

VETERINARY DIAGNOSTIC LABORATORIES

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Structure of Veterinary Diagnostic Laboratories (VDL)

- **Most VDL are associated with Universities:**
 - **Veterinary schools**
 - **Land-grant colleges – Veterinary Science or Animal Science Departments**
- **Some are linked solely to the state Department of Agriculture**
- **Some are private for-profit laboratories specializing in companion animal biopsy, microbiology and clinical pathology services**
- **Some are resources for a specific function:**
 - **Zoos and wild animal parks**
 - **State Departments of Fish and Game**

Primary Funding for VDL

Primary funding sources are:

- **Universities / Veterinary School budgets**
- **State Department of Agriculture subsidy for livestock and poultry disease testing and food product testing**
 - **Agriculture products are 11-16% of GNP (\$1.5T)**
 - **20% of workforce are in ag or related industries**
 - **22% of US production is exported**
- **Client based fee-for-service work**

Funding- Secondary sources

Secondary Sources of funds for VDL:

- **Federal-state cooperative agreements. The state is expected to bear a portion of the program cost**
- **Full fee-for-service federal contract reimbursement for program testing (BSE, AI)**
- **State, Federal and Private research grants and/or projects**
- **State Departments of Fish and Game**
- **Fee-for-service testing for state Departments of Public Health (rabies, WNV, etc)**

Functions of VDL

- **Animal disease diagnosis**
 - Performance of necropsies on wide array of animals
 - Full service microbiology and toxicology testing
- **Detection of emerging and foreign animal diseases**
- **Monitoring, surveillance and export testing**
- **Food product testing for quality & pathogens**
 - Meat, Milk, Milk Products, Water
- **Teaching**
- **Research**
- **Improvement of diagnosis and detection methods**

Daily VDL Work

- **Serology for disease monitoring, diagnosis, disease eradication (*Brucella*, *Pseudorabies*) and export**
- **Population diagnostic medicine**
- **Disease diagnosis:**
 - **Livestock, poultry and equine: most common pathology is related to abortions, diarrhea, respiratory**
 - **Small animal, pet birds and pocket pets: biopsy, clinical pathology, infectious, aging diseases (cancer, etc.)**
- **Microbiology on live animals: cultures, PCR, antigen detection methods (FA, ELISA, IHC)**

Most Common Toxicoses

<u>Livestock</u>	<u>Small Animal</u>	<u>Wildlife</u>
Nitrates	Rodenticides (anticoagulant)	Cholinesterase inhibitors
Copper, Lead	Over-the- counter meds	Lead
Insecticides (OP, carbamate)	Ethylene glycol	Anticoagulant rodenticides
Urea	Chocolate	Strychnine
Toxic Plants	Insecticides (pyrethrins)	

Salmonella Enteritidis phage type 4



Commercial Poultry infected:

Cultured 4500 egg pools (20 eggs/pool=90,000 eggs)

Necropsies of ~ 700 chickens



Research: to determine source, wild animal carriers, rapid & accurate method for poultry house litter and stream testing

SE phage type 4 -Source

Source- sewage treatment effluent upstream from ranch.
\$2.4M income loss.
\$.9M test, C&D, vaccine

