CHAPTER 5

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Animal Health Diagnostics and Veterinary Biologics

Laboratory and diagnostic services and veterinary biologics are essential components of the U.S. animal health infrastructure. This chapter describes the missions, functions, and key 2007 accomplishments of the USDA's NVSL, the Center for Veterinary Biologics (CVB), and the National Animal Health Laboratory Network (NAHLN).

NVSL provides laboratory services for USDA–APHIS. The NVSL includes four laboratories: the Diagnostic Bacteriology Laboratory (DBL), the Pathobiology Laboratory (PL), and the Diagnostic Virology Laboratory (DVL), all located in Ames, Iowa; and the Foreign Animal Disease Diagnostic Laboratory (FADDL), located on Plum Island, New York.

CVB is also headquartered in Ames, with some staff located in Riverdale, Maryland. CVB's Veterinary Biologics Program implements the provisions of the Virus-Serum-Toxin Act (VSTA) to assure that pure, safe, potent, and effective veterinary biologics are available for the diagnosis, prevention, and treatment of animal diseases.

NAHLN represents a nationwide strategy to coordinate the activities of organizations providing critical animal disease surveillance and testing. It is a cooperative effort between two USDA agencies—APHIS and the Cooperative State Research, Education, and Extension Service (CSREES)—and the American Association of Veterinary Laboratory Diagnosticians. NAHLN is comprised of Federal agencies and State and university veterinary diagnostic laboratories.

National Veterinary Services Laboratories

NVSL's mission is to safeguard animal and public health by providing quality services and resources that meet customer needs. It does this as a diagnostic laboratory and a NAHLN support organization. NVSL serves as the NAHLN reference laboratory and as the national and international reference laboratory for an increasing number of animal diseases.

More than 300 staff members work in or support the work of the four NVSL laboratories. The NVSL also works closely with the OIE to provide consultation, reagents, and training for foreign governments.

In December 2006, NVSL and an onsite Calibration Laboratory received accreditation to the International Organization for Standardization (ISO)/International Electrotechnical Commission 17025 Standard: General Requirements for the Competence of Testing and Calibration Laboratories. The ISO is the world's largest standards development organization. Accreditation recognizes the competency of the NVSL and Calibration Laboratory to perform specified tests and calibrations. In 2007, NVSL added tests and calibrations to the scope of accreditation as part of its commitment to enhancing the NVSL Quality Management System.

Diagnostic Bacteriology Laboratory

The DBL provides diagnostic assistance for bacterial and protozoal diseases by

- Conducting serologic testing for the presence of antibodies to pathogens;
- Isolating, identifying, and genotyping bacteria;
- Producing reagents needed for diagnostic testing;
- Administering proficiency tests for selected diseases; and,
- Conducting training.

The DBL is an OIE reference laboratory for leptospirosis, contagious equine metritis, and anthrax. The major functions of the laboratory include import testing of horses for equine piroplasmosis, dourine, and glanders; *Salmonella* serotyping; bovine tuberculosis and brucellosis culturing; brucellosis reagent production; Johne's disease serology and culturing; and, leptospirosis serology. The laboratory also maintains the brucellosis, bovine tuberculosis, Johne's disease, and NAHMS serum banks and the Johne's disease culture bank. The disease-specific serum banks serve as sources of well-defined sera for validating new tests as they are developed. The NAHMS serum banks are species specific and serve as a statistically valid source of sera for prevalence studies and for the validation of new tests.

Pathobiology Laboratory

PL offers a wide range of testing services. The laboratory provides training, proficiency testing, and lab oversight for those network laboratories conducting TSE testing. PL also screens blood samples of animals being transported out of the United States to detect fraudulent blood submissions.

PL testing supports APHIS–VS disease programs, such as those for bovine TB, BSE in cattle, scrapie in sheep, and CWD in deer and elk. PL supports tick surveillance by identifying ticks found on imported animals and also assists with screwworm surveillance by identifying suspected screwworm fly larvae.

The laboratory evaluates pesticides used in cattle to ensure that effective concentrations are utilized. Formaldehyde levels in veterinary biological products are also evaluated.

Diagnostic Virology Laboratory

The DVL performs diagnostic testing for numerous domestic and foreign animal viruses such as West Nile virus, vesicular stomatitis virus (VSV), HPAI virus, END virus, equine encephalomyelitis viruses (western, eastern, and Venezuelan), equine infectious anemia (EIA) virus, bluetongue virus, swine influenza virus (SIV), and PRV.

