The AVM Fall/Winter 1996 Newsletter
(Please note: Formatting has been changed for electronic presentation.)

The Association of Veterinary Microbiologists

Message from the President

My fellow Veterinary Microbiologists and AVM Members,

Salutations from the Deep South! As I write to you in late November, most folks in the northern parts of our country are experiencing some daily low temperatures below freezing; this far south, we still do the yard work in our short-sleeves.

I am humbled by the trust that you have placed in me by selecting me to guide the Association's affairs for the 1996-1997 term, our 21st year as a professional association. I pledge to serve with the best of my capabilities and judgment, for the benefit of all members. I acknowledge with great appreciation the many who have supported and contributed to the AVM with their time, treasure and talents, from the Association's "Founding Fathers" John Black and Al Pursell, up to the immediate former President Michael Justin and our valued corporate sponsors IDEXX and Symbiotics Animal Health, and our exhibitors Centaur, IDEXX, Kirkegaard and Perry Laboratories, Sensititre/Alamar, Symbiotics Animal Health, Unipath/Oxoid, Viral Antigens and VMRD.

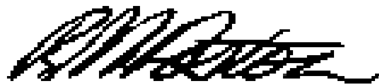
This past summer's meeting was a pleasant gathering, cast in the surprisingly delightful setting of Mobile, Alabama. Mobile projects a gracious blend of antebellum, old-town, and modern styles, with generous portions of history and southern charm. Kudos to Sarah Rowe-Rossmanith for her site organizational efforts. The meeting was quite the success judging from the spirited session discussion. It was nice seeing y'all again, especially you Yankees, who traveled so far south to attend. I particularly thank this year's newsletter coordinator Frank Austin, and all others who have contributed their session notes; together, they have made this newsletter most informative. Frank mentioned to me that he intended to editorialize some; just in case, I offer this disclaimer: Opinions expressed in the session notes of the newsletter are solely of the newsletter coordinator, and do not necessarily reflect the opinions of other AVM officers, session chairs or members. (But seriously, if we read through them, we might learn something.)

In my brief address to the Association in Mobile, I noted the growth of the Association over the past few years. We now have about 300 members. Growth is double-edged: On the upside, a greater variety of thoughts and ideas are brought into the discussion with an influx of new members. The downside is that the national meetings are becoming unwieldy; with the large numbers present at roundtable discussions, many are intimidated to participate.

To counteract the ill effects of growth, the importance of the chapter meetings is emphasized. This fall, I had the opportunity to attend the AVM Colonial States Chapter meeting in Williamsburg, VA. In addition to the round table discussions, Dr. Beth Henricson spoke on *Immunologic parameters of Intracellular Pathogenesis*, and Dr. Lloyd Lauerman entertained us with *Current Applications of PCR in Veterinary Diagnostics.* This most edifying meeting, together with the fall season's beauty and history of colonial Virginia, was a profound experience. I encourage all AVM members to become active and rigorous participants in their chapter meetings, to recapture that one-on-one intellectual engagement that existed when the AVM was young. All of us have need of continuing education in our ever-changing profession, and such opportunities for professional growth should not be missed.

Another burden of growth is the need to insure channels of communication and the flow of ideas among members. Towards this goal, we hope next spring to have another newsletter containing chapter meeting notes and some useful technical information. This will depend on resources of time and finances, and most particularly on members to contribute technical writings, articles or news items. If you have something of interest to other association members, let us hear from you. Additionally, by now, many of you have some sort of E-mail facility; if so, let us know your E-mail address so that we can publish it in a future edition of our membership directory. At Mobile, an effort was initiated to investigate the feasibility of an AVM web site, to take advantage of yet another, albeit new, vehicle of communication. Lea Dowd has kindly consented to chair the AVM Ad-Hoc Committee on Web Site Development. Her report and a draft of the proposed home page is included here for your consideration. If any association members have thoughts or ideas, particularly if you have experience with these endeavors, your input is most welcome.

I make a special appeal to the newer members who have not had convenient or frequent opportunities to attend AVM functions. Like a chain, our association is only as strong as the least of its links. If work schedules or finances preclude you from regularly attending meetings in person, please find other ways to become involved. Your ideas count the most, and your input and participation have been sorely missed! For corrections, suggestions, complements or complaints, please do not hesitate to contact me. The only bad ideas are those left unexpressed.
AVM members should take note of the *News, Events, and Items of Interest* page of this newsletter for information on upcoming events, and especially should keep in mind the next annual meeting of the AVM, the 22nd Annual Symposium on Techniques in Veterinary Microbiology, to be held in Hot Springs, Arkansas, on July 31 to August 2, 1997. Former AVM President Melody Parsley will be hosting the meeting at this popular resort. Look for the meeting information in your mailbox this spring. Let's all plan to meet this summer and "take in the waters."
Love, peace, health, and prosperity to you and your families throughout the New Year,



Rob Poston
President, Association of Veterinary Microbiologists
Louisiana Veterinary Medical Diagnostic Laboratory
LSU School of Veterinary Medicine
Baton Rouge, LA 70803
Phone 504/346-3194, Fax 504/346-3390, E-mail vmdlvr@lsumvs.sncc.lsu.edu

AVM History and Philosophy

The idea for the Association of Veterinary Microbiologists was conceived in 1972 by Alfred R. Pursell of Tifton, Georgia and John W. Black of Hopkinsville, Kentucky. The concept was based upon an informal meeting of laboratory technicians and others interested in the laboratory diagnosis of animal diseases by virologic, bacteriologic, and serologic methods. The format for the meeting was to be a round table discussion on the problems and procedures of interest to the various areas.

At the 1973 regional meeting of the American Association of Veterinary Laboratory Diagnosticians in Nashville, Tennessee, representative of several diagnostic laboratories met with Pursell and Black to discuss the advantages of the proposed association. It was agreed that such an association would be beneficial to both the participants and their laboratories.

In 1975, with the encouragement of Dr. Wade Kadel, the Director of the Hopkinsville laboratory, John Black sent notices to the microbiologists at each of the veterinary diagnostic laboratories in the southeastern United States, announcing a "Symposium on Techniques of Diagnostic Veterinary Microbiology". This symposium was sponsored by the diagnostic laboratories of Tennessee, Kentucky, and Georgia. It was held at the Mountain View Hotel in Gatlinburg, Tennessee on May 22, 1976. A total of 33 people from eleven laboratories, representing eight states were present. John Black presided over the meeting and was elected the first president of the Association. Separate sessions on virology and bacteriology and a combined session of serology were held with a moderator selected to guide the discussion. An agenda served only as a guide and each participant was free to propose additional topics for discussion.

The primary participants were the people actually doing the work at the bench. They were encouraged to present any problems and to work together on possible solutions. To establish the informality required for such an exchange the use of titles was discouraged and everyone was on a first name basis.

The objectives of the association, which were set forth in the constitution adopted in 1978 are "...to promote scientific investigations and their applications to the advancement of knowledge in this field, and to provide mutual assistance to participating laboratories in solving problems".

AVM Membership Information

Who Can Join? Any person interested in the objectives of the AVM and who is active in the field of veterinary microbiology shall be eligible for membership.

Annual Meetings. The AVM annually conducts a Symposium on Techniques in Veterinary Microbiology. In addition to the business session, the general session, and guest speakers, the principle feature of the annual meeting is the discussion sessions, typically in the "round-table" format, on the problems and procedures of interest to the various areas and disciplines of veterinary microbiology. The southeastern United States has been divided into four geographic regions for the purpose of rotating the location of the annual meeting:
Region I: Kentucky and Tennessee
Region II: Delaware, Maryland, North Carolina, South Carolina, Virginia, West Virginia

Region III: Alabama, Florida and Georgia
Region IV: Arkansas, Louisiana and Mississippi

Membership Directory: A membership directory is published every year and mailed to each member. A membership directory and membership application form can be obtained by contacting the President or Secretary of the Association.

Newsletter: A newsletter is published each year, or biannually if resources permit. It contains information on meetings, training conferences, new techniques and other items of interest.

Exhibitors and Presentations: Commercial exhibits and presentations on topics of mutual interest are welcome; their incorporation into the meeting program are left to the discretion of the local meeting site arrangements committee.

AVM Fees: Membership dues (annually) $10.00, Meeting registration $15.00, Sustaining Membership (annually) $100.00, Exhibitor's fee, $300.00.

AVM Officers and Executive Board Members, 1996-1997

President Rob Poston, Baton Rouge, LA Colonial States Representative
Vice President Theresa Love, Jackson, MS Marion Fowler, Dover, DE
Secretary/Treasurer Sherry Greer, Hopkinsville, KY Heartland Representative
Executive Advisor Al Pursell, Tifton, GA John Landgraf, Ames, IA

Past President Michael Justin, St. Joseph, MO Publications Chairperson
Past President Melody Parsley, Little Rock, AR Frank Austin, Starkville, MS
Past President Janet Mapp, Jackson, MS Meeting Site Chairperson
Historian Roxie Maddux, Hopkinsville, KY Melody Parsley, Little Rock, AR

AVM Committees

Publications Committee Frank Austin, Melody Parsley, Kay Rathman
Nominating Committee Michael Justin, Melody Parsley, Janet Mapp
By-Laws Committee John Black, John Cole, Al Pursell
Audit Committee Bill Cornell, Roxie Maddux
Meeting Site Committee Melody Parsley, Mike Parsley, Judy Clapier
Program Committee Janet Mapp, Sandy Blackwell, Theresa Love
Newsletter Advisory Committee John Cole, Sandy Baldwin
Ad-Hoc Committee for Web Page Development Lea Dowd

The AVM Colonial States Chapter

In 1985, The Colonial States Chapter was formed in Richmond, Virginia primarily from members belonging to Region II of the AVM. The CSC holds its annual meeting in Williamsburg, Virginia each November and usually sponsors a symposium on one of the microbiological disciplines (bacteriology, virology, immunology, serology, etc.) each spring. The pertinent membership information of the AVM also applies for the CSC. In years when the annual meeting of the AVM falls into Region II, the CSC annual meeting is usually held concurrently with the parent organization. CSC annual membership fees are $10, for sustaining membership, $50. Meeting registration is $15 and the exhibitor fee is $100. Those interested are invited to contact the chapter Secretary/Treasurer George Blackwell at 540/433-1638 or one of the other chapter officers.

- Colonial States Chapter President Marion Fowler, Dover, DE
- Colonial States Chapter Vice President Beth Henricson, Warrenton, VA
- Colonial States Chapter Secretary/Treasurer George Blackwell, Harrisonburg, VA

The AVM Heartland Chapter

In 1995, The Heartland Chapter was formed, and is comprised of AVM members residing in the United States north-central region, which includes the states of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin. The Heartland Chapter typically holds organizational and scientific meetings or offers wet labs in late April or early May of each year, in a format similar to the parent organization. Heartland Chapter annual dues are $5. Those interested are invited to contact the chapter Secretary/Treasurer Kathleen Strelow at 608/266-2465, or one of the other chapter officers.

- Heartland Chapter President John Landgraf, Ames, IA
- Heartland Chapter Vice President Tim Klinefelter, Ames, IA
- Heartland Chapter Secretary/Treasurer Kathleen Strelow, Madison, WI

AVM News, Events, and Items of Interest

Congratulations and good luck to the new officers of the Colonial States Chapter, elected at the AVM-CSC annual meeting on November 8th in Williamsburg, Virginia: President Marion Fowler, Vice President Beth Henricson, Secretary/Treasurer George Blackwell.

Best wishes to Andy Myrup upon his retirement from the Maryland Animal Health Diagnostic Laboratory in College Park, Maryland. Let us hope that he finds his newfound leisure time as personally fulfilling as all of us have been fulfilled by his service and commitment to the Association and the profession.
A very special note of thanks to those who presented special topics in the general session or round table sessions, particularly Dr. Gene Erickson, and Dr. Lloyd Lauerman, and especially all others who contributed their thoughts and ideas during the round table discussions.

AVM members are asked by the Program Committee to put on their thinking caps and consider possible discussion topics for this summer's meeting. If you have been involved in some new discovery, developed a new improvement or experienced difficulty with a particular technical procedure, or wonder what works best in certain circumstances, make note of it and send or phone it to Janet Mapp, Sandy Blackwell, or Theresa Love at the Mississippi Board of Animal Health Veterinary Diagnostic Lab, P.O. Box 4389, Jackson, Mississippi, 39216, 601/354-6089. Those members planning informal presentations at the discussion sessions are asked to assist organizational efforts by informing the program committee of the topic, and by providing a draft to the Publications committee to facilitate its inclusion in subsequent newsletters.

