campylobacter* spp. in chickens was first suggested by Endtz et al. (1990, 1991a (G-755, G-190)) in the late eighties in The Netherlands as isolates from chicken meats were frequently found resistant to fluoroquinolones. [van den Bogaard (B-1916) P.5 L. 29 - P.6 L. 1; Aarestrep (G-1451) P.4 L.17-20]

1383. Prior to 1995, Jacobs-Reitsma et al. (1993, 1994a (B-433)) reported that during 1992 to 1993, 33 % of 617 *Campylobacter* isolates from Dutch chickens were resistant to ciprofloxacin. [van den Bogaard (B-1916) P.6 L. 2-4]

1384. The fact that fluoroquinolone use in poultry could act as a selection pressure resulting in fluoroquinolone-resistant *Campylobacter jejuni* was first documented in vivo by Jacobs-Reitsma's research in 1994. Jacobs-Reitsma et al. found that in groups of 15 broiler chickens experimentally infected with a quinolone-sensitive *C. jejuni*, subsequent treatment with 15 or 50 mg enrofloxacin per L drinking water caused an emergence of quinolone-resistant strains during treatment (Jacobs-Reitsma et al., 1994b (B-432)). [van den Bogaard (B-1916) P.6 L. 4-9]

1385. In Jacob-Reitsma's study the resistance persisted until slaughter at an age of 43 days, 2 weeks after the last day of the 4-day treatment with enrofloxacin. Enrofloxacin treatment did not eradicate *Campylobacter* colonization of the broilers. *Campylobacters* in chicks of a control group, not receiving enrofloxacin treatment, remained fluoroquinolone-sensitive. In contrast, the treatment of the broilers with enrofloxacin during the first week of life did not select for quinolone resistance of the *Campylobacter* strain with which the birds were challenged two weeks later. [van den Bogaard (B-1916) P.6 L. 9-15]

1386. The issue of antibiotics acting as a selection pressure was discussed extensively at the Joint Advisory Committee (May 11 transcript: (B-1819 at 104, 156, 159, 194, 209, 296) and May 12 transcript: (G-219 at 118, 142, 143, 146, 153, 170, 178). It was widely acknowledged at the meeting, that any antibiotic use, including fluoroquinolones, would necessarily result in pressure that would select for resistant organisms. [van den Bogaard (B-1916) P.6 L. 15-19]

1387. Bayer, as part of its NADA, submitted the Jacobs-Reitsma paper (1994b (B-432)), among others, to CVM prior to approval of enrofloxacin. (Letter to Dr. George K. Haibel, CVM from Dianne Lavenburg, Bayer dated June 21, 1996). [van den Bogaard (B-1916) P.6 L. 21 - P.7 L. 1]

1388. On April 19, 1996, Dr. Stephen Sundlof of CVM sent a letter to Dr. Joe Gloyd, of the AVMA discussing CVM's antimicrobial monitoring efforts . [G-1003]

1389. Prior to approval of enrofloxacin, CVM acknowledged the Jacobs-Reitsma article and its premise in a letter from Dr. Stephen Sundlof to Dr. Joe Gloyd,which states "[Jacobs-Reitsma] went on to demonstrate experimentally that *Campylobacter* colonized broilers exposed to quinolones all harbored FQ resistant *Campylobacters*. What is described in the Endtz and Jacobs-Reitsma papers is consistent with other reports from Europe and England of increasing quinolone resistance in *Campylobacter* and *Salmonella* isolates from animals during the early and mid-1990's." [G-1003; van den Bogaard (B-1916) P.7 L. 1-6]

1390. Dr. Sundlof's letter to Dr. Gloyd also states that "FQ resistance has been demonstrated in both *Campylobacter* and *Salmonella* species in animals treated with FQs." [G-1003].

1391. A review of the scientific literature after the approval of enrofloxacin in 1996 does not reveal new evidence (i.e., evidence that was not already known prior to

approval) to demonstrate that fluoroquinolone use in poultry acts as a selection pressure resulting in the emergence of fluoroquinolone-resistant *Campylobacter* in poultry. Indeed, information published since the time of approval merely confirms those conclusions known at the time prior to approval. [van den Bogaard (B-1916) P.7 L.12-16]

1392. The findings of Jacobs-Reitsma et al. (1994b) have been confirmed recently by others (McDermott et al, 2002 (B-868); Luo et al., 2001 (A-190); Stapleton et al., 2001; Ridley et al., 2002, but these studies have not revealed new premises to alter 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 (B-1916) P.7 L. 17-21]

1393. 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 September 8, 1989. [Joint Stipulation 64]

1394. In late 1993 or early 1994, before fluoroquinolones were approved for use in chickens and turkeys, CVM management understood and accepted that if fluoroquinolones were used in chickens and turkeys, the likelihood existed for fluoroquinolone-resistant *Campylobacter* strains to be transferred from chickens and turkeys to humans and contribute to the development of fluoroquinolone-resistant *Campylobacter* infections in humans. [Joint Stipulation 3]

1395. The belief that the likelihood existed for fluoroquinolone-resistant *Campylobacter* strains to be transferred from chickens and turkeys to humans and contribute to the development of fluoroquinolone-resistant *Campylobacter* infections in humans was first postulated by Endtz in 1990 (G-755). [van den Bogaard (B-1916) P.8 L. 12-18]

1396. In late 1993 or early 1994, before fluoroquinolones were approved for use in chickens and turkeys in the United States, CVM management understood and accepted that articles by Endtz and others posited a temporal association between the use of fluoroquinolones in chickens in Europe and an increase in fluoroquinolone-resistant *Campylobacter* isolates from humans in Europe. [Joint Stipulation 4]

1397. Endtz et al. (1990, 1991a (G-755, B-22)) documented the prevalence of ciprofloxacin resistance among human and poultry product isolates of *Campylobacter jejuni* and *Campylobacter coli* during the years bracketing the introduction of several fluoroquinolones (norfloxacin, ciprofloxacin, ofloxacin) into human medicine and enrofloxacin into veterinary medicine. According to the authors of this study, 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. Alos according to the authors, among human *Campylobacter* isolates examined no ciprofloxacin resistance was found during 1982 to 1983 or in 1985 and the percentage of ciprofloxacin-resistant isolates increased to 8% during

1987 to 1988 and to 11% in 1989. [van den Bogaard (B-1916) P.8 L.18-24; Wegener (G-1483) P.21 L.6 -10; Endtz (G-1457) P.7 L.37 - 39]

1398. In a follow-up study in humans, the authors asserted that the prevalence of fluoroquinolone-resistant *Campylobacter* isolates amounted to approximately 25% in 1990 (Endtz et al.,1991b (B-298)). [van den Bogaard (B-1916) P.8 L. 27-28]

