Livestock Disease Diagnostic Center
Annual Report, 2009

Craig N. Carter, Director

The Livestock Disease Diagnostic Center (LDDC) strives to be one of the premier veterinary diagnostic laboratories in the United States, providing the very best and most timely services in support of the practicing veterinary profession, Kentucky animal agriculture, the signature equine industries, companion animals, and public health. As the state’s flagship veterinary diagnostic laboratory, the UK Livestock Disease Diagnostic Center’s primary goal is to develop, apply, and utilize state-of-the-art veterinary diagnostic testing methods and scientific knowledge to improve animal health and marketability, preserve the human-animal bond, and help protect and improve public health through the early and accurate identification of zoonotic diseases.

In addition to its clinical diagnostic role, the LDDC provides surveillance for emerging and endemic diseases such as West Nile virus, chronic wasting disease of deer, contagious equine metritis, bovine spongiform encephalitis (mad cow), and avian influenza. Furthermore, the laboratory is always on the watch for the emergence of foreign animal diseases such as foot and mouth disease, and classical swine fever. Animal owners use the LDDC’s services through their practicing veterinarians who have expertise in selecting, preparing, shipping, and submitting the proper specimens for testing when necessary. Laboratory findings are reported back to the submitting veterinarian who then consults with his or her clients to implement a treatment protocol or a preventative solution to disease problems on the farm.

LDDC faculty, scientists, and technical staff are specialists in essential scientific disciplines directly related to animal health to include bacteriology, clinical pathology, epidemiology, extension, molecular biology, pathology, serology, toxicology, virology and informatics. Disease diagnostic efforts are coordinated and handled by specialists in the appropriate disciplines. Complex clinical cases involving multiple sections are monitored by highly qualified case coordinators. The LDDC is organized into sections so that specialized workload/activities can be handled efficiently.
2009 Highlights:

The American Association of Veterinary Laboratory Diagnosticians (AAVLD) is the national accrediting agency for state and university veterinary diagnostic laboratories. After two years of intense preparation, the AAVLD Accreditation Team inspected the LDDC in May, 2009. In July, 2009 the LDDC Director was notified that the laboratory had achieved full accreditation for the first time in history. This achievement inspires confidence in LDDC clients and Kentucky’s animal agricultural trading partners.

Soon after receiving AAVLD accreditation, the LDDC applied for membership status in the National Animal Health Laboratory Network. This network provides surge capacity for the National Veterinary Services Laboratory in the face of serious disease outbreaks. Based on its credentials, the LDDC was approved as a full member in October, 2009. Laboratory staff are now being trained to conduct specialized testing in response to an outbreak. LDDC will receive a grant in the amount of $50,000/year minimum from the USDA to purchase equipment/reagents and to conduct any necessary training.

In September, 2008 a groundbreaking ceremony was held to signify the start of a $28.5 renovation/expansion construction project that will nearly double the square footage of the facility. During 2009, all foundation work, structural steel and much of the mechanical, electric, and plumbing work was completed. The current schedule for occupancy of the new/renovated facilities are as follows:

- New necropsy facility, occupy by June, 2010.
- New pathology offices, serology and histology labs, occupy by June, 2010

The LDDC received roughly 3,268 cases on 2009 (down roughly 8% from 2008) including almost 3000 necropsies. The caseload decrease correlates with the general economic downturn for 2009. Total tests run in each laboratory section will be listed in the individual section reports.
The LDDC implemented a new fee/test schedule in 2009 that charges per test for all equine accessions. This, and other adjustments to the schedule resulted in a $219,390 increase in fee income for 2009 (increase of 21.1%). These funds will be used to replace aging instrumentation and equipment in various laboratory sections.

LDDC successfully implemented a new Laboratory Information Management System (LIMS) and a new billing system on August 1, 2010. This state-of-the-art system is part of a statewide animal health network that will soon be complete. The new system offers new services to clients such as emailing of lab reports and web access to client data. Support for handheld devices is in the planning stages.