Upcoming Events

The AVM Colonial States Chapter will conduct a spring symposium on Saturday, April 19, 1997 at the Boars Head Inn and Sports Center in Charlottesville, Virginia. This resort complex, centered around a restored farm manor house, features many indoor and outdoor recreational opportunities. Also, this will be during "Historic Garden Week", so other springtime attractions and activities will be found in the Charlottesville area that weekend. Anticipated symposium topics are Reptile Microbiology, by Dr. Frank Austin of Mississippi State University, Starkville, MS, NCCLS Update, by Dr. Jean Cooper of the US Food and Drug Administration, Rockville, MD, and Biohazard Shipping and Packaging, by representatives of the US Postal Service, United Parcel Service, and Federal Express. A registration fee of $20 is anticipated; registration will begin at 8 am. For further details, please contact George Blackwell at 540/433-1638.

The AVM Heartland Chapter will hold its annual meeting on Friday and Saturday, April 25-26, 1997, hosted by the South Dakota Veterinary Research and Diagnostic Laboratory, South Dakota State University at Brookings, South Dakota. In addition to the business meeting and round-table discussions, Dr. Jane Hennings of the Veterinary Research and Diagnostic Laboratory will present a seminar on the PCR technique, including a wet lab and demonstration, on Friday afternoon, April 25th. First, an overview of the PCR technique will be presented, then separate sessions will be conducted on specimen extraction, on preparation of the master mixture and eliminating contamination, and on gel separation and identification techniques. The PCR technique employed by Dr. Hennings is a nested procedure that detects PRRS virus in serum, blood and tissues, and Johne’s’ Disease bacillus in fecal specimens; other applications will be discussed if interest is expressed. Meeting registration, wet lab fees, and membership dues are expected to cost $35. AVM members suggesting additional wet lab topics, or those interested needing further information, are invited to contact Connie Gates at 605/688-5689.

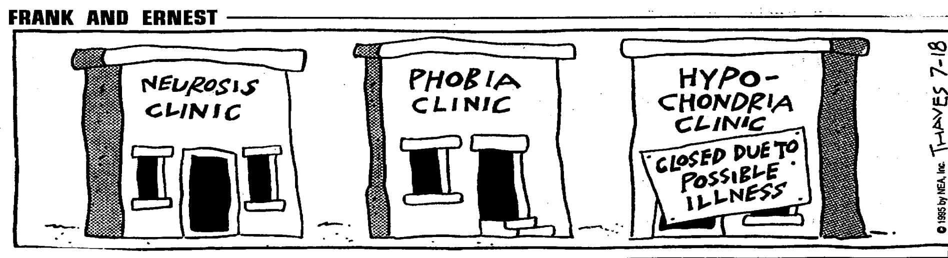
The South Central Branch of the American Association of Veterinary Laboratory Diagnosticians (AAVLD) will hold its third annual meeting on Saturday and Sunday, May 17-18, 1997 at the Louisiana State University School of Veterinary Medicine in Baton Rouge, Louisiana. The meeting will be hosted by the Louisiana Veterinary Medical Diagnostic Laboratory. Abstracts for presentations are being accepted until May 1. To submit abstracts, or for information, contact Rob Poston at LA. VMDL, Box 25070, Baton Rouge, LA 70894, or phone 504/346-3l93, fax 504/346-3390 or E-mail vmdlvr@lsumvs.sncc.lsu.edu.

The Veterinary Laboratory Association (VLA) will hold its annual meeting on May 19-21, 1997 at the Wyndham Hotel & Resort in Orlando, Florida. For information, please contact meeting coordinator Linda Zimmermann at 800/874-1983. There are additional regional VLA meetings tentatively planned for the Fall and Winter of 1997; as details are established, they can be obtained from VLA newsletter coordinator Dixie Fisher at 213/342-1600, E-mail dfisher@hsc.usu.edu, or from the Administrator of Proficiency Program Dr. Milton J. Becker at 312/754-1852.

The VLA is an organization for veterinary diagnostic laboratories. This association was formed for the purpose of providing consistency throughout veterinary diagnostic laboratories, which ultimately improves animal welfare. The association does not limit membership to any geographical area nor denote that this association is specific for any type of laboratory, being commercial, federal, state, university, or other private ownership. The association was formed in Chicago, Illinois on February 10th and 11th, 1989. Membership is open to anyone interested in the field of veterinary

laboratory medicine, to include technicians, technologists, veterinarians, veterinary pathologists, laboratory managers and directors, professional organizations and industry. Those interested in further information on the VLA are invited to contact the VLA President Joni Ray at 800/366-4184.

The AVM 22nd Annual Symposium on Techniques in Veterinary Microbiology will be held on July 31 to August 2, 1997 at the Arlington Hotel in Hot Springs, Arkansas. Meeting notices will be distributed to AVM members in the Spring of 1997. For information, please contact Melody Parsley at 501/225-5650. Individuals wanting advanced information on the amenities of the City of Hot Springs are invited to call 800/SPACITY (800/772-2489). Those planning an Arkansas vacation around the meeting can obtain tourist information from the Arkansas Parks and Tourism Commission at 800/NATURAL (800/628-8725).
The AAVLD will hold its 40th annual meeting on October 17-24, 1997 at the Galt House in Louisville, Kentucky. For further meeting details, or for membership information, please contact an AAVLD representative at 314/882-6811.



AVM Business Reports

Report of the Treasurer, July 31, 1996
Presented by AVM Secretary/Treasurer Sherry Greer to the general session of the AVM at the annual meeting in Mobile, Alabama, 1 August 1996.
BALANCE ON HAND-July 31, 1995 **$5257.53**

RECEIPTS:
Membership Fees $1365.00
Registration for 1995 Meeting $1755.00
Registration for 1996 Meeting $ 60.00
Exhibitor Fees and Contributions:
1995 Meeting: $1870.00
1996 Meeting: $1700.00 $3570.00
TOTAL RECEIPTS: **$6750.00**

EXPENSES:
Annual Meeting 1995:
River Terrace Resort $5124.55
Presidential Suite 222.00
Advisor Reimbursement 240.00
Plaques and Paperweights 436.65
Pat Black-Refund-Deposit 500.00
Name Tags 40.28
Office Supplies-Pat Black 35.52
Refreshments 68.00 $6667.00
Directory 1996:
Printing $ 196.80

Postage 255.53 $ 452.33
Program 1995:
Printing $ 120.38
Postage 127.05 $ 247.43
Program 1996:
Printing $ 187.22
Postage 138.60 $ 325.82
Miscellaneous Expenses:
Program Topics Letter $ 122.75
Fedex-Mike Justin 13.00
Policy Guide & Postage $ 42.54 $ 178.29

TOTAL EXPENSES: **$7870.87**

BALANCE ON HAND-July 31, 1996 $4136.66

Report from the Ad-Hoc Committee on Web Page Development

Presented to the President by Lea Dowd, Chairperson

So, you want to build a web page... In order to start you first must have a computer and access to the Internet. You will have to subscribe to a provider in order to have access. There are a large number of providers available, at a wide variation in price. The most commonly known are: AOL (America On Line), CompuServe, WOW (by CompuServe) and MSN (Microsoft Network). There is another provider that is classified as an Internet SLIP or PPP Account. There are definite advantages and disadvantages for each of the different providers.

When evaluating each, cost is a major factor. Most communities now have local Internet SLIP or PPP services available for a flat monthly fee with unlimited use. Random pricing in Georgia shows that these may be obtained for less than $20.00 per month. Many of these also offer home pages for no additional charge. A home page is the primary Web page for an individual, software application, or organization. Home pages can link visitors to other pages related to their site.

How to find a local service provider: 1) Use a friend's Internet account or a trial account from a commercial provider to access the World Wide Web. Search for "Internet Service providers". You will find numerous lists that you can search until you find one close to you. The Web page *http://thelist.com* has an excellent list of providers, grouped by area code. 2) You will probably want to find one that has a local access number. Services that offer "800" numbers usually charge for that number. 3) Check the business pages of your local paper. 4) Universities frequently have their own services. Check with someone in the computer department. 5) Look in the Yellow Pages under "On-line service providers".

Creating a Web page: It is not difficult to create a home page. There are many programs designed to create these pages. Two of these are Web Weaver and Astound 4.0. However, it is difficult to create a good home page. The details of creating a web page are beyond the scope of this article. However generally speaking, here is what you have to do: 1) Create the page or pages as files on your disk by using a text editor or a specialized HTML editor. 2) Test them with Netscape and other browsers to make sure that they work the way that you want. 3) Upload them to your provider's computer and put them in the appropriate place. This is wherever the provider says. 4) Update the pages often enough to keep them interesting.

There are several books also available to help create a web page. These include; *Internet Secrets, HTML For Dummies,* and *Creating Cool Web Pages with HTML.*

Some points to remember when creating a web page: 1) People connecting are using a modem. This means that great big pictures take a long time to load. Keep the pictures small or either create a small "thumbnail" version that will link to the full picture. 2) Small pages that fit in a screen or two works better than large pages. They are easier to read and load faster. You can always break up large data into five or six separate pages. 3) No Web page is complete without links to other related pages. The AVM page could link to other sources related to microbiology, virology, immunology, agriculture, universities and distributors. There are virtually thousands of related pages. 4) Once the page is created, do try to look at it with as many different browsers as possible. Make sure that the page is legible no matter what browser is used. AOL and Prodigy (close to 10 million users) use the browsers that come with their service. Others include Netscape and Internet Explorer. 5) When writing the page, remember that you should not put anything on it that you do not want the world to see. 6) Include an E-mail address for people to use for messaging, questions, problems, etc. 7) The largest effort in this project will be one of time and energy by the host (person managing the web page). However, with the computer access among members of the AVM, this should not be a problem.

The benefits of a Web page for the AVM: 1) Better communication and interaction among members. 2) Allows a forum for questions and items of interest. 3) Will be an advertisement for other microbiologists to learn of this association. 4) Could store all of the previous newsletters at this site. 5) Can place pictures of new and unusual organisms for others to see. 6) Great source for AVM members to visit and explore links.

The "Down Side" of a Web page: 1) The costs can vary from $200 a year to much more. 2) There will have to be someone responsible for answering E-mail. 3) The Web page will probably need updating at least quarterly, monthly would be even better.

Business Session Notes

The Association of Veterinary Microbiologists Twenty First Annual Symposium on Techniques in Veterinary Microbiology convened in Mobile, Alabama on August 1-3, 1996. [Editors note: Only a brief synopsis of the business session is presented here; a formal reading of the minutes of the AVM 1996 business session will occur at the next annual meeting in Hot Springs, Arkansas on July 31 1997, after which the minutes will be submitted to the AVM membership assemblage for formal approval.] The general session was formally opened at 8:45 am on August 2, 1996 by AVM President Michael T. Justin; announcements included an increase in the registration fee from $10 to $15 and a $300 appropriation for the AVM Archive.

Minutes of the Executive Board meeting, which was held on August 1, 1996, was presented by AVM Secretary/Treasurer Sherry Greer.

Sara Rowe, the 1996 Site Committee Chairperson, welcomed members to Mobile and highlighted some Mobile area attractions.

Melody Parsley, the 1997 Site Committee Chairperson, announced that the next AVM symposium will convene at The Arlington Hotel at Hot Springs, Arkansas, on 31

July 1997, and presented a striking slide show of the setting, including hotel amenities and Hot Springs area attractions.

Rob Poston of the Louisiana Veterinary Medical Diagnostic Laboratory in Baton Rouge, Louisiana, the current AVM Vice President, was elected President by the association body to serve the term of 1996-1997. Theresa Love of the Mississippi Board of Animal Health in Jackson, Mississippi was elected as AVM Vice President to serve during the same term. Sherry Greer was elected again to serve as AVM Secretary/Treasurer.

Avian Session Notes

Moderated by Ernie Wyant, British United Turkey Association, Lewisburg, West Virginia.
Prepared by Melody Parsley, NPIP Supervisor, Little Rock, Arkansas, and Bill Palin of the IDEXX Corporation, Westbrook, Maine.
[Editors note: In the midst of coverage of certain published topics, discussions of other sub-topics unfolded. To ease information retrieval, some of these ancillary topics are presented under their own heading. Additionally, other miscellaneous information is placed where it would most likely be found, regardless of the heading under which it was actually discussed.]

