REPORT OF THE COMMITTEE ON FOOD AND FEED SAFETY

Chair: Daniel E. LaFontaine, Columbia, SC
Vice Chair: Bonnie J. Buntain, Calgary, Alberta, CAN

David C. Ailor, DC; Alex A. Ardans, CA; Deanna L. Baldwin, MD; Marilyn F. Balmer, MD; Joseph L. Blair, VA; Richard E. Breitmeyer, CA; Roy D. Brister, AR; Peggy N. Carter, VA; Tony A. Caver, SC; Max E. Coats, Jr., TX; Kevin G. Custer, IA; Ria de Grassi, CA; Ignacio T. dela Cruz, MP; Reta K. Dyess, TX; Kathy D. Finnerty, NY; Robert F. Gerlach, AK; L. Wayne Godwin, FL; C. Ross Hamilton, TX; Jay Hawley, IN; Christine N. Hoang, IL; Donald E. Hoenig, ME; Tom Holder, MD; Rex D. Holt, GA; Clyde B. Hoskins, SC; Danny R. Hughes, AR; John P. Huntley, NY; Lee C. Jan, TX; Heidi D. Kassenborg, MN; Susan J. Keller, ND; Sung G. Kim, NY; Spangler Klopp, DE; Elizabeth A. Lautner, IA; Laurent O'Gene Lollis, FL; Kelli S. Ludlum, DC; John R. MacMillian, AR; Bret D. Marsh, IN; David T. Marshall, NC; Kris Mazurczak, IL; James D. McKean, IA; Katherine M. McNamara, VT; David L. Meeker, VA; Andrea Mikolon, CA; Lee M. Myers, GA; Nicole Neeser, MN; Carol A. Olmstead, MT; Kenneth E. Olson, IL; Gary D. Osweiler, IA; Gerardo Quaassdorff, VT; John R. Ragan, MD; James T. Rankin, Jr., PA; Jane F. Robens, MD; Nancy J. Robinson, Mo; Kerry A. Rood, UT; Leon H. Russell, Jr., TX; John P. Sanders, WV; Richard S. Sellers, VA; Glenn N. Slack, KY; Harry Snelson, NC; Rosemary A. Speers, VA; Philip Stayer, MS; Bruce N. Stewart-Brown, MD; James E. Stocker, NC; Stanley A. Stromberg, OK; H. Wesley Towers, DE; Liz K. Wagstrom, IA; Gary L. Waters, MT; Larry L. Williams, NE; Rob S. Williams, DC; Nora E. Wineland, CO; John F. Wortman, Jr., NM.

The Committee met on October 26, 2008 at the Sheraton Greensboro Hotel Greensboro, North Carolina, from 12:30 to 5:30 p.m. There were 13 members and 31 guests present. Chair Dan Lafontaine presided. After welcoming remarks, Dr. Lafontaine introduced this year's main topic, The Crossroads of Animal Health, Food Safety and Antimicrobial Resistance; an Update on the National Antimicrobial Resistance Monitoring System (NARMS), 1996-2007. This topic consisted of a series of presentations by antimicrobial resistance experts from the Food and Drug Administration (FDA), the United States Department of Agriculture (USDA) and the Center for Disease Control and Prevention (CDC). At the conclusion of the main topic, Dr. J. Dennis McCurdy, Center for Veterinary Medicine (CVM), FDA presented comments on the Animal Feed Safety System. The Committee's business meeting followed the scientific presentations.

FDA-CVM provided the following background information on National Antimicrobial Resistance Monitoring System (NARMS). In the United States, NARMS – Enteric Bacteria is a national public health monitoring system that tracks changes in the susceptibility of certain enteric bacteria to antimicrobial agents of human and veterinary medical importance. The NARMS program was established in 1996 by FDA-CVM as part of its overall strategy to assess the impact of antimicrobial use in food animals on public health. NARMS is a collaborative program which brings together three federal agencies; FDA-CVM, CDC, and USDA-Agricultural Research Service (ARS).

Antimicrobial resistance is a serious problem that threatens both human and animal health. In human medicine, antimicrobials are most often used to treat infectious diseases, whereas in food animals, antimicrobials are used for the prevention, control, and treatment of infectious diseases, as well as for enhancing growth and improving feed efficiency. An undesired consequence of antimicrobial use in any environment is the potential development of antimicrobial-resistant bacteria. In food animals, these bacteria can contaminate meats as well as dairy products, eggs, and indirectly produce. These resistant bacteria, and in particular resistant zoonotic pathogens, may be transferred to humans through the consumption, handling, or improper cooking of contaminated foods and may cause serious infections.

As part of the overall CVM strategy to assess relationships between antimicrobial use in agriculture and subsequent human health consequences, the NARMS program was developed in 1996 to monitor changes in susceptibility of select bacteria to antimicrobial agents of human and veterinary importance. In addition to collaboration among the three aforementioned US federal agencies, NARMS also collaborates with antimicrobial resistance monitoring systems in other countries, including Canada, Denmark, France, the Netherlands, Norway, Sweden and Mexico so that information can be shared on the global dissemination of antimicrobial resistant foodborne pathogens.