The LDDC continues to build its outreach programs around Kentucky. LDDC staffed an exhibit in Lexington for the Kentucky Cattleman Association annual meeting in January, 2009 and also in Louisville for the Kentucky Veterinary Medical Association (KVMA) annual meeting in October, 2009. The Kentucky VetLabNet listserv continues to distribute animal health bulletins and has grown to a list of over 550 LDDC clients. Over 200 field investigations were conducted by the epidemiology section on Kentucky farms, mostly in support of a research project to quantify exposure of foals to Rhodococcus equi. The LDDC still contributes articles quarterly to the KVMA journal and the Kentucky Cattleman Association Cow Country News. The LDDC Director, faculty and staff continue to deliver lectures at scientific and lay meetings and participate in the monthly Equine Diagnostic-Research Seminar Series at the LDDC.

The LDDC has been fortunate to recruit some top-notch faculty and staff in 2009. Many thanks to Deans Smith and Cox for their assistance in making this possible. The following positions have been filled:

- Dr. Michelle Bilderback, Ruminant Extension Veterinarian
- Ms. Bonnie Decker, Clinical Pathology Section Chief

Some research projects in progress during 2009 at LDDC are as follows:

- KHRC race horse breakdown project, Dr. Laura Kennedy
- Wobbler syndrome, Dr. Jennifer Janes
- Contracted Foal Syndrome, Dr. Craig Carter
- Continuous health monitoring of cattle, Dr. Craig Carter, Ms. Jackie Smith
- Animal disease cluster detection, Dr. Craig Carter
- Contagious Equine Metritis trial, Dr. Mike Donahue
- Enhanced Herpes PCR screening, Mr. Steve Sells
- Rhodococcus equi pneumonia in foals, Dr. Craig Carter & Ms. Jackie Smith
- Nocardioform placentitis study, Dr. Neil Williams, Mr. Steve Sells
- Equine uterine rupture study, Dr. Cindy Gaskill, Dr. Lori Smith, Dr. Neil Williams

Dr. (LtCol) Carney Jackson, LDDC veterinary pathologists and member of the Kentucky Air National Guard, deployed to Afghanistan with an Agricultural
Development Team in June, 2009 for a period of one year. On this deployment, he is assisting the Afghan Ministry of Agriculture, two veterinary schools and farmers by providing animal health training. His team is also involved in capacity building for animal agricultural operations in and around Kabul, Afghanistan. Dr. Jackson is scheduled to be back in his position at LDDC sometime around July, 2010.

Dr. Craig Carter, Director, LDDC assumed the role of President-Elect of the American Association of Veterinary Laboratory Diagnosticians. In this role, Dr. Carter is program chair for the annual meeting which will be held in Minneapolis, MN in November, 2010. Dr. Carter is also Executive Director of the World Association of Veterinary Laboratory Diagnosticians. In this role he oversaw the planning for a scientific symposium on diagnostic veterinary medicine in Madrid, Spain in June, 2009.

**Bacteriology/Mycology**  
_ J.M. Donahue_

The primary mission of the Bacteriology/Mycology Section of LDDC is to detect or isolate and identify pathogenic bacteria or fungi present in animals. The Section also determines the antibiotics that might be used for the treatment of specific bacterial infections. The Section is also responsible for culture of *Taylorella equigenitalis* and *T. asinigenitalis* for the federal/state CEM regulatory program in equine.

**Highlights:**
- Approximately 11,500 aerobic cultures were performed on samples submitted to the Livestock Disease Diagnostic Center; significant bacterial pathogens were found in about 50% of the samples.
- Approximately 1100 milk samples from dairy cows were tested for microorganisms that cause mastitis; over 50% were positive for pathogenic microorganisms.
- Approximately 2,500 different bacterial isolates were tested to determine the antibiotics that could be used for their treatment in exposed animals.
- Approximately 9,350 samples from equines in Kentucky were cultured for the contagious equine metritis organisms. All horses tested were negative for *T. asinigenitalis* and *T. equigenitalis*. Because of the detection of 4 positive stallions by this Section in late 2008, the number of samples received was about 33% higher than in 2008. Nationwide, a total of 22 stallions and 5 mares were confirmed to be infected. Early detection of this infection in the Quarter Horse population by this laboratory prevented this disease from becoming more widespread in the equine population of the USA.
- In cooperation with researchers at Michigan State University, the normal flora of the male genital tract of donkeys is being determined. An important preliminary finding of this study is that *Taylorella asinigenitalis*, one of the bacteria causing contagious equine metritis, is sometimes a part of the normal flora of male donkeys and that it can persist for at least 12 months in these animals.
- An investigation was undertaken with researchers at Gluck Equine Research Center to determine if the treatment of semen with antibiotics will prevent the
transmission of *T. equigenitalis* to mares bred by artificial insemination. Preliminary results indicated that the antibiotics did prevent the transmission of the disease.

