

INTRODUCTION

DIRECTOR'S MESSAGE

2002 in retrospect at the Wyoming State Veterinary Laboratory

Like our counterparts in human health, veterinary laboratories in the USA continue to deal with the aftermath of September 11, 2001. The perception that the country is vulnerable to accidental or malicious exposure to exotic and/or epidemic diseases led to several federal initiatives to address these shortfalls. All affect how veterinary diagnosticians do their jobs. They present opportunities and financial challenges for publicly-funded animal health laboratories.

One opportunity involves the implementation of a **National Animal Health Laboratory Network (NAHLN)**. The American Association of Veterinary Laboratory Diagnosticians, which is the accrediting body for veterinary laboratories in North America, proposed this initiative. The first step in its development was implemented by the USDA in 2002. The first installment comprises 12 large state laboratories (see Appendix 1). Selected laboratories signed an agreement with the USDA's national veterinary services laboratory to work cooperatively to enhance national capability for diagnosis of serious domestic and foreign animal diseases. In exchange, participating state laboratories receive federal support of up to \$2 million to apply or develop disease diagnostic capability, improve quality assurance/quality control for key tests, increase biosafety level-3 laboratory space, and enhance common laboratory data information systems. **As the only veterinary diagnostic laboratory in Wyoming, a major goal of this laboratory is to be part of the expanded NAHLN.**

To be competitive, we need to:

1. Be on track for national reaccreditation in 2005.
2. Improve our instrument and technical base, particularly for molecular diagnostics.
3. Improve test validation.
4. Work more closely with Wyoming's public health laboratory and the state veterinarian, particularly the **County Veterinary Coordinator system.**

5. Increase the number of Wyoming diagnosticians trained in foreign animal disease detection.
6. Hire a veterinary epidemiologist familiar with **geographic information systems (GIS)** and disease control data systems.
7. Continue active participation in the American Association of Veterinary Laboratory Diagnosticians.

I currently serve on the AAVLD executive, representing the northwest laboratories. Two faculty members, Drs. Williams and Cornish, are trained as foreign animal disease diagnosticians. Dr. Cornish attended the FADDL school in 2002. We intend to have at least one additional WSVL diagnostician trained in 2003. Selection of the WSVL as a part of the NAHLN will involve politicking, as funds flow uphill in politically important states like California, New York and Florida with large veterinary diagnostic laboratories. We will need political support from our clientele and institutions in Wyoming to obtain membership in the network. A key lesson learned from the foot and mouth disease outbreak in Europe in 2001 was that "surge capacity" testing is best provided by regional laboratories, and that staff must be trained and their units equipped *in advance* to deal with outbreaks of foreign animal disease outbreaks. The worst time to exchange veterinary business cards is at the start of an FMD outbreak.¹

Another consequence of 9.11 is a mandated increase in security within laboratories that deal with Select Agents, as required under the **Public Health Security and Bioterrorism Preparedness Response Act**. Select Agents are infectious and toxic agents with potential for bioterrorism. The 2002 new law imposes requirements on veterinary and human health laboratories, including penalties on individuals of up to \$250,000 for failure to comply with the law. Meeting these requirements will involve substantial changes in how the WSVL handles isolates obtained from accessions proven to contain select agents such as *Bacillus anthracis*, *Brucella abortus*, *Coxiella burnetti*, and *Francisella tularensis*. These changes carry a hefty price tag. The new

INTRODUCTION_{continued...}

law will impact our ability to store such agents for retrospective studies, and require changes in accessibility of the WSVL to the general public. These changes must be in place by **November 2003**.

A benefit of current concerns about laboratory biosecurity is that the **Wyoming laboratory directors' group** met regularly in 2002 and 2003 to address our state's **testing capabilities, redundancy, continuity of operation, and weaknesses**. A document outlining capabilities has been prepared and will be submitted shortly to the Wyoming Emergency Management Agency and state government. The major needs of the WSVL in this regard are increased laboratory space for high consequence pathogens, improved molecular biological test capability for agents of critical concern, control of public access into the building, and exchange of protocols and reagents for rapid detection of select agents with the public health laboratory. The laboratory's bacteriologist, Dr. Ken Mills, attended a workshop on validation of veterinary diagnostic tests at the annual AAVLD meeting in 2002. Validation of laboratory tests is becoming increasingly important and standardized. Ability to meet these standards will be a touchstone for laboratories seeking both national accreditation and participation in the National Animal Health Laboratory Network.

Federal mandates aside, in 2002 staff at the Wyoming State Veterinary Laboratory continued to address its basic legislative mandate to **"inquire into and maintain records of causes of contagious, infectious, and communicable diseases among livestock in this state."** During the year our staff undertook surveillance and research work on chronic wasting disease, provided diagnoses on routine accessions, conducted field investigations, and, through Drs. van Olphen and Cornish, generated important new information about the efficacy

of bovine viral diarrhea vaccines when herds are challenged by specific field strains. Details of some of these investigations are provided in this report.