The NARMS program monitors antimicrobial susceptibility/resistance in two categories of enteric bacteria recovered from food animals, humans, and retail meats. These categories are zoonotic bacterial pathogens (*Salmonella* and *Campylobacter*) and commensal bacteria (*E. coli* and *Enterococcus*). All three NARMS components (animal, human and retail meats) also characterize *Salmonella* and *Campylobacter* through use of Pulse-Field Gel Electrophoresis (PFGE) in an effort to determine genetic-relatedness between isolates.

Epidemiological and microbiological research studies are conducted within each agency or between agencies on isolates of special interest such as those of a particular serotype or expressing a particular resistance pattern. As a public health monitoring system, the primary goals of NARMS are to:

- provide descriptive data on the extent and temporal trends of antimicrobial susceptibility/resistance in zoonotic foodborne bacterial pathogens and select commensal organisms to veterinarians, physicians, public health authorities, and other stakeholders;
- provide a platform for successive epidemiology and research studies to better understand the emergence and transfer of antimicrobial resistance and the burden of illness posed by these organisms, and assist in the development of science-based strategies to contain or mitigate resistance; and
- assist the FDA in making decisions related to the approval of safe and effective drugs for humans and animals, as well as to promote judicious use of antimicrobial drugs.

This session is intended to provide attendees an update on the human, animal, and retail meat components of the NARMS program. The objectives are to: 1) present the current status of the NARMS program; 2) describe the occurrence of antimicrobial drug susceptibility/resistance among select foodborne pathogens and commensal bacteria from the three NARMS components and; 3) provide updates on the epidemiological trends of these bacteria and associated antimicrobial drug susceptibility/resistance phenotypes from1996 to 2007.

The topic was introduced by Dr. Beth Karp, Coordinator, NARMS, CVM-FDA. Her presentation was entitled, Program Overview of NARMS. NARMS is a national public health monitoring system that tracks changes in the susceptibility of certain enteric bacteria to antimicrobial agents of human and veterinary medical importance. There are four specific objectives of NARMS:

- monitor trends in antimicrobial resistance among foodborne bacteria from humans, retail meats, and animals.
- disseminate timely information on antimicrobial resistance to promote interventions that reduce resistance among foodborne bacteria.
- conduct research to better understand the emergence, persistence, and spread of antimicrobial resistance.
- assist FDA in making decisions related to the approval of safe and effective antimicrobial drugs for animals.

NARMS collects data in three different categories: human, retail meat and animals. CDC collects human data from 53 health departments nationwide which is input into the NARMS database. Retail meat data is collected by FDA-CVM through ten FoodNet sites plus the State of Pennsylvania. Animal data is collected by USDA-ARS in collaboration with USDA, Food Safety Inspection Service (FSIS). Several different microorganisms have been tested for antimicrobial resistance since 1996.

For the human component, CDC has tested:

- Non-Typhi *Salmonella* (1996) (Indicates year added)
- *E. coli* 0157:H7 (1996)
- *Campylobacter* (1997)
- *Salmonella* Typhi (1999)
- *Shigella* (1999)
- *Enterococcus* (2001)
- *E. coli* (2004)

In retail meats, the FDA has tested:

- Non-Typhi *Salmonella* (2002)
- *Campylobacter* (2002)
- *Enterococcus* (2002)
- *E. coli* (2002)

In animals, USDA tests

- Non-Typhi *Salmonella* (1997)
- *Campylobacter* (1998)
- *E. coli* (2000)
- *Enterococcus* (2003)

Laboratory testing consists first of identification, including serotyping and speciation. Microorganisms are them tested for drug susceptibility using broth microdilution. Prior to entering data into PulseNet or VetNet, pulsed-field gel electrophoresis (PFGE) serotyping is performed. Annual reports of NARMS data are being produced for each NARMS component (human, animal and retail meat). Additionally, an Executive Report is periodically produced. The Executive Report summarized, in an integrated format, NARMS data from humans, animals and retail meats.

