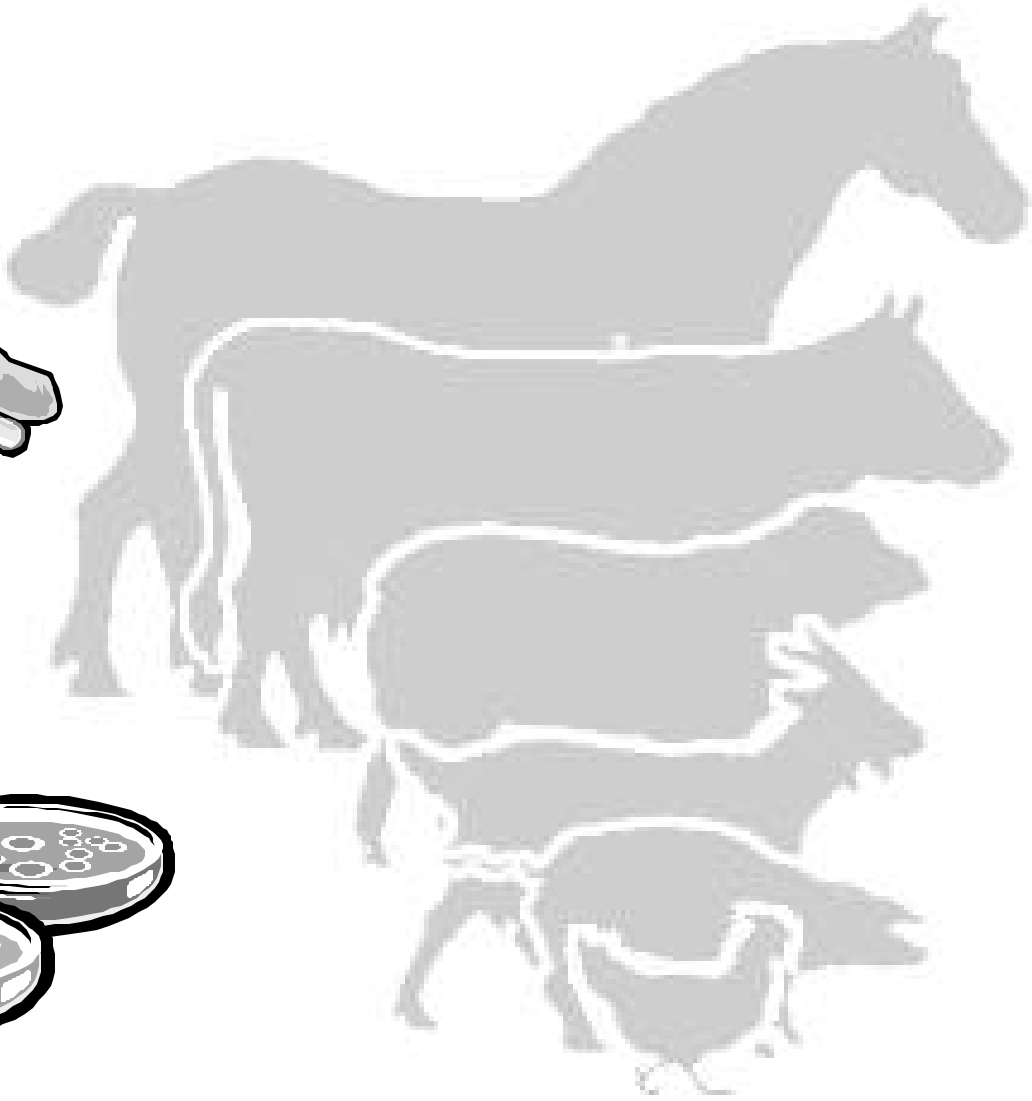
The Association of Veterinary Microbiologists

2000-2001 Newsletter



Promoting scientific investigations and their applications to the advancement of knowledge in the field of Veterinary Microbiology, and providing mutual assistance to participating laboratories in solving problems.

Message from the President

Greetings AVM Members and Associates:

First, let me say that I am both grateful and honored to have been considered for the presidency of this wonderful organization. (Sorry those of you who want a RECOUNT, but time has EXPIRED!). Very seriously, it is an honor to be your President. I am hopeful that at the end of a year, each of you will feel that it has been a positive one.