Effluent spillway below

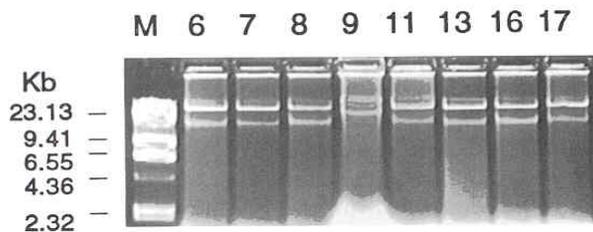
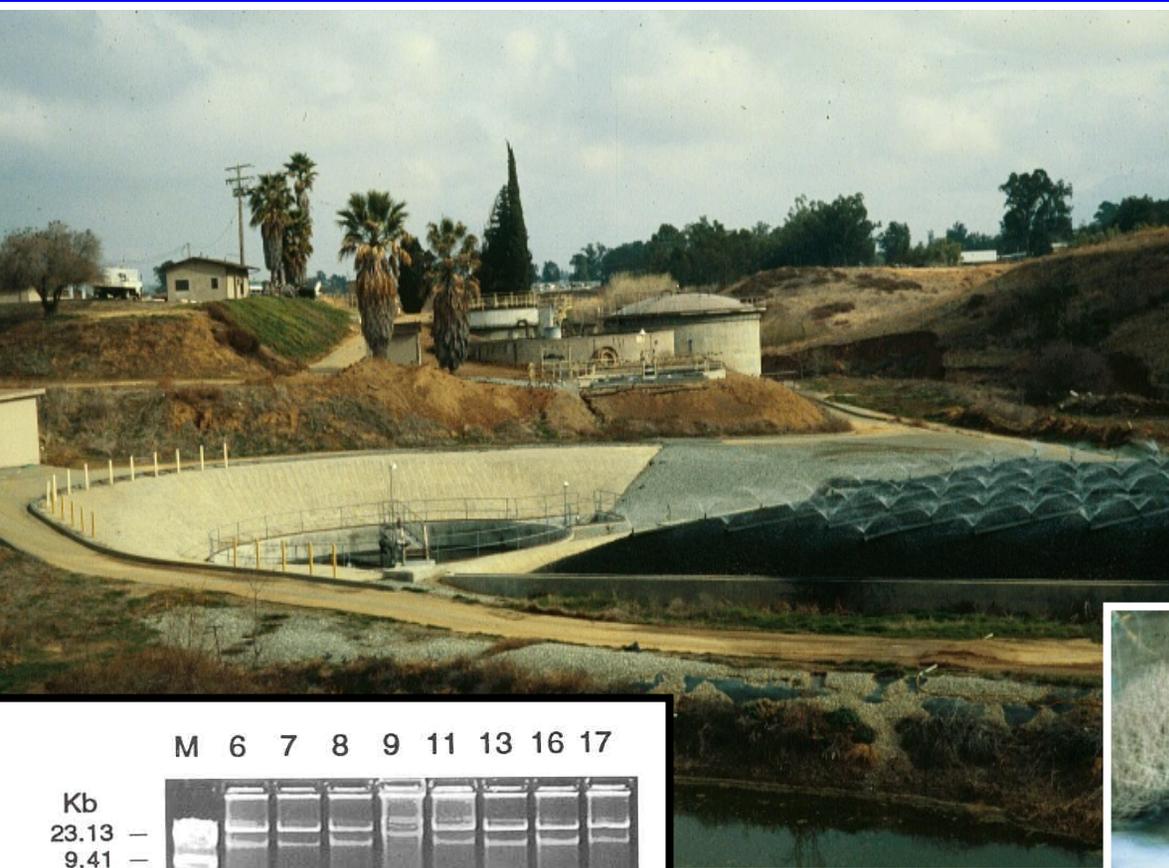


Fig. 2. Plasmid profile of *Salmonella enteritidis* isolates. Lanes: 6—creek water (upstream); 7—skunk liver; 8—chicken egg; 9—chicken liver; 11—creek water (midstream); 13—creek water (downstream); 16—mouse liver; 17—raw sewage inflow; M—molecular weight marker (lambda DNA-HindIII digest).

Identical
plasmid
profiles(left)



Foreign Animal Disease Response Exotic Newcastle Disease- 2002



Biotechnology Receiving lab (above)

Virus Isolation (above right)

END: RRT-PCR Validation

- **96-well high throughput PCR: Private-Public partnership: CAHFS, NVSL-USDA, SEPRL-ARS-USDA, Ambion, MJ Research, LLNL**
 - **Bench Validation:**
 - specificity: in silico, near neighbor & environ screens;
 - sensitivity: strain, limit of detection, quantitation;
 - precision, reproducibility, repeatability
 - **Field Validation: compared to Epidemiology/VI**
 - **Sensitivity: 0.9967 [0.9937;0.9997]**
2/926 false negative
 - **Specificity: 0.9999 [0.9999;>0.9999]**
3/65,346 false positive; post-END
- Jouden's J Statistic = 0.9966 (*3 in 1000 incorrect*)

Exotic Newcastle Disease- 2002-03

- **75,000 Real Time PCR tests in 6 months**
 - Peak 24,129 in one month, >1K/day (1 site)
 - NVSL ran an additional 6300 PCR
 - Developed and Validated RRT-PCR during END
- **15,000 Virus isolations in eggs in 12 months (at 2 lab sites), peak 2600 in one month**
 - NVSL ran additional 4700 in 12 months
- **19,000 bird necropsies, peak ~3,300/month for 3 consecutive months, >100/day (1 site)**
- **152 personnel over 12 months utilized at CAHFS, 58 were existing CAHFS employees**
 - 94 from NVSL, USDA, temps, other diagnostic labs