1. Scudamore J M, Harris, DM: 2002, Control of foot and mouth disease: Lessons from the experience of the outbreak in Great Britain in 2001. Rev Sci Tech Off Int Epiz 31: 699-710.

Donal O'Toole
Director, WSVL
July 2003



DIRECTORY OF PERSONNEL

ADMINISTRATION

Donal O'Toole, MVB, PhD, MRCPATH, Dip ECVF Director
Beth Howell Office Associate
Barbara Garrett Office Assistant Senior
Louise Smithson Office Assistant

BACTERIOLOGY/RABIES

Kenneth Mills, PhD, Supervisor
Amy Boerger-Fields, BS

CELL CULTURE LABORATORY

Lee Belden, PhD, Supervisor
Tamora Coburn

DATA MANAGEMENT

Todd Bleifuss, Programmer Analyst

ELECTRON MICROSCOPY

Alberto van Olphen, Supervisor
Carol Hearne, MS

HISTOLOGY

Todd Cornish, Supervisor
Paula Jaeger, HT(ASCP), Manager
Leslie Vieyra, BS

LIVESTOCK

Rod Rogers, AAS Vet Tech, BS, Manager

NECROPSY

Elizabeth Williams, Supervisor
Brian Parrie, BS, Technician
Helen Hsieh, BS
Samuel Vieyra, BS

PARASITOLOGY/CLINICAL PATHOLOGY

William Jolley, PhD, Supervisor
Katherine Bardsley, MS

PATHOLOGY

Elizabeth Williams, DVM, PhD, Dip ACVP
Todd Cornish, DVM, PhD, Dip ACVP

REGULATORY SEROLOGY¹

Lynn Woodard, DVM, PhD, Dip ACVM, Supervisor
Rebecca Wills, BS

SEROLOGY²

Kenneth Mills, Supervisor
Joan Edwards, BS

TOXICOLOGY

Merl Raisbeck, DVM, PhD, Dip ABVT, Supervisor
Roger Siemion, BS

TSE RESEARCH LAB

Elizabeth Williams, Supervisor
Jean Jewell, PhD

VIROLOGY

Alberto van Olphen, DVM, MS, PhD, Supervisor
Jackie Cavender, MS
Lorraine Barrows, MS

¹In 2003 this unit was renamed Regulatory and Bacteriological Serology and Dr. Mills became supervisor.

²In 2003, this unit was reamed Diagnostic Virological Serology and Dr. van Olphen became supervisor.

ADVISORY BOARD

LIVESTOCK BOARD APPOINTEES

Mr. John Morris

January - December 2003
10401 Experiment Farm Road
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307-638-6030

Mr. William Lambert

January 2003 - December 2005
P.O. Box 7
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blamb@trib.com

Mr. Ed Weppner

January 2003 - December 2004
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307-632-2750

Dr. Jim Briddle

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Department of Health
Preventive Health & Safety Division
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Cheyenne, WY 82002

Dr. Bret A. Combs

USDA-APHIS
5353 Yellowstone Road #209
Cheyenne, WY 82009

STATEMENT OF FUNDING

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CALENDAR YEAR 2002

Funding

Faculty and staff salaries.....	\$657,903
One-time equipment money (VP Finance, UW).....	\$72,298
State support.....	\$97,620
Hatch.....	\$12,000
Animal Health.....	\$33,660
Revenue.....	\$592,929



1923-1963

TESTS PERFORMED IN 2002

ACCESSIONS AND TESTS

	<u>2001</u>	<u>2002</u>
Accessions	13,008	13,954
Tests	54,650	55,319

Tests by County

<u>2001</u>		<u>2002</u>	
<u>County</u>	<u># Tests</u>	<u>County</u>	<u># Tests</u>
Albany	5,658	Albany	5,630
Big Horn	1,569	Big Horn	2,042
Campbell	1,586	Campbell	1,068
Carbon	2,117	Carbon	4,278
Converse	481	Converse	859
Crook	487	Crook	659
Fremont	4,657	Fremont	4,896
Goshen	1,689	Goshen	1,343
Hot Springs	696	Hot Springs	1,028
Johnson	1,021	Johnson	822
Laramie	2,784	Laramie	3,353
Lincoln	1,146	Lincoln	821
Natrona	1,382	Natrona	1,736
Niobrara	524	Niobrara	438
Out of State	14,786	Out of State	10,191
Park	2,766	Park	2,829
Platte	1,070	Platte	1,485
Sheridan	2,765	Sheridan	2,693
Sublette	760	Sublette	895
Sweetwater	2,848	Sweetwater	2,070
Teton	1,284	Teton	774
Uinta	323	Uinta	331
Washakie	1,006	Washakie	3,578
Weston	423	Weston	485
Not Reported	822	Not Reported	1,062
Total	<u>54,650</u>	Total	<u>55,366</u>

LABORATORY NEEDS

1. Personnel

- ▶ Endowed chair for a wildlife-livestock disease specialist.
- ▶ Veterinary extension specialist with a strong background in veterinary epidemiological investigation, data analysis, and geographic information systems (GIS).

