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

In the Matter of:	**FDA DOCKET: 00N-1571**
	DATE: July 18, 2003
Enrofloxacin for Poultry: Withdrawal of Approval of Bayer Corporation's New Animal Drug Application (NADA) 140-828 (Baytril)	

Center for Veterinary Medicine's Post Hearing Brief in Support of its Proposal to Withdraw Approval of the New Animal Drug Application for Enrofloxacin (Baytril): NADA 140-828

I. Introduction

The Center for Veterinary Medicine ("the Center" or "CVM") respectfully submits this post hearing brief in support of its proposal to withdraw approval of the new animal drug application ("NADA") for enrofloxacin, trade name Baytril (NADA 140-828).[1] As summarized herein, the evidentiary record shows that there is a reasonable basis from which serious questions about the safety of Baytril use in poultry may be inferred. Bayer Corporation ("Bayer"), therefore, has the burden to prove that the use of Baytril under the approved conditions of use in poultry has been shown to be safe. Bayer has not met and cannot meet this burden.

[1] In this brief, the trade name Baytril and the drug name enrofloxacin are used interchangeably.

II. Issues for Hearing

On October 31, 2000, CVM proposed to withdraw the approval of an NADA for the use of the fluoroquinolone ("FQ")[2] antimicrobial enrofloxacin in poultry.[3] 65 Fed. Reg. 64954 (Oct. 31, 2000). On November 29, 2000, Bayer, the sponsor of Baytril, requested a hearing on CVM's proposal. On February 20, 2002, the Commissioner of Food and Drugs granted Bayer's request for a hearing. The Animal Health Institute ("AHI") joined in the administrative hearing process by submitting a Notice of Participation pursuant to 21 C.F.R. §12.45. As set forth by the Commissioner, the issues at the enrofloxacin hearing are as follows:

> Whether new evidence shows that enrofloxacin is not now shown to be safe for use under the conditions of use upon the basis of which the application was approved. This issue includes:
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> A. Whether there is a reasonable basis from which serious questions about the safety of enrofloxacin use in poultry may be inferred, such as:
> 1. Whether enrofloxacin use in poultry acts as a selection pressure, resulting in the emergence and dissemination of fluoroquinolone-resistant *Campylobacter* spp. In (sic) poultry?
> 2. Whether fluoroquinolone-resistant *Campylobacter* spp. in poultry are transferred to humans and whether they contribute to fluoroquinolone-resistant *Campylobacter* infections in humans?
> 3. Whether fluoroquinolone-resistant *Campylobacter* infections in humans have the potential to adversely affect human health?
>
> B. Whether the use of enrofloxacin under the approved conditions of use in poultry has been shown to be safe?

A hearing was held on this matter at the Food and Drug Administration ("FDA" or "the Agency") from April 28, through May 7, 2003. This brief constitutes CVM's statement of position as required by 21 C.F.R. §12.96(a).

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[2] Enrofloxacin and ciprofloxacin (an antimicrobial drug used in humans) are both fluoroquinolones. WDT G-1482: P7, L16, L27 - P8, L1. Ciprofloxacin is a metabolite of enrofloxacin. WDT G-1482: P8, L1.

[3] The Center revised the NOOH on January 22, 2001. See 66 Fed. Reg. at 6623-4.

III. Legal Framework

A. Statutory and Regulatory Requirements

1. Federal Food, Drug, and Cosmetic Act

The Federal Food, Drug, and Cosmetic Act ("the Act") sets out the requirements for withdrawing approval of an NADA. CVM's proposal to withdraw the approval of the NADA for Baytril relies on 21 U.S.C. §360b(e)(1)(B) [Section 512(e)(1)(B)]. This section states:

> (e)(1) The Secretary shall, after due notice and opportunity for hearing to the applicant, issue an order withdrawing approval of an application filed pursuant to subsection (b) with respect to any new animal drug if the Secretary finds –
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> # # # # #
>
> (B) that new evidence not contained in such application or not available to the Secretary until after such application was approved, or tests by new methods, or tests by methods not deemed reasonably applicable when such application was approved, evaluated together with the evidence available to the Secretary when the application was approved, shows that such drug is not shown to be safe for use under the conditions of use upon the basis of which the application was approved[.]

2. Respective Burdens

CVM has the burden of coming forward with evidence that there is a reasonable basis from which serious questions about the safety of Baytril use in poultry may be inferred. Once CVM meets its burden, the burden shifts to Bayer to prove the safety of that drug. The Court in Rhone-Poulenc, Inc. v. Food and Drug Administration, 636 F. 2d 750, 752 (D.C. Cir. 1980) explained:

> In the Hess & Clark [v. FDA, 495 F.2d 975, 992 (D.C. Cir. 1974)] case we held that the "new evidence" requirement of the safety clause "plainly places on the FDA an initial burden to adduce the 'new evidence' and what that evidence 'shows'. Only when the FDA has met this initial burden of coming forward with the new evidence is there a burden on the manufacturer to show that the drug is safe."

Additionally, FDA's administrative hearing rules at 21 C.F.R. §12.87(d) (emphasis added) provide:

> [A]t a hearing involving issuing, amending, or revoking a regulation or order relating to the safety or effectiveness of a drug, device, food additive, or color additive, *the participant* who is contending that the product is safe or effective or both and who is requesting approval or *contesting withdrawal of approval has the burden of proof in establishing safety* or effectiveness or both and thus the right to approval. The burden of proof remains on that participant in an amendment or revocation proceeding.

a. Meaning of "Reasonable Basis From Which Serious Questions May Be Inferred"

The Court in Hess & Clark, 495 F. 2d at 993, a case involving the withdrawal of an NADA for diethylstilbestrol (DES), held that FDA's burden is to show: (1) whether the detected residues are related to the use of the new animal drug; and (2) if so, whether the residues, because of their composition, and in the amounts present in the tissue, present some potential hazard to the public health. The Court viewed the scope of this burden in the following way:

> We think it implicit in the statute that when the FDA proposes to withdraw an approval because new evidence shows the drug leaves residues, it has an initial burden of coming forward *with some evidence of the relationship between the residue and safety* to warrant shifting to the manufacturer the burden of showing safety.

Id. (emphasis added).

Therefore, CVM meets its burden when it provides evidence from which serious questions about the safety of a drug can be inferred; CVM need not provide conclusive evidence that the drug has not been shown to be safe. Any other interpretation would effectively serve to shift to CVM the burden to prove the new animal drug has not been shown to be safe. Applying the holding in Hess & Clark to this hearing, in order to meet its burden CVM need only present enough information to show how FQ-resistant *Campylobacter* in poultry is related to the use of FQs in poultry (and to the transmission of FQ-resistant *Campylobacter* to humans) and that the FQ-resistant *Campylobacter* presents some potential harm to the public health.

b. A Withdrawal Decision Need Not be Based Solely on "New" Evidence

Section 512(e)(1)(B) of the Act does not require that wholly new information form the basis for a decision to withdraw approval of an NADA. The plain language supports this reading. The statute provides that new evidence should be evaluated together with evidence available at the time the application was approved. This statutory language not only allows but actually requires FDA to look anew at the evidence it had when the application was approved together with subsequently available evidence. When the agency conducts this evaluation, the evidence that was available when the drug was approved can take on new importance, both when the subsequent evidence changes an initial evaluation / interpretation of data or when it provides more confidence in the results suggested by the earlier evidence.

Case law is not only in accord with this interpretation, but suggests that a re-evaluation of evidence available before an NADA is approved could also meet the statutory requirement. Bell v. Goddard, 366 F. 2d 177 (7th Cir. 1966), concerned the appeal from an Agency order withdrawing approval of an NADA under Section 505(e)(2) of the Act [21 U.S.C. §355(e)(2)].[4] The Court held that the approval of a drug can be withdrawn on the basis of a re-evaluation of existing information. The Court explained:

> In this case an extensive re-evaluation which drew together clinical experience in a manner not previously attempted and which perhaps brought its full impact to the attention of the experts for the first time, provided the basis for the Commissioner's findings. An interpretation of the statute prohibiting such a new application of existing information would do violence to the paramount interest in protecting the public from unsafe drugs.

[4] Section 505(e) applied to new animal drugs prior to the enactment of current Section 512(e).

Id. Although this opinion was interpreting Section 505(e)(2), there is no reason to interpret the Court's ruling as limited to only "clinical experience."

Further, as indicated by the Administrative Law Judge at the enrofloxacin hearing, "the fact that there is a threshold burden on the Agency doesn't necessarily constitute, quote, unquote, new evidence, but it does include a look at the evidence, a new look at the evidence which justifies a finding that the prior finding is no longer valid and therefore the burden of going forward would shift to the manufacturer." Tr. P1127, L14-21.

c. "Safe" Means "Reasonable Certainty of No Harm"

Section 201(u) of the Act provides that, for purposes of Section 512, "safe" has "reference to the health of man or animals." In determining whether a drug is "safe," Section 512 requires FDA to consider "the probable consumption of such drug and any substance formed in or on food because of the use of such drug." Section 512(d)(2)(A) [21 U.S.C. §360b(d)(2)(A)]. FQ-resistant *Campylobacter* is a substance that forms in or on the food due to the use of FQs in poultry.

"Safe," in the context of human food safety, means "reasonable certainty of no harm." The definition is derived from language in H. R. Rep. 2284, 85th Cong., 2d Sess., 4-5 (1958), defining the term "safe" as it appears in Section 409 of the Act [21 U.S.C. §348], which governs food additives. Substances formed in or on food due to the use of animal drugs were regulated under the food additive provisions in Section 409 until Congress passed the Animal Drug Amendments in 1968. The 1968 amendments merely consolidated all of the existing statutory authorities related to animal drugs into a new section (Section 512). The legislative history shows that the consolidation in no way changed the authorities or interpretation of the authorities with respect to the regulation of new animal drugs under the Act. S.R. 1308, 90th Cong., 2d Sess., 1 (1968). Thus, to give effect to Congress' intent in passing Section 512 of the Act,

"reasonable certainty of no harm" is the appropriate standard to use to determine the human food safety of new animal drugs under Section 512, including in determining whether in a withdrawal action under Section 512(e) the sponsor has met its burden to show that a drug is safe.

FDA first published regulations defining safe in 1959, when food additives and animal drugs were both regulated under Section 409 of the Act. 21 C.F.R. §121.1(i) defined safe to mean that "there is convincing evidence which establishes with reasonable certainty that no harm will result from the intended use of the food additive." 24 Fed. Reg. 2434 (Mar. 28, 1959). In 1971, after the authorities relating to new animal drugs had been consolidated into the new Section 512, FDA revised the definition of safe *with respect to food additives* to require consideration of various factors to conclude "that no significant risk of harm will result when the substance is used as intended." 36 Fed. Reg. 12093 (June 25, 1971). At that time, animal drug approvals and withdrawals were regulated under Section 512, not Section 409, and there is no regulatory history indicating whether FDA also intended to change the safety standard with respect to animal drugs to a "significant risk of harm." However, that issue is moot since FDA again adopted a reasonable certainty of no harm safety standard for food additives in 1977, and that standard remains today. 21 C.F.R. §170.3(i); 42 Fed. Reg. 14483 (Mar. 15, 1977).

To date, there has been no scientific consensus or statutory, regulatory, or judicial pronouncement of a specific level of increased antimicrobial-resistant bacterial infections in humans as a result of the use of an antimicrobial drug in animals that would be acceptable under the Act. This is true in general, and also specifically in the case of FQ use in poultry. Bayer has argued that the reasonable certainty of no harm standard does not hold sponsors or FDA to a zero risk standard. But, CVM is not arguing that "reasonable certainty of no harm" means zero risk. "Reasonable certainty of no harm" means that there is a "reasonable certainty" that any risk will not manifest itself as harm. See generally 61 Fed. Reg. 3118 (Jan. 30, 1996). CVM has

presented microbiological, epidemiological, and medical evidence that FQs select for FQ resistance in poultry, that FQ-resistant *Campylobacter* in poultry is a major source of FQ-resistant *Campylobacter* infections in humans, and that FQ-resistant *Campylobacter* infections in humans present the potential for harm in terms of extended duration of diarrhea and other complications.

B. Risk / Benefit Analysis

In Rhone-Poulenc, 636 F. 2d at 754 (1980), a case involving the withdrawal of an NADA for DES, the Court confirmed its earlier holding in Hess & Clark, 495 F. 2d at 993-994, that the Commissioner must balance the risk and benefits associated with the withdrawal of a new animal drug. However, a risk / benefit analysis should not focus on the risk of the financial loss to a sponsor or to the industry as a whole compared to the risks to human health. The Supreme Court has addressed this issue in interpreting other public health statutes. See American Textile Mfrs. Ass'n v. Donovan, 452 U.S. 490 (1981); Whitman v. American Trucking Ass'n, 531 U.S. 457 (2001). American Textile Mfrs. Ass'n, involved a challenge to the cotton dust standard promulgated under Section 6(b) of the Occupational Safety and Health Act of 1970 [29 U.S.C. §655(b)] . The Petitioners argued that the term "feasible" in the statute included a requirement to conduct a cost/benefit analysis. The Supreme Court rejected this position, holding that, "When Congress has intended that an agency engage in cost-benefit analysis, it has clearly indicated such intent on the face of the statute." American Textile Mfrs. Ass'n, 452 U.S. at 509. Here, the Act has no language indicating such intent. In American Trucking Ass'n, the Supreme Court held that the Clean Air Act bars EPA from considering implementation costs when setting appropriate National Ambient Air Quality Standards at a level to protect public health. The Court held that consideration of implementation costs

> is *both* so indirectly related to public health *and* so full of potential for canceling the conclusions drawn from direct health effects that

> it would have been expressly mentioned in §§108 and 109 had Congress meant it to be considered. Yet while those provisions describe in detail how the health effects of pollutants in the ambient air are to be calculated and given effect, See §108(a)(2), they say not a word about costs.

American Trucking Ass'n, 531 U.S. at 469 (emphasis in original). This holding applies equally to a new animal drug safety determination under the Act since consideration of costs in the safety determination is both so indirectly related to public health and so full of the potential to cancel the conclusions drawn from public health benefits.

Further, as indicated by the Administrative Law Judge's March 3, 2003, Order, "economic and environmental evidence is not relevant to the issues in this proceeding." Nor should the analysis compare the risk to human health versus the benefit to poultry health. In this case, the proper analysis would compare the risks to humans of keeping this drug on the market for use in poultry, to the benefits to humans of keeping this drug on the market for use in poultry.

C. CVM has Adduced New Evidence Not Available at the Time the NADA for Baytril was Approved

CVM has adduced a voluminous amount of evidence from which serious questions concerning the safety of Baytril may be inferred. Most of this evidence was not available to CVM at the time that Baytril was approved for use in poultry. CVM's new evidence demonstrates the rapid induction of FQ-resistant *Campylobacter* in poultry from the use of enrofloxacin in poultry, in particular, the high level of resistance that appears. This new evidence, taken together with the evidence available at the time the NADA for Baytril was approved, shows that the selection for, and multiplication of, FQ-resistant *Campylobacter* in poultry stems directly from the use of FQs in poultry and that there is no evidence of FQ resistance developing in poultry at meaningful levels without the selection pressure of FQs.

Most of the epidemiological studies, microbiological / molecular studies, and temporal data evidence is "new," that is, it was unavailable at the time FDA approved the NADA for enrofloxacin.

In addition, the evidence that was available at the time of approval has since been examined anew, in light of all of the new evidence that has emerged. The new evidence more than confirms the earlier evidence; it provides particularized information on the risks to humans of acquiring an FQ-resistant *Campylobacter* infection from poultry consumption.

The evidence of compromised patient care, treatment failures, and quantification of the adverse health impacts associated with FQ-resistant *Campylobacter* in chicken constitutes new data that did not exist at the time enrofloxacin was approved. This evidence demonstrates the adverse human health effects of FQ-resistant *Campylobacter* infections.

In his testimony, Bayer witness van den Bogaard states that scientific literature showing that FQ use in poultry acts as a selection pressure, resulting in the emergence of FQ-resistant *Campylobacter* in poultry, that became available after the approval of Baytril does not provide new evidence, but rather it merely confirms what was known at the time of the approval of Baytril. See WDT B-1916: P7, L12-21. Dr. van den Bogaard further testifies that CVM knew of the likelihood for FQ-resistant *Campylobacter* strains to be transferred from poultry to humans and contribute to the development of FQ-resistant *Campylobacter* infections in humans, and that this had already occurred in other countries and would occur in the United States, citing to studies in Europe. WDT B-1916: P8, L1 - P10, L8. Dr. van den Bogaard testified that there was information concerning the potential of FQ-resistant *Campylobacter* to adversely affect human health at the time Baytril was approved. WDT B-1916: P11, L8-10.

What van den Bogaard ignores, though, is that most of the relevant studies were conducted after Baytril was approved. For example, as CVM shows below, the evidence concerning the rapid selection for high level FQ-resistant *Campylobacter* in poultry is new; the vast majority of molecular studies were conducted after Baytril was approved; a substantial number of epidemiological studies were conducted after Baytril was approved; the temporal data

continue to prove the relationship between the approval of FQs for use in poultry and FQ-resistant *Campylobacter* in humans; studies demonstrating that FQ-resistant *Campylobacter* may lead to longer duration of illness and complications and therefore may compromise patient care are new; and new risk modeling presents evidence of the potential impact of FQ-resistant *Campylobacter* infections on pubic health. Therefore, these studies conducted after Baytril was approved serve to add to the scientific body of knowledge and give substantial scientific weight to the findings of the early studies. This new evidence sheds light on the meaning of studies that existed before the NADA for Baytril was approved. The totality of evidence, new and newly-examined, provides a reasonable basis from which serious questions about the safety of enrofloxacin use in poultry may be inferred and has changed CVM's initial determination about the safety of Baytril.

IV. CVM has Presented a Reasonable Basis to Infer Serious Questions about the Safety of Enrofloxacin Use in Poultry

CVM's proposal to withdraw the approval of the NADA for Baytril is based on CVM's determination that the use of FQs in poultry causes the development in poultry of FQ-resistant *Campylobacter*, a human pathogen; that this FQ-resistant *Campylobacter* is transferred to humans and is a significant cause of the development of FQ-resistant *Campylobacter* infections in humans; and that resistant *Campylobacter* infections are a human health hazard. 65 Fed. Reg. 64954 (Oct. 31, 2000). New evidence not contained in the application, or not available to CVM until after the application was approved, shows this drug is not now shown to be safe for use under the conditions of use upon which it was approved.

A. Selection Pressure from Enrofloxacin Use in Poultry Results in the Emergence and Dissemination of FQ-Resistant *Campylobacter* in Poultry

The evidence presented in this section demonstrates that chicken and turkey are a major source of FQ-resistant *Campylobacter*. The evidence shows that *Campylobacter* contamination

is common in all stages of poultry production, that Baytril effectively selects for FQ-resistant *Campylobacter* in poultry, and that consumers are exposed to FQ-resistant *Campylobacter* on retail poultry.

1. Poultry Industry in the United States

The poultry industry in the United States is immense. There are 8.5 billion broiler[5] chickens and approximately 270 million turkeys slaughtered in the United States each year. See WDT A-202: P3, L4; Bayer's Narrative Statement, P3. Poultry farms commonly have multiple houses on the farm, WDT B-1917: P3, L20-22; WDT B-1915: P3, L27-29, each consisting of 20,000 to 25,000 chickens, RJS 37, or 10,000 to 20,000 turkeys, RJS 38. Each poultry farm can raise multiple flocks per year. WDT G-1456: P3, L7-9.

2. Poultry are Colonized with *Campylobacter*

Campylobacter bacteria are commonly found in the intestinal tract of poultry; however, these commensal bacteria do not generally cause disease in these birds. WDT G-1459: P2, L28-30; WDT G-1484: P2, L43-44. The majority of broilers are colonized with *Campylobacter jejuni* (*C. jejuni*), see WDT G-1459: P3, L37-38, L44-49; WDT G-1454: P3, L4-5, and are generally colonized after 2 weeks of age. WDT G-1459: P2, L32-33. Colonization of turkeys starts at between 7 and 15 days of age. WDT G-1459: P4, L8-9.

For both chickens and turkeys, once the first bird in the poultry house is colonized with *Campylobacter*, the entire house quickly becomes colonized. WDT G-1459: P7, L32-33; WDT B-1908: P5, L17-18. This happens because colonized birds excrete huge numbers of

[5] Broilers are young chickens that are produced for meat.

Campylobacter in their feces and continue to do so up until slaughter. WDT G-1459: P3, L9-12, P4, L9-11; WDT B-1908: P5, L4-6, L10-12; Ex. G-1415: P19-27. There are approximately 10^7 to 10^9 (10 million to 1 billion) *Campylobacter* colony forming units (CFUs) per gram of caecal content in a colonized broiler, WDT G-1459: P2, L49 - P3, L2, P7, L28-30, and the average concentration of *Campylobacter* in a colonized turkey is between 1.2×10^4 to 1.5×10^7 CFUs per gram of caecal content, WDT G-1459: P3, L5-9, P7, L30-32.

Other birds in the poultry house are exposed to these excreted bacteria in a number of ways, including the common practice of the birds' constant pecking and eating their own and other birds' feces (coprophagia), as well as through contaminated water and feed in open systems. See WDT G-1459: P4, L13-16. These practices, in conjunction with the fact that it does not take many bacteria to colonize the poultry, Ex. G-22: P6, ensure that colonization of the entire house occurs rapidly.

3. *Campylobacter* Have Natural Mutations Conferring FQ Resistance

In *Campylobacter*, a single point mutation in the gyrase gene (gyrA) naturally occurs in approximately 1 to 5 in 100 million cells. WDT G-1465: P5, L8-10. Although this number of FQ-resistant *Campylobacter* appears quite small, the number of *Campylobacter* in a colonized chicken or turkey is quite high. See WDT G-1459: P2, L49 - P3, L2, L5-9, P7, L38-32. The result is that, in most *Campylobacter*-colonized chickens and turkeys, one would expect to find some number of *Campylobacter* organisms with mutations making them resistant to FQs. See WDT G-1465: P5, L19-24. Unlike other bacteria in which several steps are required to confer FQ resistance, only a single gyrA mutation is necessary to confer FQ resistance in *Campylobacter*. WDT G-1465: P4, L5-9. These bacteria set the stage for the "selection pressure" on FQ-resistant *Campylobacter* caused by the use of Baytril in poultry.

4. FQs are Used in Poultry Medicine

Baytril is used to control mortality associated with respiratory diseases caused by *E. coli* in chickens and *E. coli* and *P. multocida* in turkeys. Ex. A-54: P1. When a decision is made to treat poultry, Baytril is added to the drinking water of the poultry house. Thus, not only the clinically sick birds are dosed, but all the birds in the house are dosed with Baytril. WDT G-1465: P6, L46 - P7, L2. This method of medicating poultry can lead to the under-dosing of some of the animals, thus increasing the probability of selecting for FQ-resistant *Campylobacter* in both healthy and diseased birds. WDT G-1465: P7, L6-11; Ex. G-52: P29; Ex. B-868: P3.

Bayer claims that only a very small percentage of broiler flocks are dosed with this drug, citing a figure of approximately 1.6% of flocks treated between August 1995 and March 1998. Bayer's Submission of Facts, Information, and Analysis in Response to the NOOH (Feb. 21, 2001). However, treating 1.6% of the approximately 8.5 billion broilers slaughtered in the United States each year means that 136 million broilers are treated each year (8,500,000,000 x 0.016 = 136,000,000). Even using the 1999 figure of 1.1% in Bayer's Response to CVM's Interrogatories (July 26, 2002), the number of broilers directly treated each year is approximately 93,500,000.

Additionally, even if only 1.6% of broilers are directly exposed to Baytril through their drinking water, many more broilers are affected indirectly. Subsequent flocks in the same house can be exposed to the litter and manure remaining from the previous treated flocks since broiler grow-out houses are not cleaned to the floor between each flock. WDT G-1456: P3, L3-9. Further, untreated chickens are exposed to chickens treated with Baytril during transport to the slaughterhouse and at the slaughterhouse, where fecal cross-contamination of birds is common. See WDT G-1467: P8, L12 - P10, L6. Thus, whether or not a house is treated with FQs, broilers can be contaminated with FQ-resistant *Campylobacter* through transport, slaughter, or

processing. See WDT G-1467: P7, L22-28, P11, L12-17. Therefore, the number of chickens potentially exposed to FQs and FQ-resistant *Campylobacter* contamination is immeasurably high. Additionally, contaminated chicken has the potential to cross-contaminate other food, further increasing the magnitude of the problem. See WDT G-1483: P10, L9-11.