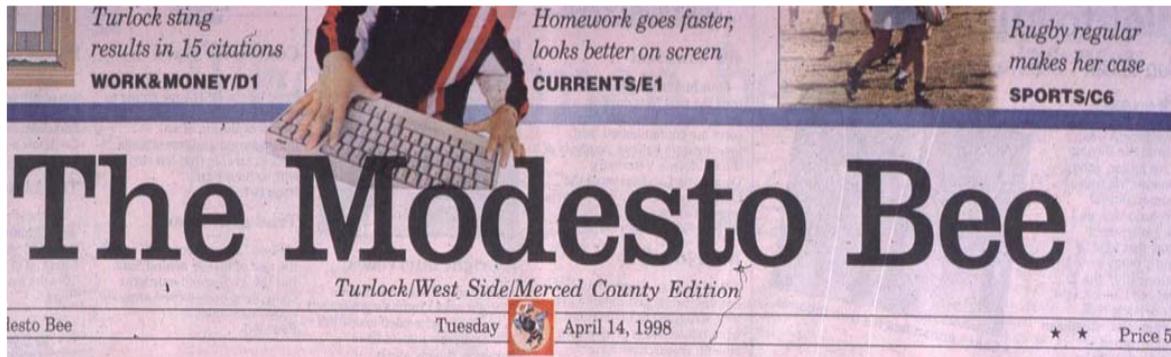
The Aftermath- National Surveillance

- **END, Avian Influenza and H5, H7 PCR assays validated by USDA-NVSL**
- **Contract fee-for-service national surveillance.**
- **Equipment provided by USDA when necessary**
- **NVSL training provided to 30 laboratories in 29 states for national surveillance**
- **Goals:**
 - **Early detection of new END and H5 or H7 AI**
 - **Prove freedom from disease (END, H5 and H7 AI)**



Botulism Type C Rendering? Milk? cattle, ducks, horses, and/or poultry yearly

**Research:
Median Lethal dose
for cattle.
Presence in milk?
More sensitive assays.**



Poisoned feed kills 400 cows

By Tim Moran
Bee staff writer

Four hundred cows have died over the past four days at a dairy west of Modesto after the cows ate contaminated feed, investigators believe.

Analysts at the California Veterinary Diagnostic Lab System at the University of California at Davis and in Tulare were working Monday to determine the exact cause.

"At this point it looks like it was one load of feed that was contaminated, and it got to a certain number of pens," said Dr. David Willoughby, district veterinarian for the California Department of Food and Agriculture.

The first dead cow was discovered at 1 a.m. Friday by dairy workers on the 1,000-cow Genasci Brothers Dairy on Blue Gum Road.

"We got a call from one of the milkers," Edwin Genasci said. "One of the cows was dead and a couple were sick. The herdsman called us and we called the veterinarian."

But the early response didn't seem to help.

"They knew they had a real serious problem. They called the veterinarian, and he got there right away," Willoughby said. The animals continued to die through the weekend, Willoughby said.

About two dozen cows that apparently didn't get as heavy a dose of the suspected feed still under observation.

Investigators have ruled out contagious disease, Willoughby said.

"It was a toxin of some kind."

See Back Page, C6

about it

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'When the sun comes out and you get a forecast of 75 degrees for five days, people drop skiing like a hot rock and head to golf courses and flower gardens and their favorite fishing streams'

— Frank Helm, Dodge Ridge owner

Wild, wet and cold

Violence may be natural

Brain dysfunction

Lead Toxicosis- Milk/Meat Risk?

Oleander Toxicosis- Milk Risk?



Oleander: Human exposures accidental, suicide, homicide⁴⁵

Carbamates- Public Health

GI upset in Humans after eating at a Thai Restaurant in CA. Methomyl Confirmed at CAHFS

- ◆ Salt “A” 1650 ppm
- ◆ Salt “B” 4800 ppm

An Outbreak of Food-Borne Illness Associated With Methomyl-Contaminated Salt

Udo Buchholz, MD,MPH; Jonathan Mermin, MD,MPH; Richard Rios, MPH; Tim L. Casagrande, REHS; Francis Galey, PhD; Mark Lee, PhD; Alfredo Quattrone, PhD; Jeff Farrar, DVM,PhD,MPH; Nico Nagelkerke, PhD; S. Benson Werner, MD

JAMA. 2002;288:604-610.