2. Laboratory space and facilities:

- ▶ Dedicated, adequately equipped, and secure Select Agent Laboratory.
- ▶ Dedicated diagnostic Transmissible Spongiform Encephalopathy Laboratory.
- ▶ More paved parking spaces at the north and west side of building.

3. Equipment:

- ▶ Thermal cyclers and DNA preparation stations.
- ▶ Dedicated TSE immunostainer (TSE laboratory).
- ▶ Automated hematoxylin-eosin stainer (histology).
- ▶ Automated slide cover-slipping unit (histology).
- ▶ Equipment for experimental large animal unit and grounds maintenance: farm truck, trailer, snowblower, and lawn mower.



Plague carcass

HIGHLIGHTS OF 2002

DISEASES

WEST NILE VIRUS ENCEPHALOMYELITIS

The disease entered Wyoming on August 18, 2002 with a case of fatal encephalitis in a horse in Goshen County. Through a collaborative arrangement established by Dr. Todd Cornish with the Wyoming Department of Health, the Wyoming State Veterinary Laboratory was positioned to map the spread of the disease in the state. A reflection of public expectations is that the West Nile virus coordinator, Mr. Terry Creekmore, was interviewed in 2002 for newspaper, radio and television pieces on 91, 21 and 12 occasions, respectively. Mr. Creekmore and Dr. Cornish gave multiple presentations throughout the state to alert horse owners to the disease. Fortunately, many owners vaccinated their animals in time. One of the few benefits of the 2002 drought was the low level of mosquito activity in much of the state. This probably contributed to the small number of laboratory-confirmed infections in people ($n = 2$), horses ($n = 94$), and birds ($n = 17$) in Wyoming, compared to adjacent states such as Nebraska, which saw >1,000 equine cases. The placement of a Department of Health employee in the WSVL to help in performing tests was an innovation. It underscores a trend in veterinary diagnostic medicine since the attacks in September 2001: human and animal health are indivisible. If states want prompt, effective surveillance for disease, it is often best done in-state, rather than a federal level, involving close liaison between veterinary and human health personnel. In addition to diagnosing the disease in horses, Dr. Cornish diagnosed fatal WNV in mountain goats. This is the first time the disease was recognized in this species.



Culex tarsalis

PIGEON FEVER

Multiple cases of this bacterial disease in horses, also known as dryland distemper, were diagnosed in Laramie, Goshen, Uinta, and Lincoln counties in 2002. The disease is due to a bacterium, *Corynebacterium pyogenes*. More than 100 horses in the Cheyenne area had symptoms of deep abscesses and multiple sores along the chest and midline. Lameness, fever, lethargy and weight loss accompanied infection, which caused a painful swelling in pectoral muscles that resembles a puffed-out pigeon breast.

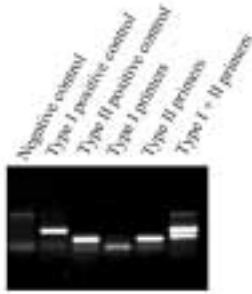
The disease usually occurs in arid parts of the West in autumn and early winter, with the highest incidence in September, October and November. The causative bacterium, which is assumed to be transmitted by flies, lives in soil and enters a horse's body through wounds, broken skin and mucous membranes. A similar organism causes caseous lymphadenitis in small ruminants, but horses appear to have their own bacterial strains. When outbreaks occur, contaminated stalls, paddocks and utensils need to be thoroughly cleaned and disinfected. Because lesions often occur on the chest, Dr. Mills speculated that horses were pushing on barbed wire to get green grass, thereby introducing the organism through small punctures. He wondered whether the current drought contributed to the 2002 outbreak. Information we gathered from colleagues in Arizona, New Mexico and Colorado supports this theory. Once the disease takes hold in a particular season, hundreds of cases can be diagnosed in a "pigeon fever year." In other years, the condition seems to disappear.

Although pigeon fever can generally run its course in a few weeks without treatment, some veterinarians recommend the use of anti-inflammatory drugs, pain relievers and antibiotics. Medication eases the discomfort of afflicted horses that suffer from chest abscesses and have difficulty walking. While the prognosis is generally good for complete recovery, some horses may have a recurrence of abscesses once treatment is stopped. Others might seem to be cured, only to develop fresh clinical signs in a matter of months. Basic research on the pathogenesis of this disease is needed. If the disease recurs in 2003, the laboratory may be interested in attempting experimental reproduction of the disease.