Bayer estimates that there were approximately 270,000,000 turkeys slaughtered in the year 2000 and that Baytril was used in less than 4% of turkeys.[6] Bayer's Narrative Statement, P3. Using 4%, approximately 10,800,000 turkeys are directly treated with Baytril each year. Again, this does not take into consideration birds that were exposed to FQ-resistant *Campylobacter* through cross-contamination during transport, slaughter or processing, or through cross-contamination of food in the kitchen. See WDT G-1483: P10, L9-11.

Moreover, it should be noted that, although the conditions of use for Baytril require it to be used by or on the order of a veterinarian, there is no limit set (as a condition of approval) on the number of poultry that can be treated with Baytril. See Ex. A-54: P1. Therefore, use will and does fluctuate depending on the geographic location of the flocks and the occurrence of respiratory disease in any given year. See WDT A-202: P10, L16-17, P11, L3-12. The approved NADA for Baytril also does not limit use to a certain percentage of poultry produced each year (i.e., current industry levels, as asserted by Bayer). Thus, one must determine whether the drug has been shown to be safe under current approved conditions of use, not under any limited use scenario.

5. FQs Used in Poultry "Select" for FQ-Resistant *Campylobacter*

It is indisputable that Baytril use selects for FQ-resistant *Campylobacter* in poultry. Not only does RJS 7 acknowledge this can occur, but multiple witnesses in this hearing, many of

[6] Bayer's Response to CVM's Interrogatories (July 26, 2002) states that a National Turkey Federation study conducted in 1997-1998 found 4% of turkeys were treated with Baytril.

whom have submitted testimony on behalf of Bayer or AHI acknowledge that it does, in fact, occur. See WDT B-1916: P5, L26-29; WDT B-1900: P48, L 4-6; WDT B-1908: P16, L23-24. Moreover, there is no evidence of FQ resistance developing in poultry at meaningful levels without the selection pressure of FQs. WDT G-1465: P6, L7-11. In fact, widespread dissemination of FQ resistance does not emerge in the absence of direct selection pressure brought about by FQ exposure. WDT G-1465: P6, L7-11.

When FQs are administered to poultry, the drug has the effect of killing most of the FQ-susceptible bacteria, including both the target pathogen (i.e., *E. coli*) and other bacteria harbored by the poultry, such as *Campylobacter*. See WDT G-1463: P7, L9-11. However, some bacteria survive. See WDT G-1465: P4, L20-24. These surviving bacteria are the mutated bacteria, described above, which then reproduce and pass on their acquired resistance to daughter bacteria. These surviving bacteria and their progeny colonize the intestinal tract previously filled by the FQ-susceptible bacteria that had been killed by the antimicrobial. See WDT G-1463: P7, L9-14; WDT G-1465: P5, L20-24. In other words, FQ treatment does not eliminate *Campylobacter* from the intestinal tract of the chickens, but rather, rapidly selects for the naturally occurring FQ-resistant mutant isolates. See WDT G-1465: P5, L20-24; Ex. G-315: P2-3; Ex. B-868: P3; Ex. G-1800: P2-3.

Laboratory tests conducted after the approval of the Baytril NADA confirm that FQ treatment at therapeutic levels quickly selects for FQ-resistant *Campylobacter*, leading to the emergence of FQ-resistant *Campylobacter* mutants which re-colonize the poultry with FQ-resistant *Campylobacter*. See Ex. G-1800: P2-3; Ex. B868. In 2001, McDermott looked at the impact of Baytril (when used according to label directions) on the development of FQ resistance in *C. jejuni* present in the gut of broiler chickens and found that, within 24 hours of Baytril treatment, the *C. jejuni* in the Baytril-treated broiler chicken gut were seven-fold more resistant,

measured by Minimum Inhibitory Concentration (MIC), to ciprofloxacin and enrofloxacin than before treatment. WDT G-1465: P3, L1-3; Ex. B-868: P2. MIC is the lowest concentration of the drug that it takes to inhibit the growth of a bacterium. WDT G-1481: P3, L4-9. Continued susceptibility testing after stopping treatment showed that FQ-resistant *C. jejuni* organisms remained in the Baytril-treated birds throughout the normal span of broiler flock. See WDT G-1465: P3, L6-7; Ex. B-868: P3-4. Notably, no FQ-resistant *Campylobacter* isolates were detected in the non-Baytril treated control group of chickens. WDT G-1465: P3, L9-11; Ex. B-868: P2-3. Thus, the resulting resistance was the direct result of Baytril exposure. WDT G-1465: P6, L8-11.

Other scientists have observed similar findings. Bayer's witness Newell observed resistance to enrofloxacin and ciprofloxacin measured by a 7-8 fold increase in MICs in all *C. jejuni* recovered 48 hours after starting Baytril treatment in chickens. WDT G-1465: P4, L38-39, Attach. P25.

In 2002, Zhang published findings that FQ-resistant *Campylobacter* emerges in chickens within 24-48 hours after Baytril treatment. Ex. G-1800: P2-3. In Zhang's experiment, enrofloxacin treatment of chickens infected with FQ-susceptible *C. jejuni* (MIC .125 μg/ml) initially reduced *Campylobacter* colonization but did not eliminate the organism from the infected chicken. Ex. G-1800: P2-3. It is also noteworthy that in Zhang's experiment the untreated control group remained sensitive to enrofloxacin, and that FQ resistance was not detected in the treated group before enrofloxacin treatment. Ex. G-1800: P2. The evidence described above, including the measurement of MIC shifts, supports the earlier findings of Jacobs-Reitsma who found that exposure to enrofloxacin within the labeled concentrations tested (15 ppm and 50 ppm) effectively selected for resistant bacteria, allowing the birds to be colonized with FQ-resistant *Campylobacter*. WDT G-1459: P7, L7-22; Ex. G-315: P2-3. The

new evidence on selection pressure adds to the body of scientific knowledge, and specifically presents new data on the shift of MICs in *Campylobacter* exposed to Baytril.

Scientists have also consistently observed that *Campylobacter* exhibit a bimodal pattern of FQ MICs; that is, they are either highly susceptible to FQs or highly resistant to FQs. WDT G-1465: P4, L44 – P5, L6; Ex. B-868: P2-3; Ex. G-1800: P2; Tr. P249, L13 - P250, L15. For this reason, arguments that the type of testing methods affect the reports on the prevalence of FQ resistance in *Campylobacter* have no merit. See Tr. P249, L13-21. Comparison of methods for determining *Campylobacter* resistance to ciprofloxacin indicates very good correlation between the E-test and agar dilution. See Ex. G-763: P8; Ex. B-170: P3; Ex. G-303: P5. This means that for those studies employing the E-test, some organisms that would have been considered intermediately susceptible or resistant by agar dilution could have been characterized as susceptible by E-test, thus underestimating the true prevalence of ciprofloxacin-resistant *Campylobacter*. Commonly used breakpoints to determine susceptibility versus resistance of *Campylobacter* to FQs are at the lower end of the MIC range (from 1 to 4 ug/ml). See WDT G-1481: P6, L42 - P7, L3; Tr. P193, L7-13. Although Ex. G-763: P8 states that the E-test yielded higher MICs at the resistant range (high range of MICs), this is irrelevant since *Campylobacter* is either highly susceptible or highly resistant and the breakpoint between the two is always at the lower end of the tested range. Moreover, there are good reasons to use the E-test, among them ease of use, and use for monitoring changes in the prevalence of ciprofloxacin resistance in *Campylobacter*. Tr. P245, L16 - P246, L3.

Similarly, the fact that no official NCCLS breakpoint[7] has been adopted for *Campylobacter* susceptibility to ciprofloxacin would not affect the designation of *Campylobacter*

[7] A breakpoint is the concentration (expressed as an MIC) that distinguishes between susceptible, intermediate and resistant bacteria. WDT G-1481: P5, L1-4.

isolates as FQ-susceptible or FQ-resistant because the bimodal nature of *Campylobacter* squarely places the organisms into two distinct, and opposite ends of the scale -- categories of very FQ-susceptible or very FQ-resistant.[8] WDT G-1465: P4, L44 - P5, L6; Tr. P249, L13 - P250, L15.

Many knowledgeable researchers in the field have used a breakpoint of greater than or equal to 4 μg/ml for determining FQ resistance in *Campylobacter*. Ex. G-1517: P4; Ex. G-337: P5; Ex. G-589: P2; Ex. G-1800: P1. CVM believes this breakpoint is reasonable and in line with what other countries' standards setting organizations have adopted. In fact, some other researchers and countries use lower breakpoints to determine *Campylobacter* resistance to ciprofloxacin. Tr. P193, L7-13; WDT G-1481: P6, L42 - P7, L2. This adds to the validity of using a greater than or equal to 4μg/ml breakpoint for determining *Campylobacter* susceptibility to ciprofloxacin. And, CVM witness Walker has testified that, in his expert opinion, a reasonable breakpoint for *Campylobacter* susceptibility to ciprofloxacin would be greater than 1 μg/ml, thus making a greater than or equal to 4 μg/ml breakpoint even more reasonable. WDT G-1481: P10, L10-14.

6. Colonization with FQ-resistant *Campylobacter* Persists until Slaughter

Experiments have demonstrated that once poultry are colonized by FQ-resistant *Campylobacter* they remain colonized for the production span of the poultry. Ex. B-868: P3-4; WDT G-1459: P3, L9-11; WDT B-1908: P5, L2-6. This is an important factor in the cross-contamination of poultry and poultry carcasses during the transportation and slaughter process. The prevalence of *Campylobacter* in live poultry varies but many studies have shown that the majority of poultry flocks are colonized with *Campylobacter*. WDT G-1459: P3, L33-39, L42 - P5, L2; Ex. G-1724: P3; Ex. G-385: P1.

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[8] This also effectively rebuts Silley's statement that an appropriate breakpoint should be 64 μg/ml. WDT B-1913: P13, L10-11.

Surveys of poultry carcasses at slaughter have also been conducted and demonstrate high levels of *Campylobacter* on poultry carcasses. See Ex. G-652: P6; WDT G-1484: P5, L7-11; Ex. G-651: P6; WDT G-1484: P5, L14-17; Ex. G-791: P1; WDT G-1484: P6, L25-27; WDT G-1464: P5, L22-23; Ex. G-37: P1.

Some recent research has focused on the antimicrobial susceptibility of *Campylobacter* found in poultry. The animal arm of the National Antimicrobial Resistant Monitoring System (NARMS) conducted surveillance testing of broiler carcass rinse samples to determine the prevalence of FQ-resistant *Campylobacter* from broiler carcasses. Results of these surveys for years 1998, 1999, 2000, and 2001 showed 9.4%, 9.3%, 10.4% and 17.6%, of *Campylobacter* organisms from broilers were resistant to ciprofloxacin, respectively.[9] WDT G-1478: P12, L6-7. Because of an outdated method of speciating *Campylobacter* used by USDA, CVM believes that, in years 1998-2000, the animal arm of NARMS underestimated the true prevalence of ciprofloxacin-resistant *Campylobacter* found in broilers at slaughter in the United States. WDT G-1465: P7, L25-34; WDT G-1478: P9, L15 - P11, L38; Tr. P175, L3-11.

Bayer witness Newell also testified to the high level of FQ-resistant *Campylobacter* in chickens. WDT B-1908: P14, L7-10. In 2000-2001, CVM witness Logue examined the antimicrobial susceptibility of *Campylobacter* from two turkey processing plants. WDT G-1464: P2, L27-30. At the smaller of the two slaughter plants (production speed of 800 turkeys per hour), 8.8% of *Campylobacter* recovered from turkeys were resistant to ciprofloxacin. WDT G-1464: P6, L14-19. At the larger of the two plants (production speed of 8000 turkeys per hour), 65.2% of the *Campylobacter* were found to be resistant to ciprofloxacin. Id.

[9] In 2001, 17.6% of *C. jejuni* were resistant to ciprofloxacin using the conventional method; 20.3% of *C. jejuni* were resistant to ciprofloxacin using the spin or optimized method. WDT G-1478: P12, L6-7.

7. Actual Exposure to FQs in Poultry Exceeds Use of FQs in Poultry

From the very first step of catching and transporting poultry to the slaughterhouse, through the very last step of packaging the poultry for retail sale, there is the potential for contamination and recontamination of poultry and poultry carcasses with bacteria, including *Campylobacter* and FQ-resistant *Campylobacter.* Many witnesses in this hearing (both for CVM and for Bayer or AHI) testified that the transportation, slaughter, and processing of poultry presents such an opportunity for cross-contamination. WDT G-1467: P8, L8 - P10, L6; WDT A-203: P7, L17 - P8, L13; WDT B-1912: P5, L2-7.

At the very beginning of the slaughter process, the crates that are used for transporting chickens and turkeys are stacked on top of each other on transport trucks, presenting the very real probability that chickens or turkeys in the lower crates will be contaminated by feces from the poultry stacked in crates on top. See WDT A-204: P46, L17-19; WDT G-1467: P8, L12-16.

The actual kill process is very often automated in a chicken slaughter plant, and less so in a turkey processing plant. WDT G-1467: P2, L16-18, P6, L37. However, in both automated and manual systems, the automated blades or hand-held knives present a point of possible contamination, especially if the neck area has *Campylobacter* on it. WDT G-1467: P8, L22-27. The blades can act to push the *Campylobacter* into the bird being slaughtered. Further, the blades are a point of cross-contamination between birds being slaughtered. WDT G-1467: P8, L22-27.

The testimony in this hearing is replete with examples of potential points of contamination within the slaughterhouse. WDT G-1467: P8, L 8 - P10, L6; WDT A-204: P46, L14 - P47, L19. A telling example is the scalding tank which often stays murky brown throughout each day from the feces in it. WDT G-1467: P8, L29-34. Plainly, the poultry slaughter process increases the likelihood that poultry carcasses leaving the slaughterhouse are

contaminated with *Campylobacter* and FQ-resistant *Campylobacter*. WDT G-1467: P11, L12-17.

8. Prevalence of *Campylobacter* and FQ-resistant *Campylobacter* on Retail Poultry

Studies have confirmed that retail poultry is a reservoir for *Campylobacter* in the United States and that other types of retail meat, most notably beef and pork, harbor very little *Campylobacter*. See WDT G-1484: P2, L46 – P3, L2; P4, L12-15; G-1466, P3, L24-26. Since campylobacteriosis is considered mostly to be a foodborne disease, WDT B-1900: P9, L42-43, the fact that other food is not nearly as contaminated with *Campylobacter* as chicken is telling.

Even early surveillance demonstrated a high level of *Campylobacter* contamination in retail chicken. Ex. G-162; Ex. G-268. More recent studies suggest that most of the retail chicken sold in the United States today is contaminated with *Campylobacter*. See Ex. G-701: P2, finding that 69% of market broilers were positive for *C. jejuni*; Ex. G-727: P 3, finding that over 70% of chicken sampled and over 14% of the turkey sampled had *Campylobacter*; Ex. G-G-541: P1, finding that 44% of the retail chicken tested positive for *Campylobacter*; Ex. G-589, finding that 88% of retail chicken sampled had *Campylobacter*, 74% had *C. jejuni* and 21% had *Campylobacter coli* (*C. coli*); Ex. G-1785: P2, finding that 82% of retail chickens were positive for *Campylobacter*; WDT G-1484: P4, L12-15, finding that 58% of retail chicken breasts and 8% of retail ground turkey were positive for *Campylobacter*. These high levels of *Campylobacter* contamination, coupled with the low levels of *Campylobacter* contamination in beef and pork, indicate the majority of foodborne campylobacteriosis must be coming from *Campylobacter* contaminated poultry. See WDT G-1484: P4, L14-15; G-727: P3.

There are additional findings that specifically concern antimicrobial susceptibility of the *Campylobacter* found in retail poultry. Antimicrobial testing has shown that approximately one quarter to one third of retail *Campylobacter*-contaminated retail chicken meat carry an FQ-

resistant strain. WDT G-1465: P7, L19-21. This, despite the contention that less than 2% of broilers are treated with Baytril.

For example, in a University of Maryland study of retail meat bought in the greater Washington D.C. metropolitan area in 1999-2000, 35% of the *Campylobacter* poultry isolates were resistant to ciprofloxacin and 41% were resistant to nalidixic acid. WDT G-1466: P3, L24-29; Ex. G-1778: P9-10. The results with respect to nalidixic acid are important because *Campylobacter* that is resistant to nalidixic acid tend to be cross-resistant with FQs. See WDT G-1482: P8, L5-13. In a 1999 Centers for Disease Control and Prevention (CDC) study of retail chicken bought in Georgia, Maryland, and Minnesota, 24% of the *Campylobacter* isolates tested were resistant to ciprofloxacin, with a total of 11% of the retail chicken tested yielding ciprofloxacin-resistant *Campylobacter*. WDT G-1484: P5, L35 - P6, L3; Ex. G-541: P1.

Similar results were found in a study conducted in the state of Iowa. Of the *C. jejuni* isolates recovered from retail chicken or turkey purchased from March 2001 to March 2002, 27% exhibited ciprofloxacin resistance. WDT G-1484: P7, L43. Likewise, 27% of the *C. coli* from retail chicken and turkey exhibited ciprofloxacin resistance.[10] See WDT G-1484: P7, L43. In Smith's Minnesota study, 20% of the retail chicken sampled had ciprofloxacin-resistant *Campylobacter*. Ex. G-589: P5.

Retail meat contamination is an extremely important factor in acquiring campylobacteriosis and/or FQ-resistant campylobacteriosis. Even the best kitchen hygiene cannot completely prevent cross-contamination from occurring in the kitchen. WDT G-1483: P10, L3-5. Multiple studies have shown kitchen hygiene to be lacking. WDT G-1475: P9, L22-27, P9, L29 - P10, L24. And, since even a drop of chicken juice can contain an infective dose of

[10] In addition, 21% of not yet speciated Campylobacter exhibited resistance to ciprofloxacin. WDT G-1484: P7, L43-44.

Campylobacter, it is easy to see how often cross-contamination can occur. See WDT G-1475: P10, L40-41. Further, while freezing and/or cooking reduces *Campylobacter* contamination, it does not eliminate it. See RJS 24; WDT G-1483: P9, L26-30, L34-35. In fact, the anatomical structure of a broiler allows insulating pockets for *Campylobacter* to survive during cooking. WDT G-1483: P9, L29-36. Therefore, if *Campylobacter* and/or FQ-resistant *Campylobacter* are present in poultry at retail, it is quite possible that an infective dose will remain on poultry at the point of consumption or cross-contaminate other foods.

B. FQ-resistant *Campylobacter* in Poultry are Transferred to Humans and Contribute to FQ-resistant *Campylobacter* Infections in Humans

Poultry is a significant risk factor for acquiring campylobacteriosis. WDT G-1483: P11, L1-2, P27, L24-27; WDT G-1457: P9, L18-19; WDT G-1470: P9, L17-18; WDT G-1475: P8, L33-36; WDT G-1452: P9, L36-40; WDT G-1473: P15, L35 - P16, L23, P20, L26-31. Strong scientific evidence supports this nearly universal conclusion.

Three broad categories of evidence comprise the scientific support relied on by CVM in proposing to withdraw the NADA for Baytril: (1) epidemiological studies; (2) microbiological and molecular studies; and (3) temporal data. The epidemiological studies show that poultry is responsible for *Campylobacter* infections (FQ-susceptible and FQ-resistant infections) in humans. The microbiological and molecular studies strengthen the results of the epidemiological studies. The temporal data corroborate the epidemiological, microbiological, and molecular evidence and provide independent support for the incontrovertible assertion that enrofloxacin administered to poultry causes the proliferation of FQ-resistant *Campylobacter* in poultry that contributes to FQ-resistant *Campylobacter* infections in humans. The evidence supporting CVM's proposal to withdraw the NADA for Baytril includes more than 17 epidemiological studies, 12 microbiological and molecular studies, and temporal data from 10 different countries.

Bayer cannot and does not wholeheartedly dispute the risk to humans posed by poultry. At least one of Bayer's experts acknowledges that, given all current evidence, a person's most likely exposure to FQ-resistant *Campylobacter* is uncooked or undercooked food that contains FQ-resistant *Campylobacter*. WDT B-1900: P9, L42-43. And, based on all of the available scientific evidence, the sole plausible conclusion is that the food in question is poultry.

1. Epidemiological Studies

A mountain of epidemiological evidence demonstrates that *Campylobacter* infections in humans are linked to poultry. Individually and collectively, epidemiological studies of *Campylobacter* infections conducted in the United States and other industrialized countries have determined that poultry, particularly chicken, is a risk factor for acquiring campylobacteriosis. WDT G-1483: P15, L30-36; WDT G-1457: P4, L12-17; WDT G-1475: P8, L5 - P9, L27; WDT G-1452: P9, L36-40. Although the epidemiological studies differ in location, technique, and sample size, they consistently indicate contact with and consumption of poultry as dominant sources of *Campylobacter* infections. WDT G-1452: P9, L36-40; WDT G-1483: P11, L1-7; WDT G-1475: P8, L23-36; Ex. G-1644: P9, P14; Ex. B-205: P3; Ex. G-1743: P8, P13. Among the repeatedly-implicated risks are: (1) consuming poultry (Ex. G-162, Ex. G-268, Ex. G-1686, Ex. G-602, Ex. G-1718, Ex. G-334, Ex. G-474, Ex. G-307); (2) consuming poultry that is raw or undercooked (Ex. G-1488, Ex. G-162, Ex. G-268, Ex. G-299, Ex. B-561, Ex. G-1681, Ex. G-182, Ex. G-307); and (3) consuming poultry in a restaurant (Ex. G-337, Ex. G-1488, Ex. G-185, Ex. G-1711, Ex. G-182). Other identified risk factors include: (1) handling raw chicken (Ex. G-300) and, (2) failing to clean food preparation / cutting board surfaces, which is a marker for cross-contamination, Ex. G-1644: P11 (Ex. G-268, Ex. G-1681).

Notwithstanding the limitations of any one epidemiological study, the sum of the studies on risk factors for campylobacteriosis leads to only one conclusion: chicken is a frequent source

of human *Campylobacter* infections in industrialized countries. WDT G-1475: P8, L5-8; WDT G-1483: P10, L38 - P11, L7, P15, L1-12. Not only do numerous epidemiological studies furnish a common theme, but molecular studies and temporal data buttress that theme. Although the results of a single epidemiological study may not necessarily permit the assertion that a disease is caused by a particular risk factor merely because the disease appears to be associated with that risk factor, Tr. P287, L14-18, the scientific evidence here points to the existence of a causal link between exposure and illness. Cf. Tr. P287, L19-22 ("[T]he judgment of causation, and it is a judgment, is based upon a body of evidence that allows people to then conclude or to have their judgment that there is a causation involved."), P445, L1-5.

Most of the epidemiological studies were designed to examine risk factors for acquiring campylobacteriosis, not specifically or exclusively FQ-resistant campylobacteriosis. If a study reveals that poultry is associated with campylobacteriosis, the study's findings relate to campylobacteriosis, whether FQ-resistant or FQ-susceptible. There is no plausible scientific reason that transmission of FQ-resistant *Campylobacter* from poultry to humans is different from transmission of FQ-susceptible *Campylobacter* from poultry to humans. WDT G-1483: P20, L18 - P21, L3. An epidemiological study evaluating risk factors for *Campylobacter* infections generally is relevant, applicable, and informative in the determination of risk factors for FQ-resistant *Campylobacter* infections.

The epidemiological studies relied on by CVM investigated risk factors for campylobacteriosis in cases of sporadic illness, which are infections unrelated to each other by a shared source of infection, WDT G-1483: P12, L18-20. Campylobacteriosis outbreaks are rare and differ epidemiologically from sporadic infections. WDT G-1475: P6, L10-16, P7, L10-22.

Because the overwhelming majority of campylobacteriosis cases in the U.S. are sporadic, the epidemiology of sporadic infections is the relevant science here.[11]

a. United States

The 1998-1999 FoodNet[12] *Campylobacter* case-control study, a large case-control study of sporadic *Campylobacter* infections, was conducted in the U.S. in FoodNet sites during a 12-month period between 1998-1999. WDT G-1452: P9, L46-48. The study was designed to examine risk factors associated with sporadic *Campylobacter* infections. Analyses include: (1) Friedman's evaluation of risk factors associated with *Campylobacter* infections; and (2) Kassenborg's evaluation of risk factors associated with FQ-resistant *Campylobacter* infections. Results from the Friedman and Kassenborg analyses demonstrate that the dominant source of domestic *Campylobacter* infections (campylobacteriosis generally and FQ-resistant campylobacteriosis specifically) in humans is poultry, particularly chicken, but also turkey. WDT G-1452: P10, L22 - P11, L1; Ex. G-1488: P3; Ex. G-228: P1; WDT G-1460: P8, L16-18; Ex. G-337: P3.