The DVL is an OIE reference laboratory for equine encephalomyelitis, EIA, HPAI, END, PRV, West Nile virus, and bluetongue virus. The lab also performs import/export testing, produces reagents/reference materials, and administers proficiency testing and laboratory certification approvals for selected diseases, including AI and END real-time reverse transcriptase polymerase chain reaction (rRT-PCR) testing in NAHLN laboratories.

Foreign Animal Disease Diagnostic Laboratory

FADDL, located at the Plum Island Animal Disease Center, is the U.S. laboratory devoted to diagnosing and researching FADs, including highly contagious FADs of livestock such as FMD.

FADDL scientists can diagnose more than 30 exotic animal diseases and perform thousands of diagnostic tests each year looking for the presence of FAD agents. Tissue and blood samples come to FADDL from veterinarians who suspect an exotic disease in domestic livestock or from animal import centers testing quarantined animals for foreign diseases. Animal health professionals in other countries also submit samples to FADDL when they need help with diagnoses or confirmation.

Additionally, FADDL is the custodian of the North American FMD Vaccine Bank. The bank, jointly owned by Canada, Mexico, and the United States, stores concentrated FMD antigen that can be formulated into vaccines should an FMD introduction occur. FADDL personnel are responsible for testing new lots of antigen and periodic testing of stored antigen for safety and potency.

FADDL also supports NAHLN with assay development and validation, training, proficiency testing, and confirmation testing for various FADs.

National Animal Health Laboratory Network

The USDA Homeland Security Office established the NAHLN as part of a national strategy to coordinate and link the testing capacities of the Federal veterinary diagnostic laboratories with the extensive

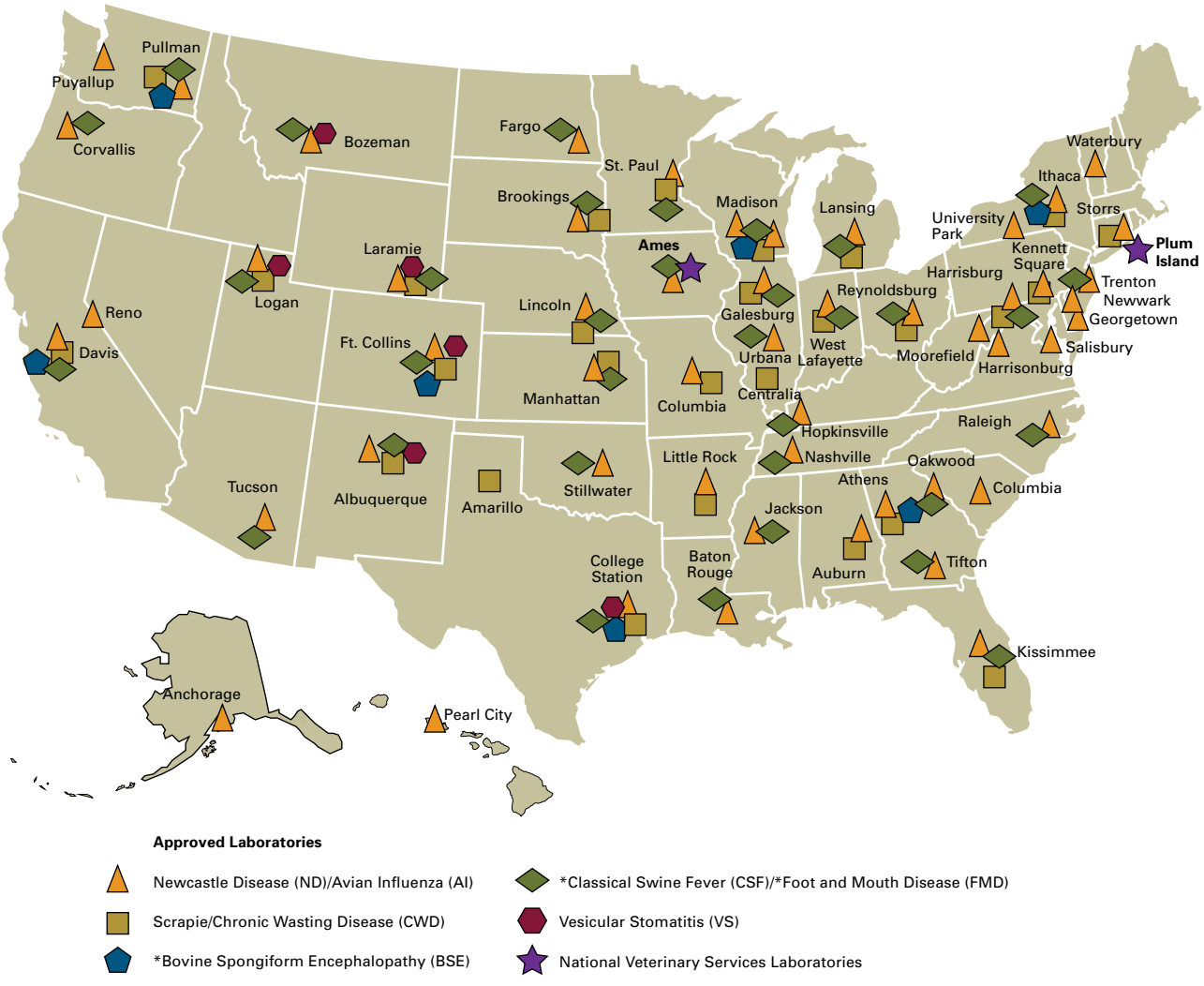
infrastructure (facilities, professional expertise, and support) of State and university veterinary diagnostic laboratories. This network enhances the Nation's early detection of, response to, and recovery from animal health emergencies, including emerging diseases and FADs that threaten the Nation's food supply and public health.