1399. Since the Endtz study, additional work on this topic has been done in the early nineties in The Netherlands and many other countries. In Spain, Perez-Trallero et al. (1993) (B-599) reported that 20 of 40 (50 %) *Campylobacter* isolates from retail poultry products obtained in 1993 were resistant to ciprofloxacin, compared to 81 of 165 (49%) human isolates obtained during the first 6 months of 1993. (G-734). [van den Bogaard (B-1916) P.9 L. 1-5]

1400. In Finland, a study on human *Campylobacter* collected in 1990 reported that 9% of the isolates were resistant to ciprofloxacin (Rautelin et al., 1991(B-625). Fluoroquinolones were not used in veterinary medicine in Finland at that time, and the authors concluded based on travel history that this quinolone resistance probably reflected the overall quinolone susceptibility of strains from other countries. [van den Bogaard (B-1916) P.9 L. 5-9]

1401. A 1993 study by Rautelin et al., reported that 17% of human isolates sampled in 1993 were resistant to ciprofloxacin, again in the absence of any fluoroquinolone use in Finnish poultry (Rautelin et al., 1993(B-881)). Subsequent analyses reported resistance rates among human isolates at 20% in 1995, 32% in 1996, and 35-37% in 1997 (see review Nachamkin et al., 2000) (B-44). [van den Bogaard (B-1916) P.9 L. 9-13]

1402. Observations as to the emergence and prevalence of fluoroquinolone resistance in *Campylobacter* isolates from humans were made prior to approval of enrofloxacin in the U.S. in several countries such as Austria (Feierl et al., 1993; 1994 (B-313), Italy (Crotti and Fonzo, 1991(B-264); Crotti et al., 1993), Japan (Itoh et al., 1995), United Kingdom (Bowler and Day, 1992 (B-223); McIntyre and Lyons,1993 (B-512)), Sweden (Sjögren et al., 1993 (B-932); Kaijser, 1994) or Canada (Harnett et al., 1995 (G-267)). [van den Bogaard (B-1916) P.9 L. 13-18]

1403. The Endtz study and the transfer of fluoroquinolone-resistant *Campylobacter* strains from animals to humans was also widely discussed at the Joint Advisory Committee meeting (May 11 transcript: (B-1819 at 61, 95, 96, 99, 102, 122, 134-135, 158, 173,195, 295); (May 12 transcript: (G-219 at 93, 95, 134, 142, 143, 171). [van den Bogaard (B-1916) P.9 L. 19-22]

1404. In addition to the extensive discussion of the Endtz study at the Joint Advisory Committee meeting, CVM acknowledged this issue in a letter drafted prior to the approval of enrofloxacin. This letter from Dr. Stephen Sundlof to Dr. Joe Gloyd stated, "Consumption of poultry is a major risk factor for the development of campylobacteriosis and salmonellosis. Poultry are believed to be the predominant

reservoir of *C. jejuni* and *C. coli.*" [G-1003; van den Bogaard (B-1916) P.9 L. 22 - P.10 L. 3]

1405. In late 1993 or early 1994, before fluoroquinolones were approved for use in chickens and turkeys, CVM management understood and accepted that fluoroquinolone-resistant *Campylobacter* infections have the potential to adversely affect human health. [Joint Stipulation 5]

1406. 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. This has been followed by an increasing occurrence of quinolone-resistant pathogenic bacteria such as *S. aureus,* coagulase negative staphylococci and *Pseudomonas aeruginosa,* and *Escherichia coli.* This was known prior to 1996 (Jones and others 1994, Kresken and others 1994, Acar and Goldstein 1997 (G-6)). [Aarestrep (G-1451) P.4 L.8-11]

1407. In 1991 Endtz et al. (1991a (B-22)) asserted that "the increasing quinolone resistance of *Campylobacter* in the animal reservoir could lead to failure of therapy if treatment of diarrhoeal disease with fluoroquinolone is used." [van den Bogaard (B-1916) P.10 L. 23-25]

1408. Endtz's papers and the potential of resistance to adversely effect human health were widely discussed at the Joint Advisory Committee meeting (May 11 transcript: (B-1819 at 61, 91, 95, 98-99) and concern over the approval of fluoroquinolones for food animals was expressed by the Infectious Disease Society, also supported by the British Society for Antimicrobial Chemotherapy, the Centers for Disease Control ("CDC"), and the Society of Hospital Epidemiologists of America (Id. at 98-99). [van den Bogaard (B-1916) P.10 L. 25 – P. 11 L. 3]

1409. CDC's opposition to the use of fluoroquinolones in animals was explicitly stated in a letter from Dr. David Satcher, CDC Director, to Dr. David Kessler, FDA Commissioner. (G-559). [van den Bogaard (B-1916) P.11 L. 4-5]

1410. Various studies prior to approval reported the emergence of quinolone resistance during fluoroquinolone treatment in humans (Altwegg et al., 1987 (B-143); Goodman et al., 1990 (B-365); Bakhtiar and Shanson, 1990 (B-172); Bernard et al., 1990 (B-193); Segreti et al., 1990;1992 (B-667); Adler-Mosca et al., 1991 (B-126); Obana et al., 1992 (A-184); Petruccelli et al., 1992 (B-605); Wiström et al., 1992 (B-850); Evans and Riley, 1992 (B-1102); Baird, 1993 (B-1135); Tee et al., 1993, 1995 (B-743); Molina et al., 1995 (B-898); Ellis-Pegler et al., 1995 (G-188); Halstensen et al.,1995 (B-885)). [van den Bogaard (B-1916) P.11 L. 10-16]

1411. In 1992, Petrucelli et al. reported that 2 of 56 patients with *Campylobacter* infection relapsed on ciprofloxacin therapy. Other studies also reported that treatment with quinolones can lead to resistance (Wistrom et al., 1992; Wretlind et al., 1992; Adler Mosca et al., 1991; Segreti et al., 1992). [Kist (B-1906) P.13 L.20 to P.14 L.2]

1412. Studies also reported that the infecting strain prior and after therapy was identical (i.e., the infecting strain developed fluoroquinolone-resistance following treatment and there was no second infection (Adler-Mosca et al., 1991 (B-127); Wretlind et al., 1992 (B-857); Giretti et al., 1993 (B-360); Gaudreau and Gilbert, 1998(B-29). [van den Bogaard (B-1916) P.11 L. 16-20]

1413. Prior to 1996, various studies demonstrated that resistance to fluoroquinolones emerges rapidly during fluoroquinolone therapy in humans. See the following studies:

- Adler-Mosca H, Lüthy-Hottenstein J, Martinetti Lucchini G, Burnens A, Altwegg M. (1991) Development of resistance to quinolones in 5 patients with campylobacteriosis treated with norfloxacin or ciprofloxacin. Eur J Clin Microbiol Inf Dis. 10:953-957. (B-127)

- Wretlind B, Stromberg A, Ostlund L, Sjogren E, Kaijser B. (1992) Rapid emergence of quinolone resistance in *Campylobacter jejuni* in patients treated with norfloxacin. Scand J Inf Dis 24:685-686. (B-857)

[Iannini (B-1905) P.5 L.13-15]

1414. Sjögren et al. (1997) (B-684) also reported "an increased frequency of in-vitro resistance of *Campylobacter* spp. isolated from [human] clinical specimens, the development of resistance sometimes being as rapid as within one day of therapy." [van den Bogaard (B-1916) P.11 L. 20-23]

1415. Reports documented up to 20% of patients relapsing due to the emerging of a resistant isolate with ciprofloxacin MICs of 32 mg/L or higher (Altwegg et al., 1987 (B-143); Goodman et al., 1990 (B-365); Adler-Mosca et al., 1991 (B-127); Bakhtiar and Shanson, 1991 (B-772); Petruccelli et al., 1992 (B-605); Wretlind et al., 1992 (B-684)). [van den Bogaard (B-1916) P.11 L. 23 -P.12 L. 3]

1416. Many experts shared their views with regard to the emergence of fluoroquinolone resistance during therapy in humans in relation to a lack of clinical benefits in reviews and other papers (Mosca and Altwegg, 1991 (B-126); Piddock, 1995 (B-609); Wiström and Norrby, 1995 (B-851); Voss and Ellis-Pegler, 2000 (B-914)). [van den Bogaard (B-1916) P.12 L.4-7]

1417. Beginning in the late 1970's it was reported that 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 (G-1477) P.2 ¶ 3]

1418. In 1992 it was reported that the clinical presentation for Guillain-Barre syndrome usually consists of a rapidly evolving generalized paralysis, frequently involving respiratory musculature, rendering patients respirator-dependent in 20-35% of the cases. [Endtz (G-1457) P.3 L.11-14]

1419. A 1995 study by Rees reported that an association with *C. jejuni* adversely affects the clinical course. [Endtz (G-1457) P.3 L.15-16]

1420. In 1995 it was reported that 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, treatment may be of significant benefit (Schønheyder et al, 1995). [Molbak (G-1468) P.3 L.21-26]

1421. Prior to 1996 it was reported that *Campylobacter* infections can be serious, in particular in vulnerable patients with underlying health problems. For example, it was reported that in immunocompromised patients who have invasive *Campylobacter* infection, treatment failure could be fatal (Schoheyder, 1995). [Molbak (G-1468) P.16 L.31-34]

1422. Prior to 1996, it wsa reported that the persons at greatest risk for invasive bloodstream infection with *Campylobacter* are the elderly and the immunocompromised. In 1984 Tauxe reported that the laboratory-based surveillance for *Campylobacter* from 1982-1986, 102/29468 or 0.03% of the infections for which this information was reported, were diagnosed by blood culture (Tauxe 1988). The proportion of infection that were in bloodstream varied with age. It 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 (G-1475) P.15 L. 4-11]

1423. A 1991 study in Los Angeles reported that persons with HIV infection are 35 times more likely to get a *Campylobacter* infection than is the general adult male population (Sorvillo 1991). [Tauxe (G-1475) P.15 L.16-18].

1424. Prior to 1996 it was reported that 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 (G-1485) P.6 L. 38 – 41]

1425. In 1995, Allos and Blaser reported that, in rare instances of extraintestinal *Campylobacter jejuni* infection, particularly among those patients with underlying immunodeficiency status, parenteral combination therapy with imipenem and gentamicin had been recommended. [Iannini (B-1905) P.5 L.6-8]

1426. In 1979, Blaser et al. reported that occasionally, a two-peaked course of illness caused by Campylobacteriosis is observed, with relapsing symptoms of diarrhea at the end of the first week. [Kist (B-1906) P.3 L.16-17]

1427. In 1986, Blaser et al. reported that extraintestinal infections (infections outside the gut) caused by campylobacteriosis are mostly septicemia/bacteremia (infection of the blood), or focal infections of *other* organs. [Kist (B-1906) P.4 L.17-20]

1428. In 1993, Skirrow et al. reported that the incidence of bacteremia is <1%, mainly in immunocompromised and elderly hosts, with an incidence in the latter reported as 0.59%. According to a 1995 report by Schonheyder et al., an incidence of 8 bacteremia cases per 1000 intestinal infections was found in Denmark. As of 1995, it was known that since most strains of *C. jejuni* and *C. coli* are susceptible to the bactericidal (killing) activity of serum, bacteremia is usually self-limited and often remains untreated; bacteremia is a prerequisite for spread of the pathogen to extraintestinal tissues; and thus, such focal infections are even rarer and are mostly presented in the literature as single case descriptions (Skirrow and Blaser, 1995). [Kist (B-1906) P.5 L.3-11]

1429. In 1984, Kist et al. reported a case of septic abortion due to *C. jejuni* or *C. coli*. [Kist (B-1906) P.6 L.9-12]

1430. A 1994 study from Norway published by Kvien et al. mentions that 11% (3 of 27 patients) of reactive arthritis cases were due to *Campylobacter*, resulting in an incidence of 3 per 1,000,000 person years in a population with 83% HLA-B27. [Kist (B-1906) P.7 L.9-11]

1431. In 1988, Ropper reported that of the 1-4 persons suffering from GBS per 100,000 population in the US, an estimated 4% were related to *C. jejuni* infection. In contrast, Ho et al. reported in 1995 that 66% of GBS cases in China are related to *C. jejuni* infection. [Kist (B-1906) P.8 L.1-4]

1432. In The Netherlands, Baytril was approved for use in poultry on April 8, 1987, and Bayer began marketing the product approximately one month later. Baytril is used in The Netherlands only when prescribed and supervised by a veterinary surgeon for specific life-threatening poultry diseases. In The Netherlands, Baytril is expensive (as in the United States) and is never used as an antimicrobial growth promoter. The conditions of use include the recommendation to determine prior to enrofloxacin therapy the susceptibility of the causative agent, as is recommended in the United States. The overall restrictions and conditions of use since the time of approval are, therefore, strikingly similar to those in the United States. [van den Bogaard (B-1916) P.12 L. 19 - P.13 L. 3]

1433. Unlike The Netherlands and the U.S., Spain (and Portugal as well) are unique among the European Union countries in that there is a high degree of uncontrolled and preventive use of fluoroquinolones in both humans and animals. [van den Bogaard (B-1916) P.13 L. 6-8]