Methods and Techniques for Serotyping Avian Infectious Bronchitis Virus

Laboratories at Purdue and Auburn Universities are performing hemagglutination (HA) and hemagglutination inhibition (HI) for avian infectious bronchitis virus (IBV). Lloyd Lauerman of Auburn University and Chinling Wang of Mississippi State University are using PCR technology. Mark Jackwood of the Univ. of Georgia at Athens has a published PCR protocol, utilized by Lauerman (see Kwon, H.M. *et al* below). The M1 sequence is used for the initial identification and the S1 for confirmation with restriction fragment length polymorphism (RFLP) [Editors Note: The concept of RFLP is explained briefly under the heading of **PCR for Rapid Identification of *Mycoplasma spp.*,** below in the bacteriology session notes]. DNA probes will distinguish the principle strain variants, including a new variant out of Delaware.

Jackwood has a new primer that is now available.

Frank Austin of Mississippi State is using a monoclonal antibody against the S2 and M proteins to detect all IBV types by IFA; three other monoclonals are used to distinguish the Massachusetts, Connecticut, and Arkansas serotypes. The protocol of Austin involves IFA testing of cryostat sections made from chorioallantoic membrane (CAM), harvested from specific pathogen free (SPF) eggs 48 hours post-inoculation. At Mississippi State, viral genetic sequences are also sought in allantoic fluid with PCR technology. The Arkansas strain cannot be detected in the presence of the Massachusetts strain using the IFA test, but is found with PCR technology. The monoclonal antibodies were acquired from S. A. Naqi of Cornell Univ. (607/253-3365).

The cost of conventional serotyping by hemagglutination inhibition (HAI) technology is not readily evident. SPF eggs are required for antigen production, which is labor intensive. In contrast, the PCR technique of Lauerman requires that the sample is subcultured three times. Following the third culture, allantoic fluid is harvested and extracted in 1:1 phenol-chloroform; which requires about three hours of hands on

time. Samples stored as the allantoic fluid or in the extracted form. Start-up costs for PCR technology is currently about ten thousand dollars for equipment, reverse transcriptase and gel electrophoresis. The PCR technique requires about four hours, time enough to run approximately 30 cycles. If a custom sequence is needed, a primer can be made by Life Technologies of Gaithersburg, Maryland for about $1.19 per nucleotide. One lab represented in this discussion charges about $200 per sample for PCR analysis. There is much interest in developing PCR technology for NDV, CAV, and AI viral diagnostics; the cost-benefit of development is being considered and contrasted to conventional technology and the demand of testing.

Recent publications indicate that recombination among IBV serotypes may produce misleading diagnostic results. Please see the following references for further information:

- W. Jia, K. Karaca, et al. A novel variant of avian infectious bronchitis virus resulting from recombination among three different strains. 1995. Arch Virol 140: 259-271.
- K. Karaca, S. Naqi, and Jgelb, Jr. Production and characterization of monoclonal antibodies to three infectious bronchitis virus serotypes. 1992. Avian Disease 36: 903-915.
- K. Karaca, S. Naqi. A monoclonal antibody blocking ELISA to detect serotype-specific infectious bronchitis virus antibodies. 1993. Vet Microbiol 34: 249-257.
- Kwon, H.M., Jackwood, M.W., J. Gelb, Jr., Differentiation of infectious bronchitis virus serotypes using polymerize chain reaction and restriction fragment length polymorphism analysis. 1993. Avian Diseases 37: 194-202.

Avian Influenza

KPL has an AI serology kit available for chickens only. There is a consideration to market a kit for testing of turkeys next quarter, but there is no prospect for development of a kit for ratite testing. Each kit has 900 tests, and is priced so that the cost per test is $.80 -$2.00, depending on testing volume. The kit shelf life is 18 months. Becton Dickinson has an antigen detection kit, but it is not labeled for use in ratites. It is anticipated that the demand for AI monitoring continue to rise, but probably not as fast as before, due to increases in test cost. The States of Arkansas, Mississippi, [and Louisiana] test individual birds for export/import/exhibition by AGID. They also have a monitoring program for *M. synoviae* (MS) at slaughter.

Evaluation of IDEXX Mycoplasma PCR Test Kit

Some labs have found "faint positives" with this kit. Some of such reactors were substantiated as positive by western blot technology by Stan Kleven of the Univ. of Georgia at Athens. Negative serology testing has been noted following certain outbreaks. *M. gallisepticum* (MG), *M. synoviae* (MS), and *M. meleagridis* (MM) positive serology controls are available from NVSL. According to KPL representatives, certain users of their MG/MS ELISA test kit have raised the cutoff point from 0.6 to 0.8, which causes their kit to lose sensitivity. Those with concerns on this point are invited to contact KPL for further information.

States vary somewhat on handling policy of MG/MS suspects. One state waits 14 days, then rebleeds and bands positive birds. For PCR verification, Lauerman developed his own primers according to published genetic sequences; he found one to three out of four pools with faint MS positive bands. Plate agglutination serology testing of these same birds became positive one to two weeks after the PCR was positive; only later did the HAI tests become positive for these birds. He used pools of five tracheal or choanal swabs without broth or agar amplification.

Ornithobacterium rhinotrachaele (ORT)
This is believed to be a secondary pathogen, not a primary cause of disease. It is often confused with *Pasteurella*. Isolation attempts lasting more than 24 hours often become overgrown. There has been little work in the U.S. with this organism. For further information, please see the following recently published article:
Travers, A.F., Concomitant ***Ornithobacterium rhinotrachaele*** and Newcastle's Disease infection in broilers in South Africa. 1996. Avian Diseases 40:488.

PCR in Bacteriology
There is concern that this technology cannot distinguish between live and dead bacteria. *H. somnus* is usually difficult to isolate, but PCR technology is able to confirm its presence. Microbiologists at the Univ. of Connecticut are developing PCR technology for *Salmonella* detection. Diagnostic and Biologic Technologies, Inc. of San Antonio offers *Salmonella* screening by PCR. Testing is done on fecal material, milk, blood, or mucosal secretions. Labs in Alabama and Georgia are gearing up to test meat samples for export to Russia. [Editors Note: The PCR technique is discussed further in the bacteriology notes under the heading **PCR for Rapid Identification of *Mycoplasma spp.*,** and in the virology notes under the heading **PCR Testing**.] For further information on commercial PCR testing for *Salmonella*, please contact:
Diagnostic and Biologic Technologies, Inc., 18585 Sigma Road, Suite 100, San Antonio, Texas 78258, Phone (800)336-3060, Fax (210)496-2517 E-mail address dbt@txdirect.net.

Laryngotracheitis
There was no mention of a commercial PCR-based diagnostic test kit for LTV, but the laboratory in Athens, Georgia has developed its own PCR technology. Histopathology was reported to be the best diagnostic method, but that understanding is contraindicated by experiences at the lab at Mississippi State; both major state testing labs in Mississippi now use DFA technology to diagnose LTV infection. The conjugate recently has been made available from NVSL, but is not yet listed in their catalog.

New Manuals and Committees
The AAAP manual is in revision and will be released within 12 months; it is expected to contain information on PCR technology. AAVLD bacteriology committee has new accreditation guidelines, which will be released at the their annual meeting in Little Rock in October 1996. AAVLD also has available a proficiency check test for veterinary determinative bacteriology.

Colibacillosis
An Alabama lab is finding quinolone resistance among *E. coli* isolates.

Salmonella Serotyping
NVSL is charging $20 for serotyping. Many labs are considering performing their own in-house Salmonella serotyping, which may prove to be expensive, costing more than $20 per test.

Microtiter Testing for *Salmonella pullorum- typhoid* (PT)
Richard Gast of the USDA Southeast Poultry Research lab at Athens, Georgia and others report that one of every twenty birds tested is positive for PT. One state lab was said to be finding some discordant tests results (certain samples test negative by

plate agglutination, positive by ELISA). No one reported PT testing requests for ratites.

Bacteriology Session Notes

Moderated by John R. Cole, Veterinary Diagnostic Laboratory, University of Georgia, Tifton.
Prepared by Frank W. Austin, College of Veterinary Medicine, Mississippi State University, Starkville.

Johne's Disease

Many tests were reported to be in use by session participants. These included:
CF 27% sensitivity (high false negatives), 90% specificity (low false positives).
AGID Low sensitivity, but high specificity.
Culture 50% sensitivity, 100% specificity.
ELISA 64% sensitivity,
PCR 35% sensitivity, 99% specificity.
DNA Probe by IDEXX - not in use.
Acid Fast Staining of rectal scraping - used prior to culture.

The double incubation procedure is used with satisfactory results by labs at Cornell and Pennsylvania Univ.'s to decrease contamination. Culture time was reported to be six weeks for high shedders, eight weeks otherwise. Herrolds media (both with and without Mycobactin J and pyruvate) is available commercially from Remel of Lexena, Kansas. Homemade media was reported to produce a little faster growth, but is difficult to make. Quality control of media is recommended; a known positive culture can be used to spike a control fecal specimen, which is cultured along with the clinical fecal specimen. The need for rapid, reliable tests was stressed.

A symposium on Crohn's disease of humans was announced to occur in Wisconsin. Topics included diagnostic technology and epidemiology of Crohn's in relation to Johne's incidence. Several articles on the topic by Whitlock were recommended. Other papers of interest include:

- J. O. Falkinham III. Epidemiology of Infection by Nontuberculous Mycobacteria. Clin. Microbiol. Rev. 1996, 9(2): 177-215.
- H.J. Van Kruiningen, B. Ruiz and L. Gumprecht. Experimental Disease in Young Chickens Induced by a *Mycobacterium paratuberculosis* Isolate from a Patient with Crohn's Disease. Can J Vet Res 1991, 55: 199-202.
- W. Bruce McNab, et al. An Evaluation of Selected Screening Tests for Bovine Paratuberculosis. Can J Vet Res 1991; 55: 252-259.
- D.C. Sockett, et al. Evaluation of Conventional and Radiometric Fecal Culture and a Commercial DNA Probe for Diagnosis of *Mycobacterium paratuberculosis* Infections in Cattle. Can J Vet Res 1992; 56: 148-153.
- D.C. Sockett, et al. Evaluation of Four Serological Tests for Bovine Paratuberculosis. J Clin Microbiol. 1992; 30(5): 1134-1139.
- J. W. B. Van Der Giessen, et al. Evaluation of the Abilities of Three Diagnostic Tests Based on the Polymerase Chain Reaction To Detect *Mycobacterium paratuberculosis* in Cattle: Application in a Control Program. J Clin Microbiol. 1992; 30(5): 1216-1219.
- G. S. Colgrove, et al. Paratuberculosis in Cattle: A Comparison of Three Serologic Tests with Results of Fecal Culture. Vet Microbiol. 1989; 19: 183-187.
- Y. G. Kim, et al. Comparison of two methods for isolation of *Mycobacterium paratuberculosis* from bovine fecal samples. AJVR 1989; 50(7): 1110-1113.

- J.J. Aduriz, et al. Lack of mycobactin dependence of mycobacteria isolated on Middlebrook 7H11 from clinical cases of ovine paratuberculosis. Vet Microbiol. 1995; 45: 211-217.

Identification of Fastidious Gram Negative Rods and Non-fermenters

This topic focused on the *Haemophilus, Actinobacillus* and *Pasteurella* group of organisms. The question was asked if this group was always considered as pathogens. Group consensus was that it is best to let the veterinarian or pathologist decide what is significant, but organisms of the HAP group generally are considered as pathogens.

API NFT was reported to be good for identification of *Pasteurella*, excluding *P. caballi*, but not for *Actinobacillus*. The BBL Crystal system, backed up by tube biochemicals, produced good results. The Sensititre system backed up with NFT was used by others. The new fermenter card for the Vitek system was not recommended. The Biolog system was liked by the few who used it. Carbohydrate base broth with crystal violet and bromcresol purple (BCP) pH indicator was used by some in attendance to discern sugar fermentation patterns of unknowns. The API Zyme system was reported to produce good results for *Haemophilus* spp. identification, which requires only four hours. Everyone agreed that tube biochemicals produced the best results. X and V factor requirements are also used in addition to tube biochemicals. Many participants in attendance admitted having problems identifying organisms in this group of bacteria.