In 2002, APHIS and CSREES launched the NAHLN by entering into cooperative agreements with 12 State and university veterinary diagnostic laboratories. These were funded by the USDA. APHIS now contracts with 54 State and university diagnostic laboratories to assist with testing and surveillance; the number of NAHLN facilities totals 58 laboratories in 45 States, which includes those 54 laboratories plus NVSL, FADDL, the DOI laboratory in Madison, Wisconsin, and the FSIS laboratory in Athens, Georgia (fig. 5.1).

NVSL provides training and proficiency testing for the NAHLN member laboratories, either annually or semiannually. Tests include standardized screening methods for NAHLN's currently targeted diseases: AI, END, FMD,CSF, BSE, CWD, vesicular stomatitis (VS), and scrapie.

NAHLN laboratories perform screening assays and forward any suspect or positive samples to the appropriate section of NVSL for confirmatory testing.

FIGURE 5.1: NAHLN network



*For specified agents, not all laboratories are currently participating in surveillance testing.

2007 Highlights in Diagnostics and Laboratory Activities

Testing Support

Brucellosis testing—The DBL modified the *Brucella ovis* test to screen rams for epididymitis to make the test more sensitive and specific. The PCR test, used to differentiate *Brucella abortus*, *B. melitensis*, *B. ovis*, and *B.suis*, was also modified to increase its ability to differentiate *B. suis* from *B. canis*. The DBL added the Western blot assay as a supplemental test to differentiate *Brucella abortus* infections from infections with *Yersinia* and other bacteria that can cross-react with *Brucella*. The DBL increased the testing available to genotype brucellosis and mycobacteria cultures to assist in the epidemiological investigation of outbreaks.

NAHMS study—The DBL provided testing support for the NAHMS Swine 2006 study, which concluded in March 2007, and the Dairy 2007 study. Testing for the Swine 2006 study included detection of porcine reproductive and respiratory syndrome antibodies and swine influenza antibody and serotyping of *Salmonella* isolates. Testing for the Dairy 2007 study included immunoglobulin and total protein determinations in calves, Johne's disease culturing, and *Salmonella* serotyping.

Salmonella serotyping—The number of *Salmonella* isolates serotyped in 2007 increased 9 percent compared to the number serotyped in 2006. The DBL staff serotyped 18,246 *Salmonella* cultures, continuing a steady increase over the last 5 years. This increase is predicted to continue due to the increased emphasis on food safety by FSIS and the Food Safety Consortium.

Pathobiology Support

Exotic parasites—PL entomologists identified several unusual exotic parasites that were submitted to the lab in 2007.

In June, 33 *Amblyomma compressum* ticks were recovered from 10 African tree pangolins from Cameroon that arrived at the San Diego, California, zoo. This tick occurs only in West and Central Africa and is found primarily on various pangolins. This is only the second documented collection of this species in the United States.

In September, entomologists identified hundreds of mites collected in Florida from a pet giant African millipede as belonging to two species, *Julolaelaps luctator* and *J. pararotundatus*. These African mites live on millipedes and become problematic only on animals in captivity.