The most recent Executive Report is available at http://www.fda.gov/cvm/narms_pg.html. Data are presented in multiple formats in the Executive Report. Some examples are *Salmonella* isolates tested, *Salmonella* serotypes, *Campylobacter* species in humans and chicken parts, antimicrobial resistance by serotype and antimicrobial class, and drug-resistant isolates by serotype. Ongoing NARMS research efforts are generally grouped into four categories:

1. methods development
   - standardized antimicrobial susceptibility testing (*e.g.*, *Campylobacter)*
   - rapid isolate testing (*e.g.*, PCR, microarrays, molecular serotyping)
   - multi-locus sequence typing (MLST) for *Campylobacter*
2. Pilot projects to examine emerging issues
   - methacillin-resistant *Staphylococcus aureus* (MRSA), *C. difficile* in retail meats
   - *Salmonella, E. coli*, and *Enterococcus* in feeds
   - *Enterococcus* strains in humans and retail meats in MD and MI
   - MLST for *Enterococcus*
3. Studies to understand the emergence and spread of resistance
   - linking NARMS susceptibility data with PFGE results
   - examining historical strains to document the emergence of resistance
   - sequencing 17 *Salmonella* genomes, including resistant strains, in partnership with J. Craig Venter Institute
   - plasmid-mediated virulence genes in *Salmonella Kentucky*
   - virulence factors in generic *E. coli* isolates
   - development of resistance in treated animals
4. Epidemiological studies
   - risk factors for acquiring resistant infections
   - public health impact of resistance

NARMS supports and collaborates with several international activities such as PulseNet International, the World Health Organization Global *Salmonella* Surveillance Initiative and the Codex Task Force on Antimicrobial Resistance.

There was a recent FDA Science Board Subcommittee Review of NARMS. The FDA Science Board Advisory Committee established a subcommittee to evaluate the NARMS program. General comments of the subcommittee were as follows: 1.) NARMS has evolved into a mission-critical tool for FDA, 2.) outstanding progress has been achieved over last decade, 3.) it should be a high priority for future support and attention, 4.) visioning, strategic and business planning processes should be considered and adopted where appropriate, 5.) consider making the program more predictive, responsive and expansive. Additionally, the subcommittee provided constructive recommendations with regard to key elements and future directions of the NARMS program. These include:

- Sampling strategies
  - use national, random sampling when possible
  - when not feasible, further stratify data or use a more targeted sampling strategy
  - encourage monitoring of commensals from healthy humans
- Research studies
  - encouraged further development and expansion
  - emphasis on hypothesis-driven and collaborative research
- International activities
  - strongly endorsed continuation and expansion of international activities, including training
- Data harmonization and reporting
  - need for an integrated database and timely reporting

NARMS has begun implementing several of these recommendations and will continue to evolve into a more valuable tool for assessing susceptibility of enteric bacteria to antimicrobial agents.

Dr. Karp's presentation was followed by Dr. Ezra Barzilay, CDC, who presented human NARMS surveillance data. Antimicrobial agents are commonly used in food animals and it is known that inappropriate use can lead to natural selection for bacterial resistance to antimicrobial agents. Additionally, food animals constitute an important reservoir of antimicrobial resistance. Resistant bacteria can be transmitted to humans through the food supply. This is most evident with pathogens, but also occurs with commensal bacteria. In order to monitor this, in 1996 FDA's Joint Advisory Committee recommended that a surveillance system be created to monitor development of antimicrobial resistance among foodborne bacteria, the NARMS. In 2003 strategic planning lead to the formation of

an integrated surveillance system wherein CDC performs human surveillance, the FDA-CVM is responsible for retail meat surveillance and the USDA-ARS conducts animal surveillance.

NARMS monitors the susceptibility of antimicrobial agents among enteric bacteria from humans, foods, and animals by collecting surveillance information on the following areas: 1) core surveillance, 2) Retail Food Survey, 3) outbreak isolates and 4) commensal organisms. Core surveillance provides a centralized source of antimicrobial resistance data from major surveillance systems using uniform methods. The Retail Food Survey monitors trends and changes in the prevalence of antimicrobial resistance among enteric bacteria isolated from four retail food commodities; ground beef, ground turkey, pork chops and chicken breasts. Outbreak isolates characterize the antimicrobial resistance attributes of bacterial pathogens isolated from foodborne disease outbreaks. Lastly, commensal organism surveillance provides ongoing monitoring for antimicrobial resistance among *Enterococci* and *E. coli*, commensal bacteria traditionally thought to cause disease in hospital settings. By providing information for action, NARMS goal is to promote the prudent use of antibiotics in veterinary settings through solidifying partnerships with food-animal producers and disseminating antimicrobial usage guidelines in agricultural settings.

Human data collection for NARMS began in 1996, with fourteen states participating. New York was added in 1999. The program had expanded to 28 states by 2002 and to all 50 states the next year. Human clinical isolates are identified by the state health departments and then submitted to the NARMS laboratory for antimicrobial resistance testing. NARMS receives every 20th non *Salmonella*, *Typhi Shigella*, and *E. coli* O157. All *Salmonella typhi, Salmonella paratyphi* A and C, *Listeria*, and non-cholera *Vibrio* are submitted. A representative sample of *Campylobacter* from ten FoodNet sites is also submitted. NARMS objectives are: 1) to monitor trends and changes in the prevalence of antimicrobial resistant enteric bacteria; 2) to determine the burden of illness of antimicrobial resistant enteric bacteria; 3) to identify and develop new intervention and mitigation strategies to help stem the increase of bacterial antimicrobial resistant; and 4) through education efforts, to promote the prudent use of antibiotics in veterinary settings.