Our 25th annual meeting in Savannah last August was a successful one, though the attendance was not one of our highest. The Avian, Virology and Bacteriology breakouts all had a generous number of interesting topics to be discussed and our exhibitor friends contributed greatly to the meeting, both at their booths and in the breakout sessions. I did find the lower attendance at such a milestone meeting a bit disheartening; because there were many friends and associates whom I didn't get to greet; but, even more because the Savannah area is such a beautiful one. It was truly a wonderland for sightseers, historians and shoppers.

The issue of decreasing meeting attendance is one that I have taken personally. I have talked with various members about this issue and the feedback has been quite varied-ranging from equal concern, to feelings that a smaller meeting is preferable and that more branches should be established to enhance localization. On another front, I was contacted by officers of the Veterinary Laboratory Association (VLA) with a proposal that the two groups coordinate meeting sites and dates, such that one would overlap or follow the other, in the same weekend, so that members with common interests in both groups could attend both in one trip. The proposal was forwarded to all Executive Board members for consideration and feedback. However, if any other member has thoughts you would like to share on this subject, feel free to do so by e-mail, letter or comment at the AVM web site. Comments and feedback regarding meeting size, and reasons, may also be submitted (e.g. sites/locations, hotel costs, economics of plane travel etc.).

Two additional areas that I personally consider of great importance are the AVM web site and becoming a tax-exempt organization. Lea Dowd, our new Secretary/Treasurer and web master, is very interested in, and very willing to continue, development of the web site. Several additions have been made in the past three months, but many more possibilities exist for even greater enhancement. These range from links to secure areas containing member information (including the directory), to publishing information channeled to the web master (from members, sustaining members, etc.) for all members to view, to direct communication between members and much, much more (well beyond MY understanding). As for the tax status of AVM, I will work with Lea and George Blackwell, who has worked on this issue previously for the Colonial States Chapter, to contact the IRS and secure the necessary forms and application details.

I am certain that I now hold the distinction of "longest AVM President's letter," so in closing I thank each of you once again, for this honor and, for your tolerance yet to come.

Jim Gary
President

AVM History and Philosophy

Drafted by Al Pursell, AVM Executive Advisor and Founding Member.

Alfred R. Pursell of Tifton, Georgia and John W. Black of Hopkinsville, Kentucky conceived the idea for the Association of Veterinary Microbiologists in 1972. 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, representatives 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". The diagnostic laboratories of Tennessee, Kentucky, and Georgia sponsored this symposium. 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 on 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.

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. Exhibitor fees are $400.00

Membership Directory: A membership directory is published each year and mailed to members. A membership directory and membership application form can be obtained by contacting the President or Secretary-Treasurer of the association.

Newsletter: A newsletter is published each year, or biannually if resources permit. Information on past and future meetings, training conferences, new techniques other items of interest are included.

Fees: Membership dues, annually, $10.00. Sustaining Members, annually, $100.00. Preregistration for the annual meeting $15.00 and $25.00 at the door.

2000-2001 AVM Officers and Executive Board Members

President	Jim Gary, St. Louis, Mo.
Vice President	Tim Klinefelter, Ames, IA
Secretary/Treasurer	Lea Dowd, Cataula, Ga.
Past President	Mike Parsley, Little Rock, Ar.
Past President	Judith Clapier, Monroe, NC.
Past President	Theresa Love, Brandon, Ms.
Executive Advisor	Albert Pursell, Tifton, Ga.
Chapter representatives:	
Colonial States	Susanna Trefsgar, Lynchburg, VA.
Heartland	Cindy Lindeman, St. Paul, Mn.
Meeting Site Chair	Rob Poston, Baton Rouge, La.
Publications Chair	George Blackwell, Richmond, VA.
Founding Member	John Black, Sevierville, TN.

Standing Committees

Annual Meeting Site Committee	Rob Poston (chair), Janet Harper, Judith Clapier (advisor)
Publications Committee	George Blackwell (chair), Lea Dowd, Connie Gates
Newsletter Advisory Committee	Sarah Rowe-Rossmanith, Anne Parkinson
Program Committee	Janet Harper (chair), Judith Clapier, Rob Poston, Theresa Love
By-Laws Committee	Albert Pursell, John Black
Audit Committee	Roxanna Maddux, Bill Cornell, Mike Parsley

Ad Hoc Committees

Web master	Lea Dowd
Chapters Advisor	Al Pursell
Historian	Roxanna Maddux
Photographer	John Black

AVM Web Site

The AVM's web site at http://www.wso.net/avm/ has been making progress. We have added two of the back newsletters in a PDF format. We are adding others as time permits. These can be easily read or printed from the web site. There have been discussions about adding the membership directory to our web site and we would sincerely appreciate feedback on this subject. It could be available to the public or protected by password.