HIGHLIGHTS OF 2002 continued...

BOVINE VIRAL DIARRHEA: TESTS AND OUTBREAKS

Bovine viral diarrhea is the most important recognized cause of reproductive wastage in beef herds in Wyoming. In 2002, the laboratory was involved in several investigations of congenital BVDV in well vaccinated herds. Several publications will result from the investigations, which were done by Drs. van Olphen and Cornish, with assistance from staff in histology, virology and serology, and two Wyoming veterinary practitioners, Drs. Jim Briddle and Dan Miller.



PCR of BVDV

One investigated herd experienced reproductive wastage of 34%, with most losses occurring as persistently infected calves. The vaccine used provides protection to Type I strains of BVDV, with lower protection to Type II strains (the product claims protection of 60% of vaccinated animals). In this case, the wild strain was a Type II strain. Documenting the field performance of widely-used products is an important service for producers and veterinarians, since



Dr. Cornish bleeding calf

As part of the investigations, the laboratory tested and helped validate an ear-notch enzyme-linked immunosorbent assay. This assay (PBS ELISA) is now our test of choice for identifying and eliminating calves under 4 months of age that are persistently infected with BVDV. Faculty in the laboratory spoke at various meetings sponsored by the Wyoming Veterinary Medical Association and several commercial companies to alert producers and veterinarians to the new test and to limitations of some commercial vaccines.

CHRONIC WASTING DISEASE

Some 22 years after Dr. E. S. Williams characterized it, chronic wasting disease (CWD) finally entered the national spotlight as an endemic disease in wildlife with potential implications for the health of livestock. The triggering event was recognition of CWD in free-ranging white-tailed deer in Wisconsin. At the 2002 annual meeting of the joint US Animal Health Association-American Association of Veterinary Laboratory Diagnosticians, CWD was the plenary session topic. Appropriately, Dr. Williams was selected to give a keynote presentation on the disease.

CWD now occurs in free-ranging cervids in Wyoming, Colorado, Nebraska, Wisconsin, New Mexico, Utah, South Dakota, Illinois and Saskatchewan. Its identification in Wisconsin persuaded national animal health leaders that this under-diagnosed disease has the potential to erode national and international confidence in ability of the US to control and map emerging diseases of animals, particularly in wildlife. In 2002, Dr. Williams and collaborators in the Wyoming Department of Game and Fish continued critical surveillance and research work on the disease. A research associate, Dr. Jean Jewell, was hired to supervise Dr. Williams' CWD research laboratory, which involved remodeling part of the laboratory's isolation building.



Dr. Jean Jewell

The laboratory helped validate a commercial enzyme linked immunosorbent test for CWD infective material in lymph nodes of deer. Dr. Williams' group collaborated in validating other commercial tests for CWD and scrapie. The recognized extent of the disease in Wyoming expanded in 2002 to include the western slopes of the Sierra Madre and Snowy Range mountains. Given the recognition of the disease in northwestern Colorado outside the official "endemic" area, the WSVL needs to continue to work closely with the Wyoming Game and Fish Department to map the extent of the disease, to assess new diagnostic tests, and to answer basic questions about the natural epidemiology of CWD.



Bio Rad ELISA plate

With her collaborators, Dr. Williams submitted several large grant proposals to federal agencies to address these questions, particularly natural routes of transmission.

CELL CULTURE LABORATORY

The cell culture laboratory is an essential for the virology and serology laboratories and for many research projects in the state veterinary laboratory. In 2002 this service was reorganized and is now operated by the department under the supervision of Dr. Lee Belden. Until now we had an arrangement with the USDA-ARS Arthropod-Borne Animal Disease Research Laboratory, which provided cells from its cell culture unit and was housed in WSVL laboratory space. The service began as a handshake agreement between the two institutes and evolved into a lease agreement with UW. In 2002 and after discussions with the USDA-ARS, we decided that the advantages of a joint facility did not outweigh the disadvantage of being able to exercise direct control over the tissue culture. Dr. Belden took over this critical service function, and cell production was transferred to his laboratory. Ms. Tamora Coburn was hired as the WSVL cell culture technician operating under Dr. Belden's supervision. There will be some logistical problems with cell transport from campus to the diagnostic laboratory in west Laramie, but this is offset by two advantages: the diagnostic laboratory now controls the quality and delivery of needed cell lines, and valuable laboratory space in the WSVL is released for other uses. We can't emphasize enough the importance of the cell culture laboratory. We appreciate Dr. Belden's willingness to accept this additional responsibility, which ensures that virology and serology services to the state are maintained.