Since its inception, NARMS has had a significant public health impact. NARMS has been a key component in completing goals from the interagency task force on antimicrobial resistance and in accomplishing CDC's mission of enhancing the surveillance and investigation of foodborne infections. Additionally, NARMS data provide information for tracking progress towards the Healthy People 2010 National Health Objective for Resistant *Salmonella* and *Campylobacter*. Some of the key findings and conclusions from a ten-year retrospective view of human NARMS data include the following:

- resistance to clinically important antimicrobial agents in non *Salmonella typhi* has increased.
- resistance to nalidixic acid (quinolone) has increased, which correlates with decreased susceptibility to ciprofloxacin
- resistance to ceftiofur (3rd generation cephalosporin) has increased, which correlates with decreased susceptibility to ceftriaxone
- *Salmonella Enteritidis* is the most common serotype among nalidixic acid-resistant non *Salmonella* typhi
- MDR-AmpC, a multidrug resistance pattern that includes ceftiofur resistance, emerged in 1998 in *Salmonella Newport* and has been detected in 14 other non *Salmonella typhi* serotypes.
- *Salmonella Newport* is the most common serotype in non *Salmonella typhi* with MDR-AmpC
- The increase in ceftiofur resistance in non *Salmonella typhi* was mainly driven by the emergence of MDR-AmpC in *Salmonella Newport*
- ACSSuT[1] in *Salmonella typhimurium* has declined, however, it has remained high at 19 percent in 2006.
- *Salmonella typhimurium* is the most common serotype in non-Typhi *Salmonella* with ACSSuT.
- ACSSuT has declined in non *Salmonella typhi,* similar to the trend observed in *Salmonella typhimurium*.
- Monitoring to detect emerging multidrug and clinically important resistance, including serotype-specific trends, is important to guide clinical care and public health interventions.

[1] ACSSuT – resistance to at least ampicillin, chloramphenacol, streptomycin, a sulfonamide and tetracycline

At the conclusion of Dr. Barzilay's presentation, comments regarding retail meat NARMS surveillance data were made by Ms. Linda English, CVM- FDA. Retail meats are monitored for antimicrobial resistant microorganisms for several reasons. The use of antimicrobial agents in food animals can result in the development of antimicrobial resistance in enteric bacteria. Meat and poultry offered for retail sale can become contaminated with enteric bacteria during slaughter. Retail meats are a direct route of exposure for consumers. Comparing enteric bacteria from retail meats to those from humans and animals permits an estimation of the contribution of enteric bacteria from meats to human illness. Additionally, NARMS retail meat data assist FDA-CVM in making decisions on the safety and effectiveness of antimicrobial drugs and support the FDA's mission as a science-based regulatory agency. NARMS surveillance of retail meats began as a pilot study in 2001 by FDA-CVM in Iowa. In 2002 Public Health Laboratories in Connecticut, Georgia, Maryland, Minnesota, Oregon and Tennessee were added. California and New York

began submitting data in 2003 while Colorado and New Mexico joined in 2004. In 2007 Maryland did not participate. To collect samples, every site visits five grocery stores per month. From each store, two packages (different brands) of chicken breasts (with bone/skin), pork chops, ground turkey and ground beef (80 percent lean) are purchased. Samples are refrigerated and cultured within 96 hours. Results are recorded on standardized specimen log sheets. All sites test for *Salmonella* and *Campylobacter* while Georgia, Oregon, Maryland and Tennessee test for *E. coli* and *Enterococcus*. Presumptive positives are forwarded to FDA-CVM for confirmation of identification, susceptibility testing, and further isolate characterization. Confirmatory testing is performed using one or more of several methods, including rapid biochemical-based methods, polymerase chain reaction (PCR) (*Campylobacter*), serotyping (all *Salmonella*) and other conventional methods (all species). Resistance to numerous antimicrobials is tested. Specific compounds tested is dependant on several factors such as organism isolated, analysis of previous years' data, human and animal NARMS data, development of new compounds, and others. The antimicrobials tested change from year to year, depending on these factors.

A summary of the 2002-2007 NARMS retail meats indicates the following:

1. both *E. coli* and *Enterococcus* occurred in a high percentage of all retail meats
2. *Salmonella* was most often recovered from poultry and *Campylobacte*r from chicken breasts but seldom from ground beef, ground turkey, and pork chops
3. *Campylobacter jejuni* was recovered two to three times more often than *C. col.*
4. resistance in *Salmonella* most often occurred to the older antimicrobials (tetracycline, streptomycin, ampicillin, and sulfasoxazole)
5. approximately 15 percent of all *Campylobacter* were resistant to floroquinolin. Almost no *C.jejun*i, but approximately 10 percent of *C. coli,* were resistant to erythromycin, azitrhromycin, clindamycin, and telithromycin. Resistance to tetracycline occurred in 40-50 percent of all *Campylobacter*
6. with a few exceptions, all *Campylobacter* were susceptible to florfenicol and gentamycin
7. multiple drug resistance occurred in both *Salmonella* and *Campylobacter*
8. NARMS retail meat surveillance provides valuable data on antimicrobial resistance in foodborne zoonotic bacteria to veterinarians, physicians, and others interested in public health