In November, mites from lung tissues of a recently deceased African rock hyrax at the Kansas City, Missouri, zoo were identified as *Pneumonyssus procavians*. This host-specific mite is commonly present in African host animals, but there are no previous records of its occurrence in the United States.

In December, entomologists found a single male tick on an African aardvark that arrived at Busch Gardens in Florida; it was identified as *Rhipicephalus praetextatus*. This tick occurs only in eastern Africa, where it parasitizes a wide variety of domestic and wild animals and may cause tick paralysis or be a minor vector for a few disease agents. This may be its first collection in the United States.

Scrapie eradication program—The PL played a key role in validating rectal biopsy as a new diagnostic procedure for the scrapie eradication program. This live-animal test will expedite identification of scrapie-infected animals and flocks and the implementation of control/eradication measures.

Virology Support

Bluetongue virus (BTV) isolates—As an OIE international reference laboratory, DVL applies new diagnostic tools as they are available to characterize previously untypeable agents. For example, NVSL recently identified several BTV isolates.

Since 1999, certain BTV isolates have been obtained or isolated that could not be identified as any of the five serotypes considered endemic to the United States (BTV-2, BTV-10, BTV-11, BTV-13, and

BTV-17). All of the isolates originated in sheep, cattle, or deer samples from Florida. Virus neutralization tests using type-specific reagents directed to serotypes identified in the Caribbean and Central American regions suggested the presence of serotypes not previously identified in the United States; however, the tests were inconclusive. Sequencing of PCR products based on newly developed primers enabled the lab to confirm the identity of the Florida isolates. NVSL also submitted several of the isolates to another OIE bluetongue reference laboratory, the Institute for Animal Health at Pirbright in the United Kingdom, for identification and/or confirmation of the NVSL results. BTV serotypes identified with these new genetic tools were BTV-3, BTV-5, BTV-6, BTV-14, BTV-19, and BTV-22.

SIV investigation—NVSL cooperates with public health agencies to investigate situations where illness in animals and people may be related. One example was an occurrence of SIV in 2007. SIV is a common cause of respiratory infection in swine. Previous sporadic human infections with SIV illustrate the zoonotic potential of the virus. Pigs can be infected with swine, human, and AI viruses and thus potentiate cross-species influenza transmission and formation of novel influenza viruses.

During 2007, pigs at an Ohio county fair developed an influenza-like illness; some individuals attending the fair developed influenza-like illness simultaneously and sought medical care. State public health officials and laboratories, the CDC, and NVSL worked together to investigate the relationship between the illnesses. Genetic sequencing and other techniques demonstrated that the virus was shared between pigs and humans at the fair.

AI wild bird surveillance—In 2007, 52 approved State/university laboratories and 1 DOI NAHLN laboratory conducted enhanced AI surveillance efforts for APHIS' VS and WS programs. These laboratories evaluate whether the AI virus is present in samples and, if so, determine whether it is an H5 or H7 subtype. Because of the potential for H5 or H7 subtypes to mutate into highly pathogenic strains, laboratory personnel forward presumptive positive samples to NVSL for confirmatory testing. NVSL then conducts additional screening and confirmatory tests with assistance from USDA's Southeast Poultry Research Laboratory to confirm genetic identification of isolated strains of the virus. Approximately 148,000 wild birds were tested in 2006 and approximately 80,000 birds were tested in 2007; samples came from all 50 States. No HPAI (H5N1) was detected.

CSF surveillance—NAHLN laboratories assist with sample collection, processing, and testing as part of USDA's surveillance plan for CSF in Puerto Rico and those States at high risk for introduction of CSF. The number of State/university NAHLN laboratories participating in surveillance testing increased to 36 in 2007.

FADDL performs confirmatory testing for CSF. During 2007, FADDL scientists developed and validated a new rRT-PCR assay with increased sensitivity and specificity, compared to current CSF assays. This new assay enhances the Nation's capability to detect a CSF outbreak rapidly.

Vesicular diseases—As of the end of 2007, personnel in 35 State/university laboratories and 1 DOI laboratory have been trained and successfully completed proficiency testing for FMD. A surveillance plan for vesicular diseases was recently developed and will be implemented in 2010.

TSEs—Personnel in eight NAHLN laboratories conduct testing for BSE and submit any inconclusive tests to NVSL for confirmation. Twenty-seven NAHLN laboratory personnel perform tests for CWD and scrapie.