Organophosphates

- Accidental Phorate exposure kills 167 dairy cows in hours**
- **Milk tested and withheld to confirm no contamination**
 - **Rendering risk? Water table? Air quality if burn?**



National Organization

American Association of Veterinary Laboratory Diagnosticians is a voluntary, member-funded organization with over 1100 members in >30 countries with ~900-950 members in U.S.

- **Publishes 6 issues/year of the Journal of Veterinary Diagnostic Investigation**
- **Annual meeting with US Animal Health Assoc.**
- **Accredits 40 laboratories/laboratory systems in 34 states and 2 Canadian provinces**
- **Unlike APHL, laboratories are not members of AAVLD only individual laboratorians are; and AAVLD receives no federal support funds**

AAVLD Accreditation

- **Accrediting labs for >30 years, initially as self-help process. 5-year re-accreditation visits**
- **In 2003, AAVLD adopted OIE, now World Organization for Animal Health, Standards for Infectious Disease Testing Laboratories which is based on ISO 17025 standards**
- **Accredited labs usually have board certified pathologists, microbiologists, toxicologists**
- **Like APHL, VDL have dwindling supply of specialists and laboratorians due to low salaries compared to Industry and Hospital labs**
- **Increasing demand for molecular biologists**

National Infrastructure

- **State and University Laboratories have been a loose knit group with common interests**
 - **AAVLD established laboratory directors email list and with USDA, national bi-monthly conference calls**
- **In 2001, AAVLD signed a Memorandum of Understanding with USDA to improve the federal-state cooperation to produce a more cohesive and comprehensive laboratory network to respond to animal health needs.**
 - **The MOU created a working group of AAVLD officers and NVSL section chiefs from Ames, IA and Plum Island Foreign Animal Disease lab.**

National Animal Health Laboratory Network (NAHLN)

- **May 2002 funds from USDA–CSREES for Homeland Security formed the pilot NAHLN:**
 - **2001 FMD in UK lead to US concerns for lab capacity**
 - **Animal Safeguarding Review in 2002 recommended a federal (NVSL)-state laboratory network**
 - **Original intent was to provide surge capacity for foreign animal disease testing (8 diseases identified)**
 - **Cooperative of State –NVSL/APHIS- CSREES**
 - **Funds for infrastructure for 12 laboratories:**
 - **Quality Assurance and BL3 facility upgrades, training, equipment, information technology, harmonization, etc.**

Impacts on NAHLN Evolution

- **2001-2002 Anthrax letters and LRN response**
 - Environmental testing by 15, non-LRN, VDL: 12 in requested by law enforcement, 9 by Public Health
- **2002 spread of Chronic Wasting Disease**
 - 2001 NVSL launched scrapie slaughter surveillance testing contracts for eradication effort
 - 2002 expansion of testing lab sites for CWD
- **2002 need for enhanced foreign animal disease surveillance (Classical Swine Fever, etc)**
 - 2003-04 need for END and AI surveillance
- **2002-2003 Exotic Newcastle Disease Outbreak**
 - Validation of assays pre-outbreak, response capacity