WYOMING LABORATORY DIRECTORS' GROUP

This group includes the directors of the public health laboratory, the analytical services laboratory, the state crime laboratory, and the state veterinary laboratory. The group met on multiple occasions in 2002 to ensure that we are familiar with each others' operations, capabilities, and needs. The group is exploring ways of

HIGHLIGHTS OF 2002 continued...

exchanging space, reagents, expertise and, where necessary, staff in the event of a disease emergency in Wyoming. The directors' group is working with the Wyoming Emergency Management Agency so that first responders will know who to contact, what to collect and where to submit samples when the time comes. Critical deficiencies in each operation should be met by the submission of a grant proposal by WEMA to the Department of Justice. The realization that we need to upgrade our ability to diagnose unexpected disease outbreaks rapidly and accurately helped focus energies and overcome traditional barriers between laboratories in the state.

NATIONAL ANIMAL HEALTH-REPORTING NETWORK

A national animal health-reporting network (NAHRN) is emerging in the United States, prompted by the September 2001 events. Historically, the strength of the publicly funded animal diagnostic laboratory system in the country has been its state-based, decentralized nature. This allowed various models for animal health diagnostic work to evolve under the aegis of the American Association of Veterinary Laboratory Diagnosticians (AAVLD), the entity that accredits laboratories. A weakness of the current system is that the quality of disease surveillance depends on willingness of sponsoring state entities, be they land-grant universities or state departments of agriculture, to invest in their public animal health laboratories. As a result, while the country has many excellent animal health laboratories, many states have a weak disease surveillance system. The USDA took its first step at establishing a NAHRN earlier in 2002 when it identified 12 veterinary laboratories for federal support. These laboratories receive money to increase national emergency preparedness, particularly laboratory information systems, quality control of test procedures, exotic disease detection capability, and safe disposal of infected tissues.

The Wyoming State Veterinary Laboratory was not among the first 12 laboratories chosen, all of which were from "Big Ag" states. An important goal in the coming years is for the WSVL to be a part of the NAHRN and to represent Wyoming in the national system.

It remains to be seen if the USDA can be persuaded that the best model for a national system is that of the human health laboratory response network (LRN), rather than designating a small number of geographically dispersed "core" laboratories. The executive of the AAVLD is engaged in discussions with the leadership of the USDA to persuade it that a true NAHRN should involve all 50 states and not depend on a small number of laboratories affiliated with veterinary schools. As a member of the AAVLD executive, other directors and I will continue to push the USDA to commit to a true national system that includes your Wyoming laboratory.

ENDOWED CHAIR IN WILDLIFE AND LIVESTOCK DISEASE

A major goal of the laboratory is to establish an endowed chair in Wildlife and Livestock disease. The WSVL is uniquely positioned for such a chair. It is one of the few diagnostic laboratories in the country where diagnosis of wildlife disease is a major part of our diagnostic caseload. We are fortunate to have considerable expertise in wildlife disease, largely through the efforts of Dr. Williams, who developed this strength since coming to Wyoming in 1983. Funding this chair will be the critical step to ensure that the next generation of wildlife disease diagnosticians and researchers is trained in the real-world environment of wildlife managers, hunters, and wildlife disease politics.



NEW LABORATORY DATA MANAGEMENT SYSTEM FOR WSVL

Nearly two years ago the WSVL conducted a survey of its clients to get their opinion of our current laboratory information system (LIMS), which contains our accession records. That system was put in place in 2000. The survey addressed facets of the system and asked users to rate its performance on a scale of 1-10. The only part of the LIMS that scored above 5 was system availability, which is not a function of the program. These responses, taken with the knowledge that the database design was flawed and incapable of correction without a major rebuild, led us to consider alternatives. In the end we decided to develop a new LIMS in-house. Todd Bleifuss, our database manager, is in charge of development. The new system should come on line in summer 2003. It will incorporate the following features, in contrast to the current system:

- A redesigned database, with referential integrity.
- A better user interface, which is designed around laboratory workflow.
- Better tracking of individual samples. Samples, rather than accession numbers, will be the fundamental unit in the new system.
- True client/server architecture as well as a 2-tier system consisting of an application server and a database server. The latter will remain hidden from the Internet for security reasons. Interaction with the database will be through the application server. End users will not require an Oracle client and will not communicate directly with the database server. In light of recent Oracle security breaches, this is important. Our present system was so vulnerable to hackers that we had to remove the database server from Internet access, which now means personnel cannot enter data except when they are on-site in the laboratory.

■ The user's client will be Internet Explorer augmented with the .NET framework. This reduces system maintenance as well as making remote access the "natural" path.

■ Clear separation of the user interface, or application layer, from the database. Optimization of the database should not require recompiling the user application. Problems that occur will be easier to pinpoint to either the interface or the database, and therefore easier to correct.

■ The user application is a .NET development, taking advantage of the latest technology.

■ Modern servers will have sufficient processing muscle and memory, as well as an appropriate RAID (a hardware redundancy sub-system) configuration.