1434. It was appreciated prior to 1996 that the conditions in Spain and Portugal have facilitated the emergence, selection and dissemination of fluoroquinolone-resistant *Campylobacter* spp. (Reina and Alomar, 1990 (B-631); Reina et al,. 1992 (B-920); Reina et al., 1994 (B-634); Jimenez et al., 1993 (B-439); Mirelis et al., 1993 (B-527); Velazquez et al., 1995 (G-671); Gomez-Garces et al., 1995 (B-364); Navarro et al., 1993 (B-559); Sanchez et al., 1994 (B-655); Saenz et al., 2000 (B-652)). [van den Bogaard (B-1916) P.13 L. 8-13; Aarestrep (G-1451) P.4 L.20-23]

1435. In was appreciated prior to 1996 that Asian countries such as Thailand also have a high degree of uncontrolled and preventive use of fluoroquinolones in both humans and animals. (Kuschner et al., 1994 (G-354); Murphy et al., 1995 (B-539); Hoge et al., 1998 (G-283)). [van den Bogaard (B-1916) P.13 L. 13-14]

1436. The situation in Spain, Portugal and Thailand is not comparable to the well controlled and prudent use in The Netherlands (and other European countries). Therefore, the situation observed in Iberia or Asia would not be expected to occur in the United States and is not relevant for purposes of comparison. [van den Bogaard (B-1916) P.13 L. 14-18]

1437. In 1987 it was reported that raw poultry meats are commonly contaminated with *Campylobacter,* with prevalence rates reported up to as high as 100%. [White (G-1484) P.2 L. 46 - P.3 L. 2]

1438. 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. Overall, *Campylobacter* was isolated from 28% of the fresh chicken meat, the highest prevalence from French chicken (49%) and the lowest from German chicken (14%). [White (G-1484) P.8 L. 12-16]

1439. 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 (G-1484) P.4 L. 24-26]

1440. The United States Department of Agriculture (USDA), Food Safety Inspection Service (FSIS) periodically analyzes samples of raw meat taken from slaughter plants for the prevalence of certain bacteria, including *Campylobacter.* In 1996, FSIS published a compilation of data for the period of July 1994 through June 1995, estimating the prevalence and levels of bacteria on broiler chicken carcasses. The results of this study indicate that approximately 88% of chicken broiler carcasses (n=1,297) were contaminated with *Campylobacter* species. [White (G-1484) P.5 L. 4-11]

1441. In 1996, FSIS published two reports: The Nationwide Ground Raw Chicken Microbiological Survey and The Nationwide Raw Ground Turkey Microbiological Survey. In the first survey, FSIS collected raw ground chicken samples (n=285) and analyzed them for the presence of bacteria of concern, including *Campylobacter.* FSIS estimated that the national prevalence of *Campylobacter jejuni/coli* on raw ground chicken would be 59.8% positive if the total volume of all the federally inspected ground chicken produced were analyzed. The second survey conducted by FSIS, the nationwide raw ground turkey survey, resulted in an estimate that 25.4% of ground turkey would be positive for *Campylobacter* if the total volume of all the federally inspected ground turkey were analyzed. [White (G-1484) P.5 L. 20-32]

1442. In 1994, 165 ground turkey samples were cultured for *Campylobacter* – 14 (8.5%) were positive for *Campylobacter jejuni/coli*, while 73 of 162 (45%) of ground chicken samples were positive (Food Safety & Inspection Service, USDA, Nationwide Raw Ground Chicken Microbiological Survey and Nationwide Raw Ground Turkey Microbiological Survey). [Gonder (A-201) P.12 L.22- P.13 L.3]

1443. [Since Approval Things Have Only Gotten Better]

1444. The two major food regulatory agencies (FDA and FSIS) in concert with CDC have declared significant reductions in food borne illness since 1996. This conclusion is based on data released on April 19, 2002 (CDC, 2002a). The reductions reflect a wide variety of initiatives by the federal government. The CDC reported reductions in campylobacteriosis of 27% and salmonellosis by 15%. [Tompkin (A-204) P.37 L.18-22]

1445. When more attention is paid to food-handling practices in restaurants and other venues outside the home, the number of fluoroquinolone-resistant *Campylobacter* infections are reduced substantially. [Kassenborg (G-1460) P.10 L.14-16]

1446. Food handling practices and consumer knowledge of microbial food safety has markedly improved over the past decade, particularly from 1993, before enrofloxacin approval, to 2001. [Tompkin (A-204) P.9 L.29-30]

1447. To the extent that CVM recognized the risk of development of fluoroquinolone-resistant *Campylobacter* in poultry, and the risk of transfer to humans prior to the 1996 approval of enrofloxacin, the risk is clearly less now than at the time of approval. [Tompkin (A-204) P.10 L.1-3]

1448. The Food and Drug Administration conducted a random digit-dial survey of a nationally representative sample of American consumers in 1988, 1993, 1998, and 2001 (Fein, Levy and Lando, 2002). The trends for both cross contamination measures and eating potentially risky foods were very similar. No improvement occurred between 1988 and 1993, and for one measure (washing hands after touching raw meat or chicken), the safety of the behavior became worse. Between 1993 and 1998, significant improvement on all of the measures of cross contamination was found, as was also the case on four of the six measures of eating potentially risky food. Then, between 1998 and 2001, most of the measures of cross contamination showed an additional but small improvement, which is an achievement after such a dramatic initial change. [Tompkin (A-204) P.10 L.9-18]

1449. Large improvements in food safety practices were seen between 1993 and 1998 (as measured by cross contamination behaviors and consumption of risky foods). These gains were maintained between 1998 and 2001 for cross contamination and for consumption except for raw seafood. [Tompkin (A-204) P.11 L.13-16]

1450. In 1994, a Joint Advisory Committee comprised of FDA's Center for Veterinary Medicine's Veterinary Medicine Advisory Committee and the FDA's Center for

Drug Evaluation and Research's Anti-Infective Drugs Advisory Committee was convened. [Joint Stipulation 21]

1451. The 1994 Joint Advisory Committee was comprised of: **Veterinary Medicine Advisory Committee Members:** Dr. Debra K. Aaron, chair, Dr. Clarence B. Ammerman, Dr. Graham Purchase, Dr. Anthony J. Johnson, Dr. Gaylord D. Paulson, Dr. Bernard J. Curran, Dr. Stanley H. Kleven, Dr. Gary D. Koritz, Ms. Sue Hudson Duran; **Anti-Infective Drugs Advisory Committee Members:** Dr. Franklyn Judson, Dr. Joseph S. Bertino, Jr., Dr. Judith K. Dunn, Dr. Henry L. Francis, Dr. Barth Reller, Dr. Roselyn J. Rice, Dr. Edwin M. Thorpe; **Consultants:** Dr. Suzanne Fitzpatrick, Dr. Thomas O'Brien, Dr. Hans Reimann, and Dr. Robert Walker. [Joint Stipulation 22]