Further Impacts on NAHLN

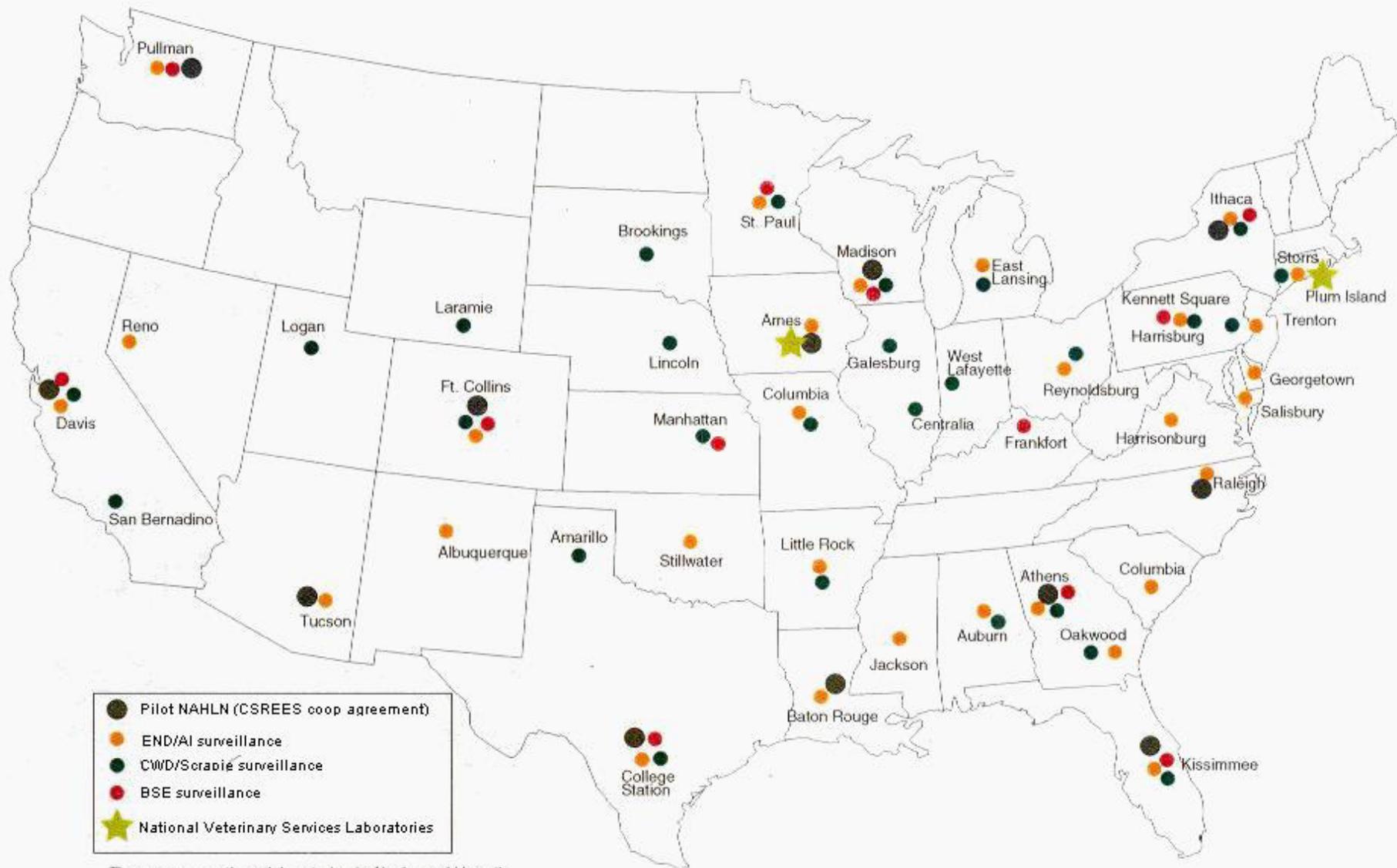
- **Increased focus on Bioterrorism threat agents**
 - Bioterrorism Preparedness Law of 2002
 - List of select agents
 - Capabilities existed in many VDL already
- **2003 Monkeypox in prairie dogs and humans**
- **2003- current: Avian Influenza in Asia, etc.**
- **Need to be cost effective and prepared:**
 - Integrate with daily activities
 - Multiplex concept for foreign and endemic diseases
 - Ongoing surveillance for diseases not just response
 - Use labs to validate foreign animal disease assays with negative field samples from regions of US

NAHLN Redefined

- **NAHLN laboratory redefined August 2004**
 - Any laboratory performing contract fee-for-service testing for USDA – expanded to 43 labs in 37 states
 - However, no infrastructure funds except pilot 12
 - NAHLN Laboratory Qualification Checklist (LQC) requires signatures of State Veterinarian, USDA Area-Veterinarian-In-Charge, Commissioner of State Dept of Agriculture and Laboratory Director
- **VDL entry into LRN at invitation of state Public Health Director for low risk environmental & food testing**
 - In 2003, 2 VDL were in the LRN (TX, NVSL)
 - October 2004: 6-7 VDL in LRN and ~8 pending

CURRENT NAHLN LABORATORIES- October 2004

43 in 37 states



There are currently no laboratories in Alaska and Hawaii.