Dr. Heather Harbottle, CVM- FDA presented, Genetic Relatedness of *Salmonella* and *Campylobacter* Isolates from NARMS. As discussed by Ms. English, *Salmonella* and *Campylobacter* are two of the most common isolates recovered from NARMS retail meat samples. Consequently, FDA-CVM is expending considerable effort on developing better and faster methods for subtype characterization of these microorganisms. The goal of this subtyping effort is to enhance NARMS' retail meat component ability to assess genetic relatedness of their isolates with those of NARMS animal and human isolates as well as other isolates in PulseNet. Subtyping is the process of analyzing multiple isolates within a given species to determine whether they represent single or multiple strains. A strain is defined as a single isolate or a group of isolates distinguishable from other isolates of the same genus and species with the use of phenotypic and/or genotypic characteristics.

Two phenotyping methods are serotyping and classifying by antimicrobial susceptibility. Serotyping is the primary typing method for *Salmonella* based on O and H antigens. There are over 2500 *Salmonella enterica* serotypes and CVM performs traditional serotyping on all retail *Salmonella* isolates received. About 37-45 different serotypes are encountered each year. Classifying by antimicrobial susceptibility involves developing phenotypic susceptibility profiles using a standard Clinical and Laboratory Standards Institute broth microdilution method. Antimicrobials tested include amoxicillin-clavulanic acid (AUG), ampicillin (AMP), cefoxitin (FOX), ceftiofur (TIO), ceftriaxone (AXO), chloramphenicol (CHL), gentamicin (GEN), kanamycin (KAN), streptomycin (STR), sulfisoxazole (FIS), tetracycline (TET), and trimethoprim-sulfamethoxazole (COT). Multiple drug resistance (MDR)-AMP is the designation used for a recognized multiple drug resistance in certain microorganisms that are resistant to AMP, AUG, FOX, TIO, CHL, STR, FIS, TET and decreased susceptibility to AXO (MIC ≥16 µg/ml). The genotyping methods used by CVM are molecular serotyping and PFGE. Molecular serotyping uses the Bioplex System which involves simultaneous multiplex analysis of up to 100 different biomolecules in a single well of a microplate in 30 minutes. Current studies involve validating molecular serotyping by traditional serotyping for all serotypes. PFGE continues to be the primary subtyping method. It is currently recognized as the gold standard for serotyping. Briefly, this process involves cutting bacterial deoxyribonucleic acid (DNA) with one or more enzymes resulting in fragments of DNA that can vary in size. This may reveal changes in the bacterial DNA, such as mutations, insertions and rearrangements. Cutting the bacterial enzyme with increasing numbers of enzymes increased the discriminatory power of PFGE, but absolute matches with other isolates is still not possible without detailed epidemiological data.

The CVM PFGE database contains PFGE fingerprints derived from cutting with two enzymes since 2002. Currently the database contains approximately 1757 *Salmonella* genotype fingerprints and 2334 *Campylobacter* fingerprints. CVM's NARMS data can be used to compare isolates in PulseNet. Several genotypically identical matches have been found. This analysis indicates that, as speed and efficiency of genotyping methods evolve, the

potential exists to compare epidemiological data with matching genotypes to assess common sources for microorganisms involved in human illness.

Microarray is a rapid detection method being tested by CVM. Microarray can detect hundreds to thousands of genes simultaneously and is customizable for specific genes of interest. Currently it is detecting 272 resistance, virulence and pathogen ID genes. It is being evaluated for identifying of all classes of antimicrobial resistance genes as well as common virulence genes. It is improving the understanding of the origin of antimicrobial resistance in retail meat, animal and human origin samples. Additionally, it supports an FDA Critical Path Initiative, Interrogating the Genomic Diversity of Enteric Pathogens Using a Novel 85 Genome *Salmonella enterica*, *Escherichia coli*, *Shigella* and *Vibrio cholera* Multi-Species Microarray. This is an intra-agency collaboration between CVM and the Center for Food Safety and Applied Nutrition. The Critical Path Initiative is FDA's effort to stimulate and facilitate a national effort to modernize the scientific process which holds the potential to improve the tools FDA uses to evaluate the safety and efficacy of human and veterinary products as well as the safety and nutrition of food and food ingredients. Examples of prospective tools are new rapid tests for biological and chemical contamination of animal-derived foods; technologies for detecting and mitigating the microbial contamination of food; and analysis technologies for assessing the safety and nutritive value of foods and food ingredients. The ultimate goal of the initiative is to stimulate the development of products needed to address urgent public health needs.