- Approximately 1750 samples from horses were tested for the presence of leptospires and tissues from 24 fetuses and/or placentas were positive.
- In conjunction with the Molecular Biology Section we are continuing to evaluate a PCR method for detecting *Crossiella equi* and *Amycolatopsis spp* in equine placentas. These bacteria are the primary cause of nocardioform placentitis in equine.
- Determined that the serovars of salmonellae involved in equine salmonellosis belong to either serogroup B or serogroup C. This information is being used to help in the formulation of a salmonella bacterin that can be used in central Kentucky.

**Clinical Pathology Section**

*Bonnie L. Decker*

The primary mission of the Clinical Pathology is to provide chemistry, hematology, urinalysis, fluid analysis, fecal parasite exams, and other testing to animal owners, veterinarians and the agriculture community. The section also provides support and testing to LDDC’s pathologists and testing related to necropsy as well as University of Kentucky equine and animal science researchers who can submit specimens to Clinical Pathology for monitoring various chemistry and hematology levels in their research animals. Clinical pathology is experiencing growth with the plan to add endocrine tests, and to add a new hematology analyzer that will allow additional animal species to be tested. Computer interface for the ACE Alera chemistry analyzer is in the works for more efficient results entry and reduction of human transcription errors. Clinical pathology is dedicated to meeting the current and future needs of the agriculture community, companion animal community and veterinarians.

**Highlights:**

- Approximately 614 chemistry test panels were performed on equine, bovine, caprine, ovine, feline, canine and other species. This represented over 10,548 individual chemistry tests.
- Approximately 502 Complete Blood counts and 505 manual differentials were performed.
- Approximately 1211 fecal specimens were submitted for fecal flotation and examination for ova and parasites. In addition, 124 specimens were submitted for cryptosporidia.
- Approximately 361 stones were submitted for chemical stone analysis. A wide variety of stones were identified with Triple phosphate (struvite) and calcium oxalate being the most common.
- Fluid analysis, protein electrophoresis, urinalysis, cytology prep, and vitreous eye fluids (necropsy) were other tests performed bringing the total tests performed in Clinical Pathology for 2009 to 13,929 tests.
Clinical Pathology completes its testing same day as receipt with a few exceptions. Cryptosporidia and protein electrophoresis require more time and are reported within 5 working days of receipt.

The department personnel consists of 1.25 FTE. A section chief with a BS MT (ASCP) and over 30 years experience in veterinary and human diagnostic laboratory testing works full time. A part time technician with a BS and 4 years experience completes the department. Other qualified LDDC personnel are available for backup and consultation.

Epidemiology
Jacqueline L. Smith

The primary mission of the Epidemiology Section is to provide animal disease surveillance, early detection of animal disease outbreaks, assist veterinarians in the investigation of serious and unusual disease problems, and conduct relevant infectious disease research. The epidemiology program is driven by state-of-the-art electronic data-gathering systems that allow for near-real time analysis and dissemination of diagnostic case information that will be useful to practitioners in treatment, prevention, and management of animal disease problems. We also provide in-depth field investigations to better characterize disease outbreaks for identifying causative etiology through the collection of diagnostic specimens and recommending diagnostic testing are provided free of charge to any farm/producer in the state of Kentucky at the request of a local client with the approval of the LDDC administration.