The 1998-1999 FoodNet *Campylobacter* case-control study was conducted in Georgia, Minnesota, Oregon, and selected counties in Connecticut, California, Maryland, and New York; the FoodNet surveillance sites represented approximately 7.7 percent of the U.S. population. WDT G-1452: P9, L46 - P10, L1; WDT G-1460: P3, L10-12. In the study: (a) all selected cases

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[11] Study results and their statistical significance are presented here in several ways: (1) odds ratios or relative risk, and (2) confidence interval or p-value. An odds ratio (OR) is the ratio of the odds of exposure in the case group to the odds of exposure in the control group. John Last, A Dictionary of Epidemiology 128 (4th. ed. 2001). A relative risk (RR) is the ratio of the risk of disease in the exposed group to the risk of disease in the unexposed group. Id. at 156. A confidence interval (CI) is an interval with a probability (e.g., 95%) that the true value of a number (e.g., mean, proportion, odds ratio) is contained within the interval. Id. at 37. A p-value (p) is the probability that the observed difference between the case and control groups (or exposed and unexposed groups) could have occurred by chance if there was no true difference between the groups. Id. at 146.

[12] FoodNet (Foodborne Diseases Active Surveillance Network) is a collaborative project among CDC, state health departments, USDA, and FDA. WDT G-1452: P2, L16-19.

with a culture-confirmed *Campylobacter* infection in the surveillance sites during the study period were attempted to be enrolled; (b) a case was defined as diarrheal illness (three or more loose stools in a 24-hour period) in a person living in a FoodNet site whose stool sample yielded a *Campylobacter* isolate and who was not part of a recognized outbreak; (c) one age-matched well control was enrolled for each case; (d) a total of 1,316 *Campylobacter* cases and 1,316 matched well controls were enrolled; and (e) cases and controls were interviewed regarding a variety of exposures in the seven days prior to the case's onset of illness. WDT G-1452: P10, L1-5, L14-15; WDT G-1460: P4, L7-20, P5, L11-17.

Friedman found that cases were 10.0 times more likely (95% CI = 6.0, 16.7) to have traveled internationally in the seven days prior to illness onset than controls (13 percent of cases traveled outside the U.S. compared with 1.5 percent of controls). The population attributable fraction for foreign travel was calculated from this finding. A population attributable fraction is the reduction in incidence of illness that would be achieved if the population had been entirely unexposed compared with its current (actual) exposure pattern. WDT G-1460: P9, L1-3. In Friedman's analysis, the population attributable fraction for foreign travel was 12 percent, suggesting that 12 percent of sporadic cases of campylobacteriosis in the U.S. are due to recent travel outside the U.S. WDT G-1452: P10, L14-20; Ex. G-1488: P11.

Friedman's final multivariate logistic regression model, which was used to determine independent risk factors for acquiring a *Campylobacter* infection among persons who did not travel outside the U.S., found that within the seven days prior to illness onset: (a) cases were 2.2 times more likely (95% CI = 1.7, 2.9) than controls to have eaten chicken in a restaurant (44 percent of cases versus 26 percent of controls); (b) cases were 2.5 times more likely (95% CI = 1.3, 4.7) than controls to have eaten turkey in a restaurant (6 percent of cases versus 3 percent of controls); and (c) cases were 1.7 times more likely (95% CI = 1.3, 2.2) than controls to have

eaten non-poultry meat in a restaurant (52 percent of cases versus 35 percent of controls). WDT G-1452: P10, L22-32; Ex. G-1488: P23. Other independent risk factors among domestically acquired *Campylobacter* cases included eating undercooked poultry. WDT G-1452: P10, L32-34; Ex. G-1488: P23.

Friedman's analysis determined that the largest population attributable fractions for domestically acquired *Campylobacter* infections were for eating chicken in a restaurant and eating non-poultry meat in a restaurant. WDT G-1452: P10, L36-44. The population attributable fraction was: (a) 24 percent (95% CI = 17%, 30%) for eating chicken in a restaurant; (b) 21 percent (95% CI = 13%, 30%) for eating non-poultry meat in a restaurant; and (c) 4 percent (95% CI = 1%, 6%) for eating turkey in a restaurant. WDT G-1452: P10, L36-41; Ex. G-1488: P23. No other exposure accounted for more than five percent of the cases. WDT G-1475: P9, L2-3. The population attributable fractions suggest that at least 28 percent of the domestically acquired *Campylobacter* infections were due to eating poultry. See WDT G-1452: P10, L36-44.

Kassenborg's findings on FQ-resistant *Campylobacter* infections were even more striking. Of the 858 persons whose FQ susceptibility status was known, 646 (75 percent) were interviewed and included in Kassenborg's analysis. WDT G-1460: P6, L5-7. Of those cases, 64 persons had an FQ-resistant *Campylobacter* infection and, of those, 58 percent (37 persons) had acquired their infection domestically. WDT G-1460: P6, L7-8, P7, L19-22, P9, L5-6.

Kassenborg's analysis focused on cases who contracted their illness in the United States and compared their exposures with exposures of their age-matched well controls. Kassenborg examined a multitude of potential risk factors; only potential risk factors that reached a certain level of statistical significance in the univariate analysis were added to the multivariate model. Tr. P606, L3-6, P615, L13-16, P617, L22 - P618, L9. Based on that criterion, four variables met

the statistical significance level and were part of the multivariate analysis: (1) eating chicken or turkey cooked at a commercial establishment; (2) eating in a non-fast food restaurant; (3) antacid use; and (4) eating non-poultry meat at home. WDT G-1460: P8, L13-16, P14.

In the final multivariate logistic regression model, Kassenborg found that eating chicken or turkey at a commercial establishment was the only risk factor that remained independently associated with FQ-resistant *Campylobacter* illness. WDT G-1460: P8, L11-18, P14. Cases with domestically acquired FQ-resistant *Campylobacter* infections were 10 times more likely to report having eaten chicken or turkey at a commercial establishment than were well controls (95% CI = 1.3, 78). WDT G-1460: P8, L18-20; P14; Ex. G-337: P15.

Eating chicken or turkey at a commercial establishment accounted for 38 percent of the domestically acquired FQ-resistant *Campylobacter* infections. WDT G-1460: P9, L3-5, P14. Twenty-two percent of all FQ-resistant infections (i.e., including, in the total, those infections that were related to foreign travel) could be attributed to eating chicken or turkey in a commercial establishment. WDT G-1460: P9, L7-8. The population attributable fraction suggests that a person's risk for an FQ-resistant *Campylobacter* infection could potentially be reduced by 22 percent if the risk associated with commercially prepared chicken and turkey were eliminated. WDT G-1460: P10, L17-19.

Results of the Effler case-control study were similar to the outcomes in Friedman's and Kassenborg's analyses.[13] Effler's multivariate analysis found that eating chicken prepared by a commercial food establishment was an independent predictor of *Campylobacter* illness (OR = 1.8, p = 0.03). WDT G-1483: P14; Ex. G-185: P3. The population attributable fraction for

[13] Effler's study was conducted in Hawaii during a five-month period in 1998 and enrolled 211 cases and 211 controls. WDT G-1483: P14; Ex. G-185: P1-2.

commercially prepared chicken was estimated to be 18 percent. Ex. G-185: P2. Eating turkey or ham was of borderline statistical significance. WDT G-1475: P9, L7-9.

Earlier foundational studies

Several earlier epidemiological studies in the United States had statistically significant findings and are considered foundational in their investigation of risk factors for campylobacteriosis. Tr. P412, L11-15. Eating poultry was identified as a risk factor for campylobacteriosis in the Harris and Deming studies; eating raw or undercooked chicken was identified as a risk factor in the Harris, Deming, and Hopkins/Olmstead studies; and handling raw chicken was associated with illness in the Hopkins/Scott study.[14] Although three of the four studies have relatively small sample sizes, sample size was not a concern because the studies were able to detect risk factors.[15]

In the Harris case-control study, consumption of chicken and cornish game hen were associated with more than a doubling of the risk of *Campylobacter* infection (chicken: RR = 2.4, 95% CI = 1.6, 3.6; game hen: RR = 3.3, 95% CI = 1.1, 9.8). The consumption of raw or rare chicken was even more strongly associated with *Campylobacter* infection (RR = 7.6, 95% CI = 2.1, 27.6). Ex. G-268: P1; WDT G-1475: P8, L10-13. Harris also found that infection among chicken eaters was associated with not washing the kitchen cutting board and other errors related to the cleaning of food preparation surfaces, which suggests that kitchen practices can easily

[14] The Harris study was conducted between April 1982 and September 1983 in Washington State and enrolled 218 cases and 526 controls. Ex. G-268: P1. The Deming study was conducted at the University of Georgia during the fall and winter quarters of the 1983-1984 academic year and enrolled 45 case-control pairs. Ex. G-162: P1. The Hopkins/Scott study was conducted in Colorado during a one-month period in 1982 and enrolled 10 cases and 15 controls. WDT G-1483: P14; Ex. G-300. The Hopkins/Olmstead study was conducted in Colorado during a 2.5-month period in 1981 and enrolled 40 cases and 71 controls. WDT G-1483: P14; Ex. G-299: P1.

[15] When there has been a failure to identify risk factors in a study with a small sample size, that failure may have been because there were not enough cases and controls to detect a difference in risk between the two groups, rather than because the potential risk factors examined were not actual risk factors. Tr. P417, L10-16.

transfer the bacteria to other foods. WDT G-1475: P9, L41-45. Harris's estimate of the population attributable fraction for chicken consumption was 48 percent. Ex. G-268: P4. Other risk factors included eating processed turkey sandwich meats. Ex. G-268: P1.

Deming's multivariate analysis identified eating fully cooked chicken and eating chicken reported to be raw or undercooked as independent risk factors for *Campylobacter* enteritis (raw/undercooked chicken: OR = 48.7, 95% CI = 2.1, 1135; fully cooked chicken: OR = 7.2, 95% CI = 1.2, 43.7). Ex. G-162: P1, P6; WDT G-1475: P8, L13-15. The population attributable fraction for eating chicken was estimated at approximately 70 percent. Ex. G-1644: P10.

In the Hopkins/Scott case-control study, *Campylobacter* infection was associated with handling raw chicken, as opposed to eating undercooked chicken, and it is likely that persons had already become infected as a result of handling chicken in the kitchen, before the chicken was cooked and eaten. WDT G-1475: P9, L34-37.

Among chicken-eaters in the Hopkins/Olmstead case-control study, eating undercooked chicken was identified as a risk factor for sporadic *C. jejuni* infection (OR = 6.27, 95% CI = 0.90, 43.84). WDT G-1483: P14; Ex. G-200: P2.

b. Other Industrialized Countries

Because the epidemiology of *Campylobacter* in the United States and Europe is comparable, studies from Europe are relevant in the evaluation of sources of *Campylobacter* infections in the United States. WDT G-1457: P7, L14-17; Tr. P408, L7-14. The European studies were conducted in the United Kingdom, Denmark, Sweden, Switzerland, Norway, and the Netherlands. Eating poultry was identified as a risk factor in the Studahl (Sweden), Neal

(U.K.), Schorr (Switzerland), Kapperud (Norway), and Oosterom (the Netherlands) studies[16]; eating undercooked chicken was associated with illness in the Neimann (Denmark) study[17]; and eating chicken in a restaurant was a risk factor in the Rodrigues (England) study[18].

Studahl's case-control study found a statistically significant association between eating chicken and having a domestically acquired *Campylobacter* infection (OR = 2.29, 95% CI = 1.29, 4.23). WDT G-1483: P14; Ex. G-602: P4. Neal's multivariate analysis found that eating chicken was independently associated with *Campylobacter* gastroenteritis (OR = 1.4, 95% CI = 1.1, 1.8). WDT G-1483: P14; Ex. G-1686: P4. In Schorr's multivariate analysis, consumption of poultry liver was an independent risk factor for *Campylobacter* enteritis (OR = 5.7, 95% CI = 1.4, 22.8). Ex. G-1718: P7. In Kapperud's multivariate analysis, eating poultry that was brought into the house raw (frozen or refrigerated) was independently associated with *Campylobacter* illness (OR = 3.20, CI = 1.17, 8.76). Ex. G-334: P3. Oosterom's case-control study found that significantly more index patients with a *C. jejuni* infection had eaten chicken meat (47 versus 29, p = 0.0002) particularly at barbecues (14 versus 2, p = 0.0015) compared with controls. Ex. G-474. Neimann's case-control study found that eating undercooked chicken was a risk factor for campylobacteriosis. WDT G-1483: P13, L8-12. Rodrigues' recent case-control study

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[16] The Studahl study was conducted in Sweden during the 12-month period in 1995 and enrolled 101 cases and 198 controls. WDT G-1483: P14; Ex. G-602: P1. The Neal study was conducted in the U.K. during a 14-month period between 1994-1995 and enrolled 313 cases and 512 controls. WDT G-1483: P14; Ex. G-1686: P1. The Schorr study was conducted in Switzerland during an 11-month period in 1991 and enrolled 167 cases and 282 controls. WDT G-1483: P14; Ex. G-1718: P1. The Kapperud study was conducted in Norway during an 18-month period between 1989-1990 and enrolled 52 cases and 103 controls. WDT G-1483: P14; Ex. G-334: P1. The Oosterom study was conducted in the Netherlands during a four-month period in 1982 and enrolled 54 cases and 54 controls. WDT G-1483: P14; Ex. G-474.

[17] The Neimann study was conducted in Denmark over a 12-month period between 1996-1997 and enrolled 217 cases and 236 controls. WDT G-1483: P13, L6 - P14.

[18] The Rodrigues study was conducted in England and enrolled 229 cases and 229 controls. Ex. G-1711: P2.

identified consumption of chicken in a restaurant as a statistically significant risk factor for campylobacteriosis (OR = 1.86, p = 0.049). Ex. G-1711: P3, P5.

Epidemiological studies conducted in Canada and New Zealand have also identified poultry as a risk factor for *Campylobacter* infection. Eating undercooked poultry was a risk factor in the Michaud (Canada), Eberhart-Phillips (New Zealand), and Ikram (New Zealand) studies; eating chicken in a restaurant was associated with illness in the Eberhart-Phillips study.[19]

In Michaud's multivariate analysis, *Campylobacter* infections were strongly associated with eating undercooked poultry (OR = 26.5, p = 0.004). Ex. G-1681. Michaud also found that failing to clean the cutting board with soap or other products was a risk factor for campylobacteriosis, which suggests that cross-contamination via the cutting board is also an important source of infection. WDT G-1475: P9, L19-27. Eberhart-Phillips' multivariate analysis found that the risk of acquiring campylobacteriosis was strongly associated with recent consumption of raw or undercooked chicken (OR = 3.71, 95% CI = 2.24, 6.13), and with recent consumption of chicken prepared in a restaurant (OR = 3.53, 95% CI = 2.17, 5.72). Ex. G-182: P4. In Ikram's case-control study, eating undercooked chicken (OR = 4.94, p = 0.05), eating poultry at a barbecue (OR = 3.00, p = 0.03), or eating poultry at a friend's house (OR = 3.18, p = 0.03) were risk factors for acquiring a *Campylobacter* infection. Ex. G-307: P1.

[19] Michaud's study was conducted in Canada during a 15-month period between 2000-2001 and enrolled 114 cases and 228 controls. Ex. G-1681. Eberhart-Phillips' study was conducted in New Zealand during a nine-month period between 1994-1995 and enrolled 621 cases and 621 controls. WDT G-1483: P14; Ex. G-182: P1. Ikram's study was conducted in New Zealand during a two-month period in 1992-1993 and enrolled 100 cases and 100 controls. WDT G-1483: P14; Ex. G-307: P1.

c. Other Findings

The risk factors for campylobacteriosis described in the studies above include a number of exposures in addition to poultry, such as drinking raw milk,[20] drinking untreated water,[21] and having contact with cats,[22] puppies,[23] or farm animals[24]. Two studies in the United Kingdom have also suggested that drinking milk from bottles damaged by birds is associated with campylobacteriosis. WDT G-1483: P14. CVM has never claimed that poultry is the sole risk factor for campylobacteriosis, and the identification of additional risk factors does not detract from the common theme that poultry is a major risk factor for campylobacteriosis. Some risk factors are uncommon in the general population (drinking raw milk) or account for usually only a small proportion of cases (contact with cats and puppies). WDT G-1475: P8, L17-20. Other risk factors are unquestionably rare (drinking milk from bottles with bird-pecked bottle caps). WDT G-1483: P15, L22-27. Although quantification of the different routes of infection may be difficult, consuming poultry has been estimated by some U.S. studies to account for roughly 50 to 70 percent of *Campylobacter* cases. See Ex. G-1644: P10; WDT G-1457: P4, L15-17.

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[20] This risk factor was identified in the Friedman analysis, the Hopkins/Olmstead study, and the Neimann study. WDT G-1452: P10, L32-34; Ex. G-1488: P23; WDT G-1483: P13, L8-12, P14.

[21] This risk factor was identified in the Friedman analysis, the Hopkins/Olmstead study, and the Studhal study. WDT G-1452: P10, L32-34; Ex. G-1488: P23; WDT G-1483: P14.

[22] This risk factor was identified in the Deming and Hopkins/Olmstead studies. WDT G-1475: P8, L13-16; WDT G-1483: P14.

[23] This risk factor was identified in the Friedman analysis and the Neal study. WDT G-1452: P10, L32-34; Ex. G-1488: P23; WDT G-1483: P14.

[24] This risk factor was identified in the Friedman analysis, the Studhal study, and the Michaud study. WDT G-1452: P10, L32-34; Ex. G-1488: P23; WDT G-1483: P14; Ex. G-1681.

Even in other studies unable to conclude that poultry is a significant risk factor for campylobacteriosis, poultry remained a possible source of infection. Epidemiological studies generally compare cases to healthy controls through data collected during interviews. In questionnaire-based investigations, it is difficult to identify risk factors if they are very common, e.g., eating chicken. Asking cases and controls if they ate chicken in a specific week could fail to identify chicken as a risk factor in the case group because most people (i.e. cases and controls) eat chicken at least once a week. See WDT G-1483: P12, L33-39. In the Schmid study, for example, the authors concluded that "poultry remains a possible, but unproved, source of human infection" because "large numbers of chicken carcasses at retail stores were contaminated with *C. jejuni*, the seasonal distribution of disease onset and percentages of chicken carcasses that were positive was similar, and serotyping did not differentiate patients' strains from chicken strains of C. jejuni." Ex. G-564: P4.

In the Adak study, occupational exposure to raw meat (e.g., through contact as a chef or butcher) was a risk factor for becoming ill from *Campylobacter*. Ex. G-10: P4-5. The exposure, although not defined, presumably included exposure to poultry because the study concluded that the finding was similar to that demonstrated in the Hopkins/Scott study, which specifically examined the handling of raw chicken. Ex. G-10: P5. A study conducted by Tenkate in Australia did not find poultry to be a risk factor for infection; however, the study population was children under three years old. Ex. G-1731. Ownership of pet chickens was statistically significantly associated with *Campylobacter* illness in these young children. Ex. G-1731: P5.

In three other studies, one conducted in the U.S. and two conducted in the U.K., poultry was not identified as a risk factor for *Campylobacter* infection in humans. However, those three studies were not "case-control" studies but were "case-comparison" studies in which exposures were compared between FQ-resistant cases and FQ-susceptible cases. If both groups have

exposure to the same food vehicle, i.e., poultry, one would not expect to find "a difference implicating that food vehicle in one group." Tr. P523, L7-8, P534, L13-20. Two of these studies reported a high percentage of poultry consumption in both the case and the comparison groups. Ex. G-589 ("a very high proportion of quinolone-resistant and quinolone-susceptible cases consumed chicken," Tr. P523, L21 - P524, L1); Ex. G-240: P6 (80 percent of ciprofloxacin-resistant and 83 percent of ciprofloxacin-susceptible cases consumed poultry). The third study, nevertheless, did report that chicken consumption was a risk for ciprofloxacin-resistant *C. jejuni* infection for a subgroup of the cases. Ex. G-1772: P3-4.

A number of epidemiological studies have suggested that, paradoxically, preparing or consuming chicken in the home may be associated with a lower risk of acquiring a *Campylobacter* infection. One possible explanation for this paradox is that repeated contact with contaminated poultry provides an acquired immunity to infection. WDT G-1457: P4, L24-25. This finding should not lead one to conclude that bringing *Campylobacter*-contaminated poultry into the home protects people against campylobacteriosis. Furthermore, this finding in no way negates the findings of all of the epidemiological studies that document that poultry is a risk factor for campylobacteriosis.

2. Microbiological and Molecular Studies

Investigation of strains of *Campylobacter* from animals, food, and humans by genetic fingerprinting and other sensitive methods for tracing sources of human infection has provided confirmation for the assertion that poultry, particularly chicken, is a source of human *Campylobacter* infections, specifically FQ-resistant *Campylobacter* infections. WDT G-1483: P17, L41 - P18, L3; WDT G-1473: P13, L41 - P14, L18. Studies have found strong similarities between *Campylobacter* strains when comparing isolates from humans with isolates from poultry. WDT G-1457: P5, L1-39; WDT G-1475: P11, L8-18. Smith's study in the U.S. made

an even more striking finding by molecularly linking domestically-acquired quinolone-resistant *Campylobacter* illness with quinolone-resistant *Campylobacter* from poultry. WDT G-1473: P13, L41 - P14, L18. The route of transmission of FQ-resistant *Campylobacter* from poultry to patient has been confirmed. WDT G-1483: P17, L41 - P18, L3; Ex. G-190: P8; Tr. P557, L15 - P558, L1.

a. Bacterial Typing Methods

Bacterial typing studies characterize bacterial isolates below the species level and are commonly used to facilitate epidemiological investigations. WDT G-1453: P4, L23-24. Serotyping compares strain similarity at the cellular level by determining the patterns of proteins and carbohydrates on the bacterial cell surface. WDT G-1475: P11, L11-12. Subtyping compares strain similarity at the genetic level; strain subtypes are called genotypes.

There are several different subtyping techniques, most of which generate a form of DNA fingerprint. Bacterial DNA fingerprinting "allows patterns of disease in the population to be seen that might otherwise be too difficult to differentiate from background disease activity," WDT G-1455: P6, L41-42, and "works by facilitating recognition of 'clusters' of disease," WDT G-1455: P7, L2. DNA fingerprinting functions to strengthen statistical associations found in epidemiological analyses. WDT G-1455: P6, L27-29.

DNA fingerprinting methods have different levels of resolution. Using a method that provides a higher level of resolution is not necessarily better for revealing information on bacterial strain relatedness. WDT G-1455: P7, L5 - P8, L8; WDT G-1453: P5, L15-16. As an extreme example, determining the complete DNA sequence of an entire bacterial genome (i.e., complete resolution) would provide too much resolution because every bacterium would appear different. Although no DNA fingerprinting method measures every difference in DNA among bacterial strains, each method examines certain markers chosen to represent differences in

bacterial strains. WDT G-1455: P9, L10-20. Both PFGE and RFLP methods[25] have been widely used to study *C. jejuni*. WDT G-1470: P8, L1-2. Other methods, including ribotyping, AFLP, and MLST have also been used.[26] WDT G-1470: P7, L34-37; WDT G-1457: P5, L8-15; Ex. G-161; Ex. G-1629.

Bayer has questioned the utility of *flaA*-RFLP subtyping because the method examines a smaller portion of the bacterial genome (only one gene) and generates fewer subtypes than do other molecular subtyping methods. Because the *flaA* gene is diverse, *flaA*-RFLP subtyping is considered to be adequately discriminating to draw conclusions about the relatedness of bacterial isolates. WDT G-1453: P5, L6-34; WDT G-1470: P8, L13-16. *Fla* typing is widely accepted and "can provide meaningful data, especially when combined with epidemiologic data." WDT G-1453: P7, L30-32.