One pointed problem is that antibiotic therapy affects organism identification on initial isolation; repeated subcultures are required to return the organism to its normal biochemical identification pattern if the patient has been on antibiotic treatment. Additionally, odd isolates are being found in the wrong species of animals; *H. parasuis* from a cow blood culture and an unidentifiable *Pasteurella* and *Actinobacillus*-like organism were mentioned as examples. As a possible explanation, it has been observed that different animal species now are being brought more frequently into close proximity with each other, especially when they are ill; new pathogens hypothetically emerge when dissimilar species repeatedly encounter each other in a common habitat, and acquire some of each other's normal flora. Non-host adapted organisms passed among aberrant animal species could undergo a change in biochemical characteristics that cause them to be difficult to recognize, resulting in misidentifications with systematized bacteria identification methods. The growing variety of animal species being tested by labs may be responsible for an increase in the number of instances that *Pasteurella* and *Actinobacillus* spp are isolated. In the search for reliable identification, many different systems have been tried by the various labs represented in this discussion.

Intestinal Anaerobe Cultures: What organisms to screen for?

The table below was constructed from the recent review article, cited below, which also contains excellent sections on diagnosis. Isolation methods commonly include CDC anaerobic blood agar with PEA, however selectivity reportedly was lost as the media ages due to the volatile nature of PEA. Another method of selection is to place a kanamycin disk at the second quadrant of a streaked CDC anaerobic blood agar plate. Oxyrase® (a catalase blocker) was observed to be only 40% successful. No one in attendance was using Cp selective agars employed in food microbiology. Many participants favored the Crystal Anaerobe system. A commercial test kit for *C. difficile* toxin now is available from Oxoid.

Ureaplasma sp.

No one in attendance was familiar with anaerobic isolation of *U. urealyticum*, which is being recovered from dogs at Texas A&M. The Manual of Clinical Microbiology states that it grows aerobically, anaerobically and in candle jars equally well. Some mycoplasmas exhibit variable growth in these atmospheres. *U. urealyticum* has optimum growth at pH 6.0. After three to four days of incubation in broth medium, the organism loses viability due to pH increase.

The significance of *U. urealyticum* in dogs, cattle, and chickens with chronic respiratory infections was questioned. Austin commented that in approximately one thousand dogs screened for mycoplasmas and ureaplasmas, he recovered *Ureaplasma* sp. only once, that from a dog with chronic prostatitis. Carrier infections by mycoplasmas in the genital tract is common: *U. urealyticum* is cultured from the vagina of 60% of normal women. The organism appears to be an effective opportunist and has been recovered from the bloodstream in pure culture from women with mild postpartum fever. Ureaplasmas appear to be a cause of nonspecific urethritis in males.

The discussion of this topic ended with an attempt to recite the formula of Modified Hayflick's medium. [Editors note: The reference to this formulation is listed below. The ATCC bacteria catalog has a formulation for Urea broth 10B for *U. urealyticum* (medium #1331) and differential agar medium A8 for ureaplasma (medium # 1332), which are very similar, if not identical, to Hayflick's. Penicillin is the principal selective agent used in mycoplasmal media. Thallium acetate, which also is often used in mycoplasma culture media, inhibits gram negative organisms but should not be used if *U. urealyticum* is suspected. Quality-control of the culture media, a necessity, is accomplished by using known positive specimens. The critical ingredient in the media is the lot of peptone, not the serum or fresh yeast extract. Specimens collected on swabs should be placed in two ml vials of transport medium, comprised of Trypticase soy broth (tested), 0.5% bovine albumin and penicillin (200 IU/ml to suppress bacterial overgrowth). If cultures cannot be inoculated upon arrival, the transport medium should be frozen at -70°C immediately. Mycoplasmas survive freezing and thawing very well provided that the medium contains protein.] Please see these references:

- L. Hayflick. Tissue cultures and mycoplasmas. Tex Rep Biol Med 1965; 23(suppl 1): 285-303.
- M.C. Shepard, C.D. Lunceford. Serological typing of *U. urealyticum* isolates from urethritis patients by an agar growth inhibition method. J Clin Microbiol. 1978; 8: 566-574.

NCCLS Update

The NCCLS document on antibiotic sensitivity testing is currently under revision, and should be complete and available sometime early in 1997. There are several levels of review. New media for the testing of *Haemophilus somnus* and *A. pleuropneumoniae* will be recommended. The revision also is expected to contain species-specific guidelines on antibiotics for veterinary use. The cost of obtaining this document was of concern; it was suggested that the AVM consider arranging a bulk purchase of the text through Jeffery Watts of UpJohn, Kalamazoo, Michigan, for approximately $15 each.

Susceptibility Testing of Anaerobes

Requests for anaerobe sensitivity testing often result from clinical treatment failure. Most participants were not performing anaerobe susceptibility testing. Some participants were including an antibiotic guideline card with their reports. Some used

a microdilution assay, done in an anaerobic chamber, either in tubes with paper disks or in 96 well plates. The E-test, which uses a gradient strip, is accepted by NCCLS but the cost is about $1.95 per antibiotic. Most participants relied on the anaerobic susceptibility sections in the following reference texts:

- G.R. Carter and J.R. Cole, *Diagnostic Procedures in Veterinary Bacteriology and Mycology*, 5th ed., Academic Press, New York, 1990.
- Barron E.J, Peterson L.R, and Finegold, S.M., eds., *Bailey & Scott's Diagnostic Microbiology*, 5th ed., Mosby Books, St. Louis, 1994.

E. coli 0157:H7 as a cause of animal food poisoning

A confirmed case of food poisoning was reported in cheetahs from Jackson, Mississippi, due to *E. coli*, which apparently causes transient diarrhea in cheetahs, then vanishes. It was isolated from the commercial meat fed to the cats. *Salmonella* also is found occasionally to contaminate commercial carnivore diets. Incidences of both have generated public health concerns. The significance of *E. coli* 0157:H7 occurrence in dogs is unknown. Downer and slaughter cows screened for 0157:H7 did not result in any significant findings. However, when calves are held off feed, the intestinal microflora apparently changes and the ability to isolate the agent increases. The cheetahs are fasted one day a week. *E. coli* 0157:H7 is sorbitol negative, which is a characteristic used to screen and separate them from other *E. coli*. The new, commercially available Rainbow agar® was reported to be difficult to read due to the variety of colors it produces. Single factor H7 antisera is available from Oxoid.

Kodak SureCell *Chlamydia* Test Kit is No Longer Available: Alternatives

The *Chlamydia* Sure Cell® test kit by Kodak is no longer available. Other kits in current use include [Clearview Chlamydia from] Wampole Laboratories (a division of Carter-Wallace, Cranbury, New Jersey) and Infection Lab. Uric acid in bird feces was mentioned to cause antibody or enzyme failure in the kits. Some participants commented that the uric acid portion of the feces could be removed to reduce failure. Alternative diagnostic measures include culturing for *Chlamydia* with the use of cell lines (as McCoy cells) or embryonated eggs prior to application of other kits. Cultured *Chlamydia* also may be stained by the Macchiavello method and viewed directly. It was stressed that all procedures should be performed in a biohazard containment cabinet due to the zoonotic potential of this agent.

[Editors Note: Elementary bodies of *Chlamydia* may be microscopically viewed in smears of clinical scrapings, organ impressions, yolk sac impressions, or cell line preparations with use of either the Macchiavello or Gimenez stains. Because the small size of elementary bodies approaches the practical working resolution of light microscopy, successful use of these stains in diagnostics requires an optimally aligned and configured microscope, consistent staining technique, and experienced evaluation. Examination of Gimenez-stained impressions of the liver and spleen of exotic birds gives a quick presumptive diagnosis of psittacosis, verifiable by culture, provided that the specimens were adequately handled to preserve infectivity. *Chlamydia* is discerned in cell line culture with either immunofluorescent or Giemsa staining; both methods highlight the *Chlamydia* inclusion body or cytoplasmic microcolony. The FA stain, performed with conjugate available from NVSL, lends antigenic group-specificity to the technique. McCoy, Vero, L-cells and possibly other cell lines may be used in culture attempts, provided that the line is maintained in media with only aminoglycoside antibiotics (i.e., streptomycin, kanamycin, gentamycin, etc.) or without antibiotics. The nutritive serum supplement in the media should be free of chlamydial antibody. The sensitivity of chlamydial culture in cell lines is enhanced with centrifugation of the specimen preparations onto the cell

monolayer, by adding cycloheximide (2 ug/ml) to the post-inoculation medium, and with at least one blind subculture.

There are other commercially available Chlamydia antigen capture immunoassay diagnostic kits, such as the Chlamydia OIA (Optical ImmunoAssay) test from BioStar of Boulder, CO, Orion Diagnostica's Chlamyset Antigen FA, OneStep Chlamydia Screen from Atlantic International Medical, Abbott Labs TestPack ELISA for *C. trachomatis* antigen and Chlamydiazyme Bead ELISA (and also the LCZ, a urine test for *C. trachomatis* based on ligase chain reaction gene probe technology). Wampole's Clearview® Chlamydia kit detects the group-specific lipopolysaccharide (LPS) of the *Chlamydiae*, but requires a specimen extraction at 80°C for 10 minutes. In contrast, the Kodak pre-treatment involves placing a swab specimen into an extraction tube containing crystalline 2-amino-2(hydroxymethyl)-1,3 propandiol 1,4 dithiothreitol (a reducing sulfhydryl compound); at minute intervals, small volumes of buffered proteolytic enzyme solution, 2-aminoethanol antigen extraction reagent, and hydrogen peroxide are added. For a kit to be useful in both human and veterinary chlamydial diagnostics, it probably should target the LPS group antigen, common to all *Chlamydiae*. Cypress Diagnostics of Leuven, Belgium offers an enzyme immunoassay (EIA) test for detection of *Chl. psittaci* specific IgG antibodies in ovine sera. No information could be found on the test kit from Infection Lab. For more information on laboratory diagnostic techniques for *Chlamydia*, please contact Rob Poston at the Louisiana Veterinary Medical Diagnostic Laboratory in Baton Rouge, 504/346-3193.]

*Bartonella (*formerly Rochalimaea*) henselae* and Cat Scratch Disease

Following the discovery of *B. henselae* as the etiologic agent of Cat Scratch Disease (CSD), the domestic cat has been proposed as a vector or reservoir of the Bartonella species, potentially infecting humans either directly through scratches, bites, or licks, or indirectly via an arthropod vector. Repeated isolation of *B. henselae* from the blood of naturally infected cats demonstrates that they remain highly bacteremic for several months, implicating cats as a major reservoir. Most cats from which *B. henselae* had been isolated appeared healthy, although infection was associated with illness in some animals. Fleas may soon be proven to be vectors; *B. quintana* infections are communicable by means of *Pediculus humanus*, the human body louse.

In attempts to culture *B. henselae,* an EDTA blood sample is plated on blood or chocolate agar and incubated for 5-28 days. Isolation rates increase if the blood is lysed before plating. The organism is not recognized by any of the available commercial identification systems; the Vitek system identifies it as *Kingella* spp. It is a small, metabolically inert gram negative rod with a coryne/brittle arrangement, forming small, thin translucent colonies. Primary isolation from the blood of infected human patients may require up to 45 days of incubation before colonies appear. A variety of published literature on the subject is available (please see below).

Following the isolation and characterization of *B. henselae*, it was realized that the routine blood culture methods in use at the time would not allow the detection of the slower growing *Bartonella* species. New procedures were therefore recommended, including the use of enriched media, prolonged incubation times, cell lysis centrifugation, and cell culture systems. The two most widely used methods are direct plating onto solid media and cocultivation in cell culture, even though separate labs using similar methods describe varying levels of success. Discordant observations have been made regarding the mean incubation time necessary to visualize the growth of bacteria and the difficulty of performing subcultures. On blood-enriched agar, *Bartonella* species are best cultivated in a humid (over 80%), CO_2 rich

(5%) atmosphere. Horse and rabbit blood are reported to be more effective supplements than sheep blood. Optimum growth results from media containing hemin at concentrations of 50 to 250 ug/ml; at 100 ug/ml, the organism becomes reactive in a number of biochemical tests in which *Bartonella* species previously had been considered inert. Freshly prepared blood-supplemented agar plates improves isolation. A liquid medium consisting of Brucella broth supplemented with 6-8% Fildes enrichment and 250 ug/ml of hemin allows the cultivation of *Bartonella* spp. The use of lysis centrifugation has been shown to increase the recovery of *Bartonella* spp. from the blood. Bovine endothelial cell lines and other endothelial cell culture systems have been shown to yield isolates when direct plating of clinical specimens onto a number of solid axenic media has not.