If you happen to come across any sites that would be of interest to other AVM members, please drop me (Lea Dowd) a note so that I can post these for other members to view. A "Chat Room" is also within our capabilities if others are interested. All suggestions would be greatly appreciated.

Microbiology Web Site List

By Linda Cox

- http://www.TrainingFinder.org/
- http://www.apha.org/
- http://resistanceweb.mfhs.edu/cit/Index.asp
- http://www.asmusa.org/
- http://www.socgenmicrobiol.org.uk/
- http://www.cdc.gov/
- http://www.medscape.com/Home/Topics/ID/InfectiousDiseases.html
- http://www.nccls.org/
- http://www.cdc.gov/ncidod/dvbid/dvbid.htm

Report from the 25th Annual AVM Symposium on Techniques in Veterinary Microbiology

August 10-12, 2000
Hyatt Regency Savannah
Savannah, Georgia

Minutes of the 2000 Executive Board Meeting

Thursday, August 10, 2000
Hyatt Regency Savannah - Savannah, Georgia
Notes compiled by George Blackwell

Members Present:
Mike Parsley - President
Jim Gary - Vice President
Theresa Love - Secretary-Treasurer, Past President, Nominating Committee
Judy Clapier - Past President, Nominating Committee
George Blackwell - Colonial States Chapter Representative
Linda Cox - Heartland Chapter Representative

Al Pursell - Founding Member, Advisor
John Black - Founding Member

Member Not Present:
Rob Poston - Past President, Nominating Committee

There being a quorum present, the meeting was convened in room 515 and called to order at 4:00 PM by President Mike Parsley.

Order of Business:

- It was determined that the registration fees of $15 and $25, exhibitor fees of $400 and dues of $10 would remain the same.
- It was determined that Al Pursell would remain as the advisor.
- It was determined that August 9-11, 2000 and Baton Rouge, Louisiana would be the time and place of the 2001 AVM Annual Meeting. Linda Cox announced that the Heartland Chapter of the AVM would like to host a national meeting. She suggested St. Paul, Minnesota as a possible meeting site.
- The Nominating Committee selections were announced as follows: Jim Gary for president, Tim Klinefelter for vice president and Lea Dowd for secretary-treasurer.
- The Site Committee Report was given by Jim Gary and Judy Clapier.
- Vice President Jim Gary began the process of selecting committee members for 2000-2001 as follows: Publications Committee - George Blackwell and Connie Gates, Newsletter Advisory Committee - Sarah Rowe and Anne Parkinson, and By-Laws Committee - Al Pursell and John Black. An Audit Committee was not selected, however, Jim mentioned that someone close to Lea Dowd would be preferable. Roxie Maddux would remain as historian and John Black as photographer. Al Pursell agreed to meet with George Blackwell and review the newsletter format.
- It was determined that the official fiscal year for the AVM would be July 1 to June 31. It was not determined when this would take effect.
- It was determined that non-paying members would be held on the membership rolls for two years; that is, one year after the year in which they were current.
- It was determined that a memorial gift of flowers be made for past secretary-treasurer, Sherry Greer. A gift limit of $50 was suggested.
- It was suggested that the AVM pursue becoming a "non-profit educational organization" for the purpose of reducing the organization's tax liability and as an incentive to contributors. George Blackwell, secretary-treasurer of the Colonial States Chapter of the AVM, agreed to gather the relevant information.
- It was mentioned that the AVM Web site needed to be better utilized. There was a call for the submission of more current information and articles of interest. It was suggested that web links be established between our web site and other sites. It was suggested that a .com web site could be established for around $15-20 per month.
- It was mentioned that the AVM needed to advertise it's meetings through the ASM and AAVLD.

There being no further business to discuss, the meeting was adjourned by President Mike Parsley at approximately 6:00 PM.