1452. The following people testified before the Joint Advisory Committee: **FDA Invited Speakers:** Thomas R. Beam, Jr., M.D., Buffalo Veterans Hospital, Andrew Beaulieu, D.V.M., FDA Center for Veterinary Medicine, Gail Cassell, Ph.D., University of Alabama at Birmingham, David C. Hooper, M.D., Massachusetts General Hospital, Stuart Levy, M.D., Tufts University Medical School, James D. McKean, D.V.M., Iowa State University, Dik Mevius, Ph.D., Institute for Animal Science and Health, The Netherlands, Clyde Thornsberry, Ph.D., Daniel Upson, D.V.M., Kansas State University, Dennis Wages, D.V.M., North Carolina State University; **Sponsored Invited Speakers:** Dr. Peter Altreuther, Bayer AG, Mike Apley, D.V.M., Ph.D., Veterinary Research and Consulting Services, Jerry Boscia, M.D., SmithKline Beecham Animal Health, Dr. Peter Coloe, Royal Melbourne Institute of Technology, Mark A. Dekich, D.V.M., Perdue Farms, Diane Fagerberg, Ph.D., Colorado Agricultural Research Enterprises, Thomas Gootz, Ph.D., Pfizer Central Research, Richard Gustafson, Ph.D., Linda M. Hanna, Sciences International, Inc., Howard Hill, D.V.M., Iowa State University, Leon D. Sabath, M.D., University of Minnesota Hospital, Craig Tucker, Ph.D., Mississippi State University; **Public Speakers:** David Bossman, American Feed Industry Association, Dr. Janis Cleland, American Animal Hospital Association, Dr. Tom Holder, National Broiler Council, Joseph McCraren, National Aquaculture Association, Dr. Rod Noel, Association of American Feed Control Officials, Joseph Pocius, National Turkey Federation, Donna Reifschneider, National Pork Producers Council, Kay Richardson, Delmarva Poultry Industry, Louise Risk, Food Animal Concerns Trust, Dr. James Sears, Academy of Veterinary Consultants, Dr. Ran Smith, National Cattlemen's Association, Dr. Peter Theran, Massachusetts Society for the Prevention of Cruelty to Animals, Richard Vulliet, Ph.D., D.V.M., University of California at Davis. [Joint Stipulation 23]

1453. At the Advisory Committee on May 11, Dr. Stuart Levy asserted, "Another generic point that I made in my introduction is that the use of antimicrobials anywhere in the world and in any particular area of the environment, whether it is in aquaculture, agriculture, food industry, hospitals, or homes, has the same effect, that is, the selection of resistant variants, whether they be plasmid-mediated or chromosomal mutations, and as long as this selection continues, these resistant strains have that selective advantage." [B-1819 at P.104]

1454. At the Advisory Committee on May 11, Dr. Clyde Thornsberry asserted, "One of the other givens I think in all this is that if you use antibiotics, you will get resistance. I do not think there is any question about that anywhere." [B-1819 at P.149]

1455. At the Advisory Committee on May 11, Dr. Clyde Thornsberry asserted, "I think I would say if you use antibiotics in humans, you are going to get resistance. If you use antibiotics in animals, you are going to get resistance. [B-1819 at P.152]

1456. At the Advisory Committee on May 11, Dr. Leon Sabath asserted, "First of all, the general principle that resistance will occur with any anti-infective with enough time. Secondly, exposing any organism in any environment to drug will promote the emergence of resistance. I think those are very well accepted." [B-1819 at P.202]

1457. At the Advisory Committee on May 11, Ms. Louise Risk asserted, "Three, can antimicrobial resistant bacteria be transferred from animals to man? To quote Dr. Threlfall, 'It can no longer be disputed that the use of antibiotics in animal husbandry has selected for drug resistant pathogens." [B-1819 at P.290]

1458. At the Advisory Committee on May 12, Dr. Thomas Beam asserted, "I would like to add some comments about the *Campylobacter* because I also foresee that to be a problem organism. ¶ The difficulty with *Campylobacter* is that the organism is carried in a large number of animals without causing symptomatic illness. . . . ." [G-219 at P.53]

1459. At the Advisory Committee on May 12, Dr. David Hooper asserted, "Back to the ... likelihood of selection of resistance. ¶ The mutations that we are talking about being selected we think occur spontaneously at some definable rate, 10 to the minus 8th, 10 to the minus 9th. So a principle determinant of whether resistant subpopulations can grow would be the size, as one factor of the bacterial inocula. So, when there are large numbers of organisms present, there is the calculable statistical chance that such a resistant organism will be present in the population." [G-219 at P.68-69]

1460. At the Advisory Committee on May 12, Dr. Hans Riemann asserted, "Now, the popular foodborne diseases, of course, cannot be dealt with by focusing on risk of development of resistant bugs. Even if we do not have resistant microbes, you still have foodborne disease. So though if you have resistant microbes, these foodborne diseases are more severe, consequent to special groups of populations like immunosuppressed people, et cetera." [G-219 at P.102]

1461. At the Advisory Committee on May 11, Dr. Thomas Beam asserted, "It is epidemiological rather than a direct link, but I think very convincing evidence that *Campylobacter*, as proposed by the authors [Endtz et al.], originated in an animal source and became part of the intestinal flora causing disease in humans." [B-1819 at P.96]

1462. At the Advisory Committee on May 11, Dr. David Hooper asserted, "Other species in human use where resistance has occurred have already been made reference to this morning, in particular the *Campylobacter* resistance rising prevalence in The Netherlands and also similar numbers reported from Finland." [B-1819 at P.165]

1463. At the Advisory Committee on May 11, Dr. Gary Koritz asserted, "Are you familiar with the article by Endtz in the Journal of Antimicrobial Chemotherapy, 1991? We have it as part of our reading material. It seems to give a suggestion of transfer of zoonotic *Campylobacter* from poultry to man in The Netherlands." [B-1819 at P.61]

1464. At the Advisory Committee on May 11, Dr. Jerry Boscia asserted, "The Endtz paper, which has been talked about several times today and one question was posed by Dr. Koritz, shows that fluoroquinolone resistance increases in the poultry. It shows that is increases in humans, but it does not show a link" [B-1819 at P.220]