Following Dr. Harbottle, animal NARMS surveillance data was reviewed by Dr. Jonathan Frye, ARS-USDA. NARMS is a collaboration with FDA-CVM, CDC, and USDA-ARS, USDA- FSIS and USDA-Animal and Plant Health Inspection Service (APHIS). USDA-ARS also has non-federal partners, including veterinary diagnostic laboratories. NARMS began in the US in 1996. It is funded through and interagency grant by the FDA. Unfortunately, funding has been level for the past three years, which, of course, equates to a decline in money each year as salaries and operating costs increase. NARMS tests isolates from on-farm and diagnostic sources when available and funding allows. However, routine testing of slaughter/processing isolates is the hallmark of the animal component of NARMS. It is a passive system, relying on the receipt of *Salmonella* isolates from FSIS. But, it remains the only comprehensive snapshot of resistance in animal production in the US. All food animal species, all sizes of plants, and all geographic areas are represented in the slaughter isolates. ARS also tests for *E.coli, Campylobacter* and *Enterococcus*, as money permits. In the past ARS received diagnostic isolates from veterinary clinics. Currently, isolates originate only from non-diagnostic sources. These include some on-farm isolates from the NAHMS, via APHIS. Predominantly, however, isolates come from slaughter samples taken by FSIS. How is the data reported? Each arm of NARMS posts yearly annual reports on their respective websites. Additionally, an executive report which combines data from all three arms is posted on the FDA website and can be linked from the other websites. A future goal is to post individual reports in a timelier manner and to have the executive reports completed within nine months of data closeout.

A data review will first focus on the multi drug resistance issue. The percentage of pan-susceptible isolates has not changed dramatically over the years and remains at approximately 50 percent. MDR, with resistance to five or more antimicrobials, appeared to go up earlier in the decade, but has declined since 2003. The same is seen for resistance to ten or more antibiotics. However, when you look by animal source, a slightly different picture emerges. More cattle isolates are pan-susceptible than isolates from other animal sources. Cattle are followed by chickens, then swine and turkey. Even though overall numbers are lower, swine and turkey have gained in percent of pan-susceptible isolates. Resistance is not only associated with animal source but also by particular serotype. Some serotypes tend to be much more resistant to more antimicrobials than other serotypes. MDR, by percentage, for the ten cattle serotypes is newport, agona, typhimurium and typhimurium variant 5-. The percent of increased resistance to nalidixic acid and/or decreased susceptibility ciprofloxacin presents an interesting situation. Human data shows a slight increase since 2002. Conversely, a decrease among animal isolates has been observed.

There are four new tools being introduced to expand the capability of NARMS. First is VetNet. The primary objective of VetNet is to capture PFGE patterns of *Salmonella* and *Campylobacter* isolates submitted to NARMS. Generic *E. coli*, *Enterococcus* and other bacterial isolates will be added over time. Then VetNet and PulseNet PFGE patterns will be compared. This will enhance the ability to investigate animal illness outbreaks and assess the possibility of linkage with food borne illness outbreaks. There are known limitations of VetNet. It evaluates only one-enzyme cuts. There is no standardized agreement of what a match really means (or what criteria to use). Band differences can be attributed to some type of molecular change – the acquisition of a plasmid in fact changes the relatedness of isolates, particularly when comparing to those that do not carry a plasmid or the same plasmid. Additionally, prior to a final interpretation, other information, including but not limited to plasmid status, presence or absence of other genes and supporting epidemiology, is required prior to determining the final level of relatedness. Another tool is an interactive database that is available on the NARMS website. There are a number of different types of graphs which allow the user to customize their searches based on the antimicrobial or organism of interest. ARS has also continued to develop techniques to improve salmonella serotyping. One of the oldest microbiological laboratory problems is serotyping. It is also slow, cumbersome, difficult and expensive. However, serotyping is

absolutely necessary for characterizing *Salmonella*. So ARS has been developing a molecular technique for determining *Salmonella* serotypes. This is based on genes identified by comparative genomic hybridizations. Through collaboration, ARS developed a multiplex PCR to detect these genes. Recently ARS adapted this to a high-throughput technique called Salmonella Multiplex Assay for Rapid Typing (SMART). It uses a single tube, fifteen-product multiplex that labels each product during PCR. This is then separated and detected by capillary analysis on a sequencer with automated scoring and serotype determination. It was tested in 2007 on a blind sampling of over 800 clinical isolates from the Washington State Department of Health. It identified over 90 percent of isolates. The few that were ambiguous could be identified by PFGE. ARS is currently beta-testing this at several clinical labs in the US and Canada and a publication should be coming out very soon. Lastly, high density tests are being developed to identify the genetic elements responsible for the phenotypes seen in NARMS isolates. This primarily uses a DNA microarray that can detect virtually every known antimicrobial resistance gene. It was designed by searching the National Center for Biotechnology Information database for all genes annotated as antimicrobial resistant associated. These were downloaded into a local database which was used to synthesize probes to detect each gene. The probes were used to construct the array. Testing is complete and it works very well. ARS is currently using it to analyze the NARMS isolates to try and find the genes causing resistance and to determine their distribution and epidemiology.