VSV—After NVSL confirms an index case of VSV, personnel from approved laboratories conduct a complement-fixation test for VSV on equid samples. Personnel from six NAHLN laboratories have been trained and proficiency-tested to conduct this test.

These laboratories are located in the region where VSV typically occurs in the United States.

Training and Preparedness

APHIS developed and implemented a "Train the Trainer" program for AI, END, FMD, and CSF rapid assays. The number of State/university and Federal laboratories approved to conduct FMD assays increased from 14 to 38 laboratories, and the number of approved laboratories conducting AI testing increased from 44 to 54. The training programs also increased the number of laboratory personnel prepared to respond to a national animal health emergency and the number of trainers available to teach others. During 2007, a training program for high-throughput testing was developed for use in two NAHLN laboratories. The program will be delivered to the remainder of NAHLN laboratories in 2008.

In September 2007, NAHLN established a working group to develop exercises and drills. The group includes representatives from large and small laboratories.

To prepare responders for the challenges likely to be encountered during an HPAI outbreak, NAHLN developed an AI tabletop exercise, which was reviewed by the NAHLN exercises and drills working group. Participants learn about laboratory issues likely to arise during an outbreak and have the opportunity to assess response and activation plans. During 2008, NAHLN laboratory personnel and other animal health professionals will participate in this exercise during facilitated sessions throughout the United States.

International Activities

APHIS has a long history of working on international efforts for animal disease identification and eradication. APHIS is working with Canadian and Mexican animal health laboratory network personnel to standardize tests used in North America for the diagnosis of AI, TB, and vesicular diseases. Under the Security and Prosperity Partnership of North America, harmonization of diagnostics has been identified as a key objective. The international partnership aims to create a safer and more reliable food supply while facilitating agricultural trade by pursuing common approaches to enhanced food safety, enhanced laboratory coordination, and information sharing.

NVSL, as part of its role as an OIE avian reference laboratory, provided training in various AI diagnostic techniques to 47 scientists from 27 countries. In addition, NVSL scientists traveled to Brazil, Kazakhstan, Tanzania, and Mexico to provide in-country training for AI diagnostics. APHIS developed and implemented similar training programs in seven countries for FMD and brucellosis.

Center for Veterinary Biologics

CVB regulates veterinary biologics (vaccines, bacterins, antisera, diagnostic kits, and other products of biological origin) to ensure that the veterinary biologics available for the diagnosis, prevention, and treatment of animal diseases are pure, safe, potent, and effective.

CVB's responsibilities include

- Reviewing biologics product license applications and associated studies,
- Issuing biologics product licenses and permits,
- Testing biologics products for purity and potency,
- Inspecting biologics product manufacturing facilities,
- Regulating the release of biologics products to the marketplace,
- Conducting postmarketing surveillance of biologics products, and
- Certifying vaccines and diagnostics for export.

CVB is comprised of two functional units—the Policy, Evaluation and Licensing (PEL) unit and the Inspection and Compliance (IC) unit. The licensing staff within CVB-PEL reviews license applications for production facilities and biological products; reviews permit applications for importation of

products; establishes licensing, testing, and permit requirements and procedures; and, reviews production methods, labels, and supporting data involved in the licensing and permit process. The IC unit is responsible for developing and implementing programs to ensure veterinary biologics are prepared and distributed in compliance with the VSTA and its subsequent regulations. Compliance with the VSTA is assured by facilities inspections, product inspections, adverse event reporting, and investigations.

As part of its mission, CVB plays a key role in many other VS activities. For example, CVB is active in soliciting bids and evaluating technical proposals for the NVS vaccine banks. Without relaxing its rigorous licensing standards, CVB expedites the evaluation of vaccines and diagnostics for national disease-eradication or disease-control programs.

2007 Biologics Highlights

Licensing activities—In 2007, CVB issued a wide variety of product licenses and permits; some were for new products critical to facilitating trade and enhancing agricultural economic activities. Responding to swine industry concerns over the emergence of porcine circovirus, CVB expedited both the licensing of new vaccines for this chronic wasting syndrome in pigs and the rapid release of vaccine into the marketplace to meet swine producers' needs.

CVB also licensed several innovative products, including the first animal cancer vaccine (a DNA vaccine for canine melanoma) and a classical swine fever diagnostic test kit for use in national surveillance. In addition, CVB implemented new policies that allow manufacturers to quickly license products as more virulent strains of influenza emerge. These policies improved CVB's ability to respond to emerging strains of influenza in horses and pigs.