Minutes from 2000 Business Meeting

August 11, 2000
8:30 AM Ballrooms D, E & F
Notes compiled by George Blackwell

AVM President Mike Parsley called the meeting to order and welcomed everyone to Savannah, Georgia. He dedicated the meeting to the memory of former AVM Secretary-Treasurer Sherry Greer. He thanked Judy Clapier and Melody Parsley for their participation and support. He thanked the exhibitors and sponsors: BD Microbiology Systems, Idexx, Synbiotics Corporation, Trek Diagnostics, Viral Antigens, VMRD and Centaur for their participation and support.

- At the request of President Mike Parsley, members and guests introduced themselves and stated their place of employment.
- The Minutes of the previous Business Meeting were accepted as read by Secretary-Treasurer Theresa Love.
- The Executive Board Report was given by President Mike Parsley. He announced that the registration fees of $15-25, exhibitor fees of $400 and dues of $10 would remain the same for 2000-2001. He also announced that Al Pursell would continue as the AVM Advisor. A call was made for the regular committee reports.
- The Treasurer's Report was accepted as read by Secretary-Treasurer Theresa Love.
- The Site Committee report was given by Rob Poston. He announced that the 2001 AVM Symposium would be held August 9-11 in Baton Rouge, Louisiana.
- The Heartland Chapter Report was given by Linda Cox. See the Heartland Chapter Report published in the 1999-2000 AVM Newsletter.
- The Colonial States Chapter Report was given by George Blackwell. See the Colonial States Chapter Report published in the 1999-2000 AVM Newsletter.
- The Publications Committee Report was given by George Blackwell. George asked that those taking notes in the microbiology sessions submit them as soon after the meeting as possible.
- The Nominating Committee Report was given by Judy Clapier. Jim Gary was nominated for the office of president, Tim Klinefelter for the office of vice president and Lea Dowd for the office of secretary-treasurer.
- Elections were conducted by President Mike Parsley. The nominees were unanimously elected by voice acclamation. After installing the new officers to their respective positions, Mike adjourned the Business Meeting.

Immediately following the business meeting, the 2000 AVM General Session was called to order by newly elected President Jim Gary. After welcoming everyone, Jim announced the 2000-2001 AVM Committee selections. Jim also noted that we need to submit articles to the web site. Observing that there was to be no speaker, he called for announcements from the floor. Woody announced that the Kissimee, Florida Laboratory had a 10-year-old Zeis electron microscope for sale at a real deal. Jim then announced that Tim Klinefelter would be moderating the Bacteriology Session. After reminding everyone to meet back at 1:00 PM and with no other announcements from the floor, Jim closed the General Session at 10:30 AM.

Treasurer's Report

Balance on Hand July 15, 1999			**$7,907.68**
Receipts:			
	Membership/Registration Fees:		
	1999 Meeting	$1,145.00	
	2000 Meeting	$2,165.56	
	Exhibitor's Fees:		
	2000 Meeting	$3,000.00	
Total Receipts:			$6,310.56
Expenses:			
	Annual Meeting 1999:		
	Wyndam Gardens Hotel	$4,760.16	
	Printing:		
	Program / Inserts	$91.75	
	Postage:		
	Program	$160.60	
	Miscellaneous:		
	Office Depot	$114.49	
	Service Charges	$53.35	
	George Blackwell (Printing)	$21.42	
	Rob Poston (Frame)	$4.19	
	Al Pursell (Advisor)	$324.00	
	Bereavement	$79.50	
	Postage	$6.17	
Total Expenses:			$5,615.63
Balance on Hand August 7, 2000			**$8,602.61**

Interim Site Selection Committee Report

Baton Rouge, 2001

The Committee is pleased to announce that the Association of Veterinary Microbiologists Twenty-sixth Annual Symposium on Techniques in Veterinary Microbiology will be held on August 2-5, 2001 at the Sheraton Baton Rouge Convention Center Hotel in Baton Rouge, Louisiana. The newly built Sheraton Baton Rouge features 300 deluxe guest rooms, a 150 seat full-service Cajun-seafood theme restaurant, two lounges, and over 14,000 square feet of function space, all connected to a 40,000 square foot glass-covered indoor atrium. Hotel amenities include an outdoor swimming pool and hot tub, on-site fitness facility, shuttle to the Baton Rouge Metropolitan Airport, and free,

convenient parking in either an open lot or garage facility. The Sheraton Baton Rouge guest rooms feature southern-style decor, full bath and shower accessories, an illuminated desk space with computer docking port, 27" TV with cable, coffee maker and condiments, iron and ironing board, and electronic security. Our contracted convention room rate is $70 per night, single or double, plus 13% tax (currently).