Informal 2003 VDL Surveys

- **35 laboratories from 33 states responded:**
 - 23 reported BSL3 lab space (200-2000 sq ft)
 - In 2002, only 8 reported BSL3 lab space
 - As of 2004 only one has BSL3 large animal necropsy
- **Food product testing, 17 labs responded:**
 - 11 routinely tested food products
 - Meat: 11 microbiology, 5 chemical testing
 - Milk: 8 microbiology, 5 chemical testing
 - Water: 6 microbiology, 5 chemical testing

Overlap Select Agents Occurring in animals in US

- *Bacillus anthracis*
- *Brucella abortus*
- *Brucella suis*
- *Clostridium botulinum* and its neurotoxins
- *Clostridium perfringens* epsilon toxin
- *Coccidioides immitis*
- *Coxiella burnetii*
- Eastern equine encephalitis virus
- *Francisella tularensis*
- Shigatoxin
- Staphylococcal enterotoxins
- T-2 toxin
- *Yersinia pestis*

VDL Select Agent Experience

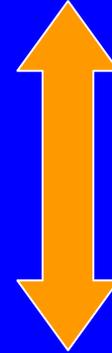
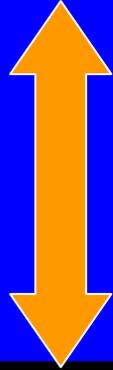
2001 survey by Dr. B. Akey, 31 VDL responding

Organism	In past 1-year	In past 5-years	Capable
<i>B anthracis</i>	19%	35%	97%
<i>F tularensis</i>	48%	74%	100%
<i>Y pestis</i>	16%	26%	90%
Botulinum	39%	48%	61%

VDL in the LRN and FERN

- **Increased recognition among Public Health of VDL capability and familiarity with select agents**
- **Active Food testing in some VDL**
- **West Nile Virus promoted understandings between public health-state veterinarian-VDL**
- **Homeland Security Presidential Directive –9**
 - **Surveillance and monitoring of animal, food and water**
 - **Expansion of LRN partnerships (food and water)**
 - **Food Emergency Response Network (4 VDL)**
 - **Food and Water Borne Disease Network**
- **NAHLN Laboratory Qualification Checklist (LQC) harmonized with LRN LQC**

National Animal Health Laboratory Network
USDA APHIS/CSREES – State Labs (AAVLD)



Laboratory Response Network
CDC & APHL
LRN Partners Workgroup
(FDA, FSIS, AAVLD, USDA,
etc)

Food Emergency Response Network- FDA & FSIS
FERN Steering Committee
**(LRN-CDC/APHL, NAHLN-
APHIS-AAVLD)**

Past Mutual Laboratory Issues

- **2002 Public Health Preparedness & BT Act...:**
 - Exemptions for formalin-fixed tissue
 - Exemptions of avirulent and vaccine strains for QC use
 - Exemptions for proficiency testing
 - Endemic disease reporting for select agents
 - Reporting and Registration, Site visits
- **Packaging and Shipping Requirements**
- **Identification criteria for select bacterial agents**
- **Biosafety in Microbiological and Biomedical Laboratories Manual – Revisions**
- **Revision of 9CFR122 regulations for Interstate import of veterinary pathogens**

Challenges for VDL Commonalities with PHL

- **Funding to improve facilities, provide training, equipment and enhance quality assurance**
- **Funding for surveillance testing**
 - **Legal authority to test for diseases not requested**
- **Proficiency testing**
- **Multi-agent rapid assays and validation**
- **Secure results communication network for state & federal laboratories and integration with state & federal emergency responders**
- **Engage all partners at state level (pandemic)**

Challenges for Laboratories (cont.)

- **Involve all laboratory partners into table top exercises at local, state, regional and national level**
- **Clarify roles and responsibilities of state/federal partners and laboratories in food-borne, human and zoonotic emergency response**
- **Clarify reporting streams for an emergency**
- **Determine actionable events and response**
- **Maintain VDL client confidentiality in order to:**
 - **Avoid damage to clients' economic well being**
 - **Avoid reduction in submissions thus compromising surveillance activities for foreign and emerging diseases**

Senate Committee Report 108-345 S-2810 – HHS Appropriations Bill

“The committee remains concerned about the emergence of new infectious diseases and the increasing zoonotic disease transmission between animals and humans... The Committee commends the CDC’s efforts to merge surveillance systems of State diagnostic labs, veterinary labs, wildlife health agencies and zoos...”