A TEACHING CLASSROOM AND NEW EQUIPMENT

In 2002, the University of Wyoming recognized the importance of on-the-job exposure of undergraduates to molecular as well as classical laboratory techniques. A former large animal surgery area that was used mostly for storage was converted into a modern classroom. A purpose-built storage area was also constructed. The laboratory received \$72,000 to replace several crucial pieces of equipment. We are currently purchasing an additional ELISA reader, a serum chemistry analyzer, a server for the laboratory data management system (LIMS), and expertise to write code for a new LIMS.

EXTERNSHIP FOR FINAL YEAR VETERINARY STUDENTS

The WSVL regularly hosts veterinary students from around the USA who wish to expand their experience by an externship at the laboratory. In 2002 we hosted **Lyle Wyninger** (final year veterinary student,

HIGHLIGHTS OF 2002 *continued...*

Colorado State University), **Katie C. Meehan** (final year veterinary student, Virginia-Maryland Regional College of Veterinary Medicine) and **Heather Mazzaccaro** (final year veterinary student, School of Veterinary Medicine, Tufts University).

ADMINISTRATIVE

STAFF AND FACULTY CHANGES

There were several important personnel changes in the Wyoming State Veterinary Laboratory in 2002. **Dr. Alberto van Olphen**, a young, energetic faculty member - crucially, one with a strong sense of humor - was hired from Purdue University to be the laboratory's virologist. Dr. van Olphen quickly expanded the laboratory's capabilities for bovine viral diarrhea. He established a close collaborative working relationship with Dr. Todd Cornish to work on BVDV. One of Dr. Lynn Woodard's favorite sayings is: "The cheapest form of research is field investigation." Drs. van Olphen and Cornish demonstrated the truth of this by documenting and helping control natural outbreaks occurring in several vaccinated herds.



Dr. Alberto van Olphen

For the first time the laboratory contracted with a board-certified veterinary pathologist, **Dr. Charlotte Quist**, to help deal with the flow of pathology case material until the vacant pathology position is filled (anticipated fill is July 2003). We appreciate the patience of clients in allowing us to do this. Dr. Quist helps to take some, but by no means all, work pressure off the two pathologists.

The laboratory hired new staff members.

Ms. Katherine Bardsley joined the department in October as a parasitology/clinical pathology technician. She is no stranger to the laboratory as she did her Master's thesis work with Dr. Bill Jolley in the early 1990s. Ms. Bardsley will help in expanding the range of clinical pathology tests, including endocrine assays

Ms. Lorraine Barrows was hired on the laboratory's income account to beef up BVDV testing capability, and to improve turnaround times. Ms. Barrows worked in a research laboratory dealing with high-level (BSL-3) pathogens. Her arrival in January 2003 provides the laboratory with additional expertise in these agents.

As interim director for much of 2002, **Dr. Ken Mills** successfully completed a reclassification system for the laboratory's technical personnel. This reform eliminated salary inequities visited upon our highly trained staff by a previous classification system. That system made minimal allowance for special skills and training. Thanks to Dr. Mills and Dean Galey, staff are now accorded a separate classification system as Laboratory Technicians I, II and III. The more realistic salary ranges allow the laboratory to compete with human hospitals for the talent that, above all else, makes or breaks successful diagnostic laboratories.

After an external search, **Dr. Donal O'Toole** took over as laboratory director in September 2002. A national search was undertaken to fill the vacancy for a veterinary pathologist. The new pathologist is expected to be on board by September 2003. Until that time, Drs. Williams and Cornish will do the work of the three pathologists, supplemented by Dr. Quist.

NOTABLE INVESTIGATIONS

BVDV in vaccinated herds

Several episodes of BVDV in herds vaccinated with Pfizer's BoviShield were investigated and the presence of wild-strains established. A rapid and simple laboratory test for diagnosing persistent infections in valves was tested and validated.



Collecting samples in the field

Paramyxovirus outbreak in Laramie pigeons

An outbreak of central nervous disease in large numbers of wild pigeons in downtown Laramie was investigated. The cause was pigeon paramyxovirus-1, which is related to, but distinct from, Newcastle disease, an exotic and highly pathogenic disease of poultry.

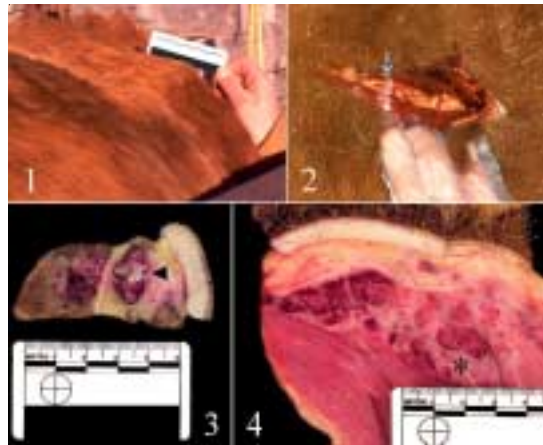
New congenital encephalopathy in cattle

A novel encephalopathy of newborn cattle was investigated in Nebraska. The disease is morphologically indistinguishable from maple syrup urine disease (MSUD), but typical genetic and biochemical features of the disease were absent. We appreciate the work of Dr. Lynn Steadman in helping characterize this disease.