FlaA-RFLP subtyping has been evaluated in light of instability in *fla* genes that could cause the emergence of other subtypes through the exchange of *fla* DNA sequences within and among strains. If the *flaA* gene is unstable, the recombination between *fla* genes would probably create additional *flaA* gene sequences. WDT G-1453: P6, L8-18. It would be more likely that strains originally alike would become different rather than that strains originally different would become alike. WDT G-1453: P6, L20-23. Such an increase in the diversity of *flaA* types would decrease the likelihood of finding a substantial overlap between *Campylobacter* isolates from

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[25] Pulsed-field gel electrophoresis (PFGE) examines DNA sequences that could occur on any part of the bacterial genome. WDT G-1455: P9, L37 - P10, L3. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), specifically of the *flaA* gene (*flaA*-RFLP), examines DNA sequences in the flagellin (or flagellar) gene, which codes for the bacteria's motility appendage (the flagellum). WDT G-1455: P9, L23-27.

[26] AFLP is amplified fragment length polymorphism; MLST is multilocus sequence typing.

poultry and humans. In other words, this putative weakness is not likely to cause a finding of strain overlap where there is none.

b. Bacterial Typing Studies

A multitude of typing studies conducted in numerous countries using various typing methods shows substantial overlap between *Campylobacter* isolates from poultry and humans. Twelve studies from eight countries using six bacterial typing methods correlated human and poultry strains of *Campylobacter* isolates and found a high degree of strain similarity. The typing studies have consistently isolated bacterial types from human *Campylobacter* isolates that are related to *Campylobacter* isolates from poultry.

Not unexpectedly, the microbiological and molecular studies do not reveal a 100 percent overlap between the poultry and human *Campylobacter* isolates. Aside from factors related to study design or sampling and testing methods, one reason for the lack of complete overlap is that all *Campylobacter* strains from poultry may not be equally capable of causing illness in humans. WDT G-1453: P6, L27-33, P7, L41-43. Another reason for differences in poultry and human strains of *Campylobacter* is, as CVM acknowledges, that poultry is not responsible for 100 percent of all *Campylobacter* infections. Nevertheless, even if the population of *Campylobacter* strains is not identical in humans and poultry, concluding on that basis that poultry is not the source of most *Campylobacter* infections would be wrong. WDT G-1453:P6, L36-38.

Using *flaA*-RFLP, Smith (Minnesota) found that 92.3 percent of isolates (12 of 13 isolates) from humans with domestically acquired quinolone-resistant *C. jejuni* infections had subtypes that were also found in quinolone-resistant isolates from domestically produced chicken (retail samples). WDT G-1455: P10, L7-10. This association by itself implicates chicken as "a likely source" of quinolone-resistant *C. jejuni* in humans. WDT G-1455: P10, L9-10. Further strengthening this implication is the additional finding that, in comparison to isolates from

humans with a domestically acquired quinolone-resistant *C. jejuni* infection, isolates from humans with either a domestically acquired quinolone-susceptible *C. jejuni* infection or a foreign travel-associated *C. jejuni* infection had a low overlap in strain subtypes with quinolone-resistant isolates from domestic chicken products. WDT G-1455: P10, L10-15. Patients with domestically acquired quinolone-resistant *C. jejuni* infections were 15 times more likely to have a *C. jejuni* subtype that was also found among quinolone-resistant *C. jejuni* isolates from domestic chicken products than were patients with domestically acquired quinolone-susceptible *C. jejuni* infections. WDT G-1473: P14, L8-12; Ex. G-589: P6. Patients with domestically acquired quinolone-resistant *C. jejuni* infections were 22.3 times more likely to have a *C. jejuni* subtype that was also found among quinolone-resistant *C. jejuni* isolates from domestic chicken products than were patients with foreign travel-associated quinolone-susceptible *C. jejuni* infections. WDT G-1473: P14, L12-16; Ex. G-589: P6.

It is unlikely that a third source common to both poultry and humans accounts for the molecular subtyping results. When asked on cross-examination whether a possible third source could be responsible for his findings, CVM witness Smith stated:

> In my opinion, it's not likely at all that there's a common third source. You have to kind of use common sense and go by what's logical – that resistant campylobacter is on the chicken and people are eating the chicken. So that's by far – that's the most likely explanation.
>
> You don't necessarily need to be looking for some proposed third source when a direct link is available.

Tr. P557, L15 - P558, L1.

Using PCR-RFLP of both the *flaA* and *flaB* genes, Clow (United Kingdom) found 14 of 62 genotypes from approximately 1500 isolates were common to both chicken and human strains and represented 77 percent of the human strains isolated. WDT G-1455: P10, L18-22; WDT G-1453: P6, L40-41; Ex. B-250: P4. Clow suggested that the majority (61 percent) of genotypes found only in human isolates was represented by only one isolate and could be a reflection of

instability in the flagellin genes, Ex. B-250: P5, rather than a true mismatch between human and poultry isolates. Therefore, "at least 77% of human [*Campylobacter*] infections could potentially be explained by poultry exposure."[27] WDT G-1453: P6, L43-44 (emphasis added).

A molecular subtyping study in Finland of human and chicken (retail and at slaughter) isolates revealed overlapping genotypes among human and chicken strains. WDT G-1458: P7; Ex. B-380; Ex. G-264. The results were corroborated with three subtyping methods (PFGE, AFLP, and ribotyping) applied in combination with serotyping. WDT G-1458: P7; Ex. B-380. In Iceland, researchers subtyped a portion of the *flaA* gene called the SVR DNA sequence and reported that "[c]onsistently, Campylobacter with identical *flaA* SVR DNA sequences were found in broilers and in human disease." Ex. G-771; WDT G-1475: P12, L41-42. The researchers also detected a temporal relationship between the time of isolation of particular genotypes from broilers and the successive appearance of those genotypes in human disease. WDT G-1475: P12, L39-41; Ex. G-771. Using PFGE and *fla* typing, Fitzgerald (United Kingdom) found that turkeys were a reservoir of *Campylobacter* subtypes found in human clinical isolates. WDT G-1470: P9, L8-11; Ex. G-218: P3, P5.

A comparison of serotypes in the Netherlands of human and animal isolates of *C. jejuni* showed that four of the five most common types present in human isolates were also common in poultry and that there was little overlap between human and swine serotypes. Ex. G-1698: P4; WDT G-1475: P11, L44 - P12, L6. A study in Denmark observed a large overlap of in distribution of *Campylobacter* serotypes between human serotypes and serotypes found in broiler

[27] Studies with similar findings include: (a) an MLST study of *C. jejuni* isolates from humans and animals mainly from the United Kingdom or the Netherlands that found an overlap between subtypes found in chicken and human disease isolates, Ex. G-1629: P5; (b) a study in the Netherlands using AFLP analysis that found an overlap between strains from poultry and strains from human patients, Ex. G-176: P5; and (c) a study in Canada using PFGE to test broiler chicken *Campylobacter* isolates and isolates from sporadic cases of human *Campylobacter* illness within the same geographic area that found 20 percent of the human isolates had genotypes that were genetically related to poultry genotypes, WDT G-1457: P5, L33-35; Ex. G-1684.

chicken and cattle isolates. WDT G-1457: P5, L17-22; Ex. G-459: P8. The study explained that the results initially suggest that both cattle and broilers could be important sources of *Campylobacter* infection in humans. WDT G-1457: P5, L17-22; Ex. G-459: P8. However, when taking into consideration the high prevalence of *Campylobacter* in retail poultry in Denmark, the study concluded that poultry (as opposed to cattle) is likely to be a major source of campylobacteriosis. Ex. G-459: P8. A later study in Denmark of *C. jejuni* isolates also found an overlap in genotypes between poultry and human *Campylobacter* isolates. WDT G-1457: P5, L36-39; Ex. G-494: P5.

Researchers in Taiwan conducted an evaluation specifically of quinolone-resistant *Campylobacter* isolates from poultry and from humans. WDT G-1457: P5, L26-28; Ex. G-1775: P2. When combining PFGE and *flaA*-RFLP methods, the study found that the quinolone-resistant genotypes that were identified in nearly 40 percent of the human isolates were also identified in the poultry isolates. WDT G-1457: P5, L29-30; Ex. G-1775: P3-4. As implied by the data from Taiwan, "nearly 40% of [quinolone-resistant] human isolates may derive from poultry sources[,]" and the molecular evidence shows that "domestic poultry products are important sources of human infections associated with quinolone-resistant campylobacters." Ex. G-1775: P4.

3. Temporal Data

Temporal data from ten countries -- Belgium, Iceland, Norway, Sweden, Finland, Australia, the Netherlands, Spain, the United Kingdom, and the United States -- provide additional support for the conclusion that poultry is a source of campylobacteriosis, specifically FQ-resistant campylobacteriosis. Intervention studies and data showing the correlation between enrofloxacin approval dates and the increase in FQ-resistant campylobacteriosis levels all support the epidemiological, microbiological, and molecular evidence of the role of poultry in the occurrence and rise of FQ-resistant *Campylobacter* infections in humans. The pattern of

public health consequences encountered in the United States is similar to that experienced in other countries.

a. Intervention Studies

Intervention studies, which may be intentional or result from a natural event, present an opportunity to assess a cause-and-effect relationship by modifying a risk factor in the population. Interventions primarily or exclusively aimed at poultry have reduced the incidence of human *Campylobacter* infections by 40 to 70 percent. WDT G-1483: P20, L4-8. Three national intervention studies in Belgium, Iceland, and Norway document that in these countries poultry, specifically chicken, constitutes a major source of human campylobacteriosis.

For a four-week period in 1999, all Belgian chicken and eggs were withdrawn from the Belgian market because livestock had been given dioxin-contaminated feed. WDT G-1457: P4, L29-31; Ex. G-672. Belgium's 40 percent decline in campylobacteriosis lasted four weeks, which coincided precisely with the same four-week period that Belgian poultry was unavailable for consumption. WDT G-1457: P4, L31-36; WDT G-1483: P18, L16-24; Ex. G-672: P2-3. After Belgian poultry returned to the market, the level of *Campylobacter* infections returned to "normal." Ex. G-672: P3; WDT G-1483: P18, L17-24.

Iceland experienced a sharp increase in human *Campylobacter* infections between 1997 and 1999, which coincided with an increase in the marketing of fresh, rather than frozen, chicken products. WDT G-1483: P18, L25-27. Iceland implemented a control program to test flocks for *Campylobacter* a week prior to slaughter; chicken products from *Campylobacter*-positive flocks were frozen before sale, which is known to reduce (but not eliminate) contamination with *Campylobacter*. WDT G-1483: P19, L3-4. Following introduction of Iceland's strategy in early 2000, the incidence of domestically acquired campylobacteriosis was reduced by approximately 70 percent. WDT G-1483: P19, L4-13; WDT G-1475: P17, L29-39; Ex. G-791.

Norway initiated a *Campylobacter* action plan in 2002 based on the same principles as Iceland's plan of freezing *Campylobacter*-contaminated poultry before sale. WDT G-1483: P19, L14-15. After implementation, Norway observed a nearly 50 percent reduction in domestically acquired campylobacteriosis in the first 39 weeks of 2002 compared to the same time period in 2001. WDT G-1483: P19, L15-L18.

b. Increase in FQ-resistant Campylobacteriosis after Enrofloxacin Approval

There is a commonly observed relationship between introduction of enrofloxacin and an increase in FQ-resistant *Campylobacter* isolated from humans. This relationship indicates that FQ use in poultry is the source of FQ-resistant *Campylobacter* infection in humans. Many countries, including the United States, have experienced a temporal association between the approval of poultry FQs and a rise in FQ-resistant *Campylobacter* infection levels in humans. WDT G-1473: P15, L45 - P16, L4; WDT G-1458: P8-9; WDT G-1451: P4 (Fig.1); WDT G-1457: P7, L19 - P9, L6; Ex. G-191: P4-6; Ex. G-586: P1-4. The correlation between enrofloxacin use and FQ resistance rates has been acknowledged by Bayer. Ex. B-454 (admitting, in a 1997 e-mail between Bayer employees, that "[r]esistance rates in Denmark are low because relatively few [sic] Baytril is being used").

In many countries, the introduction of FQs (specifically enrofloxacin) in veterinary medicine (specifically for poultry use) has been followed by an increase in FQ-resistant *Campylobacter* in humans. The increase begins shortly after the licensing of enrofloxacin for use in poultry. These countries experience the emergence of increasing levels of FQ resistance at different times; the varying times of onset are consistently associated in each country with that country's licensing of FQs for food animals. These observations strongly support the conclusion that veterinary use of FQs in poultry, and not the medical use of FQs in humans, is the driving force behind the rise in FQ-resistant *Campylobacter* infections in humans. WDT G-1483: P23,

L5-15; WDT G-1451: P4, L28-32; WDT G-1473: P16, L4-12. Countries in which enrofloxacin has not been introduced for use in poultry have not undergone a similar sequence of events.

Countries without enrofloxacin

Finland, Australia, and Sweden report low levels of FQ-resistant *Campylobacter* in indigenous (not associated with foreign travel) infections in humans. WDT G-1457: P8, L45 - P9, L6; WDT G-1470: P7, L16-19; Ex. B-421; Ex. B-255; Ex. G-66; WDT 1458: P3-4; Ex. G-524; Ex. B-934; Ex. G-579. FQ-resistant *Campylobacter* infections in these countries have been linked to foreign travel, and in some cases, specifically to travel to countries bearing high levels of FQ-resistant campylobacteriosis. WDT G-1457: P9, L2; Ex. G-66; WDT G-1458: P3-4; Ex. G-524: P2-3; Ex. G-578: P3. In each of the three countries, ciprofloxacin had been registered for use in humans since the late 1980s (1987 in Finland and Australia; 1988 in Sweden, RJS 54, 66, 64), and has not caused a pattern of increasing FQ-resistant *Campylobacter* infections.

Countries with enrofloxacin

FQ-resistant *C. jejuni* was first recognized in Europe in the late 1980s and was attributed to the introduction of FQs for veterinary use. The Netherlands, Spain, and the United Kingdom licensed enrofloxacin for use in poultry in 1987, 1990, and 1993, respectively. Prior to enrofloxacin licensing in each respective country, there was little or no FQ resistance detected in *Campylobacter* isolates from humans. The Netherlands and Spain experienced an increase in FQ-resistant *Campylobacter* infections within one to two years after enrofloxacin licensing. In the United Kingdom, data gathered from 3.5 to four years after licensing of enrofloxacin show an increase in FQ-resistant campylobacteriosis. Further, these countries also found that the level of FQ resistance in *Campylobacter* isolated from humans continues to rise after the emergence of the initial signature increase. The United States is experiencing the same pattern of increase in FQ-resistant *Campylobacter* in humans.

Enrofloxacin was registered for poultry use in the Netherlands in 1987. RJS 61. Before 1987, FQ-resistant *Campylobacter* was absent in poultry and humans. WDT G-1457: P7, L30-33. In 1988/1989, FQ resistance was observed in poultry and human strains. WDT G-1457: P7, L37-38. By 1989, the prevalence of FQ-resistant *Campylobacter* rose to 14 percent in isolates from poultry and 11 percent in isolates from humans. WDT G-1457: P7, L37-39; Ex. G-190: P4. In demonstrating the link between the introduction of enrofloxacin and the increase in FQ-resistant *Campylobacter* in poultry and humans, this study ruled out the possibility that another veterinary quinolone (flumequine) or human FQs (e.g., ciprofloxacin or norfloxacin) could have been responsible for the development of FQ resistance in poultry and humans as described above. WDT G-1457: P7, L39 - P8, L12; Ex. G-190: P7-8. One report from the Netherlands indicated that, by 1997, the proportion of FQ-resistant *Campylobacter* from human isolates rose to 29 percent. Ex. G-586: P2.

In Spain, enrofloxacin was registered for poultry use in October 1990; ciprofloxacin was registered in 1988. RJS 63. Before 1990, the prevalence of FQ-resistant *Campylobacter* in humans was between zero and three percent. WDT G-1457: P8, L16-17. Since approval of enrofloxacin, the level of FQ resistance in Spain skyrocketed in the presence of widespread use. The sharpest increase in FQ resistance occurred in the first year after enrofloxacin was introduced. WDT G-1457: P8, L19-20. Two studies of samples from a mainly pediatric population determined that ciprofloxacin-resistant *C. jejuni* rose to 13 percent in 1990 and to 30 percent in 1991, Ex. G-529: P2, and to 48 percent in 1993, Ex. G-532: P2. Another study with a majority of samples from pediatric cases determined that ciprofloxacin-resistant *C. jejuni* rose to 51 percent in 1991. Ex. G-557: P3. Because quinolones are not recommended for use in the pediatric population, Ex. G-557: P3, human quinolones are unlikely to have caused the striking increase in ciprofloxacin resistance among *C. jejuni* isolates. A later study in Spain revealed that

ciprofloxacin resistance in *C. jejuni* isolates rose to 63 percent in 1992, 73 percent in 1993, and 88 percent in 1994. Ex. G-544: P2. See also Ex. G-491; Ex. G-671; Ex. G-734; Ex. G-549: P2.

In the U.K., enrofloxacin was registered for poultry use in November 1993; ciprofloxacin had been registered since February 1987. RJS 65. There was no emergence in human *Campylobacter* isolates of increasing ciprofloxacin resistance associated with ciprofloxacin approval. Between 1991 and 1994, the United Kingdom studies reported low levels (under five percent) of ciprofloxacin resistance in *Campylobacter spp.* isolated from humans, and attributed some of the resistance to travel outside of the U.K. or to consumption of imported chicken. Ex. G-77; Ex. G-407; Ex. G-240; WDT G-1457: P8, L22-25. Of 5,401 isolates of *C. jejuni* collected from humans in England and Wales during 1997, 10.5 percent were resistant to ciprofloxacin. Ex. G-634: P2-3. The prevalence of ciprofloxacin-resistant *C. jejuni* isolated from humans in England and Wales increased to 19 percent between 2000 and 2001, and of these infections, 46 percent were not acquired through foreign travel. WDT G-1457: P8, L27-30; Ex. G-1772: P2-3.

The United States, too, has experienced a temporal relationship between the approval of FQs for use in poultry and the rise in the level of FQ-resistant *Campylobacter* infections in humans. WDT G-1470: P7, L11-14; WDT G-1473: P20, L13-15. U.S. studies nationwide as well as state-specific have demonstrated this relationship. Veterinary FQs were approved in the U.S. in August 1995 (sarafloxacin) and October 1996 (enrofloxacin). RJS 47, 39. Prior to approval of enrofloxacin, there was little FQ resistance detected in isolates from human *Campylobacter* illness in the U.S. Ever since enrofloxacin approval, the level of FQ resistance in human isolates of *Campylobacter* in the United States has been rising, as discussed below.

The U.S. historically experienced a lack of resistance to nalidixic acid, an analog of the chemical building block of the FQ class of drugs. WDT G-1453: P2, L24-25. In fact, resistance to nalidixic acid during the 1980s was rare enough that, in 1988, the use of nalidixic acid was

still recommended for the speciation of *Campylobacter spp.* WDT G-1453: P3, L1-12. A study published in 1988 found only two of 42 *C. jejuni* and none of 25 *C. coli* isolates from humans were resistant to nalidixic acid. WDT G-1453: P3, L3-6; Ex. G-1609: P3-4. Resistance to ciprofloxacin was even more uncommon. Ex. G-191: P5. In a national county-based study conducted between 1989 and 1990, only 1 of 332 *Campylobacter* isolates from humans was resistant to ciprofloxacin. Ex. G-624; Ex. B-589; Ex. G-1803.

Nachamkin's study susceptibility-tested 130 *C. jejuni* isolates and 12 *C. coli* isolates from patients with gastroenteritis who were seen between 1982 and 1992 in outpatient clinics at the University of Pennsylvania Medical Center. Ex. G-440: P2-3. None of those isolates was resistant to ciprofloxacin. WDT G-1470: P6, L31-36; Ex G-440: P3. A subsequent Nachamkin study susceptibility-tested 297 *C. jejuni* isolates collected from patients (mostly outpatients) treated between 1995 and 2001 within the University of Pennsylvania Health System. WDT G-1470: P6, L36-40; Ex. G-1490. The level of ciprofloxacin resistance dramatically increased from a low in 1996 of 8.3 percent to a high in 2001 of 40.5 percent. WDT G-1470: P6, L36-45; Ex. G-1490.

Smith found that the percentage of *C. jejuni* isolates from Minnesota residents that were resistant to nalidixic acid statistically significantly increased from a low of 1.3 percent in 1992 to a high of 10.2 percent in 1998. WDT G-1473: P7, L9-12; Ex. G-589: P3. When the Smith study focused on trends in domestically-acquired infections in Minnesota residents in 1996-1998, the increase of domestically acquired FQ-resistant *C. jejuni* was also statistically significant. WDT G-1473: P12, L17-24; G-589: P5. Smith determined that, at most, 15 percent of FQ-resistant *C. jejuni* infections could have been due to prior FQ therapy in humans during this time. WDT G-1473: P15, L15-30; Ex. G-589: P6.

The findings derived from Smith's 1996-1998 Minnesota data are particularly reliable and generalizable. According to Bayer witness Burkhart, Minnesota data on FQ-resistant *Campylobacter* from 1996-1998: (1) is a source of U.S. multi-year data; (b) derives from a well-defined denominator; (c) is not based upon non-random sampling of reported cases; and (d) has captured data on foreign travel and prior FQ use. WDT B-1900: P16, L40-44. The 1996-1998 Minnesota data "are probably the most robust multiyear dataset in the U.S., and perhaps the world, containing information on foreign travel and prior [FQ] use." WDT B-1900: P17, L12-14. Finally, the 1996-1998 Minnesota data "are likely to be the most valid data in the US that are available to study this issue [of increasing domestically acquired FQ-resistant *Campylobacter*]." WDT B-1900: P46, L1-2.

A national surveillance system has documented the substantial burden of ciprofloxacin-resistant *Campylobacter* in the United States population since approval of enrofloxacin. WDT G-1452: P8, L9-11, P17, L9-10; Ex. G-1487. Data from human NARMS[28] demonstrate that a high proportion (approximately one-fifth) of human *Campylobacter* isolates in the U.S. is resistant to ciprofloxacin. WDT G-1452: P8, L11, P9, L1-2; Ex. G-1487. Human NARMS further demonstrates that ciprofloxacin-resistant *Campylobacter* infection in the U.S. population is increasing. WDT G-1452: P7, L25 - P9, L13, P17, L17; WDT G-1468: P8, L25.

Human NARMS used a multivariate model to account for the regional variation and increasing population size in the surveillance program.[29] A multivariate statistical analysis

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[28] NARMS (National Antimicrobial Resistance Monitoring System) is a collaborative project among CDC, participating state health departments, USDA, and FDA. WDT G-1452: P3, L26-27, L36; Ex. G-749. Human NARMS began testing of *Campylobacter* isolates in 1997. Participating clinical laboratories isolate foodborne enteric bacteria usually from diagnostic specimens collected from ill persons; these isolates are ultimately forwarded to the CDC for susceptibility testing. WDT G-1452: P3, L32-33, L38-40, P7, L38-40.

[29] In human NARMS, the percent of *Campylobacter spp.* isolates resistant to ciprofloxacin was 13 percent (28 of 217) in 1997, 14 percent (48 of 345) in 1998, 18 percent (58 of 319) in 1999, 14 percent (46 of 324) in 2000, and 19 percent (75 of 387) in 2001. WDT G-1452: P8, L9-11; Ex. G-1487.

demonstrates that the proportion of human *Campylobacter* isolates in the United States resistant to ciprofloxacin was two and a half times higher in 2001 than it was in 1997. WDT G-1452: P8, L23-38, P9, L2-5; Ex. G-1487. The trend between 1997 and 2001 of an increasing prevalence of ciprofloxacin resistance among human *Campylobacter* isolates is statistically significant (95% CI = 1.4, 4.4).[30] WDT G-1452: P8, L35-38, P9, L3-6. Despite the lower risk of getting a *Campylobacter* infection in 2001 compared with 1996, the risk of getting an FQ-resistant infection has increased over approximately the same time period; therefore, the 27 percent decrease in *Campylobacter* incidence is more than outweighed by the 61 to 98 percent increase in proportion of *Campylobacter* infections that are FQ-resistant. WDT G-1468: P8, L39-42.

Other considerations

Similar to the respective experiences of Finland, Australia, Sweden, the Netherlands, Spain, and the United Kingdom, treatment of humans with ciprofloxacin is not responsible for the increasing level in the United States of FQ-resistant campylobacteriosis. Ciprofloxacin has been approved for use in the U.S. since 1987 without emergence of FQ resistance in *Campylobacter* in humans. Ex. G-191: P5. In fact, other quinolones (e.g., nalidixic acid) have been used in human medicine since the mid 1960s and have not caused a pattern of rising FQ-resistant *Campylobacter*. WDT G-1453: P2, L1-2. In the 1998-1999 FoodNet *Campylobacter* case-control study, Kassenborg did not find that FQ use in humans contributed directly to the observed FQ resistance. WDT G-1460: P6, L22 - P7, L4, P10, L22.