An adapted isolate of *B. henselae* may be inoculated onto Vero cell line monolayers, maintained without antibiotics, for the production of antigen substrate for IFA serology testing. Antigen slides are made by pelleting and resuspending infected cells in PBS with 0.5% bovine serum albumin; antigen suspension then is spotted onto slides in 50 ul volumes. Slides are dried, fixed in acetone, and placed in frozen storage. Serological IFA testing of cats for antibodies to *B. henselae* follows standard procedures, except that preincubation of the test serum with uninfected Vero cells (10^6 cells per ml for 30 minutes at 37^0C) removes much of the nonspecific staining. Infected Vero cells contain clusters of small coccobacilli in the cytoplasm and around the periphery of the cell membrane. Some extracellular coccobacilli may be seen. Cat sera may be initially screened at a 1:64 dilution; positive reactors have titers greater than or equal to 256.

[Editors Note: In the generation of the notes on this topic, Alma Roy of the Louisiana Veterinary Medical Diagnostic Laboratory, Baton Rouge, contributed information from her research endeavors.] For more information, please see:

- M. Maurin and D Raoult. *Bartonella (Rochalimaea) quintana* Infections. Clin Microbiol Rev. 1996; 9(3): 273-292.
- Welch D. F., et al. Bacteremia due to *Rochalimaea henselae* in a child: practical identification of isolates in the clinical laboratory. J Clin Microbiol 1993; 31: 2381-2386.
- Welch D. F., et al. *Rochalimaea henselae* sp nov. As a cause of septicemia, bacillary angiomatosis, and parenchymal bacillary peliosis. J Clin Microbiol 1992; 30: 275-280.
- Koehler JE, Glaser CA, Tappero, JW. *Rochalimaea henselae* infections: a new zoonosis with the domestic cat as reservoir. JAVMA l994; 271:531-535.
- Koehler JE, et al. Isolation of *Rochalimaea* sp. from cutaneous and osseous lesions of bacillary angiomatosis. N Engl J Med 1992; 327: 1625-1631.

Laboratory Policies Concerning Primates

For several years, participants have expressed concern over primate specimens. Most labs are not equipped to handle zoonotic pathogens such as the hepatitis viruses, tuberculosis, simian herpes and others. There is little expertise or educational background among lab personnel or veterinarians at this forum regarding primate diagnostics or diseases. Many university-based veterinary teaching hospitals and clinics no longer accept primate patients. Because of lower costs, many human hospitals have the practice of sending research primate specimens to veterinary diagnostic labs. Whether work at an institution is with primates or just their specimens, it is governed by human regulations, according to the Federal Center for Disease Control (CDC). [Editor's Note: Since the summer meeting, the Mississippi State University administration has accepted a proposal, drafted by Dr. Austin, from its College of Veterinary Medicine concerning the handling of primate specimens. For

members wanting details on this policy to use as a guide in forming their own, please contact Frank Austin at MSU, Starkville, 601/325-3432.] The regional primate centers perform all procedures under Biosafety Level 3 conditions. For facilities lacking either the expertise, certification, or appropriate biocontainment, it is strongly recommended that primate specimens are not accepted. Please refer to the following resources for information on primate laboratory diagnostics:

- Yerkes Primate Research Center, Emory University, Atlanta, GA 30322 (U.S Postal Service)
- Yerkes Primate Research Center, 954 Gatewood Rd, Atlanta, GA 30329 (Fed Ex packages). Contact Dr. Harold McClure at 404/727-7724
- Tulane Primate Center, 18703 Three Rivers Rd, Covington, LA 70433, Contact Dr. Gary Baskin at 504/892-2040 (formalin-fixed tissues only, no dissections).
- Virus Reference Laboratory, Inc., 7540 Louis Pasteur, San Antonio, TX 78229, (performing non-human primate virology and viral serology). Contact Drs. S.S. Kalter or Richard L. Heberling at 210/614-7350, fax 210/614-7355.

Identification of Gram Positive Rods

Three new identification systems for gram positive rods will be available over the next few months from IDS, Crystal, and Sensititre.

Reptile Salmonella

Recent popular news media attention on this topic has resulted in increased public concern and specimen submission. Experiences at the laboratory of MSU-CVM suggest that the 90% incidence of Salmonella in iguanas, as reported by talk-show media, is greatly exaggerated. Reptiles (greater than 6,300 species) are quite susceptible to salmonellosis, but not of the high incidence as has been reported for iguanas.

A review of the literature reveals several important facts. Asymptomatic carrier infection and intermittent fecal shedding was published to be as high as 90% in communally housed reptiles at the National Zoological Park in Washington, D.C., and among small green pet turtles, the trade of which was curtailed in the U.S. during the 1970s due to the *Salmonella* concern. Greater than a million iguanas are sold in the U.S. pet trade each year and greater than 90% die before adulthood due to the stress of inadequate husbandry. Only four human deaths and approximately twenty cases have been associated with iguanas since 1990; each patient was associated with a high risk group, implicating predisposing factors. A wide variety of *Salmonella* serotypes have been isolated from asymptomatic reptiles, including many that are rarely isolated from other animals. The CDC and other researchers have termed these unusual isolates "reptile-associated" serotypes. Austin mentioned that in his experience common mammalian serotypes are easily isolated with standard techniques; in reptiles, they cause a fulminating and ultimately fatal disease. Cambre (reference listed below) reported better isolation from asymptomatic reptiles using GN broth rather than selenite or tetrathionate broth. Serial cultures may be necessary to recover *Salmonella* from subclinically infected reptiles. Many specimens submitted to labs are of environmental origin and are probably best cultured using tetrathionate broth. The CDC has published recommendations for preventing the transmission of *Salmonella* from reptiles to humans; on this and other information, please see:

- CDC. Iguana-associated salmonellosis-Indiana. 1990, MMWR 1992;41:38-9.
- CDC. Lizard-associated salmonellosis-Utah. MMWR 1992;41:610-1.
- CDC. Reptile-associated salmonellosis-Selected States, 1994-1995. MMWR 1995;44(17): 347-350.
- Chiodini RJ, Sundberg JP. Salmonellosis in reptiles: a review. AVM J Epidemiol 1981;113:494-9.

- Cambre RC, Green DE, Smith EE, et al. Salmonellosis and arizonosis in the reptile collection at the National Zoological Park. J AVM Vet Med Assoc 1980:9:800-3.

Gram Negative Bacteria as Normal Flora in Pet Birds

Some participants found difficulty assessing gram stains from cloacal swabs and fecal specimens; such specimens contain many artifacts. A group consensus was that all gram negative organisms should be considered abnormal flora in pet birds. Typically, more than 5-10% of flora cultured from sick birds is gram negative bacteria. Several participants have frequently encountered large mucoid colonies that may be a *Bacillus* spp. or a *Stomatococcus* spp. In addition, a variety of metabolically inert gram negative rods, both Oxidase positive and Oxidase negative, are being cultured.

How Nomenclature is Decided and How to Keep Up with It

Binomial nomenclature is the scientific method of naming the genus and species of the various life forms. This method of systematically naming life forms was first applied in 1753 by Linnaeus. Numerous rules have evolved since that time and are known as the International Rules of Nomenclature. Separate rules exist for botany, zoology and microbiology due to the inherent differences in the various kingdoms of life. Taxonomy is the study of the classification of life according to their similarities and differences. Phylogeny is the study of the evolutionary history of an organism. A reconsideration of bacterial nomenclature began about 25 years ago with the development of polyphasic taxonomy, the aim of which is the integration of phenotypic, genotypic, and phylogenetic information to gain a consensus on classification; nomenclature, taxonomy, and phylogeny must be consistent with one another. In the last decade, developments in nucleic acid sequencing have contributed to the reorganization of bacterial phylogeny, taxonomy, and nomenclature, which typically are reported in the International Journal of Systematic Bacteriology. There may never be a definitive classification of bacteria because, in taxonometric bacteriology as in all experimental sciences, scientific progress is linked to and made possible through technological progress. Please refer to the following references:

- P. Vandamme, B. Pot, M. Gillis, et al. Polyphasic taxonomy, a consensus approach to bacterial systematics. 1996. Microbiol Rev 60(2): 407-438.
- M. Goodfellow and A. G. O'Donnell. 1993. Handbook of new bacterial systematics. Academic Press Ltd., London.
- N. A. Logan. Bacterial systematics. 1994. Blackwell Scientific Publications, London.
- F. Priest and B. Austin. Modern bacterial taxonomy. 1993. Chapman & Hall, Ltd., London.
- K. J. Towner and A. Cockayne. Molecular methods for microbial identification and typing. 1993. Chapman & Hall, Ltd., London.

Staphylococcus Coagulase Testing

This discussion focused on the use of rabbit plasma verses commercial latex kits by Difco and Oxoid. Rabbit plasma in the tube and the slide test were methods used most; very few used the latex kits. It was recommended that plasma reactions be checked both at four hours and after overnight incubation. *S. intermedius* and *S. hyicus* are not consistent in tube coagulase reactions; several participants reported that many negative tube reactions had been seen with these organisms, the identities of which were confirmed with a Staph Ident strip. Some participants felt that

interference from antibiotic treatment is the cause. Coagulase is a virulence factor in disease production.

B. canis Testing

The *B. canis* serology test kit (the rapid slide agglutination or RSAT from Pitman Moore) is currently out of production; the discussion centered on alternative methods for the serological diagnosis of canine brucellosis. In addition to the RSAT, there is the tube agglutination, 2-mercaptoethanol tube agglutination, agar-gel immunodiffusion (AGID), indirect fluorescent antibody (IFA), and enzyme-linked immuno-specific assay (ELISA) tests. Some participants made their own antigen for the tube agglutination and AGID tests, while others used the commercially available IFA slides (from VMRD, Pullman, WA) and referred AGID testing to the lab at Cornell Univ. The group consensus was that all positive test reactions require confirmation. Reagents to perform the ELISA test should be available soon from NVSL; for information on it, please call NVSL at 515-239-8315.

Cryopreservation of Bacterial Isolates

At the request of several participants, Dr. Austin was asked to publish his method of cryopreserving clinical isolates.

Following isolation and identification, several similar colonies are chosen from the original isolation plate, to prevent cloning out of virulence factors; these are subcultured to a fresh blood agar plate. In this method, the isolate is preserved following the second passage *in vitro*. The freezing medium consists of sterile brain-heart infusion broth (BHIB) containing 25% glycerol (Sigma, # G-9012, Glycerol 99%). The glycerol is added to the BHIB just prior to use and lightly vortexed. In our experience, the glycerol is sterile from the manufacturer. Do *not* autoclave the BHIB containing the glycerol; autoclaving affects the glycerol in the medium and decreases viability. Subcultured growth (from one entire plate) is harvested from the agar surface using sterile cotton swabs and transferred to the freezing medium (5.0 ml) in a sterile plastic tube. This creates a heavy suspension. The growth is allowed to equilibrate for 30 minutes at room temperature. This is a critical step that allows time for the hygroscopic effect of glycerol to remove water from the cells. After equilibration, 1.0 ml of the suspension is aseptically transferred to sterile 2.0 ml cryovials (Corning, #25704, 2.0 ml) and frozen at -70^0 C. Once frozen, the vials are never thawed until use because freeze-thawing decreases viability. To subculture from the cryovial, approximately 10 ul of slush ice is aseptically removed from the top of the cryovial using a sterile short-stemmed Pasteur pipette and transferred to a fresh agar plate as an inoculum. The only organisms found not adaptable to this procedure are the *Campylobacter* spp. Anaerobes can be preserved in a similar fashion, but are grown in thioglycollate broth to which glycerol is then added. Before the original isolate is discarded, an aliquot of the frozen stock should be thawed and plated to check for viability.

PCR for Rapid Identification of *Mycoplasma spp.*

This topic was presented by Lloyd Lauerman of Charles S. Roberts Veterinary Diagnostic Laboratory at Auburn University. The key for the PCR technique is having primers of a unique known sequence, which are selected from the 16S rRNA (actually the small ribosomal subunit DNA) of the organism. Genetic sequences that could serve as possible species-selecting primers are found by comparing sequences of various *Mycoplasma* spp.; regions are sought that are unique to the *Mycoplasmas* but vary between the species. The product of PCR is termed the amplicon and the primers dictate the length of the amplicon.