Vaccination-associated lameness in cattle

A herd episode of lameness in cattle in Nebraska following vaccination with two commercial products containing a proprietary adjuvant, Extend III, was investigated. Similar lesions were reproduced experimentally at the laboratory. The USDA's Center for Veterinary Biologics was informed about iatrogenic disease caused by the two

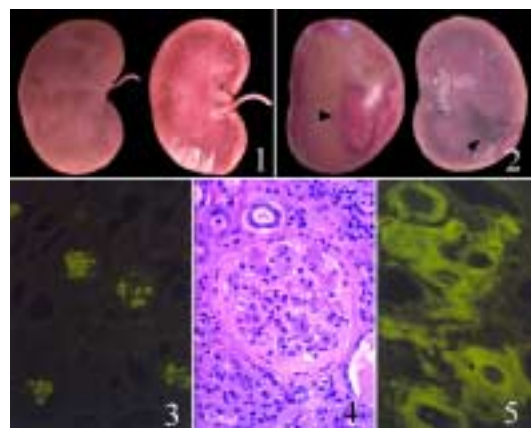
products (Scour Bos-4 and PiliShield +C, Grand Laboratories, Inc.).



Images of injection site reaction due to commercial vaccine in cattle. 1. Gross appearance of swelling in hip. 2. Leakage of vaccine material (white) from injection site in cow that died of other causes. 3. Gross appearance of muscle at injection site. 4. Gross appearance of muscle at injection site; white areas correspond to scar tissue formation.

Wasting and infertility syndrome in rabbits

Reports of a wasting and infertility syndrome in rabbits was investigated. Owners claimed the problem was due to a specific brand of rabbit chow. Investigations established that rabbits were affected by renal amyloidosis, complicated terminally by thromboembolism. There was no evidence that feed was a causative factor.



Images of renal amyloidosis in rabbits dying of a wasting syndrome. 1. Capsular and incised appearance of kidney with disseminated fibrosis. 2. Capsular and incised appearance of kidney with focal infarction (arrowhead). 3. Autofluorescent amyloid in glomeruli of affected rabbit. 4. Glomerular amyloidosis. 5. Autofluorescent amyloid surrounding tubules in renal medulla.

NOTABLE INVESTIGATIONS

continued...

Pigeon fever in Wyoming horses

As noted elsewhere in this report, cellulitis-myositis due to the bacterium *Corynebacterium pyogenes* occurred on multiple properties in 2002, particularly in the SE part of the state. We appreciate Dr. J. D. Fox's willingness to sample horses and share his experience with this disease, which is poorly understood and would benefit from research on transmission routes.



Horse with pigeon fever swelling

Arsenic toxicosis in a large group of cattle

The toxicology unit assisted in an episode of mortality in a large group of cattle. The proximate cause of death was arsenic toxicosis, probably malicious. A legal case is pending.

SECTION REPORTS

BACTERIOLOGY AND RABIES

Total of bacteriology accessions	1,745
Total numbers of rabies accessions	696
Positive skunks	9
Positive bats	9
Total number of tularemia accessions	34
Positive cases	1
Total number of plague accessions	32
Positive cases	1

Antibiotic sensitivities

Species	
Canine	190
Equine	140
Bovine	64
Feline	35
Rabbit	7
Porcine	7
Ovine	6
Caprine	4
Chameleon	3
Avian (misc.)	2
Other species	9

Mastitis sensitivities

Bovine	18
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Rabies-infected drainages (skunk strain) in Wyoming (blue area)

VIROLOGY

Virology tests	2001	2002
IFA/FA	809	1150
Virus isolation	1030	2652
BVDV rapid ELISA	769	4118
BVDV PBS ELISA	Not offered	897

TRANSMISSION ELECTRON MICROSCOPY

Negative staining by species	2001	2002
Avian	10	29
Bison	5	4
Bovine	236	144
Canine	49	56
Caprine	2	0
Domestic (pets)	3	12
Equine	32	8
Feline	19	12
Non-animal	0	14
Ovine	6	11
Porcine	3	6
Wildlife	48	55

SECTION REPORTS continued...