The NARMS group's formal presentations were concluded with Dr. Frye's second presentation entitled, Extended-Spectrum β-lactam Resistance Among *Salmonella.* Human data in this presentation is courtesy of Jean Whichard at the CDC. β-lactams are divided into four general groups: the penicillins, the cephalosporins, the monolactams and carbapenems. They all posses the four member β-lactam ring and have various modifications to their R groups. These R-group modifications change the chemical properties of the β-lactams and affect solubility, stability, bioavailability and degradation by the host. Thus these changes in chemical properties result in the spectrum of clinical activity due to transport and access to their targets in the bacteria and resistance to β-lactamases produced by the bacteria. How do β-lactams work? The β-lactam is mistaken by the enzymes that build the cell wall for one of its components. When the enzymes try to catalyze the reaction, the four member ring of the β-lactam is broken and bound irreversibly to the enzyme, inactivating it. Eventually the growing cells wall weakens causing the cell to lyse. β-lactamases work by cleaving the β-lactam ring, inactivating the antibiotic. To help combat this, sometimes β-lactams are mixed with β-lactamase inhibitors like clavulanic acid. An example is Augmentin which is ampicillin and clavulanic acid. These work by binding to and inhibiting the β-lactamases. This is kind of a molecular arms race with the bacteria.

NARMS has been testing susceptibility of *Salmonella* to the following β-lactams: cephalothin, ceftriaxone, ceftiofur, cefoxitin, ampicillin, and amoxicillin/clavulanic acid. In 2004 we decided to do a study on β-lactam resistance in *Salmonella* animal isolates. ARS reviewed the data and found that resistance to the various β-lactams was increasing over the past 5 years. An earlier study of NARMS animal isolates from 1997-1998 had identified the bla_{CMY-2} β-lactamase gene as responsible for resistance. A similar increase had been seen in human isolates of *Salmonella*. ARS decided to focus on ceftiofur resistance because of the importance of 3rd generation cephalosporins in treatment of infections and its wide spread use in animals. In all animal groups from 1999 to 2003, 34,411 *Salmonella* were isolated and about 11 percent of those were resistant to ceftiofur. Cattle have the highest level of resistant isolates. The majority of the resistant cattle isolates were clinical samples from ill animals. *Salmonella newport* stood out as having the greatest ceftiofur resistance with over 77 percent of animal isolates resistant. Almost all of these were also isolated from cattle. The ARS NARMS group finally looked at the cause of ceftiofur resistance and found that by PCR assay of 125 representative isolates, over 80 percent had the bla_{CMY-2} gene detected. The group then took a closer look at some strains and found that the bla_{CMY-2} gene was located on a large, self- transmissible MDR plasmid. A summary of the data shows that ceftiofur resistance in animal isolates had increased over time during the study. Cattle were the dominant source of resistant isolates and were mostly from diagnostic samples. *S. newport* was the dominant ceftiofur resistant serotype. The resistance mechanism was the bla_{CMY-2} gene and was linked to the MDR-AMPC plasmid.

Because of the results of the previous study, the next study focused on resistance in cattle. ARS was especially concerned about the possibility of extended spectrum β-lactamases being present in animals. These markers had spread widely in human and animal isolates in other parts of the world and had begun to be found in humans in the U.S. To do this ARS took a close look at all 3,984 cattle slaughter isolates from 2000-2004. These were all from healthy animals. They were first screened for resistance and selected any with reduced susceptibility to ceftriaxone for further analysis. These were then tested for extended-spectrum β-lactamase (ESBL) phenotype, PCR analysis for several β-lactamase genes, and PFGE analysis for genotyping. All 3,984 isolates collected were susceptible to 4th generation cephalosporins. None of the 97 selected for further analysis had an ESBL phenotype which is defined by a greater than two-fold reduction in minimum inhibitory concentration (MIC) to ceftazidime when clavulanic acid is added to the assay. No ESBL genes were detected by PCR in the 97 isolates, and almost all isolates had the cmy-2 gene detected. All of the 97 isolates had the MDR-AMP phenotype with resistance to a variety of other

antimicrobials. Most of the 97 isolates were *S. newport* or *S. a*gona and these were clonal in nature, with the *Newport* being wide spread while the Agona were mostly from the northeastern region.