1465. At the Advisory Committee on May 11, Dr. Thomas Beam asserted, "Finally, I want to address a particular human medicine concern and that deals with special populations. I have already mentioned the young child and the elderly as causes for concern for enteric pathogens both because of the frequency of disease, as well as the clinical implications of acquiring that illness. ¶ This is an evaluation of *Campylobacter* infections in patients with AIDS. It was conducted in the City of Los Angeles ... The average incidence of *Campylobacter* in AIDS patients is 39-fold increased over the population at large. That is, we have a highly vulnerable population to *Campylobacter* infections, and the presumption is that these *Campylobacter* cases were derived from foodstuffs." [B-1819 at P.97-98]

1466. At the Advisory Committee on May 11, Dr. Thomas Beam asserted, "The Counsel of ISDA [Infectious Diseases Society of America] and the Antibiotic Use and Clinical Trials Committee express grave concern about the potential approval of the addition of fluoroquinolones to animal feeds for either therapeutic or subtherapeutic purposes. The link between resistant bacteria and animals and humans has been convincingly demonstrated. The quinolones have certain unique applications in humans that should not be subject to compromise by environmental contamination. ¶ In support of this statement, I have received letters from the British Society for Antimicrobial Chemotherapy, the Centers for Disease Control, and the Society of Hospital Epidemiologists of America reinforcing this grave concern expressed by the Infectious Diseases Society." [B-1819 at P.99]

1467. At the Advisory Committee on May 11, Dr. James Hughes' statement was read which asserted, "The National Center for Infectious Diseases of the Centers for Disease Control and Prevention is concerned about the potential use of quinolones in animal feeds.... [F]luoroquinolone resistance in *Campylobacter jejuni* occurs at relatively high frequencies both *in vitro* and in patients who are being treated for diarrheal disease caused by this pathogen." [B-1819 at P.101]

1468. At the Advisory Committee on May 11, Dr. Gail Cassell asserted, "In a paper actually published in 1992 by Tauxe, et al., he indicates that the greatest risk factor for human *Campylobacter* infections is not only the consumption of chicken, which has now been documented in several other studies, but also exposure to pets with diarrheal disease. ¶ Also in studies by Deming, et al., performed in college students in Georgia, in fact they also show that not only is consumption of chicken one of the greatest risk factors for *Campylobacter* infections, but also in fact that exposure to cats is a significant risk factor." [B-1819 at P.123-124]

1469. At the Advisory Committee on May 11, Dr. Thomas Gootz asserted, "To finish, I would like to show some data from a study that we published with John Segreti from Chicago. These were pre and post-therapy isolates from one of the first quinolone trials with ciprofloxacin to treat adult non-traveller's diarrhea. All the time I have left is simply to say that the pre-therapy isolate was very sensitive to quinolones while the post-therapy isolate was not. ... Now, I simply point to these studies because I think this shows that clearly we can get the selection of resistance in patients treated with fluoroquinolones." [B-1819 at P.187]

1470. The significance of poultry as a source for human infections seems to vary depending on several factors, such as the contamination level of Campylobacters in poultry, meat processing conditions, meat product types available at retail stores, and behavior of the consumer/food handler in handing and cooking raw poultry meats. [Hanninen (G-1458) P.7 ¶10]

1471. *C. coli* is more often recovered from retail turkey samples than *C. jejuni*. [Meng (G-1466) P.3 L.17-18]

1472. Transfer of fluoroquinolone-resistant *Campylobacter* from chickens and from turkeys to humans is not the only cause of fluoroquinolone-resistant *Campylobacter* infections in humans. [CVM Response to Bayer's Interrogatory 9]

1473. Human use of fluoroquinolones can lead to fluoroquinolone-resistant *Campylobacter*. [CVM Response to Bayer's Interrogatory 10 and 45]

1474. CVM does not have any facts or data demonstrating any increase in fluoroquinolone-resistant *Campylobacter* loads in chickens or turkeys at the point of consumption since fluoroquinolone approval for use in chickens and turkeys. [CVM Response to Bayer's Interrogatory 27]

1475. CVM does not have any facts or data that allow quantitation of the change in incidence rates of fluoroquinolone-resistant campylobacteriosis in humans caused by fluoroquinolone use in chickens and turkeys. [CVM Response to Bayer's Interrogatory 31]

1476. Before enrofloxacin was approved, CVM believed that there existed a temporal relationship between the use of fluoroquinolones in chickens and an increase in fluoroquinolone-resistant *Campylobacter* isolates from humans. [CVM Response to Bayer's Interrogatory 37]

1477. Some time prior to May 11, 1994 CVM first understood that fluoroquinolone-resistant *Campylobacter* infections have the potential to adversely affect human health. [CVM Response to Bayer's Interrogatory 52]

1478. CVM does not have any facts or data demonstrating any increase in the rate or extent of complications (including but not limited to Guillian-Barre syndrome) from infections caused by fluoroquinolone-resistant *Campylobacter* as compared to infections caused by fluoroquinolone-susceptible (non-resistant) *Campylobacter.* [CVM Response to Bayer's Interrogatory 60]

1479. Fluoroquinolone-resistant *Campylobacter* infections in humans existed in the United States prior to 1995. [CVM Response to Bayer's Interrogatory 79]

1480. CVM does not have any facts or data to demonstrate that there was little to no fluoroquinolone-resistance in humans *Campylobacter* isolates prior to the approval of fluoroquinolones for use in poultry. [Burkhart (B-1900) P.8 L.4-28]

1481. Fluoroquinolone-resistant C. coli lacking a Thr-86 substitution have been reported. [Meng (G-1466) P.4 L.28-29]

1482. Chlorine/Hypochlorite/Chloramines are compounds which are unproven, yet suspect as agents able to select for gyr-A spontaneous mutants. [Silley (B-1913) P.9 L.1-3]

1483. The CmeABC efflux pump is known to contribute to the intrinsic resistance of *Campylobacter* to fluoroquinolones as well as other antimicrobials and chemicals. [Silley (B-1913) Attachment 1 P.40 ¶ 1 & B-1209]

1484. The frequency of occurrence of resistant *Campylobacter* sps may be overestimated and this erroneous data may lead FDA to conclude that certain veterinary antibiotics have a greater impact on human health than they actually do. [Silley (B-1913) Attachment 1 P.40 ¶ 2]

1485. The vast majority of authors have not even considered the principles laid down in the NCCLS Guideline M37-A2 with regard to how one evaluates for *Campylobacter* the utility of an appropriate method for determining its utility to a test antimicrobial compound. [Silley (B-1913) Attachment 1 P.45 ¶ 4]

1486. There are no recommended antibiotic breakpoint concentrations (or an agreed susceptibility testing method) for *Campylobacter* spp." [Silley (B-1913); citing Piddock et. al., 2000, Attachment 1 P.46 ¶ 2]