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[30] When restricting the multivariate logistic regression model to only *C. jejuni* isolates, the proportion of *C. jejuni* isolates resistant to ciprofloxacin in 2001 was 2.2 times higher (95% CI 1.2, 4.0) than the proportion of *C. jejuni* isolates resistant to ciprofloxacin in 1997. WDT G-1452: P8, L35-44; Ex. G-1487. No remarkable changes in the analysis were observed in either multivariate model (all *Campylobacter* or restricted to *C. jejuni*) when the cases from Connecticut (a state where the prevalence of ciprofloxacin resistance was high) were excluded from the model. WDT G-1452: P8, L46-47.

More fundamentally, humans are not natural reservoirs of *Campylobacter*. WDT G-1483: P20, L12-13. In the United States, as in other developed countries, human-to-human transmission of *Campylobacter* infection is low. WDT G-1470: P5, L13-15; Ex. G-1644: P13. This method of transmission simply could not account for the level of FQ-resistant *Campylobacter* infections observed in humans. Because of the absence of significant person-to-person transmission, it is reasonable to conclude that a "significant proportion of fluoroquinolone-resistant Campylobacter is reaching people via poultry." WDT G-1457: P9, L24-26.

C. FQ-resistant *Campylobacter* Infections in Humans Have the Potential to Adversely Affect Human Health

FQ-resistant *Campylobacter* infections in humans lead to compromised patient care and treatment failures. Patients who suffer from compromised care and experience treatment failures are adversely affected. The evidence in this section addresses the loss of the ability to empirically treat gastroenteritis, including campylobacteriosis, because of FQ-resistant *Campylobacter*; the increase in the duration of diarrhea associated with FQ-resistant *Campylobacter* infections; and a quantification of the number of people potentially adversely impacted by FQ-resistant *Campylobacter* in chicken. The evidence discussed in this section demonstrates the deleterious effect FQ-resistant *Campylobacter* infections can have on the public health.

1. Compromised Patient Care and Treatment Failures

Between 1.4 and 2.4 million people suffer from campylobacteriosis each year. WDT G-1452: P7, L5-14; Ex. G-410; Ex. G-1486. The symptoms of *Campylobacter* infections are similar to symptoms of other bacterial diarrheal diseases, such as those caused by *Salmonella*, *Shigella*, and some *E. coli* bacteria. WDT G-1477: P2 ¶3. Symptoms of these illnesses include

fever, headache, abdominal pain, and diarrhea, which can be bloody or dysentery-like. WDT G-1485: P6, L25-28; WDT G-1477: P2 ¶3.

Studies have demonstrated that antimicrobial treatment of gastroenteritis, including campylobacteriosis, is more effective if initiated early in the illness. WDT G-1469: P4, L22 - P5, L3; WDT G-1457: P6, L34-35; Ex. G-707; Ex. B-1127. For this and other reasons, most physicians initiate treatment to mitigate symptoms and decrease associated complications without the results of a stool culture to identify the cause of a patient's diarrheal illness. WDT G-1477: P2-3 ¶4; WDT G-1485: P8, L14-16, L19-33. This "empiric treatment" is the opposite of "directed therapy" or therapy where the exact causative agent is known. WDT G-1485: P11, L34-36. Bayer witness Pasternack testified that empiric treatment for community-acquired enteritis has become commonplace over the past 15 years. WDT B-1909: P18, L10-11.

Results of a recent survey conducted in Maryland confirmed that most physicians do not order a stool culture. In that survey only 43% of physicians seeing patients for diarrhea reported that they ordered a stool culture for their most recent patient. WDT G-1469: P5, L12-15. Even if a physician does order a stool culture, most will start therapy with an antibiotic before the results are available. WDT G-1485: P10, L28-30.

Although most physicians and treatment guidelines agree that patients with mild symptoms of enteritis presumed to be bacterial do not require antimicrobial treatment, many patients do require treatment with antimicrobials. WDT G-1485: P10, L1-7, P11, L11-18. *Campylobacter* infections can incapacitate otherwise healthy adults. WDT G-1469: P4, L9-12. In particularly vulnerable patients, the course of illness is even more severe. WDT G-1477: P2 ¶3. Complications, including reactive arthritis, Guillain-Barré syndrome, and blood stream infections, can occur. Id.

Common criteria for the antimicrobial treatment of human *Campylobacter* infections 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. RJS 42; WDT G-1485: P11, L11-18. The objective of antimicrobial treatment for this group of people is to reduce the rate of recurrence and prevent complications. WDT G-1485: P7, L8-16.

Although macrolides, such as erythromycin, are considered by many to be the preferred treatment for known cases of campylobacteriosis, FQs are the preferred agents for empiric therapy because they are active against all major causes of bacterial diarrhea, and erythromycin is not effective for the treatment of other common causes of gastroenteritis, such as *Salmonella*, *Shigella*, or pathogenic *E. coli* bacteria. WDT G-1457: P6, L44 - P7, L3; WDT G-1477: P2 ¶4, P4 ¶10; WDT G-1469: P5, L21 - P6, L2. Bayer witness Iannini testified that the broad spectrum of activity offered by FQs makes these compounds attractive candidates for use where empiric therapy is indicated. WDT B-1905: P4, L3-4.

Ciprofloxacin is a drug of choice for empiric treatment of gastroenteritis. WDT G-1485: P13, L42-45. Commonly used treatment guidelines recommend the use of ciprofloxacin to empirically treat bacterial gastroenteritis. Id.; WDT G-1485: P11, L44 - P12, L7; WDT G-1477: P3 ¶6; WDT G-1469: P5, L18-23, P6, L2-5; Ex. G-261; Ex. G-244. Not only is ciprofloxacin effective against most bacteria causing gastroenteritis, including *C. jejuni*, WDT G-1477: P4, ¶10, it also has fewer side effects than erythromycin, including fewer gastric distress side effects, WDT G-1485: P12, L7-12, P13, L31-33, an important consideration when treating gastric illness. No other oral drug is currently available with comparable activity and toxicity profile. WDT G-1457: P7, L7-9.

There has been a significant increase in the number of *Campylobacter* isolates resistant to antibiotics. WDT G-1485: P14, L6-9. Patients infected with antimicrobial-resistant *Campylobacter* are less likely to benefit from treatment than patients infected with antimicrobial-susceptible *Campylobacter*. WDT G-1485: P14, L11-13. The increase of FQ-resistant *C. jejuni* makes treatment more difficult for infected patients and diminishes the opportunity for treatment that will reduce the severity and duration of their symptoms. WDT G-1485: P14, L38-42.

The FoodNet, Smith, and Neimann/Molbak studies all showed an increased duration of diarrhea in FQ-treated patients infected with FQ-resistant *Campylobacter* strains when compared to FQ-treated patients infected with FQ-susceptible *Campylobacter* strains. In these studies, FQs appear to be less efficacious against FQ-resistant *Campylobacter*, thus prolonging the diarrheal illness. WDT G-1452: P15, L12 - P16, L7; WDT G-1473: P20, L31-38; WDT G-1468: P19, L15-37; Ex. G-589; Ex. G-1489. The FoodNet study also showed an increased duration of diarrhea in patients with FQ-resistant *Campylobacter* infections when compared to patients with FQ-susceptible *Campylobacter* infections, but who were <u>not</u> treated with FQs. This additional finding suggests the possibility that FQ-resistant *Campylobacter* may have some intrinsic factor or factors that make them more virulent than FQ-susceptible *Campylobacter*. WDT G-1452: P16, L27-28. The mean or median increase in duration of diarrhea associated with an FQ-resistant *Campylobacter* infection found in the studies varies from one to six days depending on whether persons were treated with antidiarrheal medication and/or antimicrobial agents. The increase in duration of diarrhea for a particular patient with FQ-resistant *Campylobacter* infection may be higher.

Two researchers conducted three separate analyses on duration of diarrhea in patients enrolled in the 1998-1999 FoodNet *Campylobacter* case-control study. Two analyses were conducted by Nelson (nee McClellan) and one analysis was conducted by Marano. Nelson's

more recent analysis found that, compared to persons with an FQ-susceptible *Campylobacter* infection, persons with an FQ-resistant *Campylobacter* infection are likely to have diarrhea for a longer duration. WDT G-1452: P15, L12 - P16, L7; Ex. G-1489. Marano's analysis and Nelson's earlier analysis revealed similar results. WDT G-1468: P19, L23-33; Ex. G-394; Ex. G-780; Ex. G-1367. In Nelson's more recent analysis, of the 858 persons with a *Campylobacter* infection whose FQ susceptibility status was known, 740 persons were included. WDT G-1452: P15, L23. The mean duration of diarrhea was 8 days (range, 2 to 21 days) for the 82 (11%) persons with FQ-resistant *Campylobacter* infections and 7 days (range, 1 to 60 days) for the 658 persons with FQ-susceptible *Campylobacter* infections ($p = 0.1$). WDT G-1452: P15, L26-29; Ex. G-1489.

Nelson then conducted stratified analyses, based on treatment with antidiarrheal medication and antimicrobial agents. The mean duration of diarrhea among the 421 (57%) persons who did not take antidiarrheal medications (loperamide, diphenoxylate, or a prescribed antidiarrheal medication) for their illness was 9 days (range, 2 to 21 days) for the 39 patients with FQ-resistant *Campylobacter* infections and 7 days (range, 2 to 60 days) for the 382 patients with FQ-susceptible *Campylobacter* infections ($p = 0.05$). WDT G-1452: P15, L31-36; Ex. G-1489. The mean duration of diarrhea among the 67 of 421 (16%) persons not taking an antidiarrheal medication who also did not take an antimicrobial agent for their illness was 12 days (range, 8 to 20 days) for the 6 persons with FQ-resistant infections and 6 days (range, 2 to 21 days) for the 61 persons with FQ-susceptible infections ($p < 0.01$). WDT G-1452: P15, L36-40; Ex. G-1489. Of the 740 persons included in the analysis, the mean duration of diarrhea among the 128 (17%) persons who took FQs and no other antimicrobial agent or antidiarrheal medication for their illness was 8 days (range, 3 to 14 days) for the 17 patients with FQ-resistant

infections and 6 days (range, 2 to 31 days) for the 111 patients with FQ-susceptible infections (p = 0.08). WDT G-1452: P15, L42-46; Ex. G-1489.

The Smith study showed that the duration of diarrhea was statistically significantly longer for patients infected with quinolone-resistant *C. jejuni* than for patients with quinolone-sensitive *C. jejuni*. WDT G-1473: P10, L32-34. Specifically, the Smith study found that FQs were not as effective in treating patients with quinolone-resistant infections as they were in treating patients with quinolone-sensitive infections. WDT G-1473: P10, L35-37. Among patients who were treated with an FQ after the collection of stool specimens, the duration of diarrhea was statistically significantly longer for the patients with quinolone-resistant *C. jejuni* infections (median, 10 days) than for the patients with quinolone-sensitive *C. jejuni* infections (median, 7 days) (p = 0.03). WDT G-1473: P10, L31-35; Ex. G-589: P5.

Smith determined that, in his study population, FQs were the most popular choice of antibiotics for treating patients with *Campylobacter* infections, including those with quinolone-resistant infections. WDT G-1473: P10, L21-22; Ex. G-589. Overall, 110 of the 130 patients (85%) with resistant *C. jejuni* infections were treated with an antibiotic. WDT G-1473: P10, L22-25. Sixty-nine of 106 patients (65%) with resistant *C. jejuni* infections who received antibiotic treatment received an FQ. WDT G-1473: P10, L25-26. Thus, many patients with quinolone-resistant infections suffered a significantly longer course of illness because the antibiotic provided to them (an FQ) did not work against resistant *C. jejuni*. WDT G-1473: P10, L38-40.

The Neimann/Molbak case-control study also showed an increased duration of diarrhea for persons with FQ-resistant *Campylobacter* infections. WDT G-1468 P19, L23-33; Ex. G-455. Of the patients who received treatment with FQs, 5 patients were infected with a FQ-resistant *Campylobacter* strain and 31 patients were infected with a FQ-susceptible *Campylobacter* strain.

The median duration of diarrhea among FQ-treated patients was 14 days for patients with resistant infections and 9 days for patients with susceptible infections ($p = 0.13$). WDT G-1468: P19, L23-33.

A recent study from the United Kingdom compared cases infected with FQ-resistant *C. jejuni* to cases infected with antimicrobial-sensitive *C. jejuni*. WDT G-1468: P19, L37-40; Ex. G-1772. The analysis, which was stratified by foreign travel, found that there was no difference with regard to the mean duration of illness between cases infected with ciprofloxacin-resistant strains and cases infected with strains sensitive to all antimicrobials. WDT G-1468: P19, L37-40; Ex. G-1772. But the results of this study cannot be compared with the FoodNet, Smith, and Neimann/Molbak studies because the U.K. analysis was not stratified by any treatment, including FQ treatment, and does not detract from their findings. WDT G-1468: P19, L37-38, P20, L1-2.

Bayer argues that, in case-control studies analyzing the duration of diarrhea, foreign travel is a confounder for FQ-resistant *Campylobacter* infections. Tr. P292, L16-20. But foreign travel is not a confounder, as demonstrated by both Smith and Nelson. By definition a confounder must be both (1) an independent risk factor for the outcome and (2) associated with the exposure. Tr. P462, L15-16. In the Smith study, foreign travel was not statistically significantly associated with duration of diarrhea and, therefore, did not meet the first criterion for being a confounder. Tr. P559, L3-7. Thus, people who acquired infections during foreign travel should not have been (and were not) excluded as confounders from the analysis. Id.

Nelson included antidiarrheal medication in a multivariate model to account for the strong effect of antidiarrheal medication on diarrhea. Tr. P463, L7-14. The inclusion of antidiarrheal medication in the model revealed that foreign travel is not a confounder. Tr. P466,

L2-4. Foreign travel is "not an independent risk factor for the outcome [i.e., the duration of diarrhea] . . . it's just a proxy for taking antidiarrheal medication." Tr. P464, L8-10.

Further, as described above in the FoodNet case-control study, the mean duration of diarrhea among the 67 persons not taking an antidiarrheal medication who also did not take an antimicrobial agent for their illness was 12 days for persons with FQ-resistant infections and 6 days for persons with FQ-susceptible infections ($p<0.01$). None of the 67 persons traveled internationally. Tr. P465, L3-5. Clearly then, international travel could not have had any effect on this analysis, which found a marked difference in duration of diarrhea between people having FQ-resistant infections compared with people having FQ-susceptible infections. Tr. P465, L5-10.

Antibiotic therapy for *Campylobacter* enteritis significantly reduces the chances that a patient will have a relapse. WDT G-1485: P13, L14-16. Relapse of illness occurs in about 5-10% of persons who do not receive treatment. WDT G-1477: P2 ¶3. Moreover, at least one expert has suggested that antibiotic therapy, which shortens the duration of illness, might decrease the stimulation of the immune system and prevent some complications. WDT G-1475: P4, L31-40.

The duration of a person's *Campylobacter* illness may be related to the likelihood of developing reactive arthritis. See WDT G-1475: P4, L13-29. Up to 1.7% of persons with *Campylobacter* infections develop an acute arthritis that affects the joints of the ankles, knees, and hands. WDT G-1475: P4, L15-19. The arthritis may last for weeks or months. WDT G-1475: P4, L15-18. The 2001 Neal study in the United Kingdom found that 13% of persons with *Campylobacter* infections developed symptoms of reactive arthritis. WDT G-1475: P4, L19-22. Neal found that the arthritis symptoms were correlated with the duration of gastroenteritis. WDT G-1475: P4, L23-24. Persons with reactive arthritis were 2.7 times more likely to have had acute

gastroenteritis that lasted more than 15 days than persons without joint symptoms; this difference was statistically significant. WDT G-1475: P4, L24-25. This study suggests that prolonged illness increases the risk that a person will develop reactive arthritis. WDT G-1475: P4, L27-28.

FQ-resistant *Campylobacter* infection is potentially life-threatening for patients who have a higher risk of complications from *Campylobacter* enteritis. WDT G-1485: P14, L42-44. Because physicians presume that the empiric treatment of bacterial enteritis with ciprofloxacin will be effective, WDT G-1485; P15, L19-23, ciprofloxacin-resistant *Campylobacter* dramatically complicates treatment, WDT G-1485: P15, L35-37. CVM witness Thielman summarized this well, stating: "Failure of *Campylobacter* infections to respond to a fluoroquinolone antibiotic can be devastating; not only may the illness be prolonged, but patients may then be prone to further complications including death." WDT G-1477: P4-5 ¶11.

Because most physicians do not wait the few days to one week necessary to identify the bacteria causing the patient's symptoms before initiating antimicrobial therapy, see WDT G-1469: P5, L5-6, it is important to preserve the ability to use an effective, broad spectrum antimicrobial, such as ciprofloxacin, for the empiric treatment of suspected bacterial gastroenteritis. In some countries physicians appear to have already lost the ability to use empiric treatment because of the high level of FQ-resistant campylobacteriosis. See WDT B-1906: P11, L3-5. The rise in the level of FQ-resistant *Campylobacter* infections is threatening the ability of physicians to use this important drug to treat gastroenteritis empirically, and increasing the potential adverse human health effects.

2. Quantification of the Potential Adverse Health Effects of FQ-Resistant *Campylobacter*

On January 5, 2001, after a public review and after accommodating industry concerns, CVM released its revised final risk assessment report [31] entitled "The Human Health Impact of Fluoroquinolone Resistant Campylobacter Attributed to the Consumption of Chicken." Ex. G-953. This report describes a risk assessment model used by CVM to estimate the likelihood of human health impact of FQ-resistant *Campylobacter* from FQ-resistant *Campylobacter* on poultry. On the basis of the risk assessment model, CVM estimated that, in 1998, the mean number of people in the United States who acquired FQ-resistant *Campylobacter* infections associated with consumption of chicken and who subsequently received treatment with a FQ was 8,678 people and, in 1999, this number was 9,261. Ex. G-953: P63-64. The risk assessment's 5th and 95th percentile (the lower and upper) estimates for 1998 were 4,758 and 14,369, respectively, and for 1999 were 5,227 and 15,326, respectively. Ex. G-953: P63-64. The implication of the mean estimate is that, in a year like 1999, it would be expected that approximately 9,261 people could receive ineffective medical treatment for their campylobacteriosis and suffer a longer, more severe illness because of FQ-resistant *Campylobacter* attributed to the use of FQs in poultry.

a. Calculations in CVM's Risk Assessment

In part at the suggestion of the animal drug industry, CVM developed a quantitative risk assessment model to estimate the risk to human health from antibiotic-resistant foodborne pathogens associated with the domestic use of antimicrobials in food producing animals. WDT G-1478: P15, L39 - P16, L5. Specifically, CVM developed a model to relate the prevalence of FQ-resistant *Campylobacter* infections in humans associated with the consumption of chicken to the prevalence of FQ-resistant *Campylobacter* in chicken. Ex. G-953: P6.

In calculating an estimate of the human health impact of FQ-resistant *Campylobacter* attributed to the consumption of chicken in the United States, the model uses several valid, available data sets from various sources. The model starts with data on the United States population and FoodNet enteric/invasive disease data to determine the nominal mean culture-confirmed cases of campylobacteriosis reportable to health departments in the United States. Ex. G-953: P23, Table O.1. Then, the model calculates the probabilities that: (a) a person with campylobacteriosis seeks medical care; (b) the individual is requested to provide a stool sample; (c) the individual provides a stool sample; (d) the stool sample is tested; and, (e) the stool sample is positive for *Campylobacter*. Id.

Next, the model calculates the probability that a *Campylobacter* case is attributable to chicken and the probability that a *Campylobacter* case that is attributable to chicken is an FQ-resistant case. Ex. G-953: P24, Table O.1. Once those probabilities are estimated, the model determines the probability that an individual with FQ-resistant campylobacteriosis will seek medical care. Id. The risk assessment then models the probability that a person with FQ-resistant campylobacteriosis attributable to chicken who seeks care is prescribed an antibiotic, and that the antibiotic is an FQ. Id. Ultimately, the risk assessment calculates the nominal mean number of FQ-resistant campylobacteriosis cases attributable to chicken, that seek care, and that are prescribed an FQ. Id.

CVM's risk assessment also models the prevalence of *Campylobacter* on broiler carcasses and the subsets associated with that prevalence, including the prevalence of FQ-resistant *Campylobacter*. Id. After also modeling the total U.S. per capita consumption of boneless, domestically reared chicken (in pounds), the risk assessment ultimately comes up with the total

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[31] The previous version of CVM's risk assessment was published on October 18, 2000.

consumption of boneless, domestically reared chicken (in pounds) contaminated with FQ-resistant *Campylobacter* in the United States. Id.

The model then calculates the ratio between the: (1) nominal mean number of FQ-resistant campylobacteriosis cases in the United States attributed to chicken, who sought care and who were treated with an FQ ("the outcome"); and (2) total consumption of boneless, domestically reared chicken (in pounds) contaminated with FQ-resistant *Campylobacter* ("the exposure"). Ex. G-953: P16. The ratio, a proportionality constant identified by the symbol K_{res}, makes it possible to predict the human health impact under certain hypothetical conditions. Id. K_{res} represents the probability that a pound of FQ-resistant *Campylobacter*-contaminated chicken meat will result in a case of FQ-resistant campylobacteriosis. Ex. G-953: P76.

b. CVM's Predictive Model is Appropriate for its Needs

In general, the purpose of conducting a risk assessment is to provide decision-makers with information, WDT G-1480: P2, L20-23, and to answer questions about an existing or hypothetical hazard, WDT G-1479: P2, L8-9. According to the Society for Risk Analysis, risk analysis uses informed judgment and plausible interpretations in the process of making predictions about the unknown. WDT G-1480: P2, L29-47.

There is no single process for conducting a risk assessment, WDT G-1479: P2, L9-10; i.e., all risk assessments do not need to follow a rigid formula. Thus, Bayer's and AHI's concerns about following certain required steps in risk analysis are unfounded. The specific risk assessment model to be used depends on the question being asked. WDT G-1479: P2, L10-11, L24-26. Put another way, the form of the risk assessment is driven by the decision-makers' needs. WDT G-1480: P3, L33-35. The tendency in risk analysis is to use the simplest approach consistent with the level of precision needed, WDT G-1479: P2, L38-39, which is exactly what CVM did.

CVM needed a risk assessment model that could consistently address complicated antimicrobial resistance risk issues. WDT G-1480: P5, L47 - P6, L3. In light of these issues, CVM wanted a risk assessment model that could easily and regularly be monitored to ensure the model's accuracy and validity. Ex. G-953: P7-8. CVM chose to construct a risk assessment model with parameter values and structural assumptions that could be readily verified by comparing predicted and observed values. Ex. G-953: P9, P75.

CVM also sought to construct a risk assessment model that could predict how the level of FQ-resistant *Campylobacter* infections in people would change if there were a reduction in the levels of FQ-resistant *Campylobacter* in poultry. WDT G-1480: P5, L41-47. CVM required a method that could be easily updated with new, federally-collected, scientific data on the number of FQ-resistant *Campylobacter* infections in people and the number of those ill people who were likely to be treated with a FQ. WDT G-1480: P5, L47 – P6, L3.

CVM created a predictive risk assessment model that suits its needs and serves its public health responsibilities. "[F]or any prevalence of resistant *Campylobacter* on carcasses, the model predicts the resultant human health impact." WDT G-1480: P6, L15-17. In other words, CVM's model can predict how changes in the amount of consumption and the prevalence of *Campylobacter* contaminated meat will impact future levels of human health. WDT G-1480: P8, L7-10. CVM's model allows updates of data and resulting risk estimates annually and, therefore, can track changes in risk. WDT G-1480: P10, L36-38; Ex. G-953: P10.