2009 Highlights:

- Research farm visits: for R. equi – 180 (30 farms, 6X each), approx 30min per farm plus drive time
  (90 hrs of visits, 47 hrs drive times logged) -137hrs
- Research farm (Crestwood) for R.equi -141 (47 foals, 3X each) each approx 30 min plus drive time
  (70.5 hrs of visits, 8 hrs drive time logged) – 78.5hrs

**TOTAL RESEARCH VISITS -321, 215.5 hrs**
- Surveys – 2 (requiring 30-40hrs each for prep, send out, analysis and follow-up), R. equi and customer satisfaction survey.
- Phone calls asking for questions, suggestions, recommendations – 112
- Stats requests: (from LDDC faculty, UK faculty, state or federal officials, or local vets ) -19 (1-10hrs each)
- Graphics requests: 8 (2-10hrs each)
- Reportable disease reports sent: 51 weekly reports (approx 1hr each week)
Molecular Diagnostics

S. Sells

Diagnostic PCR assays are being increasingly utilized because of their speed and specificity. Nucleic acid based tests are now used so that unknown organisms can be identified, closely related organisms can be differentiated, and small numbers of pathogens can be detected in complex samples. Specimens such as blood, swabs, feces, etc are accepted directly from clinicians and also from the Pathology, Virology and Bacteriology sections of this and other diagnostic facilities.

Highlights:

- Approximately 4,750 specimens submitted for PCR testing.
- The most requested tests included equine herpesvirus type 1 and EHV1 pathotyping (over 1250), EHV4 (125), Streptococcus equi subsp. equi (over 1,150), Crossiella equi (1,200) and Amycolatopsis species (1,200), Clostridium perfringens (55), Lawsonia intracellularis (190), Neorickettsia risticii Potomac Horse Fever (120). BVDV (170), Moraxella bovis (50), and Mycoplasma bovis (65).
- This section continues to be responsible for providing the majority of Kentucky’s arbovirus (mosquito-borne virus) testing for an environmental risk analysis program with the Kentucky Department for Human Health.
- In cooperation with researchers at Michigan State University, the normal flora of the male genital tract of donkeys is being determined. An important preliminary finding of this study is that Taylorella asinigenitalis, one of the bacteria causing contagious equine metritis, is sometimes a part of the normal flora of male donkeys and that it can persist for at least 12 months in these animals.
- An investigation was undertaken with researchers at Gluck Equine Research Center to determine if the treatment of semen with antibiotics will prevent the transmission of T. equigenitalis to mares bred by artificial insemination. Preliminary results indicated that the antibiotics did prevent the transmission of the disease.
- In conjunction with the Bacteriology Section we are using PCR methods for detecting Crossiella equi and Amycolatopsis spp in equine placentas. These bacteria are the primary cause of nocardioform placentitis in equine.

Pathology

Neil M. Williams

The Livestock Disease Diagnostic Center pathology section is composed of 8 faculty pathologists, 3 post-doctoral scholars (pathology residents), 4 full time necropsy technicians, 4 part time necropsy student workers, and 5 histology technicians. The section performs complete necropsy examinations on submitted animals, histopathology on necropsy cases and surgical biopsies, and cytological examinations. As part of the comprehensive necropsy examination, additional laboratory tests are ordered by the pathologist case coordinator to aid in confirming a diagnosis. The abnormal findings on
necropsy are correlated with other laboratory tests, including microscopic examination of the tissues, and a comprehensive report is prepared for every pathology case.

**Necropsy**: A postmortem examination (necropsy) is conducted to identify any pathologic changes in the tissues that would indicate disease, injury, or any other abnormal process resulting in illness.

<table>
<thead>
<tr>
<th>Total Necropsy Cases</th>
<th>3,152</th>
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<tbody>
<tr>
<td>Avian</td>
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<tr>
<td>Bovine</td>
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<tr>
<td>Caprine</td>
<td>52</td>
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<tr>
<td>Equine</td>
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<tr>
<td>Ovine</td>
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<td>Porcine</td>
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<tr>
<td>Small Animal</td>
<td>464</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>43</td>
</tr>
</tbody>
</table>

**Histopathology**: Tissues are prepared and processed to produce glass slides for microscopic examination conducted by the pathologists. Tissues from the necropsy and surgical biopsy cases were processed and 32,771 microscopic slides produced. In addition to the routine hematoxylin and eosin stained tissue sections, special and immunohistochemical stains were done resulting in 3,375 slides produced for the purpose of identifying microscopic organisms/agents the may cause disease or tissue antigens the define or identify cell structures.