1487. Fluoroquinolone susceptible *Campylobacter* sps. can undergo de novo mutation during the course of human therapy with fluoroquinolones for enteric disease resulting in the selection for fluoroquinolone resistant mutants during treatment. [Silley (B-1913) Attachment 1 P.48 ¶ 7]

1488. For fluoroquinolones, the best clinical outcomes are associated with peak/MIC ratios >/= 10. [Silley (B-1913) Attachment 1 P.50 ¶ 2]

1489. If a high enough peak to MIC ratio can be achieved then not only will the parent organism be killed but also the "resistant" mutant. [Silley (B-1913) Attachment 1 P.51 ¶ 1]

1490. Peak to MIC ratios can easily exceed 10 in the gastrointestinal tract of patients with Campylobacters that have an MIC of 32 when patients are treated with 500mg ciprofloxacin BID. [Silley (B-1913) Attachment 1 P.51 ¶ 1, 2]

1491. *Campylobacter* infection occurs in the gastrointestinal tract. (G-444)

1492. Given the high levels of ciprofloxacin reported in the gastro-intestinal tract it is not surprising that clinical cure can be demonstrated for organisms with an MIC of 32 ug/ml. [Silley (B-1913) Attachment 1 P.52 ¶ 1]

1493. A proportion of the isolates tested in the NARMS program have been shown to be impure cultures, this will lead to a degree of misinterpretation of the data. [Silley (B-1913) Attachment 1 P.55 ¶ 4]

1494. It is highly inappropriate to consider that *Campylobacter* spp. With an MIC of 4 ug/ml will be clinically resistant to ciprofloxacin. [Silley (B-1913) Attachment 1 P.55 ¶ 6]

1495. Available data supports a breakpoint of 64 ug/ml. Such a breakpoint would need to be substantiated in accordance with NCCLS guidelines. [Silley (B-1913) Attachment 1 P.56 ¶ 2]

1496. The NCCLS breakpoint for two different bacteria to the same antimicrobial may be very different. [Walker (G-1481) P.5 ¶ 10]

1497. Testing methods not endorsed by NCCLS and interpretive criteria that are not set by NCCLS may be of questionable value. [Walker (G-1481) P.9 ¶ 13]

1498. There is no evidence that at the time of approval of enrofloxacin for use in turkeys or chickens, CVM considered in determining that enrofloxacin was safe for its intended use any benefits to human health from the use of enrofloxacin in chickens or turkeys.

1499. There is no evidence in Notice of Opportunity for Hearing for enrofloxacin for use in chickens or turkeys (65 Fed. Reg. 65954 (October 31, 2000)), that CVM considered benefits to human health from the use of enrofloxacin in chickens or turkeys, when it proposed to withdraw the approval of enrofloxacin.

1500. There is no evidence in Notice of Hearing for enrofloxacin for use in chickens or turkeys (67 Fed. Reg. 7700 (February 20, 2003)), that CVM considered benefits to

human health from the use of enrofloxacin in chickens or turkeys, when it proposed to withdraw the approval of enrofloxacin.

1501. There is no evidence in CVM's Narrative Statement filed to Docket No. 00N-1571 on February 20, 2002, that CVM considered benefits to human health from the use of enrofloxacin in chickens or turkeys, when it proposed to withdraw the approval of enrofloxacin.

1502. There is no evidence in the Vose Risk Assessment (G-953), in the testimony of CVM's risk assessment experts Bartholomew, Travis, or Vose (G-1478, p. 16, ln. 27-30; G-1454, G-1479, G-1480), or in the Written Direct Testimony of CVM's Deputy Director's description of the output of Vose Risk Assessment (G-1478, P.16, ln. 27 through P.17, ln. 9) that CVM considered or evaluated benefits to human health from the use of enrofloxacin in chickens, when it conducted a risk assessment of the potential risks to human health from use of enrofloxacin in chickens.

1503. There is no evidence in the Vose Risk Assessment (G-953), in the testimony of CVM's risk assessment experts Bartholomew, Travis, or Vose (G-1478, P.16, ln. 27-30; G-1454, G-1479, G-1480), in CVM's Deputy Director's description of the output of Vose Risk Assessment (G-1478, P.16, ln. 27 through P.17, ln. 9) or in the evidentary record that CVM conducted a risk assessment of the potential risks to human health from use of enrofloxacin in turkeys, including that CVM considred or evaluate in any such risk assessment risk assessment benefits to human health from the use of enrofloxacin in turkeys.

1504. Dr. Tollefson is the deputy director of the Center for Veterinary Medicine, and has served in that position since February 2001. From January 1997 until February 2001 Dr. Tollefson was director of the Office of Surveillance and Compliance in CVM. (G-1478, P.2, l. 4-9).

1505. 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 data from the NARMS efforts. In Dr. Tollefson's opinion the evidence requires CVM to act to stop the poultry use of fluoroquinolones. (G-1478, P.19, l. 40 – P.20, l. 1)

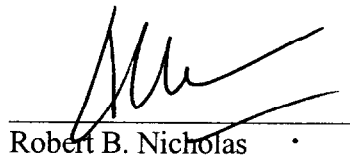
1506. The articles listed in Appendix A were all published prior to the approval of enrofloxacin in October 1996.

1507. The following witnesses are experts in their respective fields as described in their Written Direct Testimony and are qualified as experts to testify as to the matters set forth in their Written Direct Testimony submitted on December 13, 2002:

- Gregory Burkhart
- Tony Cox

- Roger Feldman
- John Glisson
- Charles Haas
- Paul Iannini
- Manfred Kist
- Tom Martin
- Diane Newell
- Mark Pasternack
- James Patterson
- Michael Robach
- Scott Russell
- Peter Silley
- John Smith
- Terry TerHune
- Anthony van den Bogaard
- Dennis Wages
- Steven Woodruff
- Robert Harris
- Richard Carnevale
- Bardley DeGroot
- Eric Gonder
- Charles Hofacre
- Ronald Prucha
- Bruce Tompkin

Respectfully submitted,



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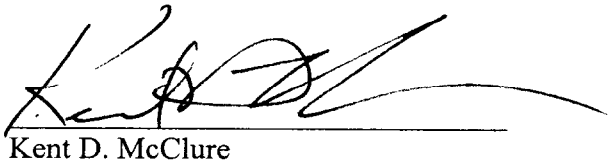

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CERTIFICATE OF SERVICE

I hereby certify that an original and one copy of Bayer Corporation's and Participant Animal Health Institute's Joint 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 Bayer Corporation's and Participant Animal Health Institute's Joint Proposed Findings of Fact was 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 Bayer Corporation's and Participant Animal Health Institute's Joint Proposed Findings of Fact was e-mailed and mailed via first-class mail, postage pre-paid, this 17th day of March 2003 to:

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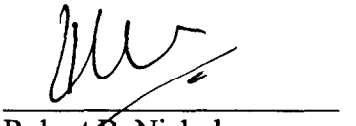

Robert B. Nicholas
Counsel for Bayer

APPENDIX A

CVM

Exhibit	Author	Date	Title
A-73	Animal Health Institute	1987	Submission to Docket 98D-0969 - Risk Assessment on the Human Health Impact of FQ Resistance Campylobacter Associated with the Consumption of Chicken
A-169	Barbara Geilhausen, Heidi Schutt-Gerowitt, Stojanka Aleksic, Rudiger Koenen, Gottfried Mauff, Gerhard Pulverer	1996	Campylobacter and salmonella contamination of fresh chicken meat. Campylobacters, Helicobacters, and Related Organisms 1996; 105-108
B-15	Blaser, M.; D. Taylor and R. Feldman	1983	Epidemiology of Campylobacter jejuni infections, p. 157-176. In Epidemiologic reviews, vol. 5. Johns Hopkins University School of Hygiene and Public Health
B-36	Jacobs-Reitsma, W. F.	1992	Campylobacter and quinolone resistance, p. 79-84. In M. H. Hinton (ed.), The role of antibiotics in the control of foodborne pathogens. In: Flair no. 6, Vol. 7
B-170	Baker CN	1992	The E-Test and campylobacter jejuni. Diagnostic Microbiology and Infectious Diseases 1992; 15(5); 469-472
B-187	Benjamin J; Leaper S; Owen RJ; Skirrow MB	1983	Description of campylobacter laridis, a new species comprising the nalidixic acid resistant thermophilic campylobacter (NARTC) group. Current Microbiology 1983; 8 231-238
B-196	Berndtson E; Tivemo M; Engvall A	1992	Distribution and numbers of campylobacter in newly slaughtered broiler chickens and hens. International Journal of Food Microbiology 1992; 15(1-2); 45-50
B-213	Blaser MJ; Taylor DN; Feldman RA	1983	Epidemiology of campylobacter jejuni Infections. Epidemiologic Reviews 1983; 5 157-176
B-252	Cohen M; Tauxe R	1986	Drug-resistant salmonella in the United States: An R Nepidemiologic perspective. Science 1986; 234 964-969

B-288	Doyle MP; Jones DM	1992	Foodborne transmission and antibiotic resistance of campylobacter jejuni. Campylobacter jejuni: Current status and future trends 1992; 45-48
B-384	Hariharan H; Wright T; Long JR	1990	Isolation and antimicrobial susceptibility of campylobacter coli and campylobacter jejuni from slaughter hogs. Microbiologica 1990; 13(1); 1-6
B-387	Harris NV; Thompson D; Martin DC; Nolan CM	4/1986	A survey of campylobacter and other bacterial contaminants of pre-market chicken and retail poultry and meats, King County, Washington. American Journal of Public Health April 1986; 76(4); 401-406
B-412	Hopkins R; Scott A	1990	Handling raw chicken as a source for sporadic campylobacter jejuni infections. Journal of Infectious Diseases 1990; 148(4); 770
B-589	Patton CM; Nicholson MA; Ostroff SM; Ries AA; Wachsmuth IK; Tauxe RV	1993	Common somatic O and heat-labile serotypes among campylobacter strains from sporadic infections in the United States. Journal of Clinical Microbiology 1993; 31(6); 1525-1530
B-626	Rees JH; Soudain SE; Gregson NA; Hughes RAC	1995	Campylobacter jejuni infection and Guillain-Barre syndrome. New England Journal of Medicine 1995; 333(21); 1374-1379
B-826	Wang Y; Huang WM; Taylor DE	1/5/1993	Cloning and nucleotide sequence of the campylobacter jejuni gyrA gene and characterization of quinolone resistance mutations. Antimicrobial Agents and Chemotherapy 1993; 37(3); 457-463
B-832	Waterman SC; Park RWA; Bramley AJ	1984	A search for the source of campylobacter jejuni in milk. Journal of Hygiene(Lond) 1984; 93(2); 333-337
B-901	Frank J. Sorvillo; Loren E. Lieb; Stephen H. Waterman	1991	Incidence of Campylobacteriosis Among Patients with AIDS in Los Angeles County. Journal of Acquired Immune Deficiency Syndromes, Vol. 4, No. 6, 1991, 598-602
B-1127	Eduardo-Salazar-Lindo, M.D.; R. Bradley Sack, M.D.; Elsa Chea-Wood, M.D.; Bradford A. Kay, Dr. P.H.; Zoila A. Piscoya, M.S.; Raul Leon-Barua, M.D.; August Yi, M.D.	8/1986	Early treatment with erythromycin of Campylobacter jejuni-associated dysentery in children. The Journal of Pediatrics. Aug. 1986, p. 355-360

B-1195	Nujiten, Piet	1992	Molecular Characterization and Analysis of Campylobacter Jejuni Magellin Genes & Proteins
G-9	Acuff, G.R., Vanderzant, C., Hanna, M.O., Ehlers, J.G., Golan, F.A., Gardner, F.A.	1986	Prevalence of *Campylobacter jejuni* in Turkey Carcass Processing and Further Processing of Turkey Products.
G-10	Adak, G.K., Cowden, J.M., Nicholas, S., Evans, H.S.	3/27/1995	The Public Health Laboratory Service National Case-Control Study of Primary Indigenous Sporadic Cases of *Campylobacter* Infection.
G-28	American Society of Microbiology.	7/6/1994	Report of the ASM Task Force on Antibiotic Resistance.
G-67	Black, R.E., Levine, M.M., Clements, M.L., Hughes, T.P., Blaser, M.J.	3/1988	Experimental *Campylobacter jejuni* Infection in Humans.
G-70	Blaser, M.J.	7/25/1992	*Campylobacter* and Related Species.
G-121	Charvalos, E., Tselentis, Y., Hamzehpour, M.M., Kohler, T., Pechere, J.C.	1/17/1995	Evidence For an Efflux Pump in Multidrug-Resistant *Campylobacter jejuni.*
G-157	Davies, J	4/15/1994	Inactivation of Antibiotics and the Dissemination of Resistance Genes.
G-162	Deming, M.S., Tauxe, R.V., Blake, P.A., Dixon, S.E., Fowler, B.S., Jones, T.S., Lockamy, E.A., Patton, C.M., Sikes, R.O.	1987	*Campylobacter* Enteritis at a University: Transmission From Eating Chicken and From Cats.
G-180	DuPont, H.L., Ericsson, C.D., Robinson, A., Johnson, P.C.	4/27/1987	Current problems in Antimicrobial Therapy for Bacterial Enteric Infection.
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