The hotel structure is part of a complex that includes the Argosy Casino riverboat, and is within a short walking distance to the Baton Rouge Riverfront Park, home of the Louisiana Naval War Museum and Nautical Historic Center, the USS Kidd, the Louisiana Arts and Sciences Center, the Baton Rouge Riverside Centroplex, Repentance Fountain, and Louisiana's recently restored Old State Capitol Building. The current state capitol complex is located just a few blocks north of the hotel site. The hotel is in downtown Baton Rouge at the east foot of the I-10 bridge over the Mississippi River, and is easily accessible by interstate travelers. The Sheraton Baton Rouge is located at 102 France Street, Baton Rouge, Louisiana 70802. For further hotel information, please contact the Sheraton Baton Rouge at 225/242-2600, or fax 225/242-2601. For additional details on the AVM 2001 Annual Meeting at Baton Rouge, please contact Rob Poston at 225/LSU-9777 or 225/578-9777.

Colonial States Chapter Annual Report

The Colonial States Chapter of the Association of Veterinary Microbiologists (CSC-AVM) was formed in 1985 in Richmond, Virginia primarily from members belonging to the southeastern region of the United States. These states include Delaware, Maryland, Virginia, West Virginia, North Carolina and South Carolina. The CSC-AVM 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 for the AVM also applies for the CSC-AVM. In those years when the annual meeting of the AVM falls into the Colonial States region, the CSC annual meeting is usually combined with that of the parent organization.

Interim report: Winter 2001
With a mailing list of one hundred fifteen, the Colonial States Chapter continues to be an active part of the AVM. Our 2000 Fall Symposium hosted twenty-four active members, two speakers, and six exhibitors and sponsors, for a total participation of thirty-two. This shows a greater than 50% increase in participation from our 1998 Williamsburg meeting and twice the participation we had at the 1999 Annapolis meeting. We expect to sustain this trend as the CSC-AVM hosts it's 2001 Spring Symposium on Techniques in Veterinary Microbiology and continues to gain new members as we did this November.

The 2000 CSC-AVM Fall Symposium - Williamsburg, Virginia

Administrative Board Meeting The CSC-AVM Administrative Board Meeting was called to order by President Suzy Trefsgar on Thursday, November 2, 2000 at 5:10 PM. Copies of the Minutes of the 1999 Administrative Board Meeting had been distributed to the members by the Secretary-Treasurer prior to the meeting. A quorum being present, they were approved as written. The usual topics of business were discussed and voted upon, advisors elected, committee reports given and goals established for the new year. The Spring Symposium was tentatively set for April or May 2001 in a location to be named at a later date. The 2001 Fall Symposium was tentatively set for November 8-10 in either Richmond or Williamsburg, Virginia. The meeting was adjourned at 7:03 PM. (A

detailed copy of the Minutes of the 2000 Administrative Board Meeting is available from George Blackwell.)

Business Meeting President Suzy Trefsgar called the meeting to order at 8:37 AM. After thanking everyone for coming, she also thanked Beth Henricson and Marion Fowler for their work on the 1999 AVM Meeting in Annapolis, Maryland. Finally, Suzy thanked all the CSC-AVM exhibitors and sponsors for their support. Participants introduced themselves and gave their place of employment. Secretary-Treasurer George Blackwell gave the minutes of the previous Business Meeting and the 1999-2000 Treasurer's Report. They were approved as read. President Suzy Trefsgar gave the Administrative Board Meeting Report and then called for the committee reports.

As Chair of the Site Committee, Suzy reminded the participants of the scheduled times, session sites and called for questions from the floor. Publications Committee Chair, George Blackwell, listed the articles and reports submitted to the AVM Publications Committee for inclusion in the 2000 AVM Newsletter. President Suzy Trefsgar then read the By-Laws Committee Report as submitted by Chair, Lynn Lewis. The proposed changes concerned: ARTICLE III, Section 4 to include fax and E-mail communications among Administrative Board members; ARTICLE VI, Section 4 to eliminate need for the presentation of scientific papers at the annual meetings; and ARTICLE VIII, Section 8 to allow for the addition of fax and E-mails as a method of transacting business by the general membership.