Basically, the procedure involves the separation of DNA strands of the organism at 94^0C. The complimentary strands are then annealed at a low temperature, then the temperature is raised to 74^0C for two to four minutes so that a polymerase enzyme can duplicate that strand. This process is repeated through many cycles to amplify the product. The amplicon is then separated in an agarose gel and bands are visualized with UV light.

Primer selection involves computer alignment of 16S rRNA gene sequences of the various *Mycoplasma spp* to discern variable regions unique for each specie. In addition to the primer(s), other ingredients are the nucleotide cocktail and polymerases. Necessary equipment include a thermocycler, a vortex mixer, a microfuge, and electrophoresis equipment. Depending on the protocol, the PCR procedure can be completed in as little as three hours, or up to six to eight hours, with sensitivity as good as culture and excellent specificity.

Many labs represented in the discussion are using the PCR technique as a diagnostic tool. examples of its use include *Leptospira*, *M. paratuberculosis*, TB, *Salmonella* serotypes, *Bordetella*, *Chlamydia*, and *E. coli* 0157/H7. It is expected that this technique will be used more frequently for the difficult and slow growing organisms.

Because some primers are difficult to find and identify, an alternative procedure is to use PCR with restriction fragment length polymorphism (RFLP). PCR is used to amplify the entire genome of an organism. Restriction enzymes recognize and cut the amplicon at certain specific palindromic sequences. Different restriction enzymes are used to cut the PCR product into various fragment lengths, each restriction enzyme acting at a different cut site, producing a unique fragment cut pattern. The fragments are then separated into different bands and visualized in electrophoresis. Identifications are made when band patterns are compared with those of controls.

Lauerman used these techniques to determine that ostrich mycoplasmas are not related to the Mycoplasmas of common poultry species (MM, MS and MG). Six new putative species were recognized through his efforts. To date, they have not been shown to be clinically significant in poultry. The ostrich mycoplasmas were experimentally introduced to poultry, in which no disease developed. They have not been passed in ostriches.

Miscellaneous Topics

Mega Regs: What's Happening? The Food Safety and Inspection Service (FSIS) has put out a 842 page document on Hazard Analysis Critical Control Points (HACCP) regarding meat inspection. Many labs may be asked to participate or conduct regulatory testing. *Food and Chemical News* (published by CRC Press, 202/544-1980) is a good weekly publication providing in-depth information regarding regulation of food, including additives, microbiology, standards, contaminants, and feed. The subscription cost is $1,097 per year, therefore visit your library. *Food and Chemical News* has a current advertisement for a HACCP Management Manual.

Blood Cultures. Most participants did not recognize contamination as a significant problem.

Run-off Waters from Processing Plants. Among the participants, only the State Diagnostic Lab in Jackson, Mississippi has been asked to do water testing, in which coliforms are the most significant concern. MPN, plate counts, Vitek's bactometer (electrical impedance quantification of growth), and automated grid counting were all suggested as possible methods.

BBL Prompt Inoculation System: Consistency in Sensitivity Testing. No problems were reported.

Vetlim and Vitek Conversion Program. None were reported to be available yet.

Virology Session Notes

Moderated by John Black, American BioResearch, Seymour, Tennessee
Prepared by Rob Poston, Louisiana Veterinary Medical Diagnostic Laboratory, Baton Rouge, Louisiana.

Use and Interpretation of PRRS Viral Antibody ELISA

This topic was presented by Gene Erickson, Rollins Animal Disease Diagnostic Laboratory, Raleigh, North Carolina. Notes included here are in supplement to the distributed handout.

IDEXX ELISA technology has been adapted for use in a routine swine herd health and productivity application. The data format is similar to that used for poultry, that is, antibody levels (as defined by S/P ratios) of individuals within a herd are categorized into groups. A computer program analyzes data from antibody assays, totals the number of individuals for each reactor group, and generates a herd profile.
PRRS ELISA reactors were sorted into six groups by S/P ratios; estimated endpoint titer ranges were given for each grouping:
Group 0 <0.4 Group 3 1.51-2.00
Group 1 0.40-1.0 Group 4 2.01-2.50
Group 2 1.01-1.50 Group 5 >2.5

Qualitatively, ELISA and IFA agree on the appearance of antibody. Quantitative correlation between the techniques is not perfect, and is somewhat dependent on viral strain selection. The ELISA technique displayed a 99.7% specificity, in comparison with immunoperoxidase.

Profile examples were presented: Nursery pigs (age three to nine weeks) from a vaccinated herd displayed little antibody (all were group 0 or 1). Nursery pigs with active virus circulation grouped in 2, 3, 4, or 5, higher in older pigs. A profile of naive feeder pigs receiving a single dose of vaccine displayed progressively higher groupings as time proceeded through 28 days post-vaccination. A profile of four aggressively vaccinated sow herds showed groupings predominantly of 0 , 1, or 2. In a vaccinated herd with an outbreak of reproductive disease, individuals profiled in the 2-5 range. A variety of groupings were discerned in a vaccinated sow herd with improper gilt backgrounding. A profile of vaccinated boar studs receiving four vaccinations per year develop low antibody groupings (0-1).

In addition to recognizing, profiling, and monitoring troubled herds, PRRS viral antibody ELISA testing would have obvious use in the screening of incoming and replacement stock. The typical breakdown of PRRS virus control occurs when an infected gilt is introduced into the herd; virus is shed, quickly infects other susceptible pigs, and overwhelms herd immunity. Although young pigs recover, their development is stunted and lacks uniformity. The respiratory syndrome resulting from PRRS virus infection imparts a high economic cost.

Following natural infection with PRRS virus, pigs develop antibodies to the virus within seven to 14 days, but continue to shed the virus seven to eight weeks after infection. Shedding ceases when the pig responds to the virus with cell-mediated immunity, which develops in that same time frame (seven to eight weeks after infection). Naturally infected animals will revert to a negative antibody status within four to six months but remain resistant to reinfection by the homologous strain of PRRS virus for at least a year. A persistent carrier state is known for other arteriviruses, i.e., equine arteritis virus, and it is very likely that this will be shown for PRRS virus as well.

Two modified live virus vaccines are available for PRRS. One, RespPRRS, has been available for over a year and has used extensively in growing pigs and adult swine. The other product, from Schering, has just been licensed by the USDA for sale. A typical sow and boar vaccination regimen has been the administration of four doses per year. A breeding cycle vaccination strategy is another popular regimen in sows; vaccination is given during lactation and at a second time during the middle trimester of pregnancy. Half or more of the sows and boars in herds stabilized under this program will be seronegative by ELISA. The seronegative immune state is most likely due to hyperimmunization of the cell mediated immune response, which clears the vaccine virus from a vaccinated animal shortly after administration; the animal does not then need to mount an anamnestic humoral response. Antibody levels for positive animals in such herds are usually low, usually less than 1.00, but sometimes up to 1.50. When this antibody profile is obtained for a properly vaccinated sow herd under a four dose per year strategy, it indicates excellent immunity. Such a profile in a well managed herd is no surprise; it is reassuring news for the conscientious producer and herd veterinarian.

After revaccination, antibody response seems to be attenuated among vaccinated adults in a stable breeding herd; possibly greater than 20% of animals in a well maintained, vaccinated herd may be seronegative. An ideal herd average S/P ratio target would be 1.00. Although most vaccinees eventually revert to become seronegative, they probably remain protected by non-humoral immunity.
Regular monitoring is needed to be certain that a new strain of PRRS virus has not entered the herd. When that happens, typically 90% or more of the sows will become positive, with antibody levels ranging up to 2.50. This unique property occurs with the introduction of heterologous field virus, which rapidly spreads through the vaccinated herd. There is a wide antigenic spectrum of PRRS viruses in nature, with those least similar to the vaccine strain the most likely to stimulate the strongest humoral response. PRRS ELISA cannot easily differentiate between infection and vaccination.

Nonvaccinated pigs and sows or boars with S/P ratios >2.25 may be viremic at the moment of sampling and probably have been infected recently. Sows or boars with ratios of 3.5 to >5.0 are believed to be recently infected. Immature swine in this S/P range will likely have a protracted viremia, unique to growing pigs. Adults in stabilized breeding herds (with negative nurseries) generally exhibit S/P ratios <2.5. Vaccinated naive feeder pigs can develop ratios above 3.5 due to vaccination itself.

Piglets (three to four weeks old) typically show little maternal antibody (S/P ratios from 0.7 to negative) when there is little PRRS virus in circulation among breeding adults in the herd; most piglets will be negative by six weeks of age. Levels >1.8 are probably from viremic pigs that were weaned into the nursery. As viremia spreads within a nursery, S/P ratios will increase; many pigs at seven weeks of age will display ratios >1.0. In an infected nursery, most pigs nine to ten weeks old will be positive for antibody, and are likely to be shedding virus.

When transferred to the finishing floor with other infected pigs, previously uninfected pigs will become infected and seroconvert. In the finishing floor environment, the mean S/P ratio rarely rises above 1.8, presumably because of building design and the continuous turn-over of animals.

In further discussion on PRRS virus, substrate slides for PRRS IFA serology were said to be best made from an adapted strain of PRRS virus in MARC-145 cells (a clone of Ma104 cells). A relatively crowded monolayer of cells is infected and allowed to develop CPE. Infected cells are removed with a swab and resuspended. Spot slides are seeded with this suspension. Optimum PRRS virus antigen presentation depends on the use of the proper MOI, which must be discerned by titration.

PRRS virus is related to other Arteriviruses (family *Togaviridae*), Equine Viral Arteritis (EVA) virus of horses and Lactic Dehydrogenase-Elevating Virus (LDH) virus of mice. Little practical comparative data is available on these viruses. A killed vaccine is in use for EVA in horses. Sonication of infected EVA cultures increases the number of infectious units.

PCR Testing

Lloyd Lauerman of the Charles S. Roberts Veterinary Diagnostic Laboratory at Auburn Univ. presented a polymerase chain reaction (PCR) test format for IBV diagnosis and biotyping. This method uses reverse transcriptase (RT), transforming viral RNA into DNA for analysis. All primers and restriction endonucleases for this technique are commercially available.

Tracheal swabs from infected chickens were processed and inoculated into chicken embryos by the allantoic sac route. Each case was subcultured through chicken embryos three times. Allantoic fluid was harvested from the last passage and used in PCR analysis. Nucleic acid was extracted from the harvested allantoic fluid with phenol/chloroform/isoamyl alcohol. The extracts were concentrated with column chromatography. cDNA was made with RT supplied in a Life Technologies superscript kit. A general PCR was performed to amplify the amount of cDNA. Amplicons were cut by three different endonucleases, and resolved by electrophoresis. Virus identification and strain differentiations were made from the pattern of gene segments appearing on the stained electrophoretic gel. Technical refinements may permit the resolution between Massachusetts and Connecticut strain isolates, the distinction of which is of paramount concern in defining vaccination regimens. Gene extraction directly from clinical material for this PCR protocol was not successful.

This same approach was successfully employed in detection of Newcastle's Disease Virus (NDV) and Chicken Anemia Virus (CAV), using egg-passaged isolates and commercially available primers. Although technical details may vary with the type of virus, the gene amplification and detection portion of this method is performed within 24 hours.

Other miscellaneous points on the PCR techniques in diagnostics were mentioned. A multiplex PCR method to detect swine viruses from clinical materials was mentioned being under development.

Bob Robison of the Maryland Department of Agriculture Animal Health Lab in Salisburg, Maryland mentioned using a PCR method to detect EEE virus from mosquito pools in surveillance efforts. The protocol included a double primer and labeled probes, with labeled hybrids detected by dot-blot.

The NVSL routinely uses a PCR technique to detect BTV in leukocytes. This approach has an advantage over culture in that it is not influenced by circulating antibody. BTV PCR can detect positive reactions months after the last positive culture from an infected animal. NVSL also has experimental protocols for the detection of BVD I & II, and MCF viruses by PCR.