**Biopsy**: Abnormal areas or lesions are often removed surgically or a portion biopsied from live animals and sent to the laboratory for determination of the type of process and recommended treatment and potential prognosis. These tissue specimens are processed and microscopic slides prepared for the pathologists to examine by microscopy. Tissue specimens representing 1367 cases were processed and examined. A report with diagnosis was produced for each case.

**Cytology**: Preparations of cells harvested from abnormal lesions or abnormal fluids are placed on microscopic slides and stained for examination under the microscope by the pathologists. Cytopathological examinations were performed, a diagnosis made, and a report generated for 141 cases.

**Quality Assurance/Quality Control**

*Mary Harbour*

The goal of the Quality Assurance Program is continuous improvement of service to clients and to ensure quality results. The design of the program is on based American Association of Veterinary Diagnostic Laboratory (AAVLD) requirements, International Standards Organization (ISO) guidelines and Organization of International Epizootics (OIE). The Livestock Disease Diagnostic Center Quality Program also helps fulfill the
university’s mission of improving service delivery while achieving excellent human relations (internally and externally), sound leadership, and effective communications.

Besides the continuous improvement of service, the Quality Assurance Section prepared the laboratory for AAVLD accreditation. The accreditation site visit occurred in May 2009 and for the first time LDDC was given full AAVLD accreditation. Quality Assurance will continue to monitor and update policies and procedures to meet the AAVLD Requirements. The laboratory also became a part of National Animal Health Laboratory Network (NAHLN) and QA section will assist in implementing all policies and procedures involved.

**Serology**

*Meg Steinman*

The Serology Section provides accurate and timely results for both diagnostic and regulatory testing. The results generated provide veterinarians and regulatory personnel with data upon which to base their decisions. Serology also performs numerous tests for movement of animals within the United States and for international export purposes. The serology section utilizes a variety of testing methodology. In 2009 the serology started screening for avian influenza by ELISA allowing for a quicker turnaround time. This section continues to see the number of specimens from the poultry industry increase. Below is a sampling of the higher volume tests that are performed in this section.

**Some Testing Highlights:**

Tests done on multiple species:
- Leptospirosis testing: 8,899
- Brucella antibody: 6,437 (includes testing on dairy milks)
- Toxoplasmosis: 64

Equine:
- Contagious equine metritis – 1,128
- Equine infectious anemia – 39,224
- Leptospirosis panel: 3,056

Poultry:
- *Mycoplasma gallisepticum* plate agglutination: 56,197
- *Mycoplasma synoviae* plate agglutination: 56,223
- *Salmonella pullorum* plate agglutination: 19,425
- *Avian influenza* antibody testing: 15,371

Bovine:
- Anaplamosis antibody: 198
- Bovine Leukemia virus antibody: 452
- Bluetongue antibody: 215
- Neospora: 195
- Johnes antibody: 1,170
Canine and feline:
- Fungal serology:
  - Histoplasma: 262
  - Blastomyces: 297
  - FeLV/FIV: 17/16

Swine:
- Pseudorabies: 84
- PRRS: 9

**Toxicology**
*Cynthia L. Gaskill*

The primary mission of the Toxicology Section at the LDDC is to provide toxicological diagnostic testing capabilities and consultations to Kentucky veterinarians, LDDC pathologists and pathology residents, county extension agents, livestock producers, and pet owners. A large variety of toxicological tests are available through the Toxicology section, including assays for metals and minerals in tissues, feed, water, and soil; organic compounds including a multitude of pesticides, drugs and other chemicals; biological toxins such as plant toxins, toxic insects, and bacterial and fungal toxins; and numerous other toxins. Consultation services include assistance with appropriate sample collection and submission recommendations; determination of appropriate tests to be performed; interpretation of analytical results; therapeutic advice; differential diagnoses; residue considerations; and other general toxicological information.