CVM's model is not a farm-to-fork risk assessment model for several reasons. First, a farm-to-fork analysis would have resulted in greater data gaps because such analysis would require numerous assumptions that could not be supported by data. WDT G-1480: P10, L26-29; WDT G-1479: P17, L8-17. Second, a farm-to-fork analysis would be very costly to maintain because new studies and data would be required to reflect changes in husbandry, transportation,

processing, and human behavior. WDT G-1480: P10, L30-32. Third, farm-to-fork analysis requires a dose-response model. WDT G-1480: P10, L33-35. A dose-response model was inappropriate for CVM's purposes because there is not sufficient dose-response data on *Campylobacter* and because there is no generally agreed upon analysis. WDT G-1480: P10, L33-35; WDT G-1479: P17, L8-10. Fourth, although CVM's model updates the K (pounds of FQ-resistant *Campylobacter*-contaminated poultry at slaughter, WDT G-1480: P7, L10) and p (proportion of FQ-resistant *Campylobacter* cases that fail to benefit from treatment, WDT G-1480: P7, L45-46) values annually so that changes concerning the consumer's risk per pound of contaminated meat can be tracked, WDT G-1480: P10, L36-38, the complexity of a full microbial food safety risk assessment requires considerably more data in terms of both its structure and model parameter values for validation. Ex. G-953: P10. Finally, CVM did not need a model that would pose reduction strategies and estimate effects of such strategies over which CVM has no authority or control, e.g., a farm-to-fork risk assessment. WDT G-1480: P5, L43-45; P10, L5-8. CVM has limited options in the case of an approved application for a new animal drug (i.e., CVM can withdraw the approval of the new animal drug application or prohibit extra-label use), WDT G-1480: P5, L43-45, as well as in the case of a pending application for a new animal drug (i.e., CVM can approve the application under specific use restrictions or deny the application). A more intricate risk assessment model, such as a farm-to-fork model, would not have yielded additional information that was useful to CVM and would have increased the likelihood of error in the model's calculations. WDT G-1480: P10, L6-8, 10-13, 26-29, 33-35.

c. CVM's Risk Assessment Model Used Reasonable Assumptions

The assumptions made in CVM's risk assessment were reasonable, valid, and well explained. All assumptions and limitations of the data used in the model are explained in CVM's risk assessment. Ex. G-953: App. B.

CVM's risk assessment assumes that, after removing FQ-resistant *Campylobacter* cases associated with prior FQ use and foreign travel, the remaining FQ-resistant *Campylobacter* cases are attributable to chicken. Ex. G-953: P103; WDT G-1454: P9, L18-33. The validity of this assumption is supported by scientific literature concerning poultry as a source of *Campylobacter* and the implausibility of other possible scenarios. Ex. G-953: P56-57. In fact, because CVM's risk assessment does not account for cases of campylobacteriosis from secondary chicken contamination, the model is likely to underestimate the overall chicken-related risk of acquiring a *Campylobacter* infection. Ex. G-953: P102.

The Rosenquist article indicates that the risk managers in Denmark initiated a risk assessment focusing on *Campylobacter* in chicken products because of an increase in the number of human enteric infections of campylobacteriosis. Ex. G-1788: P2. Their decision to conduct a risk assessment focusing on *Campylobacter* in chicken was based on the prevalence of *Campylobacter* in retail chicken and the results of eight case-control studies demonstrating that eating and/or handling chicken were key risk factors for campylobacteriosis. Ex. G-1788: P2. In addition to the eight case-control studies cited by Rosenquist, a number of other studies, as discussed above, support the conclusion that poultry is the most important source of human campylobacteriosis. WDT G-1483: P11, L1-4; WDT G-1457: P4, L12-13, 15-17; WDT G-1457: P4, L27 - P5, L39.

CVM's risk assessment assumes that there is a constant proportionality between the pounds of contaminated poultry meat produced a year and the expected rate of human illnesses. WDT G-1480: P7, L30-34. This assumption is reasonable. The draft risk assessment from Denmark demonstrated the exact proportionality that CVM assumed, i.e., if the prevalence of contaminated meat increases by some factor, then the incidence of human illness will also

increase by that factor. WDT G-1480: P7, L34-40. This analytical corroboration offered CVM additional assurance that the assumption of constant proportionality is valid.

CVM's model is based on a linear assumption, i.e., that the risk to human health is directly proportional to the pounds of contaminated chicken consumed. CVM's model thus assumes that there is a causal relationship between the amount of chicken contaminated with *Campylobacter* consumed and incidence of campylobacteriosis and, similarly, between the amount of chicken contaminated with FQ-resistant *Campylobacter* consumed and the incidence of FQ-resistant *Campylobacter* in humans. Ex. G-953: P81-83. Even Bayer witness Cox originally agreed that CVM's linear assumption was plausible. Tr. P1089, L1-8. Furthermore, during cross-examination, Cox agreed with CVM's assumption that *Campylobacter* illnesses are not related to the microbial load distribution but to the prevalence of contaminated chicken carcasses, i.e., the linear relationship between the prevalence of contaminated chicken carcasses and number of *Campylobacter* illnesses. See Tr. P972, L20 - P975, L7.

d. Data Used in CVM's Risk Assessment are Robust

CVM's model used data compiled by federal agencies to estimate the number of people adversely affected by FQ-resistant *Campylobacter*. Ex. G-953. CDC used prevalence data from its FoodNet surveillance system to provide CVM with an estimate of the number of cases of campylobacteriosis that were reported in the United States each year. WDT G-1454: P5, L38-43. The number of laboratory-diagnosed *Campylobacter* cases reported to public health officials represents but a fraction of the many *Campylobacter* infections that occur in the United States. WDT G-1452: P6, L47 - P7, L2. To calculate the total number of *Campylobacter* infections, CDC adjusted the FoodNet incidence to account for underreporting. Id. at P7, L4-14. Furthermore, the human isolates sent to CDC for *Campylobacter* testing as part of NARMS

constituted a statistically valid subset of the culture-confirmed cases reported to FoodNet. WDT G-1454: P6, L11-14.

Data on the amount of chicken consumed were retrieved from the USDA Economic Research Service. WDT G-1454: P6, L17-18. Data on the proportion of resistant isolates in chicken were retrieved from the USDA Agricultural Research Service, which conducts susceptibility testing through the animal arm of NARMS, on *Campylobacter* isolated on chicken collected by the Food Safety Inspection Service. WDT G-1454: P6, L18-21; WDT G-1478: P9, L4-12.

CVM's model estimated the percentage of human cases of campylobacteriosis attributable to chicken by applying the best available data at that time. When CVM conducted its risk assessment, the Harris 1986 and Deming 1987 case-control studies were the best supported attributable fraction estimates available. WDT G-1454: P14, L25-26. CVM used the studies for input values to determine the proportion of all cases of campylobacteriosis attributable to chicken. WDT G-1454: P8, L13-15; Ex. G-268; Ex. G-162. In the Harris study, the population attributable fraction was 48.5%, and in the Deming study the population attributable fraction was 66.7%. Tr. P772, L18-20; Ex. G-268; Ex. G-162. CVM modeled uncertainty between those fractions because it did not know which fraction was the better estimate, and developed a distribution that had a mean value of 57% and that included lower and higher values than the fractions from the studies. Tr. P772, L21 - P773, L5.

Interestingly, Bayer witness Cox argues that CVM's 57% attributable fraction for cases of campylobacteriosis should be lower. WDT B-1901: P15, 20-22, 24, 36, 38, 55, 56, 58, and 77. But Cox's argument undermines his position that chicken consumption is not a significant risk factor for FQ-resistant *Campylobacter* cases among humans. As explained in CVM witness Bartholomew's testimony, if the proportion of <u>all</u> *Campylobacter* cases attributable to chicken in

the risk assessment goes down, then the proportion of FQ-resistant *Campylobacter* cases attributable to chicken in the risk assessment goes up. WDT G-1454: P15, L46 – P17, L32. This is because the estimates of FQ-resistant *Campylobacter* cases in the CVM risk assessment obtained by subtraction of other sources of resistance are likely to be nearer to the real values of resistance among humans attributed to FQ use in poultry than values for all *Campylobacter* cases. WDT G-1454: P15, L41- P17, L32. Given a known value for the proportion of resistance among humans attributable to FQ use in poultry, a lower attributable fraction for the total cases (both susceptible and resistant) would mean that the prevalence of resistance among human cases attributed to domestically-produced chicken is substantially higher than the estimate in CVM's risk assessment. WDT G-1454: P15, L50 - P16, L3.

e. CVM's Risk Assessment Process was Transparent

CVM produced draft versions of the risk assessment model that anyone with access to Microsoft Excel could run. WDT G-1480: P6, L26-28. CVM also placed downloadable versions of the risk assessment model on CVM's internet homepage. WDT G-1480: P6, L28-30. CVM clearly and openly stated the assumptions and limitations of the model. Ex. G-953: App. B. CVM provided a public comment period and held a conference to elicit comments concerning the assessment. WDT G-1480: P6, L30-33. CVM addressed the comments received in the final version of the risk assessment model and report. WDT G-1480: P6, L34-36.

VI. Enrofloxacin Under the Approved Conditions of Use in Poultry Has Not Been Shown to be Safe

The evidence summarized above meets CVM's initial burden to present evidence from which serious questions about the safety of Baytril use in poultry may be inferred, thus shifting to Bayer the burden to show that Baytril has been shown to be safe under the approved conditions of use in poultry.

A. Bayer's Attack on NARMS Data is Baseless

Bayer's attempts to diminish the import of or contradict the findings from human NARMS have been unavailing. Bayer has argued that NARMS cannot be generalized to the U.S. because, Bayer alleges: (1) the sampling scheme is not representative of the general population; (2) sampling is not done proportionately throughout the year to account for any seasonal variation in FQ-resistant *Campylobacter*; (3) NARMS does not direct the participating clinical laboratories to use specific isolation and identification techniques for *Campylobacter*; and (4) participating sites have not followed the NARMS protocol. Bayer is wrong on all counts. Regardless of any limitation inherent in public health surveillance systems in general or NARMS in particular, the human NARMS data provide a consistent and reliable collection of information on FQ resistance in *Campylobacter* infections in humans. Tr. P437, L1-5. Human NARMS data can be generalized to the United States population, and they show that the prevalence of FQ-resistant campylobacteriosis in the United States is increasing. Tr. P437, L8-11.

Because NARMS is conducted within the FoodNet sites, WDT G-1452: P3, L46-47, Bayer's focus on NARMS generalizability lies in the representativeness of FoodNet. FoodNet surveillance data are generalizable to the U.S. population for the purpose of understanding the epidemiology of foodborne illness. WDT G-1452: P4, L21-26; Ex. G-769. This statement has been borne out by continuing CDC evaluations of FoodNet to estimate the similarity between the participating and non-participating sites and to assess the generalizability of FoodNet data. Tr. P311, L13-21; Tr. P315, L3-7; Tr. P320, L10-20; Tr. P322, L2 - P323, L1; Tr. P325, L8-12. It is disingenuous for Bayer to allege that FoodNet is not an accurate portrayal of *Campylobacter* infections in the U.S. population when it is the FoodNet data on which Bayer relies to assert that *Campylobacter* incidence has declined in the U.S. by 27 percent between 1996 and 2001. See Bayer PFOF 648, 655 (filed Mar. 17, 2003).

Furthermore, the NARMS sampling scheme permits the extrapolation of data to the United States. Tr. P358, L10-17. The prevalence of FQ-resistant *Campylobacter* observed in NARMS is "a representation of the national prevalence of [f]luoroquinolone-resistant Campylobacter." Tr. P362, L13-14. Any seasonal variation in FQ-resistant *Campylobacter* is not believed to skew the approximation of NARMS data to the national prevalence of FQ resistance in *Campylobacter*. Cf. Tr. P382, L6-10, L13-15. For example, according to a Bayer witness, seasonal variation explains only a small proportion of the number of resistant isolates submitted by Minnesota to the human NARMS surveillance program, beyond what can be expected based on overall resistance measured for the state. WDT A-200: P24, L1-7.

It is immaterial that NARMS does not direct the participating clinical laboratories to use specific isolation and identification techniques for *Campylobacter*. All clinical laboratories that are CLIA[32]-certified (all clinical labs in the U.S. that receive reimbursement from the government are CLIA-certified) follow standard procedures for isolating *Campylobacter*. Tr. P343, L4-12. All CLIA-certified clinical laboratories also use CLIA-certified procedures for identifying *Campylobacter*. Tr. P344, L12-19. CDC has surveyed the NARMS sentinel laboratories and confirmed that they are following, and have followed, CLIA-certified procedures for isolation and identification of *Campylobacter*. Tr. P343, L18-21, P344, L21 - P345, L2.

Finally, Bayer assumes that, if a participating FoodNet site did not submit a *Campylobacter* isolate to NARMS, then the site did not follow the NARMS protocol. As CVM witness Angulo explained during cross-examination, however, it is reasonable to expect that a NARMS-participating clinical laboratory within FoodNet may not have had any *Campylobacter* isolates in a particular month and, therefore, would not have submitted any isolates to NARMS.

Tr. P373, L15-22. Simply put, if an isolate does not exist, then there is nothing to submit. Tr. P374, L3-7.

B. Bayer's Claim that Most FQ-resistant Campylobacteriosis is Waterborne is Incorrect

Bayer witness Patterson's written direct testimony is beset with irrelevant and incorrect statements. First, much of Patterson's testimony addresses *Campylobacter* outbreaks instead of sporadic cases of campylobacteriosis. WDT B-1910: P5, L8-14, P9, L8-12, P10, L9-11, P13, L23-24, P14, L3-14, P16, L5-9, P21, L6-7, L11-22, P22, L1-13, L19-22, P23, L8-22, P24, L1-19, P25, L10 - P26, L4, L16-20. However, most cases of campylobacteriosis are sporadic in nature, WDT G-1452: P9, L18-19; WDT B-1912: P10, L7-8; WDT A-204: P14, L13; WDT G-1457: P3, L45-46; WDT B-1906: P3, L7; WDT B-1911: P5, L17, and even Patterson acknowledges this in his testimony. See WDT B-1910: P22, L7-18.

Second, Patterson's comparison of the numbers of foodborne versus waterborne campylobacteriosis cases is, at best, misleading. Patterson states that "[C]urrent best estimates of the annual incidence of U.S. waterborne infection due to *Campylobacter* range up to almost 500,000 per year (see, e.g., Morris and Levin, 1995; Mead, et al., 1999)." WDT B-1910: P27, L9-11. However, a review of the articles Patterson cited (Ex. B-927 and Ex. G-410) does **not** support his testimony. In fact, according to Ex. B-927: P8, there were approximately 2.1 million total cases of campylobacteriosis, only 15% of which were attributed as waterborne. According to Ex. B-927: P9, 15% of the total cases equal 320,000, far fewer than the "almost 500,000" cases Patterson asserts are waterborne.

Patterson uses a 60,000 figure as an estimate of foodborne campylobacteriosis cases. WDT G-1910: P27, L15-16. However, this 60,000 figure is a figure based on actual reported cases, not an estimate of total cases of foodborne attributed campylobacteriosis. In order to get a

[32] CLIA stands for Clinical Laboratory Improvement Amendments.

number appropriate to compare to the 320,000 figure above, one first must multiply the 60,000 reported cases by 38, since CDC has estimated that the true incidence of campylobacteriosis is 38 times the reported cases. Ex. G-410: P12. Using Patterson's 60,000 figure, and multiplying it by 38, there would be 2,280,000 cases of foodborne related campylobacteriosis. Thus, if Patterson made a fair comparison, he would have found that waterborne cases of campylobacteriosis (320,000) fall far behind foodborne cases of campylobacteriosis (2.28 million). Ex. B-927: P9; Ex. G-410. Even with the slight difference in the estimates of total campylobacteriosis (Ex. B-927: P8 uses a 2.1 million total figure and Ex. G-410: P5 uses a 2.4 million total figure), it is clear that Patterson's assertion that most campylobacteriosis is waterborne is incorrect.

Patterson then builds on his faulty comparison of 320,000 waterborne cases versus 60,000 foodborne cases stating that "[i]f recreational water as a waterborne route of campylobacteriosis has parity with drinking water, then the waterborne *Campylobacter* infections approach one million cases per year . . . ." WDT B-1910: P27, L16-18. Patterson's assumption is unsupported. But, accepting Patterson's faulty hypothetical scenario for purposes of argument, even if recreational water doubled the estimate in Ex. B-927: P9, it would only equal 640,000 total cases; this number is still far below the estimate in both B-927 and G-410 of over 2 million cases of foodborne campylobacteriosis each year, and is even far below the CDC's estimate of 1.4 million cases of campylobacteriosis in 1999. WDT G-1452: P7, L13-14.

Third, in many places, Patterson's testimony relies on: (a) antimicrobials or antimicrobial resistance instead of specifically referring to FQs or FQ-resistance, WDT B-1910: P6, L13-19, P8, L8-22, P9, L3-8, P13, L2-10, P14, L12-14, P15, L7-8; (b) bacteria in general instead of *Campylobacter* specifically, WDT B-1910: P3, L7-14; and, (c) gastroenteritis instead of campylobacteriosis, WDT B-1910: P13, L23-24, P21, L18-22.

C. Bayer's Reliance on Cox's Testimony is Groundless

Testimony should be truthful, accurate, and non-misleading. Cox's testimony is none of these. For a variety of reasons, Cox's testimony is not credible. For example:

- Cox's opinion appears to change depending on who is asking for it. Cox agreed with CVM's risk assessment when he was a consultant to CVM but changed his views when he became a consultant to AHI. When Cox was a consultant to CVM, Cox agreed with the methodology of CVM's risk assessment. Tr. P866, L3-9, P871, L20 - P872, L13. In fact, at a December 1999 meeting with CVM, Cox stated that CVM's approach was good and sensible and that the assumptions were well-documented. Tr. P866, L3-9, P869, L13-15, P872, L6-13; Ex. G-1810. Shortly after the December 1999 conference with CVM, Cox became a consultant to AHI. Tr. P881, L13-22. In his written direct testimony, Cox now describes CVM's risk assessment as "meaningless" and "technically deficient." WDT B-1901: P25 ¶2. But in the December 1999 meeting with CVM, Cox had explicitly agreed with CVM's "big K," i.e., the aggregation of the end sequences into one probability that a pound of *Campylobacter* contaminated chicken will result in a case of campylobacteriosis. Tr. P877, L15 - P878, L4; Ex. G-1810: P143, L15-21; Ex. G-953: P12. Moreover, Cox acknowledged that the "big K" principle that he had agreed with in 1999 exists in CVM's 2001 published risk assessment. Tr. P879, L4-9. Cox's testimony does not permit one to distinguish his opinion from his interest.
- Cox's testimony misrepresents the text of the articles on which he relies. The quality of Cox's testimony is below minimally acceptable standards. Cox's written direct testimony purported to quote from the Rosenquist article. WDT B-1901: P16 ¶3. But, in his quote of the article, Cox had: (a) deleted pertinent text from the article without indicating the deletion; (b) inserted text from other parts of the article without indicating the addition; and

(c) inserted a period without indicating that the text in the article continued. Tr. P947, L11 - P961, L8. Cox admitted that the way he quoted the Rosenquist article is representative of the way he quoted scientific articles throughout his written direct testimony. Tr. P968, L3-17. Cox also admitted that his use of quotation marks in his written direct testimony does not necessarily mean that the quoted words can actually be attributed verbatim to another source. Tr. P1003, L7-21. Cox's written direct testimony is additionally compromised because it provides the results of an analysis he has conducted but is vague about the methods used and fails to indicate the strength of the conclusions he draws from it, thereby misleading the reader as to the importance or validity of his analysis. Tr. P1069, L6 - P 1074, L14. Although Cox testified during cross examination that his analysis was "just exploratory" and not "serious data analysis," id., Cox quoted his analysis without such caveat many times in his written direct testimony, WDT B-1901: P17, 20, 27, 37, 38, 71, 73, as well as in a journal article he authored, Ex. B-1252: P3.

- Cox's testimony misrepresents the findings of various studies. For example, in his testimony Cox cites the studies of Effler, Rodrigues, Friedman, Eberhart-Phillips, and Kassenborg to support his notion that restaurant dining is the major cause of campylobacteriosis, rather than chicken consumption. WDT B-1901: P30. But contrary to Cox's assertion, each of these studies actually does find that chicken consumption outside the home, not restaurant dining in and of itself, is a major cause of campylobacteriosis. Tr. P889, L22 - P892, L5, P894, L13 - P900, L11, P901, L18 - P910, L18; Ex. G-185, Ex. G-1711, Ex. G-1488, Ex. G-182, Ex. G-337.
- Cox's testimony misrepresents CVM's risk assessment. For example, Cox states that CVM's model "incorrectly assumes that risk is proportional to prevalence of 'contaminated' chicken *servings* ingested . . . ." WDT B-1901: P16 ¶3 (emphasis added). But CVM's risk

assessment model was based on the overall consumption of chicken, not chicken servings, which Cox later admitted during cross-examination. Tr. P443, L21 - P444, L22. Cox also claimed that CVM's risk assessment model is "based on technically deficient concepts such as . . . 'average exposure for an average individual' . . . ." WDT B-1901: P25 ¶1. But after much discussion, Cox admitted that he was not arriving at this concept based on CVM's risk assessment model itself. Tr. P1002, L2-10.

- Despite the fact that his model is labeled "final report," Cox stated that "it is not . . . [a final model] by a mile." Tr. P1024, L5-9; Ex. A-17. Moreover, Cox denied the relevance of parts of his 2001 model, despite the fact that his written direct testimony relies on that model, and does not identify which of his models is in fact the final model. Tr. P1024, L10-20, P1026, L13 - P1027, L6.
- Cox's testimony is in irreconcilable conflict with itself. On the one hand, Cox claims that there is no causal relationship between *Campylobacter* in chickens and *Campylobacter* in humans but, on the other hand, he claims that banning Baytril will result in an increase in *Campylobacter* cases in humans as a result of increased levels of *Campylobacter* in chickens. WDT B-1901: P85 ¶1. Either chickens are an important source of *Campylobacter* or they are not: Cox cannot logically argue both scenarios.

D. Bayer's Reliance on Russell's Study is Misplaced

Bayer's reliance on Russell's study to try and show that Baytril provides a benefit to human health is misplaced. Russell's study purports to show that withdrawal of the NADA for Baytril would lead to greater pathogen contamination in the slaughterhouse because air sacculitis positive chickens (i.e., ones that haven't been effectively treated) are smaller and have weaker intestines than healthy chickens and, therefore, the intestines of these birds are more likely to break during automated evisceration. See, WDT B-1912: P19, L18 – P26, L14; WDT B-1912:

Attach. 1. Russell's findings are far from conclusive. First, this was one study, and there is no evidence of similar studies with similar results. Second, Russell's study presented mixed results (in only 2/5 of the replicates were decreased body weight in air sacculitis positive birds statistically significant). WDT B-1912: P23, L3-4. Third, the study looked only at Baytril treatment versus treatment with two other drugs and only in a limited geographical area. WDT B-1912: P20, L3-4. This limited study cannot be taken as representative of results looking at all available drugs (including those available under the Act as amended by the Animal Medicinal Drug Use Clarification Act (AMDUCA)) on a nationwide basis. There is no indication that levels of air sacculitis caused by *E. coli* infection in birds remain static from year to year. WDT B-1914: P19, L5-7. And, it appears that the levels of infection vary from geographic area to geographic area. WDT B-1917: P11, L1-2; WDT B-1914: P19, L21. Finally, Bayer is attempting to use the results of this limited study in a way that ignores the ability of the chicken industry to change to address public health concerns. For instance, Bayer assumes that the automated nature of the chicken processing industry is static. It is not. Smaller chickens can be sent through a separate kill line or manually slaughtered and processed and line speeds can be reduced, reducing the probability of processing errors.

E. Bayer's Argument that Baytril is the Only Effective Drug to Treat Poultry is Faulty

There are available alternatives to Baytril. CVM witness Tollefson testified to the availability of alternative treatments, and that under the extra label use provisions of AMDUCA, other antimicrobials can be used. WDT G-1478: P18, L34-46. Moreover, the determination of whether Baytril is safe does not depend on the availability of alternative drugs.

VII. Conclusion

The evidentiary record of this hearing is replete with "new" evidence that, taken together with evidence available to CVM when Baytril was approved, provides a reasonable basis from

which serious questions about the safety of Baytril use in poultry can be inferred. CVM has met its burden to adduce this evidence and what it shows, shifting to Bayer the burden to demonstrate that the use of Baytril under the approved conditions of use in poultry has been shown to be safe. Bayer has not met its burden in this case. Therefore, CVM respectfully requests that the Administrative Law Judge find that new evidence, evaluated together with the evidence available to CVM when the NADA for Baytril was approved, shows that Baytril use in poultry is not shown to be safe for use under the conditions of use upon the basis of which the application was approved, and that the Administrative Law Judge order the withdrawal of the NADA for Baytril.

Appendix A

Proposed Findings of Fact

1. Baytril is the trade name for enrofloxacin (NADA 140-828).

2. Baytril is used to control mortality associated with respiratory diseases caused by *E. coli* in chickens and *E. coli* and *P. multocida* in turkeys. Ex. A-54: P1.