Although there are currently no commercial test kits for veterinary diagnostic application based on PCR methodology, most reagents used in PCR protocols are commercially available. Symbiotics is considering to provide primers to be used in PCR diagnostic techniques. Targeted infectious agents are FIP virus, FeLV, *Bartonella henselae* (Cat-Scratch Fever agent), *Mycobacterium tuberculosis*, *avium*, and *bovis*, *Ehrlichia canis*, Rocky Mountain Spotted Fever (*Rickettsia rickettsi*), Rabies virus, *Yersinia*, *Chlamydia psittaci*, and *Borrelia*. If interest develops, additional reagents may be made available. Howard Jones of Symbiotics asked for samples from primate *Mycobacterium tuberculosis* reactors. Symbiotics is developing a PCR technique that distinguishes between true TB reactors and those exposed to *M. avium* or *bovis*. Those interested in donating specimens are invited to contact Dr. Jones at 800/247-1725.

Preliminary trials with a PCR technique to detect Johne's Disease bacillus (*Mycobacterium paratuberculosis*) reveals that PCR is not necessarily more sensitive than culture, but it is much quicker.
The existence of a genetic sequence unique to all pathogenic FIP virus strains was mentioned; attempts are underway to characterize it for use in a PCR format.

New Tests and Changes in Current Products

At this point, attending representatives of commercial interests were asked to mention recent or projected additions or improvements to their product lines:

Symbiotics: EIA viral antibody AGID test kit, based on a recombinant p26 antigen (Removal of tissue culture residue from the antigen formulation is expected to yield fewer non-specific reactions.) Parvovirus antigen ELISA test kit for fecal specimens; Feline Leukemia Virus antigen colloidal gold test kit for STAT testing; Canine Dirofilaria antigen ELISA test kit that removes the transfer step from specimen pretreatment; Canine brucellosis antibody RSAT using a new in-house antigen formulation. (At a future time when the kit is ready for trials, field samples will be solicited from interested labs.)

VMRD: EIA viral antibody AGID test kit, based on a recombinant p26 antigen; E. coli K-99 test kit; *Ehrlichia* and *Rickettsia* slides and conjugates.

Centaur: Endotoxemia latex agglutination test kit for rapid use in horses; Semen potency test kit (Fertile Check) for bulls, stallions, boars, and rams, based on a dye reduction indicator.

KPL: Avian influenza viral antibody ELISA test kit; Chick anemia virus testing service.

Also mentioned were the *Giardia* and *Cryptosporidium* kits produced by Alexxon of Sunnyvale, California, and *Ehrlichia* and *Rickettsia* slides made by Lee Fuller of California.

Bluetongue Antibody ELISA Test Kits

Currently, DiagXotics of Wilton, Connecticut (The BluePlate Special™) and Veterinary Diagnostic Technology of Wheat Ridge, Colorado produce microwell BTV

antibody ELISA test kits. The ELISA format offered by VMRD was mentioned to be currently too sensitive and lacks specificity in that it also detects antibody to EHD virus. The BTV FA conjugate from VMRD also has this property, but that could be considered as an advantage in a screening test. In general, hyperimmune serum is capable of cross-reacting between BTV and EHD virus, but the heterologous type will be far less potent and dilute out before the homologous reagent end-points. Other accepted technologies to detect BTV antibodies are the Complement fixation (CF) test, the AGID test, and Neutralization test, the latter of which is used for serotyping isolates.

Confirmatory Heartworm Testing

No one in attendance reported using alternative laboratory technology, i.e., western blot, IFA, or PCR, to confirm heartworm antigen test kit reactors. Heartworm infestation is usually confirmed by gross necropsy examination. It was noted that *Dirofilaria immitis* typically does not fully develop in cats, yet veterinary diagnostic laboratories are receiving an increasing number of requests for heartworm testing in cats. [Editors Note: Feline sera can be assayed for both antibodies and antigen of *Dirofilaria immitis* by Animal Diagnostics of St. Louis, Missouri, 314/647-3348.]

On the broad topic of diagnosis of parasitological infections, it was noted that many commercial diagnostic serology reagents give non-specific reactions, similar to that seen with rheumatoid factor. Wampole Laboratories and Alexxon offer test kits to detect Giardia and Cryptosporidium antigen.
Most antigen preparations in fungal AGID test kits are derived from yeast phase antigen; those using mycelial phase antigen are prone to give multiple line reactions, as seen with Aspergillus testing.

Procedure Manuals

The question of whether the association should be involved in publishing procedure manuals has been deferred in the past. The unvoiced consensus seemed to be there was a need to offer members some written test guides. One suggestion was that the newsletter could include one to two procedures with each issue, in lieu of a formal procedure handbook published by the AVM. Most laboratories following good laboratory practices (GLP) probably already have a procedure book. The AVM can suggest the latest technique improvements in its published procedures, which members could later choose to incorporate into their existing laboratory procedure books. Laboratories that excel in a specific testing field could share their procedures with other members by publication in the newsletter.

Quality Control Manuals

Here again is the question of whether the AVM desires to become active in publishing quality control (QC) manuals or participate in the development of QC efforts. Currently, private and professional agencies are developing guides for Good Laboratory Practices (GLP) certification. The Veterinary Laboratory Associates (VLA) offers check test samples and panel evaluations for certain testing services to participating laboratories. The South Central Branch of the AAVLD is currently developing a QC testing service for serology and virology. Those present who have participated in this trial reported inconclusive findings; different laboratories have been using different technologies for given assays, yielding results that are difficult to compare.

For those regulatory testing services, it is felt that if a laboratory has passed an annual federal check test to become a certified test site, it should be assumed that the lab is performing adequately. For such testing services, this understanding

obviates the need for documentation and expense involved with closer scrutiny and constricted regimentation of laboratory operations.

An example is seen in VSV neutralization testing; either MDBK or Vero cell lines may be used, but the Vero line allows the method to be slightly more sensitive. However, proper use of either will enable a lab to pass the check test for VSV neutralization testing, so specifying a certain cell type is unnecessary. The laboratory may use whichever cell is convenient and should not be forced to bear the expense of maintaining another cell line for which it has no other need. QC measures should strive to avoid redundancy in order to minimize laboratory operational overhead.

Most labs have written protocols for each major test procedure, which usually corresponds to a test method code or description that appears on the result report.

Testing for FIP Virus Types I and II, TGE Virus in Pigs, and Other Coronaviruses

Most labs typically screen for FIP with the ELISA test kit, then use IFA to verify. Certain veterinarians and breeders, however, will specify IFA testing for their patients. ELISA will detect antibody to both types of FIP virus. It is not thought necessary to verify FIP ELISA positive reactions against both serotypes, unless high testing volume and client specification dictate otherwise. Substrate slides for IFA serological testing for both types of FIP virus are commercially available from VMRD. When antibody appears to FIP type II, the cat is generally exhibiting illness. A small portion of FIP-infected cats, which are positive on the ELISA screening test, will be negative on high dilution IFA confirmation. The IFA technique should not be the sole criteria of laboratory diagnosis because of possible false negative reactions. Such traits are classic characteristics of an immune-mediated coronavirus infection and disease.

Diagnosis of TGE virus (another coronavirus) in pigs is often done virologically, by either direct FA of intestinal sections or smears of enterocyte scrapings, or by electron microscopic examination of stool or intestinal contents. Because of differences in antigen content and viral particle concentrations at the time of sampling, it may be possible to receive a positive reaction with one technique (FA or EM) but not the other in any given case; this is not to be construed as discordant reactions, because different entities are sought with each technique: The FA detects cell-bound viral antigen in enterocytes, while EM finds intact viral particles free in the intestinal lumen. Although infected cells quickly exfoliate, it is possible to discern infected cells on the tips and sides of intestinal villi after detectable levels of viral particles have been flushed from the intestine. It is also possible to find coronaviruses by EM in the intestine of a given case, even though FA attempts on the tissue were negative; the tissue submitted for testing may not have had active lesions, or that infection was by another coronavirus antigenically different from classic TGE virus. Porcine epidemic diarrhea (PED), yet another pig coronavirus distinct from TGE virus, is a significant disease problem in Europe, but is unknown in the U.S.

There are a plethora of coronaviruses that infect pigs, causing anything from severe diarrhea or respiratory problems to subclinical or asymptomatic infection. The differentiation of respiratory and enteric pig coronaviruses is of some diagnostic interest; it is thought that certain research groups are developing monoclonals for differentiation. Generally, pig respiratory coronaviruses, but not TGE virus, will grow on primary swine kidney cells. TGE virus will grow on the ST cell line. For laboratory diagnosis of respiratory coronavirus infection, direct FA conjugate to TGE virus can be used on tracheal cell smears, although this method cannot be expected to detect

all possible respiratory coronavirus infections. As certain bovine coronaviruses, pig coronavirus infects trachea epithelium with little or no pathology.

As cooler weather approaches, "winter dysentery" or bovine enteric coronavirus infection will be noticed with more frequency. No one in attendance noted changes during the previous season in the rate at which bovine enteric coronavirus is found in clinical specimens.

Bovine respiratory coronavirus infection probably is not sought as much as other bovine respiratory viruses. If its pathogenesis parallels respiratory coronavirus infections of other species, bovine respiratory coronaviruses may be involved in asymptomatic or subclinical infections, with occasional moderate to severe sequelae in certain individual animals. It may be under-appreciated in its economic impact if it predisposes animals to serious secondary bacterial infections, as *Pasteurella* and *Hemophilus*.

Changes in Viral Nomenclature

The pig coronaviruses highlight the difficulty and confusion of classifying viruses according to the diseases they cause. Other examples occur among the Herpesviruses: What was once considered classic IBR viral encephalitis is now thought of as BHV-5 infection. DN-599, another bovine herpesvirus, was once classified as an alpha herpesvirus, but now is considered as a gamma herpesvirus, although some properties of DN-599 are not consistent with other gamma viruses.

Among the Equine Herpesviruses, two types dominate veterinary concern, one (EHV-1) causing abortion and the other causing the viral respiratory illness known as Equine Rhinopneumonitis. The respiratory virus was once commonly referred to as ERV but now is specified as EHV-4 by some, or as a variant of EHV-1 by others. What was once considered the encephalitic manifestation of ERV infection is now thought of as neurotropic EHV-4 infection. The two viruses are essentially indistinguishable by conventional serologic techniques and require monoclonals or gene probes for differentiation. In general, the abortifacient virus is occasionally recovered with lab cell lines in diagnosis, but the respiratory virus is rarely so. EHV-2 is Equine Cytomegalovirus, a Beta herpesvirus, and causes respiratory illness. EHV-3, another Equine Alpha herpesvirus, is recognized as the causative agent of coital exanthema.

Many of the newer taxonometric associations are genetically-based. Medical and veterinary virologists have need to edify themselves on contemporary viral classification, which is becoming progressively independent of the medical or diagnostic perspective. [Editors Note: For a brief explanation of microbiological nomenclature, please see the section titled **How Nomenclature is Decided and How to Keep Up with It** in the Bacteriology session notes.]

New Morbilliviruses

An equine morbillivirus has been discovered in Australia and is believed to be dangerously zoonotic; several humans deaths were reported to have occurred 72 hours after onset of signs. Fruit bats are a suspected reservoir. A lethal epidemic among lions of central Africa was found to be due to another morbillivirus, which is closely related, if not identical, to Canine Distemper Virus (CDV). It has been known since the mid 1960's that the lion is considered within the natural host range of CDV. (Please review the reference below). A separate lethal epidemic, as of yet of undetermined etiology, has occurred among the exotic cat population of King's Dominion Amusement and Zoological Park, near Richmond, VA.

- Gorham, J.R., The epizootiology of distemper, 1966, JAVMA 149,610.

Equine Infectious Anemia Testing

In addition to the traditional double radial agar gel immunodiffusion (AGID) test method in the Ouchterlony format, there are two ELISA test kits that meet federal approval for serological testing of horses for EIA virus for regulatory purposes, the IDEXX CELISA and the Centaur SA-ELISA. Both ELISA kits are microwell tests and require minimum technical equipment, a micropipet device that measures 50 and 100 microliter volumes, a wash bottle, a sink, and biohazard disposal. Both tests can be run on serum or plasma. To expedite testing, a centrifuge to separate blood would be helpful, but not necessary if time is allowed for the blood to separate on its own. No spectrophotometer is needed to read the test reactions; essentially both tests can be sight read against a white background with the unaided eye. A spectrophotometer is useful to quantify and classify threshold reactors. Each kit contains 96 break-away microwells, so that 32 single tests may be run, one sample in each of 32 separate runs, or 94 sera can be run simultaneously in a single test run (allowing for a positive and negative control well in each run). As with most ELISA tests, both kits have critical washing steps that must be done thoroughly. Both ELISA formats are understood to be more sensitive than the standard AGID test. Both kits have lengthy shelf lives of four to eight months, but need to be kept refrigerated between uses.