Animal data through 2007 shows that *Salmonella newport* has been declining both in number and MDR phenotype. While predominantly isolated from cattle, *S. newport* continues to be isolated form other sources. In 2007, cattle were followed closely by turkey. Another interesting observation is that the number of pan-susceptible isolates appears to be increasing. Of the 50 isolates in 2007, 52 percent were resistant to five or more antimicrobials but 48 percent were pan-susceptible whereas in 2006 only 19 percent were pan susceptible. The reasons for this shift are unknown, but have been observed among other serotypes over time. How does this compare to current human data? Human data shows that *S. newport* was going up in the percentage of total *Salmonella* since the beginning of the decade. However, if we look at the level of multi-drug resistance, among *S. newport*, we noted that it also went up in the beginning of the decade but then began declining, just as we saw in animal isolates. The multi-drug resistance in humans and in animals seems to be linked to the MDR-AMPC phenotype. Similar to our work in animal isolates, this has also been associated with large plasmids in the human isolates and a paper describing this has just been published by the CDC. To summarize, a large proportion of multi-drug resistance was due to *Salmonella Newport*, but other serotypes were also involved. This was associated with cattle isolates, especially diagnostic isolates. Resistance was due to an MDR-AMPC plasmid, which is found not just in *S. newport*. The same was true for human isolates. Prevalence has been going down over time in both human and animal *S. newport* isolates. However, the MDR-AMPC plasmids have been found in other serotypes and may be spreading.

Ongoing and future planned studies are focused on the following areas:

- Continued surveillance for β-lactam resistance (esp. ESBLs) in animal isolates (dairy cattle next target). ARS will use many of the same techniques discussed above to continue this work.
- Sequencing and genomics of MDR-AMPC (and other) plasmids. To complete this work we have our antimicrobial resistance gene microarray to which probes for four of the major *Salmonella* MDR plasmids have been added.
- Identification of resistance genes in MDR isolates collected over the ten years of NARMS (*Salmonella* and *E.coli*). The microarray will be used for these studies
- Epidemiology of resistance genes. This will be done by a meta-genomics approach with appropriate statistical analysis.

Following Dr. Frye's remarks, Dr. Lafontaine introduced Dr. J. Dennis McCurdy, CVM- FDA, who provided an overview of the Animal Feed Safety System (AFSS). The FDA began modernizing its animal feed safety program in 2003. The new program, the AFSS, is being designed to be comprehensive, preventive, and risk-based so that the FDA and collaborating states can ensure the safety of feed intended for food animals and pets, as well as the safety of human food derived from food-producing animals. The AFSS has six components:

- ingredients and the approval process;
- limits for animal feed contaminants;
- process control for the production of feed ingredients and mixed feed;
- reporting of unsafe feed;
- regulatory oversight; and
- education and outreach.

These are all presented in the AFSS Framework Document, which is available on the FDA-CVM Web site (www.fda.gov/cvm). The Framework Document also identifies gaps for each component as well as the manner in which the FDA intends to address each gap. To incorporate the concepts of risk-assessment in AFSS, the AFSS Team developing the program has drafted a risk-assessment tool. The tool evaluates the hazard, the health consequences for humans and animals and the exposure potential. A risk scoring algorithm has been developed which is currently based on the relative level of health consequences times the exposure potential. The team is continuing to collect data that can be used for the model and is working to validate the model. AFSS has also been revised to include provisions of the FDA Amendments Act of 2007 concerning the safety of pet food and feed ingredients. The AFSS initiative fits well into FDA's overarching Food Protection Plan, which was designed to integrate all federal, state, and local food safety and food defense (counterterrorism) programs in the United States. AFSS and the Food Protection Plan have many cross-cutting principles. Detailed information on the AFSS project is available at the following link: http://www.fda.gov/cvm/AFSS.htm

Committee Business:

After the scientific presentations, Chair Lafontaine opened the Committee's business meeting. In May 2008, the United States Animal Health Association (USAHA) Executive Committee (EC) approved the merger of the Committees on Food Safety and Feed Safety. USAHA recommended that the merged Committee consider three

items: 1) continue to address both food and feed safety issues within its agenda, based on current topics relevant to animal health. 2) explore the establishment of a Feed Safety Subcommittee to monitor and address emerging issues in feed safety. 3) revise the mission statement to reflect the topics [feed and food safety]. This year's program fulfilled the first recommendation and the Committee will continue to do so in future years. Members of the former Committee on Feed Safety who were in attendance were polled by Chair Lafontaine for comments regarding the formation of a Feed Safety Subcommittee. The consensus was that a Subcommittee is unnecessary as long as the newly formed Committee continued to address issues of interest to the feed safety community. Regarding the third recommendation, the Chair offered a proposed combined mission statement to the membership. Discussion from the floor resulted in several changes. After incorporating the recommended changes, the following was approved as the new mission statement:

The purpose of the Committee on Food and Feed Safety is to serve as a focal point for consideration of food safety and feed safety issues within USAHA. The Committee should recommend food/feed safety policies to protect animal and human health and be active in all areas of food/feed safety concerning foods of animal origin. Further, the Committee should provide a national forum for debate on minimizing chemical, microbial and physical contamination in the feed of food producing animals and provide specific recommendations, using the latest available knowledge to enhance the safety of animal feeds.