HISTOLOGY

	1999	2000	2001	2002
Total number of blocks processed	18,448	19,363	22,083	32,193
Hematoxylin and eosin sections:	19,184	20,014	23,083	33,080
Special stains:	1,755	1,884	1,272	1,341
Immunohistochemistry	4,543	4,157	11,266	18,325
BVDV:				2,400
Scrapie:				5,168
CWD:				10,532

Histology section has shown the most consistent and large increases in tests done. Much of this is due to growth in immunohistochemistry work, which increased 400% in four years. BVDV immunohistochemistry (IHC) on aural skin contributed substantially to the growth in histology accessions in 2002. With the recent transfer of BVD testing on aural skin to virology section, this work should decline in 2003 although the ear notch IHC service continues to be offered. The manager of Histology section, Paula Jaeger, spent a week at Dr. Daniel M. Albert's ocular pathology laboratory in the Department of Ophthalmology and Visual Sciences at the University of Wisconsin Medical School to develop additional expertise sectioning and staining eyes. One histotechnologist, Leslie Vieyra, was trained in operation of the immunostainer by the Ventana company and will take the certification examination to become a certified histology technician. The laboratory hired a third histotechnologist to keep up with volume of accessions. New equipment in the histology laboratory included a third ergonomic microtome, an embedding center, and two additional modules for the Ventana immunostainer. We are currently seeking to acquire a DAKO immunostainer. Other desirable pieces of equipment are an automated coverslipping unit and an automated HE stainer. Creation of a separate laboratory for work on transmissible spongiform encephalopathy TSE tissues will need to be undertaken soon in order to address concerns about safe handling of TSE tissues.

REGULATORY SEROLOGY

TEST	2000	2001	2002
Equine infectious anemia	12,088	12,652	11,743
Brucellosis	41,817	48,841	47,570
Pseudorabies	4,145	3,768	2,916
Bluetongue	686	170	443
Anaplasmosis	969	565	285
Bovine leukosis	1	17	4

EIA testing declined slightly because of a reduction in samples from BLM horses. The slight decline in brucellosis testing may be an artifact since we continued to get samples in 2003 from some sale barns where the samples were drawn in 2002. These will be rolled into the numbers for 2003.

SECTION REPORTS continued...

DIAGNOSTIC SEROLOGY

	<u>2001</u>	<u>2002</u>
Total number of tests:	6,639	5,390
BVDV Type I and II serum neutralization		
Bovine	633	2,032
Ovine	6	2
Wildlife	488	194
Brucella canis	45	43
Brucella ovis	939	636
CAE (AGID)	142	143
Canine adenovirus (wildlife)		
Ovine	18	6
Caprine	31	20
CDV (SN)		
Canine	4	6
Wildlife	735	549
Canine adenovirus (wildlife)	113	
EHD (AGID)		
Cattle	1	3
Wildlife	401	93
EHV-1 (SN)	77	46
FIP	64	68
FeLV	14	9
FeLV-FIP	59	60
IBR (SN)		
Cattle	259	170
Wildlife	363	95
Johnes (AGID)		
Ovine	1	1
Bovine	22	122
Caprine	1	0
Wildlife	233	94
Leptospira (5 serovars)		
Ovine	4	0
Bovine	72	144
Canine	5	2
Equine	10	1
Wildlife	418	134
PI-3		
Bovine	13	9
Wildlife	430	114
RSV		
Bovine	193	25
Wildlife	410	114

Part of the decline in wildlife testing in 2002 is due to the absence of samples that year from Alaska. The main increase in testing was attributable to BVDV testing in cattle (300% increase) due to field investigations of the disease.

SECTION REPORTS continued...

CLINICAL PATHOLOGY-PARASITOLOGY

<u>TEST</u>	<u>2002</u>
Total fecal analyses:	930
Flotations	576
Cryptosporidia examinations	142
Baermannizations	87
Fluke screens	22
Giardia fecal ELISA	103
Serum chemistry panels	240
CBCs	311
Urinalyses	55
Cytology	198
Heartworm serology	101
Toxoplasma serology	101
Referrals (EPM, thyroids, etc.)	53
IDs (nematodes, plant cells, etc.)	36
Water MPAs	19
Tritrichomonas	
Cultures	6,512
Positives	75

TOXICOLOGY

Total cases:	274
Samples analyzed:	911
Total number of analyses:	4,398

Among the cases diagnosed in 2002 were a large episode of **aldicarb** (Temik) toxicosis, malicious poisoning involving **Paris Green** in cattle, and an outbreak of **botulism** in mules due to exposure to a dead porcupine on the feed ground. An episode of **lead poisoning** in calves was linked by isotopic analysis to lead in a battery. **Copper deficiency/molybdenosis** occurred in a herd of cows that had classical overt signs, including hair coat changes and pathologic fractures. An unusual cause of poisoning in a dog was due to the bird repellent **4-aminopyridine**. There were two confirmed cases of **lead poisoning in raptors**. The toxicology laboratory was involved in losses that were thought to be due to a petroleum pipeline spill in South Dakota., The problem proved to be due to **highly saline water** that was run through the system between different lots of petroleum products.

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Appendix 1: NAHRN network