Inspection activities—CVB released more than 16,000 veterinary biological serials, comprising upwards of 80 billion doses, into the marketplace. In addition, CVB conducted more than 110 inspections of both domestic and international production facilities. Although most of these were in-depth inspections of licensed facilities, some were select agent inspections, observations of product efficacy studies, and antigen-bank inspections. CVB also issued more than 2,600 licensing and inspection certificates and more than 850 certificates for the export of veterinary biologics.

ISO certification—CVB successfully achieved ISO 9001 standards certification in 2007. This certification provides external recognition that CVB's business practices meet international standards for quality products, customer satisfaction, individual accountability, and process improvement. Operation manuals and memoranda of understanding with service providers were revised to meet current standards. CVB certification is specific for business processes involved with

- Reviewing prelicensing data,
- Issuing establishment and product permits and licenses,
- Inspecting facilities,
- Producing testing aids,
- Evaluating products,
- Writing standards and procedures for product release, and
- Overseeing compliance of firms that produce or distribute veterinary biologics in the United States.

Strategic diagnostics and vaccines support—CVB provided expertise on vaccine and diagnostic kits for a variety of pathogens, including Rift Valley fever, AI, and FMD, at international conferences and working group meetings. As a result, new diagnostic and vaccination strategies to aid in early detection of FAD incursions have been developed and implemented. For example, CVB provided expertise for the inspection and risk analysis of biocontainment manufacturing facilities in the Czech Republic. This activity was part of a program to prepare the United States to respond to the need for wild-type H5N1 avian and human influenza vaccine in the United States. CVB's continued involvement in the North American FMD Vaccine Bank and the NVS has resulted in multiple contracts for a variety of products and has expanded the emergency supply of AI vaccine. CVB ensures that more than 600 million doses remain potent, effective, and available for use in the event of an AI outbreak.

International activities—CVB's involvement in the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) led to the development of several new technical guidelines relating to animal safety and post-licensing monitoring of product performance. Once implemented, these will serve as new international standards and allow for increased trade with Japan and the European Union. In September 2007, as part of its participation in VICH, CVB hosted the Pharmacovigilance Expert Working Group meetings in Washington, D.C. The meetings were attended by representatives from the veterinary medicinal products industry and regulatory agency representatives from Japan, the European Union, Australia/New Zealand, Canada, and the United States.

CVB provided expertise for audits conducted by Brazilian regulatory officials at several U.S. vaccine manufacturers. This collaboration provided Brazil with confidence in CVB's regulatory system and helped promote the export of U.S. products. CVB hosted a Japanese veterinary biologics regulatory official in an extended training program. CVB also provided expertise and training at a joint CVB/Institute for International Cooperation in Animal Biologics program, aimed at educating foreign officials on U.S. regulatory processes. More than 135 delegates from 21 countries participated.

CVB was represented at the Global Animal Health Conference held at the European Medicines Evaluation Agency in London. The primary objective of the conference was to promote a dialogue among key stakeholders in global animal health in the field of veterinary medicines. Approximately 140 representatives and officials from more than 20 countries attended. The attendees represented regulatory agencies, industry, international organizations, academia, and the research community.

Ames Modernization Project

NVSL, CVB, and the USDA's ARS National Animal Disease Center are modernizing their facilities and consolidating operational support in Ames, Iowa. The result will be USDA's largest animal health center, providing world-class animal health research, diagnosis, and product evaluation. This new center is known as the National Centers for Animal Health.

The Ames modernization project has four main components:

- Phase 1 of the consolidated laboratory facility was completed in September 2004. This facility includes bio-safety level 2 and 3 laboratories for pathobiology and diagnostic bacteriology work.
- Phase 2 is the consolidated laboratory facility, which will include additional bio-safety level 2 and 3 laboratories, caged animal facilities, and space for administrative, office, conference, and support services. Construction is scheduled for completion in early 2009.
- The high-containment large-animal housing and training facility, dedicated in July 2007, includes 22 rooms for animals such as bison, cattle, horses, and swine. It also includes two necropsy areas, one with training space.

- The low-containment large-animal housing facility, where diagnosticians, scientists, and ARS researchers work on livestock diseases, is scheduled to be completed in 2008.

The finished complex will include almost 1 million square feet of safe, energy-efficient, modern facilities with state-of-the-art capabilities for research, diagnosis, and biological product evaluation.