**2009 Highlights:**
- Initiated a student internship program between the LDDC Toxicology section and Eastern Kentucky University Forensic Sciences program. Our first intern completed her internship and remains employed as a part-time technician supported in part by the Kentucky Higher Education Assistance Authority (KHEAA) work-study program.
- Installation of new, state-of-the-art analytical instrumentation was completed. Instrumentation includes inductively coupled plasma mass spectrometer; gas chromatograph/mass spectrometer; high-performance liquid chromatograph; and ion chromatograph among other updates and improvements.
- Method development and validation for all new analytical test procedures has been initiated and is progressing smoothly.
- New quality control measures have been instituted to ensure results are accurate, reproducible, and meaningful.
- Several toxicological research projects are in progress in collaboration with internal and external researchers.

In 2009, the toxicology section received samples from more than 1,000 cases, with most cases involving multiple samples such as various tissues, forages, or other samples, often involving multiple animals and with multiple test requests per case. Due to the lack of unique test codes in the old LIMS system for many of the tests performed in the Toxicology section, actual numbers of each test performed cannot be obtained by a computer data search. This problem has been corrected with the institution of the new
LIMS system and data retrieval will be dramatically improved in 2010. The most common tests requested include metal and mineral quantifications in tissues such as liver and kidney; screening of rumen and stomach contents for organic compounds and drugs; analysis of tissues for pesticides, and evaluation of forages and feeds for nitrate content, mycotoxins, ionophores, cyanide, and other feed-related toxins.

**Virology**

*Neil Williams*

The Virology Section of the Livestock Disease Diagnostic Center provides diagnostic virology support to the laboratory pathologists, the Commonwealth and USDA Veterinarians, and the livestock producers and pet owners of Kentucky and other states.

**2009 Highlights**

- This section provides 40 different tests including fluorescent antibody tests, serologic tests for detection of antibodies to viruses, virus isolation tests for cattle, horses, sheep, pigs, goats, cats, dogs, and birds, and tests for the detection of viral antigens. The section maintains 11 tissue culture cell lines that are used routinely.
- The section performed 29,170 tests during 2009. Of this total, 14,239 were virus neutralization tests for the detection of viral antibodies to meet Regulatory requirements for the equine industry.
- Cattle producers have continued to utilize the screening test to detect animals persistently infected with bovine viral diarrhea virus in their herds. The laboratory tested 8,632 animals this year. Identification and removal of affected animals helps producers reduce illness, loss of production, and death of their herd animals and add value to one of our most important commodities.
Livestock Disease Diagnostic Center Faculty
2009

Bryant, U.K., DVM, Assistant Professor

Bolin, D.C., DVM, PhD, DACVP, Associate Professor

Carter, C.N., DVM, MS, PhD, DACPVM, DSNAP, Professor and Director (R)

Cassone, L.M.C., BS, DVM, Assistant Professor

Donahue, J.M., PhD, Professor

Gaskill, C.L., DVM, PhD, Associate Professor

Hong, C.B., DVM, PhD, DACVP, Professor

Jackson, C.B., DVM, DACVP, DACPVM, Associate Professor

Loynachan, A.T., BS, DVM, PhD, Assistant Professor

Kennedy, L.A., DVM, Assistant Professor

Vickers, M.L., PhD, Associate Professor

Williams, N.M., DVM, PhD, DACVP, Professor and Associate Director
Books and Book Chapters:


Refereed Journal Publications


Other Research


*Gaskill, C.L.*. Large animal toxicology update: new and unusual causes of poisoning. 31st Annual Morehead Clinic Days Veterinary Conference, Morehead KY, June 2009

*Gaskill, C.L.*. Companion animal toxicology update: new and unusual causes of poisoning. 31st Annual Morehead Clinic Days Veterinary Conference, Morehead KY, June 2009


Faculty member M.L. Vickers contributed to one article and 2 Genbank Registers in Gluck.

Staff member S.F. Sells contributed to one article and 2 Genbank Registers in Gluck.