The specifics of the three proposed By-Laws changes were read to the membership and a voice vote was taken on each. All proposed changes to the By-Laws were approved as read. Nominating Committee Chair Earnest Wyant proposed that the following names be submitted to the membership for consideration: Suzy Trefsgar for President, Liz Steele for Vice President and Shelley O'Brien for Secretary-Treasurer.

There being no special announcements or unfinished business, President Suzy Trefsgar called for any new business. George Blackwell suggested that Howard Jones, President of the VLA, address the membership concerning his proposal to AVM President Jim Gary that the AVM and VLA hold concurrent meetings.

Specifically, Howard suggested that because there was an overlap in membership and interests, these two organizations could better serve their respective members by utilizing the same facilities. Holding separate, but concurrent meetings would assure greater attendance at both functions and would thereby encourage greater sponsorship and exhibitor attendance. The concurrent AAVLD/USAHA meetings were mentioned as an example of how this could work to both organization's advantage.

Al Pursell then voiced his concerns to the membership. He noted that the VLA's main concern is quality assurance and continuing education whereas the AVM's main function is to serve as a venue for round table veterinary microbiology problem-solving and discussion groups. He emphasized that all registration, sponsorship and exhibitor fees and meeting times would have to be kept separate. He cautioned that holding these two meetings on a national level might prove both confusing and cumbersome to both memberships, and suggested that it might work better on a regional level with the chapter meetings.

There being no other new business, Suzy turned the meeting over to Secretary-Treasurer George Blackwell for the election of the 2000-2001 officers. There being no nominations from the floor,

those names submitted by the Nominating Committee were approved for selection by the membership. Suzy Trefsgar, Liz Steele and Shelley O'Brien were unanimously elected by voice acclamation and officially installed into their respective offices.

President Suzy Trefsgar thanked everyone for their support and called for any announcements from the floor. There being no other announcements, she adjourned the business meeting at 9:16AM.

General Session Immediately following the Business Meeting, President Suzy Trefsgar opened the General Session by announcing the 2000-2001 goals and advisors as delineated by the Administrative Board. She then selected and announced the standing committee members for the upcoming year as follows:

Site Committee - Liz Steele, Suzy Trefsgar and Shelley O'Brien, George Blackwell
Publications Committee - George Blackwell, Liz Steele
By-Laws Committee - Lynn Lewis and Mary Lynn Rowland
Nominating Committee - Earnie Wyant, Marion Fowler and Beth Henricson
Audit Committee - Lisa Crofton and Liz Steele

Following these announcements, Suzy introduced the first speaker. Dr. Bruce Akey, Chief of the Office of Laboratory Services, Virginia Department of Agriculture and President of the AAVLD gave an amusing and very informative presentation entitled, THE FUTURE OF VETERINARY DIAGNOSTIC LABS - YOUR PSYCHIC HOTLINE (reprinted in this issue.) Following a short break for exhibits, Dr. Susan Sumner, Department of Food Science and Technology at Virginia Tech addressed the question uppermost in all our minds just before we adjourned for lunch, HOW DO YOU KNOW THERE HAS BEEN A FLY IN YOUR SOUP? This well thought out and enjoyable presentation answered everything we wanted to know about food microbiology (but were afraid to ask.)

After an alfresco lunch at the King's Arms in Colonial Williamsburg, the participants met for a joint microbiology round table discussion of the avian, bacteriology and virology topics covered at the 26th Annual AVM Symposium held in Savannah, Georgia. Members adjourned at 5:00PM and reconvened at 8:30AM on Saturday to complete the agenda. For making the CSC-AVM Fall Meeting possible, the membership wishes to thank our exhibitors -- Dr. Howard Jones of Synbiotics Corporation and Mike Street of Centaur, Inc., and our sponsors -- John Lawrence of Idexx, Dr. Miladin Kostovic of VMRD, Inc. and Mark Carpenter of BD Microbiology Systems. We especially thank Bob Studholme and Dr. Bob Crandell of Viral Antigens, Inc. for sponsoring the Friday evening Social Session.