3. There are 8.5 billion broiler chickens and approximately 270 million turkeys slaughtered in the United States each year. See WDT A-202: P3, L4; Bayer's Narrative Statement, P3.

4. *Campylobacter* bacteria are commonly found in the intestinal tract of poultry; however, these commensal bacteria do not generally cause disease in these animals. WDT G-1459: P2, L28-30; WDT G-1484: P2, L43-44.

5. The majority of broilers are colonized with *Campylobacter jejuni* (*C. jejuni*). See WDT G-1459: P3, L37-38, L44-49; WDT G-1454: P3, L4-5.

6. For both chickens and turkeys, once the first bird in the poultry house in colonized with *Campylobacter*, the entire house quickly becomes colonized. WDT G-1459: P7, L32-33; WDT B-1908: P5, L17-18.

7. There are approximately 10^7 to 10^9 (10 million to 1 billion) *Campylobacter* colony forming units (CFUs) per gram of caecal content in a colonized broiler. WDT G-1459: P2, L49 - P3, L2, P7, L28-30.

8. In turkeys, the average concentration of *Campylobacter* is between 1.2×10^4 to 1.5×10^7 CFUs per gram of caecal content. WDT G-1459: P3, L5-9, P7, L30-32.

9. It does not take many bacteria to colonize the poultry. Ex. G-22: P6.

10. In *Campylobacter*, a single point mutation in the gyrase gene (gyrA) naturally occurs in approximately 1 to 5 in 100 million cells. WDT G-1465: P5, L8-10.

11. In most *Campylobacter*-colonized chickens and turkeys, one would expect to find some number of *Campylobacter* organisms that are resistant to fluoroquinolones (FQs). See WDT G-1465: P5, L19-24.

12. Medicating poultry via drinking water can lead to the under-dosing of some of the animals, thus increasing the probability of selecting for FQ-resistant *Campylobacter* in both healthy and diseased birds. WDT G-1465: P7, L6-11; Ex. G-52: P29; Ex. B-868: P3.

13. Between 136,000,000 and 93,500,000 broilers are directly treated with Baytril each year.

14. Untreated chickens are exposed to chickens treated with Baytril during transport to the slaughterhouse and at the slaughterhouse, where fecal cross-contamination of birds is common. WDT G-1467: P8, L12 - P10, L6.

15. Whether or not a house is treated with FQs, broilers can be contaminated with FQ-resistant *Campylobacter* through transport, slaughter, or processing. See WDT G-1467: P7, L22-28, P11, L12-17.

16. Approximately 10,800,000 turkeys are directly treated with Baytril each year.

17. Baytril use selects for FQ-resistant *Campylobacter* in poultry. See RJS 7; WDT B-1916: P5, L26-29; WDT B-1900: P48, L 4-6; WDT B-1908: P16, L23-24.

18. Widespread dissemination of FQ resistance does not emerge in the absence of direct selection pressure brought about by FQ exposure. WDT G-1465: P6, L7-11.

19. When FQs are administered to poultry, it has the effect of killing most of the FQ-susceptible bacteria, including both the target pathogen (i.e., *E. coli*) and other bacteria harbored by the poultry, such as *Campylobacter*. See WDT G-1463: P7, L9-11. However, some bacteria survive. See WDT G-1465: P4, L20-24. These surviving bacteria are the mutated bacteria, which then reproduce and pass on their acquired resistance to daughter bacteria. These surviving bacteria and their progeny colonize the intestinal tract previously filled by the FQ-susceptible bacteria that had been killed by the antimicrobial. See WDT G-1463: P7, L9-14; WDT G-1465: P5, L20-24.

20. FQ treatment does not eliminate *Campylobacter* from the intestinal tract of the chickens, but rather, rapidly selects for FQ-resistant mutant isolates. See WDT G-1465: P5, L20-24; Ex. G-315: P2-3; Ex. B-868: P3; Ex. G-1800: P2-3.

21. Laboratory tests conducted after the approval of Baytril confirm that FQ treatment at therapeutic levels quickly selects for resistant *Campylobacter*, leading to the emergence of FQ-resistant *Campylobacter* mutants which re-colonize the poultry with FQ-resistant *Campylobacter*. See Ex. G-1800: P2-3; Ex. B868.

22. McDermott found that, within 24 hours of Baytril treatment, the *C. jejuni* in the Baytril-treated broiler chicken gut were seven-fold more resistant, measured by Minimum Inhibitory Concentration (MIC), to ciprofloxacin and enrofloxacin than before treatment. WDT G-1465: P3, L1-3; Ex. B-868: P2.

23. MIC is the lowest concentration of the drug that it takes to inhibit the growth of a bacterium. WDT G-1481: P3, L4-9.

24. In McDermott's study, continued susceptibility testing showed that FQ-resistant *C. jejuni* organisms remained in the Baytril-treated birds throughout the normal span of broiler flock. WDT G-1465: P3, L6-7; Ex. B-868: P3-4.

25. In McDermott's study, no FQ-resistant *Campylobacter* isolates were detected in the non-Baytril treated control group of chickens. WDT G-1465: P3, L9-11; Ex. B-868: P2-3.

26. In McDermott's study, the resulting resistance was the direct result of Baytril exposure. See WDT G-1465: P6, L8-11.

27. Newell observed resistance to enrofloxacin and ciprofloxacin measured by a 7-8 fold increase in MICs in all *C. jejuni* recovered 48 hours after starting Baytril treatment in chickens. WDT G-1465: P4, L38-39, Attach. P25.

28. Zhang found that FQ-resistant *Campylobacter* emerges in chickens within 24-48 hours after Baytril treatment. Ex. G-1800: P2-3.

29. In Zhang's experiment the untreated control group remained sensitive to enrofloxacin, and FQ resistance was not detected in the treated group before enrofloxacin treatment. Ex. G-1800: P2.

30. Jacobs-Reitsma found that exposure to enrofloxacin within the labeled concentrations tested (15 ppm and 50 ppm) effectively selected for resistant bacteria, allowing the birds to be colonized with FQ-resistant *Campylobacter*. WDT G-1459: P7, L7-22; Ex. G-315: P2-3.

31. The emergence, selection, and mechanism of FQ resistance in bacteria is characteristic of the bacterium and not the host animal in which resistance is selected. WDT G-1463: P4, L9-11.

32. *Campylobacter* exhibit a bimodal pattern of FQ MICs; that is, they are either highly susceptible to FQs or highly resistant to FQs. WDT G-1465: P4, L44 – P5, L6; Ex. B-868: P2-3; Ex. G-1800: P2; Tr. P249, L13 - P250, L15.

33. Comparison of methods for determining *Campylobacter* resistance to ciprofloxacin indicates very good correlation between the E-test and agar dilution. Ex. G-763: P8; Ex. B-170: P3; Ex. G-303: P5.

34. There are good reasons to use the E-test; among them are ease of use, and usefulness for monitoring changes in the prevalence of ciprofloxacin resistance in *Campylobacter*. See Tr. P245, L16 - P246, L3.

35. The fact that no official NCCLS breakpoint has been adopted for *Campylobacter* susceptibility to ciprofloxacin would not affect the designation of *Campylobacter* isolates as FQ-susceptible or FQ-resistant because the bimodal nature of *Campylobacter* squarely places the organisms into two distinct, and opposite ends of the scale, i.e., categories of very FQ-susceptible or very FQ-resistant. See WDT G-1465: P4, L44 - P5, L6; Tr. P249, L13 - P250, L15.

36. A breakpoint is the concentration (expressed as an MIC) that distinguishes between susceptible, intermediate and resistant bacteria. WDT G-1481: P5, L1-4.

37. Many knowledgeable researchers in the field have used a breakpoint of greater than or equal to 4 μg/ml for determining FQ resistance in *Campylobacter*. Ex. G-1517: P4; Ex. G-337: P5; Ex. G-589: P2; Ex. G-1800: P1.

38. A 4 μg/ml for determining FQ resistance in *Campylobacter* is a reasonable breakpoint and in line with what other countries' standards setting organizations have adopted. See Tr. P193, L7-13; WDT G-1481: P6, L42 - P7, L2.

39. Once poultry are colonized by FQ-resistant *Campylobacter* they remain colonized for the production span of the poultry. Ex. B-868: P3-4; WDT G-1459: P3, L9-11; WDT B-1908: P5, L2-6.

40. The majority of poultry flocks are colonized with *Campylobacter*. WDT G-1459: P3, L33-39, L42 - P5, L2; Ex. G-1724: P3; Ex. G-385: P1.

41. Surveys of poultry carcasses at slaughter demonstrate high levels of *Campylobacter* on poultry carcasses. See Ex. G-652: P6; WDT G-1484: P5, L7-11; Ex. G-651: P6; WDT G-1484: P5, L14-17; Ex. G-791: P1; WDT G-1484: P6, L25-27; WDT G-1464: P5, L22-23; Ex. G-37: P1.

42. Results of the animal arm of NARMS surveys for years 1998, 1999, 2000, and 2001 showed 9.4%, 9.3%, 10.4% and 17.6%, of *Campylobacter* organism from broilers were resistant to ciprofloxacin, respectively. WDT G-1478: P12, L6-7.

43. Because of an outdated method of speciating *Campylobacter* used by USDA, the animal arm of NARMS underestimated the true prevalence of ciprofloxacin resistant *Campylobacter* found in broilers at slaughter in the United States between 1998-2000. WDT G-1465: P7, L25-34; WDT G-1478: P9, L15 - P11, L38; Tr. P175, L3-11.

44. In 2000-2001, Logue looked at the antimicrobial susceptibility of *Campylobacter* from two turkey processing plants. WDT G-1464: P2, L27-30. At the smaller of the two slaughter plants (production speed of 800 turkeys per hour) 8.8% of *Campylobacter* recovered from turkeys were resistant to ciprofloxacin. At the larger of the two plants (production speed of 8000 turkeys per hour), 65.2% of the *Campylobacter* were found to be resistant to ciprofloxacin. See WDT G-1464: P6, L14-19.

45. The transportation, slaughter, and processing of poultry presents an opportunity for cross contamination. WDT G-1467: P8, L8 - P10, L6; WDT A-203: P7, L17 - P8, L13; WDT B-1912: P5, L2-7.

46. During transport to the slaughterhouse, chickens and turkeys are stacked on top of each other on transport trucks, presenting the very real probability that chickens or turkeys in the lower crates will be contaminated by feces from the poultry stacked in crates on top. WDT A-204: P46, L17-19; WDT G-1467: P8, L12-16.

47. In both automated and manual systems, the automated blades, or hand-held knives present a point of possible contamination, especially if the neck area has *Campylobacter* on it. WDT G-1467: P8, L22-27.

48. There are many points of contamination within the slaughterhouse. WDT G-1467: P8, L 8 - P10, L6; WDT A-204: P46, L14 - P47, L19.

49. The scalding tank often stays murky brown throughout each day from the feces in it. WDT G-1467: P8, L29-34.

50. The poultry slaughter process increases the likelihood that poultry carcasses leaving the slaughterhouse are contaminated with *Campylobacter* and FQ-resistant *Campylobacter*. See WDT G-1467: P11, L12-17.

51. Retail poultry is a reservoir for *Campylobacter* in the United States and other types of retail meat, most notably beef and pork, harbor very little *Campylobacter*. See WDT G-1484: P2, L46 – P3, L2; P4, L12-15; WDT G-1466: P3, L24-26.

52. A majority of the retail chicken sold in the United States today is contaminated with *Campylobacter*. See Ex. G-701: P2; Ex. G-727: P 3; Ex. G-541: P1; Ex. G-589; Ex. G-1785: P2; WDT G-1484: P4, L12-15; WDT G-1484: P2, L46 – P3, L2.

53. The high levels of *Campylobacter* contamination in poultry coupled with the low levels of *Campylobacter* contamination in beef and pork, indicate the majority of foodborne campylobacteriosis must be coming from *Campylobacter* contaminated poultry. See WDT G-1484: P4, L14-15; Ex. G-727: P3.

54. Antimicrobial testing has shown that approximately one quarter to one third of retail *Campylobacter*-contaminated retail chicken meat carry an FQ-resistant strain. WDT G-1465: P7, L19-21.

55. In an University of Maryland study of retail chicken bought in the greater Washington D.C. metropolitan area in 1999-2000, 35% of the *Campylobacter* poultry isolates were resistant to ciprofloxacin and 41% were resistant to nalidixic acid. WDT G-1466: P3, L24-29; Ex. G-1778: P9-10.

56. *Campylobacter* that is resistant to nalidixic acid tend to be cross resistant with FQs. See WDT G-1482: P8, L5-13.

57. In a CDC study of retail chicken bought in Georgia, Maryland and Minnesota, 24% of the *Campylobacter* isolates tested were resistant to ciprofloxacin, with a total of 11% of the retail chicken tested yielding ciprofloxacin-resistant *Campylobacter*. WDT G-1484: P5, L35 – P6, L3; Ex. G-541: P1.

58. In a study conducted in the state of Iowa, of the *C. jejuni* isolates recovered from retail chicken or turkey purchased from March 2001 to March 2002, 27% exhibited ciprofloxacin resistance. WDT G-1484: P7, L43. Likewise 27% of the *C. coli* from retail chicken and turkey exhibited ciprofloxacin resistance. See WDT G-1484: P7, L43.

59. In Smith's Minnesota study, 20% of the retail chicken sampled had ciprofloxacin resistant *Campylobacter*. Ex. G-589: P5.

60. Even the best kitchen hygiene cannot prevent cross contamination from occurring in the kitchen. WDT G-1483: P10, L3-5.

61. A drop of chicken juice can contain an infective dose of *Campylobacter*. WDT G-1475: P10, L40-41.

62. Freezing and/or cooking reduces *Campylobacter* contamination but does not eliminate it. See RJS 24; WDT G-1483: P9, L26-30, L34-35.

63. The anatomical structure of a broiler allows insulating pockets for *Campylobacter* to survive during cooking. WDT G-1483: P9, L29-36.

64. Poultry is a significant risk factor for acquiring campylobacteriosis. WDT G-1483: P11, L1-2, P27, L24-27; WDT G-1457: P9, L18-19; WDT G-1470: P9, L17-18; WDT G-1475: P8, L33-36; WDT G-1452: P9, L36-40; WDT G-1473: P15, L35 - P16, L23, P20, L26-31.

65. The epidemiological studies show that poultry is responsible for *Campylobacter* infections (FQ-susceptible and FQ-resistant infections) in humans. The microbiological and molecular studies strengthen the results of the epidemiological studies. The temporal data corroborate the epidemiological, microbiological, and molecular evidence and provide independent support for the incontrovertible assertion that enrofloxacin administered to poultry causes the proliferation of FQ-resistant *Campylobacter* in poultry that contributes to FQ-resistant *Campylobacter* infections in humans.

66. Individually and collectively, epidemiological studies of *Campylobacter* infections conducted in the United States and other industrialized countries have determined that poultry, particularly chicken, is a risk factor for acquiring campylobacteriosis. WDT G-1483: P15, L30-36; WDT G-1457: P4, L12-17; WDT G-1475: P8, L5 - P9, L27; WDT G-1452: P9, L36-40.

67. Although the epidemiological studies differ in location, technique, and sample size, they consistently indicate contact with and consumption of poultry as dominant sources of *Campylobacter* infections. WDT G-1452: P9, L36-40; WDT G-1483: P11, L1-7; WDT G-1475: P8, L23-36; Ex. G-1644: P9, P14; Ex. B-205: P3; Ex. G-1743: P8, P13.

68. Among the repeatedly-implicated risks are: (1) consuming poultry (Ex. G-162, Ex. G-268, Ex. G-1686, Ex. G-602, Ex. G-1718, Ex. G-334, Ex. G-474, Ex. G-307); (2) consuming poultry that is raw or undercooked (Ex. G-1488, Ex. G-162, Ex. G-268, Ex. G-299, Ex. B-561, Ex. G-1681, Ex. G-182, Ex. G-307); and (3) consuming poultry in a restaurant (Ex. G-337, Ex. G-1488, Ex. G-185, Ex. G-1711, Ex. G-182). Other identified risk factors include: (1) handling raw chicken (Ex. G-300) and, (2) failing to clean food preparation / cutting board surfaces, which is a marker for cross-contamination, Ex. G-1644: P11 (Ex. G-268, Ex. G-1681).

69. The scientific evidence here points to the existence of a causal link between exposure and illness. Cf. Tr. P287, L19-22.

70. There is no plausible scientific reason that transmission of FQ-resistant *Campylobacter* from poultry to humans is different from transmission of FQ-susceptible *Campylobacter* from poultry to humans. WDT G-1483: P20, L18 - P21, L3.

71. The 1998-1999 Foodborne Diseases Active Surveillance Network (FoodNet) *Campylobacter* case-control study, which is the largest case-control study of sporadic *Campylobacter*

infections, was conducted in the U.S. in FoodNet sites during a 12-month period between 1998-1999. WDT G-1452: P9, L46-48.

72. Results from the Friedman and Kassenborg analyses demonstrate that the dominant source of domestic *Campylobacter* infections (campylobacteriosis generally and FQ-resistant campylobacteriosis specifically) in humans is poultry, particularly chicken but also turkey. WDT G-1452: P10, L22 - P11, L1; Ex. G-1488: P3; Ex. G-228: P1; WDT G-1460: P8, L16-18; Ex. G-337: P3.

73. A population attributable fraction is the reduction in incidence of illness that would be achieved if the population had been entirely unexposed compared with its current (actual) exposure pattern. WDT G-1460: P9, L1-3.

74. Friedman's final multivariate logistic regression model, which was used to determine independent risk factors for acquiring a *Campylobacter* infection among persons who did not travel outside the U.S., found that within the seven days prior to illness onset: (a) cases were 2.2 times more likely (95% CI = 1.7, 2.9) than controls to have eaten chicken in a restaurant; (b) cases were 2.5 times more likely (95% CI = 1.3, 4.7) than controls to have eaten turkey in a restaurant; and (c) cases were 1.7 times more likely (95% CI = 1.3, 2.2) than controls to have eaten non-poultry meat in a restaurant. WDT G-1452: P10, L22-32; Ex. G-1488: P23.

75. In Friedman's analysis, the population attributable fractions suggest that at least 28 percent of the domestically acquired *Campylobacter* infections were due to eating poultry. See WDT G-1452: P10, L36-44.

76. In the final multivariate logistic regression model, Kassenborg found that eating chicken or turkey at a commercial establishment was the only risk factor that remained independently associated with FQ-resistant *Campylobacter* illness. WDT G-1460: P8, L11-18, P14. Cases with domestically acquired FQ-resistant *Campylobacter* infections were 10 times more likely to report having eaten chicken or turkey at a commercial establishment than were well controls (95% CI = 1.3, 78). WDT G-1460: P8, L18-20; P14; Ex. G-337: P15.

77. In Kassenborg's analysis, eating chicken or turkey at a commercial establishment accounted for 38 percent of the domestically acquired FQ-resistant *Campylobacter* infections. WDT G-1460: P9, L3-5, P14.

78. In Kassenborg's analysis, twenty-two percent of all FQ-resistant infections (i.e., including, in the total, those infections that were related to foreign travel) could be attributed to eating chicken or turkey in a commercial establishment. WDT G-1460: P9, L7-8. The population attributable fraction suggests that a person's risk for an FQ-resistant *Campylobacter* infection could potentially be reduced by 22 percent if the risk associated with commercially prepared chicken and turkey were eliminated. WDT G-1460: P10, L17-19.

79. Results of the Effler case-control study were similar to the outcomes in Friedman's and Kassenborg's analyses. See WDT G-1483: P14; Ex. G-185: P3.

80. Eating poultry was identified as a risk factor for campylobacteriosis in the Harris and Deming studies; eating raw or undercooked chicken was identified as a risk factor in the

Harris, Deming, and Hopkins/Olmstead studies; and handling raw chicken was associated with illness in the Hopkins/Scott study. Ex. G-268: P1; Ex. G-162: P1, P6; Ex. G-200: P2; WDT G-1475: P8, L10-15, P9, L34-37; WDT G-1483: P14.

81. Because the epidemiology of *Campylobacter* in the United States and Europe is comparable, studies from Europe are relevant in the evaluation of sources of *Campylobacter* infections in the United States. WDT G-1457: P7, L14-17; Tr. P408, L7-14.

82. Eating poultry was identified as a risk factor for campylobacteriosis in the Studahl (Sweden), Neal (U.K.), Schorr (Switzerland), Kapperud (Norway), and Oosterom (Netherlands) studies; eating undercooked chicken was associated with illness in the Neimann (Denmark) study; and eating chicken in a restaurant was a risk factor in the Rodrigues (England) study. Ex. G-602: P4; Ex. G-1686: P4; Ex. G-1718: P7; Ex. G-334: P3; Ex. G-474; Ex. G-1711: P3, P5; WDT G-1483: P13, L8-12, P14.

83. Eating undercooked poultry was a risk factor for campylobacteriosis in the Michaud (Canada), Eberhart-Phillips (New Zealand) , and Ikram (New Zealand) studies; eating chicken in a restaurant was associated with illness in the Eberhart-Phillips study. Ex. G-1681; Ex. G-182: P4; Ex. G-307: P1.

84. The identification of additional risk factors does not detract from the common theme that poultry is a major risk factor for campylobacteriosis. Some risk factors are uncommon in the general population (drinking raw milk) or account for usually only a small proportion of cases (contact with cats and puppies). WDT G-1475: P8, L17-20. Other risk factors are unquestionably rare (drinking milk from bottles with bird-pecked bottle caps). WDT G-1483: P15, L22-27.

85. Although quantification of the different routes of infection may be difficult, consuming poultry has been estimated by some U.S. studies to account for roughly 50 to 70 percent of *Campylobacter* cases. Ex. G-1644: P10; WDT G-1457: P4, L15-17.

86. Investigation of strains of *Campylobacter* from animals, food, and humans by genetic fingerprinting and other sensitive methods for tracing sources of human infection has provided confirmation that poultry, particularly chicken, is a source of human *Campylobacter* infections, specifically FQ-resistant *Campylobacter* infections. WDT G-1483: P17, L41 - P18, L3; WDT G-1473: P13, L41 - P14, L18.

87. Studies have found strong similarities between *Campylobacter* strains when comparing isolates from humans with isolates from poultry. WDT G-1457: P5, L1-39; WDT G-1475: P11, L8-18.

88. Smith's study in the U.S. made a striking finding by molecularly linking domestically-acquired quinolone-resistant *Campylobacter* illness with quinolone-resistant *Campylobacter* from poultry. WDT G-1473: P13, L41 - P14, L18.

89. The route of transmission of FQ-resistant *Campylobacter* from poultry to patient has been confirmed. WDT G-1483: P17, L41 - P18, L3; Ex. G-190: P8; Tr. P557, L15 - P558, L1.

90. DNA fingerprinting functions to strengthen statistical associations found in epidemiological analyses. WDT G-1455: P6, L27-29.

91. Both pulsed-field gel electrophoresis (PFGE) and polymerase chain reaction restriction fragment length polymorphism, specifically of the *flaA* gene (*flaA*-RFLP), methods have been widely used to study *C. jejuni*. WDT G-1470: P8, L1-2. Other methods, including ribotyping, amplified fragment length polymorphism (AFLP), and multilocus sequence typing (MLST) have also been used. WDT G-1470: P7, L34-37; WDT G-1457: P5, L8-15; Ex. G-161; Ex. G-1629.

92. Because the *flaA* gene is diverse, *flaA*-RFLP subtyping is considered to be adequately discriminating to draw conclusions about the relatedness of bacterial isolates. WDT G-1453: P5, L6-34; WDT G-1470: P8, L13-16. *Fla* typing is widely accepted and "can provide meaningful data, especially when combined with epidemiologic data." WDT G-1453: P7, L30-32.

93. Twelve studies from eight countries using six bacterial typing methods correlated human and poultry strains of *Campylobacter* isolates and found a high degree of strain similarity. The typing studies have consistently isolated bacterial types from human *Campylobacter* isolates that are related to *Campylobacter* isolates from poultry. Ex. G-589: P6; Ex. B-250: P4-5; Ex. G-1629: P5; Ex. G-176: P5; Ex. B-380; Ex. G-264; Ex. G-1684; Ex. G-771; Ex. G-218: P3, P5; Ex. G-1698: P4; Ex. G-459: P8; Ex. G-494: P5; Ex. G-1775: P2-4; WDT G-1455: P10, L7-15, L18-22; WDT G-1473: P14, L8-16; WDT G-1453: P6, L40-41, L43-44; WDT G-1457: P5, L17-22, L26-30, L33-39; WDT G-1458: P7; WDT G-1475: P12, L39-42; WDT G-1470: P9, L8-11, P11, L44 - P12, L6.