The older of the two formats, CELISA, is based on the p26 viral core protein of EIA virus (as is the standard agar gel Coggins test) and is highly specific. If EIA viral antibody appears in a sample, it competes with antibody on the test well surface for enzyme-conjugated antigen. Because of the competitive step in this assay, positive reactors appears colorless and negative reactors develop a blue color. The kit uses distilled or deionized water for its washing step. Including blood separation, the CELISA requires 2.5 hours to run. This kit is manufactured by IDEXX of Westbrook, Maine. [Editor's Note: IDEXX has reformulated the CELISA test kit to shorten the test run length to 45 minutes; these kits will be available soon.]

The newer format, the SA-ELISA, is a direct antibody capture test using microwells coated with a synthetically manufactured protein, based on an invariable portion of the p45 envelope protein of EIA virus. The advantage of the synthetic antigen is that, in the eventuality of a gene-deleted EIA virus vaccine, the kit antigen can be adjusted to detect only the antibody that reacts to the deleted protein, which appears only in field strains; thus, vaccination would not interfere with testing, surveillance, and eradication programs. Even though this kit detects antibody against a different viral component than the other formats, discordant reactions between the ELISA kits and the AGID tests are rare: During virtually all natural infections of EIA virus, antibody is typically made against all viral components, and antibodies reacting against the viral envelope proteins are usually the most abundant. The SA-ELISA is the quickest of the two; including serum separation, it requires about 45 minutes to run. There is an extra incubation step in the SA-ELISA, but incubation steps are only ten minutes long and are done at ambient temperature. A positive reaction appears blue-green, which turns dark yellow after the stop reagent is added; it is still easy to read by sight. This kit has its own wash concentrate, which must be diluted to working concentration before the test is run. The kit labels indicate that SA-ELISA kit has a slightly longer shelf life than the CELISA kit. The kit is manufactured by Viral Antigens for Centaur of Overland Park, Kansas.

EIA viral antibody AGID test kits are marketed by IDEXX of Westbrook, Maine, Symbiotics of San Diego, California, and VMRD of Pullman, Washington; all utilize recombinant technology to produce the p26 EIA antigen contained in the test kits.

EIA viral antibody is also detected by Western blot and IFA techniques, both of which are not officially recognized for regulatory testing.

On another note, a question was asked regarding the federal policy and ultimate goal of EIA testing. The U.S. Government does not involve itself with control or eradication of EIA in horses; it provides resources and assists States in what ever goal each of them pursues, whether control, eradication, or both. The highest incidence of EIA virus in the U.S., about 1% of all horses, is found in Louisiana and Florida.

The AGID agar formulation was discussed. Some mentioned favorable results when agarose was used instead of Noble agar; others saw no difference. Most in attendance had not compared the two types of agar, neither have they explored potential differences between BBL and Difco Noble Agar.

[Editors Note: Two weeks following the AVM annual meeting in Mobile, one of three "Hotzone Project" sessions was held in Baton Rouge, Louisiana. This project was conducted by Chuck Issel and Sheila Cook of the Univ. of Kentucky, Dept. of Veterinary Science, and funded by the USDA. It compared and contrasted results obtained with the standard approved EIA test reagents and kits against a special set of serum samples, many of which were weak or partial reactors. This was a technique "tune-up" for the participants, several of whom were AVM members. Participants have the privilege of referring equivocal reactors and trace-back samples to the Univ. of Kentucky, where other techniques as western blot and PCR will be applied. Regarding the AGID technique, it was found that by using 17 ml of agar (instead of 15 ml as suggested in the official protocol) in a 100 x 17 mm plate, weak reactors were interpreted more easily. The CELISA format seemed to detect early antibody responses slightly better than SA-ELISA format. Presumably, one of the long range goals of this effort would be to discern the factors contributing to the low-level endemic condition and persistence of EIA virus in the 18 States contiguous with the Mississippi River valley and the Gulf of Mexico (The "Hotzone"), despite decades of testing and control.]

New Cell Cultures, Media and Serum

There are few cell lines available for avian viral techniques; most employ embryonated hen's eggs or chick fibroblast primary cells. The Quail Fibroma cell line is of limited diagnostic use. No one reported any success at transforming and immortalizing chicken cell cultures.

Most in attendance still use HEPES buffer in cell culture media of general application.

Different media formulations have been attempted to lengthen the longevity of the BT cell line, with no ideal mixture found. BT cells are suitable for the isolation of BVD virus from clinical specimens, however sensitivity will vary depending on virus and cell type.

The EBTr cell line (Embryonic Bovine Trachea, ATCC CCL 44) is not as easily handled as other cell lines and forms a monolayer unattractive in appearance, but they are reportedly susceptible to PI-3 virus in clinical viral culture attempts. According to the ATCC catalog, this cell line also is suitable for the isolation of IBR and BVD viruses. Most in attendance use MDBK cells in bovine virological techniques; they are versatile, easy to split and maintain. Few participants reported using primary cell cultures in their diagnostic virology protocols.

In the isolation of bovine viruses, horse serum is a good alternative to other cell culture growth supplements of bovine origin. Most pooled bovine-origin cell culture

nutritive products may still contain antibody to viruses, despite processing. To avoid potential antibody interference in virological methods, bovine cell cultures are maintained with the standard bovine additives, but conditioned with horse serum (10%) before they are used in viral isolation attempts. Horse serum is added, in lieu of bovine serum, in the post-inoculation medium as well.

A reliable method of maintaining BVD-free cells is the use of a serum supplement comprised of a mixture of fetal calf serum and bovine serum. Antibodies in the bovine serum will neutralize any potential BVD viral contamination in the fetal calf serum. Of course, this regimen cannot be used during bovine virus isolation attempts or in serological methods due to the presence of antibodies.

Virus neutralization serology for BVD antibody requires an antibody-free nutritive supplement, such as horse serum, Harmon Technologies SPF Donor Bovine Serum, or serum from a carefully screened lot.

There was a question whether products from Harmon Technologies of Gowrie, Iowa were still BVD-free. In the past, Harmon was represented at AVM annual meetings, but not this year, so the question could not be addressed at the time. [Editors Note: Telephone contact with Harmon Technologies on October 16, 1996 related that BVD-free Donor Bovine Serum is still available and under production, with past problems of potential contamination resolved. It can be ordered directly from Harmon Technologies at 515/879-2475.]

As gamma irradiation techniques improve, irradiated fetal calf serum is finding more acceptance as a growth and nutritive supplement in cell culture handling and propagation procedures. When properly done, irradiation inactivates all contaminating agents, including BVD virus, in serum without loss of nutritional potential or growth factor function. Irradiated serum is often supplied in bottles of amber color, which is possibly a by-product of the bulk irradiation process.

Isolation of small animal viruses was discussed. FHV and Feline Caliciviruses can be recovered from clinical material in the CrFK cell line. Isolation of CPV and CHV can be attempted with CrFK and MDCK cell lines. Caliciviruses have been found in dogs; their presence is currently of unknown significance.

An as of yet unidentified viral isolate has been recovered from the brain of a dog, and is suspected to be a Retrovirus, according to the nature of the histopathological changes seen in the brain tissue. The isolation was done in a cell line of dog bone marrow origin (DBM). Particles, approximately 60 nm in diameter, were discerned in infected cell culture extracts with electron microscopy. Cytomorphic analysis, chloroform sensitivity, and reverse transcriptase assays are pending.

The ED (Equine Dermis) cell line, as most primary cell cultures and established cell lines, prefers adult serum for propagation and maintenance. In general, when cell culture is used in defined protocols, fetal serum is desirable to support quality control and to avoid possible contamination by viruses and antibody.

Several lab representatives in attendance use embryonated eggs in diagnostic virological applications, typically for the isolation of Influenza virus, Bluetongue virus, Poxvirus, and avian viruses. EEE virus isolation attempts in ovoculture may be slightly more sensitive than in cell culture. The MDCK cell line may be difficult to trypsinize and disperse, especially when mature, but is useful in the propagation of Influenza viruses; in roller culture, with medium containing a low concentration of trypsin, MDCK cells possibly are more sensitive than egg inoculation for the isolation of Influenza virus.

Swine influenza virus (SIV, the principle agent of "Thumping Pig Disease") may be isolated with the ST cell line. Subculture of primary isolates of SIV may be facilitated by adding a low concentration trypsin to the inoculation medium. The presence of influenza virus in inoculated cells can be substantiated by hemadsorption. There is an ELISA test kit commercially available to detect antibodies to equine influenza virus (EIV).

CAE virus in goats is recoverable in goat turbinate cell culture and ovine choroid plexus cell culture, the latter of which is also suitable for the isolation of certain other ovine viruses. Serology is the current approach to assist in the diagnosis of CAE virus in goats. CAE AGID reagents are available from Veterinary Diagnostic Technology of Wheat Ridge, Colorado. CAE viral antibodies are also discerned with IFA technology, and are found, with unknown significance, in a high percentage of asymptomatic animals.

Diagnostic Virology of Exotic Species

This update was presented by Woody Fraser of the Florida Animal Disease Diagnostic Lab at Kissimmee, Florida.

A cytopathic agent has been isolated from a swab pool taken from tapirs. BVD virus has been discovered in llamas, which are also susceptible to Equine Herpesvirus type 1. Iridoviruses are common in fish, especially Large Mouth Bass, which usually are infected without disease. Iridoviruses often cause pathogenic changes in other fish species. Infectious problems may be spread in an aquaculture facility by dogs ranging between the fish tanks or ponds. Many common diseases of shrimp are often caused by viruses. Last year's much publicized Manatee die-off is now thought to be due to the red tide; a viral etiology was never substantiated. There was an unconfirmed report of isolation of Herpes Simplex virus from a hedgehog.

Circovirus infections of pigeons are common and can be discerned by electron microscopy of bursal tissue, in which inclusion bodies are found upon histopathological examination. It is not believed that the pigeon circovirus cross-reacts with that of psittacine bead and feather disease. For control of appearance and recognition by electron microscopy, circoviruses can be extracted from disrupted PK-15 cells, which frequently are found to be endogenously contaminated.

BVD Virus, Revisited

With vigilance and good virological technique, it is possible to keep cell cultures free of BVD viral contamination. [Editors Note: BVD-free technique is described above, under the heading **New Cell Cultures, Media and Serum**.]

American BioResearch (ABR) of Seymour, Tennessee now offers a direct FA conjugate that detects both types I and II of BVD virus. BVD virus on substrate slides will detect antibody to both types of BVD virus, but the homologous type will be favored. In the preparation of hyperimmune serum to BVD virus, it can be discovered that there is little anamnestic cross-response between the two types.

In the laboratory diagnosis of Border Disease of goats or sheep, BVD virus can be isolated from the same type of clinical material from sheep or goats as BVD is cultured from cattle; isolates possess similar cultural properties as non-cytopathic BVD virus from cattle.

Requests for New Tests

There was a request for a commercial source of antibody against Torovirus, to assist in the differentiation of virus from debris during electron microscopic examination. When compared to the diagnosis of Rotavirus and Coronavirus, it was noted that Torovirus seems to attach a different portion of the intestinal villi.

A request was made for diagnostic aids for Orf virus or Parapox virus. It was noted that the distinctive morphology of the Parapox viruses can be recognized with electron microscopic examination of fresh scabs or culture fluids. Direct FA against Parapox virus works well to substantiate identification.
ABR nearly has completed development on new direct FA conjugates for Feline Calicivirus and Group A Rotavirus.

Acknowledgments

The AVM Fall/Winter 1996 Newsletter was edited by Frank Austin and Rob Poston. Major section notes were contributed by Melody Parsley and Bill Palin. Proofreading was done by Theresa Love, C.C. Wu, and the Newsletter Advisory Committee. The cover graphics was prepared with PrintShop Deluxe. Appreciation is expressed to Alma Roy of the Louisiana Veterinary Medical Diagnostic Laboratory for her contribution to the *B. henselae* section, and to Kathleen Harrington of the Dept. of Epidemiology and Community Health at the Louisiana State Univ. School of Veterinary Medicine for her assistance with graphics generation and text scanning. A special note of thanks goes to all those who have made suggestions, clarified points, and contributed miscellaneous notes to make topic coverage as complete as possible.