94. Even if the population of *Campylobacter* strains is not identical in humans and poultry, concluding on that basis that poultry is not the source of most *Campylobacter* infections would be wrong. WDT G-1453:P6, L36-38.

95. Using *flaA*-RFLP, Smith (Minnesota) found that 92.3 percent of isolates (12 of 13 isolates) from humans with domestically acquired quinolone-resistant *C. jejuni* infections had subtypes that were also found in quinolone-resistant isolates from domestically produced chicken (retail samples). WDT G-1455: P10, L7-10. This association by itself implicates chicken as "a likely source" of quinolone-resistant *C. jejuni* in humans. WDT G-1455: P10, L9-10. Further strengthening this implication is the additional finding that, in comparison to isolates from humans with a domestically acquired quinolone-resistant *C. jejuni* infection, isolates from humans with either a domestically acquired quinolone-susceptible *C. jejuni* infection or a foreign travel-associated *C. jejuni* infection had a low overlap in strain subtypes with quinolone-resistant isolates from domestic chicken products. WDT G-1455: P10, L10-15.

96. There is a negligible possibility that a third source common to both poultry and humans accounts for the molecular subtyping results. See Tr. P557, L15 - P558, L1.

97. As implied by the data from Taiwan, "nearly 40% of [quinolone-resistant] human isolates may derive from poultry sources," and the molecular evidence shows that "domestic poultry

products are important sources of human infections associated with quinolone-resistant campylobacters." Ex. G-1775: P4.

98. Temporal data from ten countries -- Belgium, Iceland, Norway, Sweden, Finland, Australia, the Netherlands, Spain, the United Kingdom, and the United States -- provide additional support for the conclusion that poultry is a source of campylobacteriosis, specifically FQ-resistant campylobacteriosis. Intervention studies and data showing the correlation between enrofloxacin approval dates and the increase in FQ-resistant campylobacteriosis levels all support the epidemiological, microbiological, and molecular evidence of the role of poultry in the occurrence and rise of FQ-resistant *Campylobacter* infections in humans. Ex. G-672; Ex. G-791; Ex. G-191: P4-6; Ex. G-586: P1-4; WDT G-1457: P4, L29-36, P7, L19 - P9, L6; WDT G-1483: P18, L16-27, P19, L3-18, P23, L5-15; WDT G-1475: P17, L29-39; WDT G-1473: P15, L45 - P16, L12; WDT G-1458: P8-9; WDT G-1451: P4 (Fig.1), L28-32.

99. Interventions primarily or exclusively aimed at poultry have reduced the incidence of human *Campylobacter* infections by 40 to 70 percent. WDT G-1483: P20, L4-8.

100. Three national intervention studies in Belgium, Iceland, and Norway document that in these countries poultry, specifically chicken, constitutes a major source of human campylobacteriosis. Ex. G-672; Ex. G-791; WDT G-1457: P4, L29-36; WDT G-1483: P18, L16-27, P19, L3-18; WDT G-1475: P17, L29-39.

101. Many countries, including the United States, have experienced a temporal association between the approval of poultry FQs and a rise in FQ-resistant *Campylobacter* infection levels in humans. WDT G-1473: P15, L45 - P16, L4; WDT G-1458: P8-9; WDT G-1451: P4 (Fig.1); WDT G-1457: P7, L19 - P9, L6; Ex. G-191: P4-6; Ex. G-586: P1-4.

102. The correlation between enrofloxacin use and FQ resistance rates has been acknowledged by Bayer. Ex. B-454 (admitting, in a 1997 e-mail between Bayer employees, that "[r]esistance rates in Denmark are low because relatively few [sic] Baytril is being used").

103. In many countries, the introduction of FQs (specifically enrofloxacin) in veterinary medicine (specifically for poultry use) has been followed by an increase in FQ-resistant *Campylobacter* in humans. The increase begins shortly after the licensing of enrofloxacin for use in poultry. These countries experience the emergence of increasing levels of FQ resistance at different times; the varying times of onset are consistently associated in each country with that country's licensing of FQs for food animals. These observations strongly support the conclusion that veterinary use of FQs in poultry, and not the medical use of FQs in humans, is the driving force behind the rise in FQ-resistant *Campylobacter* infections in humans. WDT G-1483: P23, L5-15; WDT G-1451: P4, L28-32; WDT G-1473: P16, L4-12.

104. Countries in which enrofloxacin has not been introduced for use in poultry have not undergone a similar sequence of events. Finland, Australia, and Sweden report low levels of FQ-resistant *Campylobacter* in indigenous (not associated with foreign travel) infections in humans. WDT G-1457: P8, L45 - P9, L6; WDT G-1470: P7, L16-19; Ex. B-421; Ex. B-255; Ex. G-66; WDT 1458: P3-4; Ex. G-524; Ex. B-934; Ex. G-579. FQ-resistant *Campylobacter* infections in these countries have been linked to foreign travel, and in some cases,

specifically to travel to countries bearing high levels of FQ-resistant campylobacteriosis. WDT G-1457: P9, L2; Ex. G-66; WDT G-1458: P3-4; Ex. G-524: P2-3; Ex. G-578: P3.

105. The Netherlands, Spain, and the United Kingdom licensed enrofloxacin for use in poultry in 1987, 1990, and 1993, respectively. RJS 61, 63, 65. Prior to enrofloxacin licensing in each respective country, there was little or no FQ resistance detected in *Campylobacter* isolates from humans. Ex. G-77; Ex. G-407; Ex. G-240; WDT G-1457: P7, L30-33, P8, L16-17, P8, L22-25.

106. The Netherlands and Spain experienced an increase in FQ-resistant *Campylobacter* infections within one to two years after enrofloxacin licensing. Ex. G-190: P4, P7-8; Ex. G-529: P2; Ex. G-532: P2; Ex. G-557: P3; WDT G-1457: P7, L37 - P8, L12, P8, L19-20. In the United Kingdom, data gathered from 3.5 to four years after licensing of enrofloxacin show an increase in FQ-resistant campylobacteriosis. Ex. G-634: P2-3. Further, these countries also found that the level of FQ resistance in *Campylobacter* isolated from humans continues to rise after the emergence of the initial signature increase. Ex. G-586: P2; Ex. G-544: P2; Ex. G-491; Ex. G-671; Ex. G-734; Ex. G-549: P2; Ex. G-1772: P2-3; WDT G-1457: P8, L27-30.

107. The United States, too, has experienced a temporal relationship between the approval of FQs for use in poultry and the rise in the level of FQ-resistant *Campylobacter* infections in humans. WDT G-1470: P7, L11-14; WDT G-1473: P20, L13-15.

108. The U.S. historically experienced a lack of resistance to nalidixic acid, an analog of the chemical building block of the FQ class of drugs. WDT G-1453: P2, L24-25. Resistance to ciprofloxacin was even more uncommon. Ex. G-191: P5. In a national county-based study conducted between 1989 and 1990, only 1 of 332 *Campylobacter* isolates from humans was resistant to ciprofloxacin. Ex. G-624; Ex. B-589; Ex. G-1803.

109. Smith found that the percentage of *C. jejuni* isolates from Minnesota residents that were resistant to nalidixic acid statistically significantly increased from a low of 1.3 percent in 1992 to a high of 10.2 percent in 1998. WDT G-1473: P7, L9-12; Ex. G-589: P3. When the Smith study focused on trends in domestically-acquired infections in Minnesota residents in 1996-1998, the increase of domestically acquired FQ-resistant *C. jejuni* was also statistically significant. WDT G-1473: P12, L17-24; G-589: P5.

110. The 1996-1998 Minnesota data "are probably the most robust multiyear dataset in the U.S., and perhaps the world, containing information on foreign travel and prior [FQ] use." WDT B-1900: P17, L12-14. Finally, the 1996-1998 Minnesota data "are likely to be the most valid data in the US that are available to study this issue [of increasing domestically acquired FQ-resistant *Campylobacter*]." WDT B-1900: P46, L1-2.

111. A national surveillance system has documented the substantial burden of ciprofloxacin-resistant *Campylobacter* in the United States population since approval of enrofloxacin. WDT G-1452: P8, L9-11, P17, L9-10; Ex. G-1487.

112. Data from the human National Antimicrobial Resistance Monitoring System (NARMS) demonstrate that a high proportion (approximately one-fifth) of human *Campylobacter* isolates in the U.S. is resistant to ciprofloxacin. WDT G-1452: P8, L11, P9, L1-2; Ex. G-

1487. Human NARMS further demonstrates that ciprofloxacin-resistant *Campylobacter* infection in the U.S. population is increasing. WDT G-1452: P7, L25 - P9, L13, P17, L17; WDT G-1468: P8, L25.

113. In human NARMS, the percent of *Campylobacter spp.* isolates resistant to ciprofloxacin was 13 percent (28 of 217) in 1997, 14 percent (48 of 345) in 1998, 18 percent (58 of 319) in 1999, 14 percent (46 of 324) in 2000, and 19 percent (75 of 387) in 2001. WDT G-1452: P8, L9-11; Ex. G-1487.

114. A multivariate statistical analysis demonstrates that the proportion of human *Campylobacter* isolates in the United States resistant to ciprofloxacin was two and a half times higher in 2001 than it was in 1997. WDT G-1452: P8, L23-38, P9, L2-5; Ex. G-1487. The trend between 1997 and 2001 of an increasing prevalence of ciprofloxacin resistance among human *Campylobacter* isolates is statistically significant (95% CI 1.4, 4.4). WDT G-1452: P8, L35-38, P9, L3-6.

115. Despite the lower risk of getting a *Campylobacter* infection in 2001 compared with 1996, the risk of getting an FQ-resistant infection has increased over approximately the same time period; therefore, the 27 percent decrease in *Campylobacter* incidence is more than outweighed by the 61 to 98 percent increase in proportion of *Campylobacter* infections that are FQ-resistant. WDT G-1468: P8, L39-42.

116. Human NARMS data can be generalized to the United States population; these data show that the prevalence of FQ-resistant campylobacteriosis in the United States is increasing. Tr. P437, L8-11.

117. The prevalence of FQ-resistant *Campylobacter* observed in NARMS is "a representation of the national prevalence of [f]luoroquinolone-resistant Campylobacter." Tr. P362, L13-14.

118. Other quinolones (e.g., nalidixic acid) have been used in human medicine since the mid 1960s and have not caused a pattern of rising FQ-resistant *Campylobacter*. WDT G-1453: P2, L1-2.

119. Humans are not natural reservoirs of *Campylobacter*. WDT G-1483: P20, L12-13.

120. Because of the absence of significant person-to-person transmission, it is reasonable to conclude that a "significant proportion of fluoroquinolone-resistant Campylobacter is reaching people via poultry." WDT G-1457: P9, L24-26.

121. Between 1.4 and 2.4 million people suffer from campylobacteriosis each year. WDT G-1452: P7, L5-14; Ex. G-410; Ex. G-1486.

122. The symptoms of *Campylobacter* infections are similar to symptoms of other bacterial diarrheal diseases, such as those caused by *Salmonella*, *Shigella*, and some *E. coli* bacteria. WDT G-1477: P2 ¶3.

123. Most physicians initiate treatment to mitigate symptoms and decrease associated complications without the results of a stool culture to identify the cause of a patient's diarrheal illness. WDT G-1477: P2-3 ¶4; WDT G-1485: P8, L14-16, L19-33.

124. *Campylobacter* infections can incapacitate otherwise healthy adults. WDT G-1469: P4, L9-12.

125. Complications of *Campylobacter* infections include reactive arthritis, Guillain-Barré syndrome, and blood stream infections, can occur. WDT G-1477: P2 ¶3.

126. Common criteria for the antimicrobial treatment of human *Campylobacter* infections 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. RJS 42; WDT G-1485: P11, L11-18.

127. While macrolides, such as erythromycin, are considered by many to be the preferred treatment for known cases of campylobacteriosis, FQs are the preferred agents for empiric therapy because they are active against all major causes of bacterial diarrhea, and erythromycin is not effective for the treatment of other common causes of gastroenteritis, such as *Salmonella*, *Shigella*, or pathogenic *E. coli* bacteria. WDT G-1457: P6, L44 - P7, L3; WDT G-1477: P2 ¶4, P4 ¶10; WDT G-1469: P5, L21 - P6, L2.

128. There has been a significant increase in the number of *Campylobacter* isolates resistant to antibiotics. WDT G-1485: P14, L6-9.

129. The FoodNet, Smith, and Neimann/Molbak studies all showed an increased duration of diarrhea in FQ-treated patients infected with FQ-resistant *Campylobacter* strains when compared to FQ-treated patients infected with FQ-susceptible *Campylobacter* strains. Ex. G-1489; Ex. G-589; WDT G-1468: P19, L23-33.

130. Nelson's analysis of the 1998-1999 FoodNet *Campylobacter* case-control study found that the mean duration of diarrhea was 8 days (range, 2 to 21 days) for the 82 (11%) persons with FQ-resistant *Campylobacter* infections and 7 days (range, 1 to 60 days) for the 658 persons with FQ-susceptible *Campylobacter* infections ($p = 0.1$). WDT G-1452: P15, L26-29; Ex. G-1489.

131. Nelson's analysis of the 1998-1999 FoodNet *Campylobacter* case-control study found that the mean duration of diarrhea among the 421 (57%) persons who did not take antidiarrheal medications for their illness was 9 days (range, 2 to 21 days) for the 39 patients with FQ-resistant *Campylobacter* infections and 7 days (range, 2 to 60 days) for the 382 patients with FQ-susceptible *Campylobacter* infections ($p = 0.05$). WDT G-1452: P15, L31-36; Ex. G-1489.

132. Nelson's analysis of the 1998-1999 FoodNet *Campylobacter* case-control study found that the mean duration of diarrhea among the 67 of 421 (16%) persons not taking an antidiarrheal medication who also did not take an antimicrobial agent for their illness was 12

days (range, 8 to 20 days) for the 6 persons with FQ-resistant infections and 6 days (range, 2 to 21 days) for the 61 persons with FQ-susceptible infections ($p < 0.01$). WDT G-1452: P15, L36-40; Ex. G-1489.

133. Nelson's analysis of the 1998-1999 FoodNet *Campylobacter* case-control study found that of the 740 persons included in the analysis, the mean duration of diarrhea among the 128 (17%) persons who took FQs and no other antimicrobial agent or antidiarrheal medication for their illness was 8 days (range, 3 to 14 days) for the 17 patients with FQ-resistant infections and 6 days (range, 2 to 31 days) for the 111 patients with FQ-susceptible infections ($p = 0.08$). WDT G-1452: P15, L42-46; Ex. G-1489.

134. The Smith study showed that the duration of diarrhea was statistically significantly longer for patients infected with quinolone-resistant *C. jejuni* than for patients with quinolone-sensitive *C. jejuni*. WDT G-1473: P10, L32-34.

135. The Smith study showed that among patients who were treated with an FQ after the collection of stool specimens, the duration of diarrhea was statistically significantly longer for the patients with quinolone-resistant *C. jejuni* infections (median, 10 days) than for the patients with quinolone-sensitive *C. jejuni* infections (median, 7 days) ($p = 0.03$). WDT G-1473: P10, L31-35; Ex. G-589: P5.

136. The Smith study showed that many patients with quinolone-resistant infections suffered a significantly longer course of illness because the antibiotic provided to them (an FQ) did not work against resistant *C. jejuni*. WDT G-1473: P10, L38-40.

137. The Neimann/Molbak case-control study showed the median duration of diarrhea among FQ-treated patients was 14 days for patients with resistant infections and 9 days for patients with susceptible infections ($p = 0.13$). WDT G-1468: P19, L23-33.

138. Antibiotic therapy for *Campylobacter* enteritis significantly reduces the chances that a patient will have a relapse. WDT G-1485: P13, L14-16. Relapse of illness occurs in about 5-10% of persons who do not receive treatment. WDT G-1477: P2 ¶3.

139. The 2001 Neal study in the United Kingdom found that 13% of persons with *Campylobacter* infections developed symptoms of reactive arthritis. WDT G-1475: P4, L19-22.

140. FQ-resistant *Campylobacter* is potentially life-threatening for patients who have a higher risk of complications from *Campylobacter* enteritis. WDT G-1485: P14, L42-44.

141. On January 5, 2001, CVM released its revised final risk assessment report entitled "The Human Health Impact of Fluoroquinolone Resistant Campylobacter Attributed to the Consumption of Chicken." Ex. G-953.

142. On the basis of the CVM's risk assessment model, CVM estimated that, in 1998, the mean number of people in the United States who acquired FQ-resistant *Campylobacter* infections associated with consumption of chicken and who subsequently received treatment with a FQ was 8,678 people and, in 1999, this number was 9,261. Ex. G-953: P63-64. The

risk assessment's 5th and 95th percentile (the lower and upper) estimates for 1998 were 4758 and 14,369, respectively, and for 1999 were 5,227 and 15,326, respectively. Ex. G-953: P63-64.

143. CVM developed a risk assessment model to relate the prevalence of FQ-resistant *Campylobacter* infections in humans associated with the consumption of chicken to the prevalence of FQ-resistant *Campylobacter* in chicken. Ex. G-953: P6.

144. CVM's risk assessment model calculates the probabilities that: (a) a person with campylobacteriosis seeks medical care; (b) the individual is requested to provide a stool sample; (c) the individual provides a stool sample; (d) the stool sample is tested; and, (e) the stool sample is positive for *Campylobacter*. Ex. G-953: P23, Table O.1.

145. CVM's risk assessment calculates the nominal mean number of FQ-resistant campylobacteriosis cases attributable to chicken, that seek care and that are prescribed an FQ. Ex. G-953: P24, Table O.1.

146. After also modeling the total U.S. per capita consumption of boneless, domestically reared chicken (in pounds), CVM's risk assessment ultimately comes up with the total consumption of boneless, domestically reared chicken (in pounds) contaminated with FQ-resistant *Campylobacter* in the United States. Ex. G-953: P23, Table O.1.

147. CVM's risk assessment model calculates the ratio between: (1) the nominal mean number of FQ-resistant campylobacteriosis cases in the United States attributed to chicken, who sought care, and who were treated with an FQ ("the outcome"); and (2) the total consumption of boneless, domestically reared chicken (in pounds) contaminated with FQ-resistant *Campylobacter* ("the exposure"). Ex. G-953: P16.

148. The purpose of conducting a risk assessment is to provide decision-makers with information, WDT G-1480: P2, L20-23, and to answer questions about an existing or hypothetical hazard, WDT G-1479: P2, L8-9.

149. There is no single process for conducting a risk assessment. WDT G-1479: P2, L9-10.

150. CVM chose to construct a risk assessment model with parameter values and structural assumptions that could be readily verified by comparing predicted and observed values. Ex. G-953: P9, P75.

151. CVM required a risk assessment with a method that could be easily updated with new, federally-collected, scientific data on the number of FQ-resistant *Campylobacter* infections in people and the number of those ill people who were likely to be treated with a FQ. WDT G-1480: P5, L47 - P6, L3.

152. CVM's risk assessment model can predict how changes in the amount of consumption and the prevalence of *Campylobacter* contaminated meat impacts future levels of human health concerns. WDT G-1480: P8, L7-10.

153. CVM's risk assessment model allows updates of data and resulting risk estimates annually and, therefore, can track changes in risk. WDT G-1480: P10, L36-38; Ex. G-953: P10.

154. A more intricate risk assessment model, such as a farm-to-fork model, would not have yielded additional information that was useful to CVM and would have increased the likelihood of error in the model's calculations. WDT G-1480: P10, L6-8, 10-13, 26-29, and 33-35.

155. All assumptions and limitations of the data used in the model are explained in CVM's risk assessment. Ex. G-953: App. B.

156. CVM's risk assessment assumes that, after removing FQ-resistant *Campylobacter* cases associated with prior FQ use and foreign travel, the remaining FQ-resistant *Campylobacter* cases are attributable to chicken. Ex. G-953: P103; WDT G-1454: P9, L18-33.

157. CVM's risk assessment assumes that there is a constant proportionality between the pounds of contaminated poultry meat produced a year and the expected rate of human illnesses. WDT G-1480: P7, L30-34.

158. CVM's risk assessment model assumes that there is a causal relationship between the amount of chicken contaminated with *Campylobacter* consumed and incidence of campylobacteriosis and, similarly, between the amount of chicken contaminated with FQ-resistant *Campylobacter* consumed and incidence of FQ-resistant *Campylobacter* in humans. Ex. G-953: P81-83.

159. CVM's risk assessment model used data compiled by federal agencies to estimate the number of people impacted by FQ-resistant *Campylobacter*. Ex. G-953. CDC used prevalence data from its FoodNet surveillance system to provide CVM with an estimate of the number of annual cases of campylobacteriosis that were reported in the United States. WDT G-1454: P5, L38-43. Data on the amount of chicken consumed were retrieved from the USDA Economic Research Service. WDT G-1454: P6, L17-18. Data on the proportion of resistant isolates in chicken were retrieved from the USDA Agriculture Research Service as part of its *Campylobacter* susceptibility testing, through the animal arm of NARMS, on chicken collected by the Food Safety Inspection Service. WDT G-1454: P6, L18-21; WDT G-1478: P9, L4-12.

160. CVM's risk assessment used the Harris 1986 and Deming 1987 case-control studies for input values to determine the proportion of all cases of campylobacteriosis attributable to chicken. WDT G-1454: P8, L13-15; Ex. G-268; Ex. G-162.

161. CVM addressed comments received from the public in the final version of the risk assessment model and report. WDT G-1480: P6, L34-36.

162. CVM's risk assessment was based on reliable, scientific data. WDT G-1480: P6, L40-42.

163. Bayer witness Patterson incorrectly compared of the numbers of foodborne versus waterborne campylobacteriosis cases in his written direct testimony. WDT B-1910: P27, L9-11; Ex. B-927 and Ex. G-410.

164. Alternative treatments other than Baytril are available to treat poultry respiratory diseases. WDT G-1478: P18, L34-46.

165. The determination that Baytril is safe is in no way dependant upon the availability of alternative drugs.

Proposed Conclusions of Law

1. CVM has met its burden of adducing evidence that there is a reasonable basis from which serious questions about the safety of Baytril use in poultry may be inferred.

2. Bayer Corporation ("Bayer") has the burden to prove that the use of Baytril under the approved conditions of use in poultry has been shown to be safe.

3. Bayer has not met its burden to prove that the use of Baytril under the approved conditions of use in poultry has been shown to be safe.

4. In order to meet its burden CVM need only present enough information to show how FQ-resistant *Campylobacter* in poultry is related to the use of FQs in poultry (and to the transmission of FQ-resistant *Campylobacter* to humans) and that the FQ-resistant *Campylobacter* presents some potential harm to the public health.

5. Section 512(e)(1)(B) of the Act does not require that wholly new information form the basis for a decision to withdraw approval of an NADA.

6. Section 512(e)(1)(B) contemplates a re-evaluation of evidence that was available at the time an NADA was approved. Bell v. Goddard, 366 F. 2d 177 (7th Cir. 1966).

7. "Safe," in the context of human food safety, means "reasonable certainty of no harm."

8. There is microbiological, epidemiological, and medical evidence that FQs select for FQ resistance in poultry, that FQ-resistant Campylobacter is a major source of FQ-resistant Campylobacter infections in humans, and that FQ-resistant Campylobacter infections in humans present the potential for harm in terms of increased duration of diarrhea and other complications.

9. The proper risk / benefit analysis compares the risks to humans of keeping this drug on the market for use in poultry to the benefits to humans of keeping this drug on the market for use in poultry.

10. The Commissioner cannot consider economic benefits in determining the safety of a new animal drug. See Whitman v. American Trucking Association, 531 U.S. 457 (2001).

11. Extended duration of diarrhea constitutes harm under the Act.

12. Complications of FQ-resistant campylobacteriosis constitutes harm.

13. A risk assessment is not necessary to demonstrate harm.

Respectfully Submitted:

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Enrofloxacin Hearing
Docket No: 00N-1571

CERTIFICATE OF SERVICE

I hereby certify that an original and one copy of the foregoing Center for Veterinary Medicine's Post Hearing Brief was hand delivered this 18th day of July, 2003, to:

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

I also certify that a copy of the Center for Veterinary Medicine's Post Hearing Brief was hand delivered and e-mailed this 18th day of July, 2003, to:

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

I also certify that a copy of the Center for Veterinary Medicine's Post Hearing Brief was mailed and e-mailed this 18th day of July, 2003, to:

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