The 2001 CSC-AVM Spring Symposium

The Colonial States Chapter will host its Spring Symposium on Techniques in Veterinary Microbiology in April or May of 2001. Proposed sites include Lynchburg or Roanoke, Virginia. For more information on this upcoming meeting or to get on our mailing list, contact Shelley O'Brien (SObrien@vdacs.state.va.us) or Suzy Trefsgar (Strefsgar@vdacs.state.va.us) at The Virginia Department of Agriculture - Office of Laboratory Services, 4832 Tyreeanna Road in Lynchburg, Virginia 24504. Phone: 804-947-2518

Respectfully Submitted,
George Blackwell
avmgeorge@aol.com

Heartland Chapter Annual Report

The Heartland Chapter of the Association of Veterinary Microbiologists was formed in 1995 and is comprised of those members residing in the north-central region of the United States. These states include Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota, Wisconsin and Ohio. The Heartland Chapter typically holds organizational and scientific meetings and we offer labs around the last weekend of April each year, in a format similar to the parent organization. Heartland Chapter annual dues are $5.00. Those interested in membership are invited to contact the chapter Secretary-Treasurer or one of the other chapter officers.

The Heartland Chapter meeting of the AVM was held April 27-29, 2000 in Manhattan, Kansas. All day workshops were held on April 27th. A workshop entitled "Veterinary Anaerobic Bacteriology for the Clinical Laboratory" was conducted by Spencer Jang from UC Davis. Dr. Sanjay Kapil conducted another workshop, "New Developments in Diagnostic Virology". Presentations were given at Dr. Kapil's workshop by Kim Austin, Cindy Chard-Bergstrom and Adriane Bustamante. On Saturday, April 29th, after our regular meeting ended, Trek Diagnostic held a workshop on the new update for their system.

On April 28th the keynote speakers were Dr. Jerry Jaax and Dr. Nancy Jaax. They were both involved with the Reston outbreak of Ebola virus while working at the United States Army Medical Research Institute of Infectious Diseases (USAMRID). This story was reported in the book the Hot Zone by Richard Preston. The Jaaxes gave an excellent presentation about Ebola and bioterrorism in general.

Following the keynote speakers the business meeting was held. Cindy Lindeman from the University of Minnesota Veterinary Diagnostic Lab was elected as President-elect. Next year's meeting will be held in St. Paul, MN. Roundtable discussions were held Friday afternoon and Saturday morning. The Bacteriology group was moderated by Kathy Strelow and the Virology roundtable was moderated by John Landgraf.

About 80 people attended the meeting. All states in the Heartland Chapter were represented. Some visitors from Kentucky attended because of the Trek Diagnostic workshop. Other states attended the meeting for the first time. These included Nebraska, Colorado, Oklahoma, and California. There seemed to be some interest from the new people in starting a western chapter. It would be great to see our organization continue to grow. The meeting was a definite success due to the great turn out and input by many of those in attendance. As always it was great to meet new friends, visit with old friends and learn about the microbiology that concerns us most.

Avian Session Notes

Moderator, Janet Harper, Cobb-Vantress
Notes compiled by Sara E. Rowe, Alabama Vet Diagnostic Lab,
transcribed and edited by George Blackwell, CSC-AVM

Ornithobacterium rhinotracheale (ORT) Revisited.
It was reported that Minnesota performs serological testing for ORT.

It is better to culture from tracheas of birds in the early stages of respiratory disease and easier to recover the organism if cultured before an outbreak. ORT is often found in turkey lungs when thinking of Pasteurella. It is usually seen in cooler weather, as in the fall. There was one report of ORT isolated from a broiler with synovitis. In this case, the organism was recovered in pure culture from the hock. ORT may be seen in mixed cultures, usually by the second day.

ORT is a fastidious organism. Blood agar (BA), BA with Gentamicin or BA with Gentamicin and Novobiocin are the preferred culture media. Incubate overnight in 5% CO2.

Pasteurella and Pasteurella-like Organisms From Chickens.
Some labs see these organisms out of the trachea of chickens. (see bacteriology notes)

New Experiences with Diagnostic PCR.
PCR vs. culture: It was reported that there are often MG/MS positive PCR results on birds where culture results are negative for MG/MS. PCR is used as a diagnostic technique for MG/MS and as part of a screening program for commercial poultry flocks. The samples are usually tracheal swabs. PCR is used along with serological testing such as the MG/MS ELISA and confirmatory HI's. It is not unusual to get PCR positive and HI negative. PCR is more sensitive than HI and results can be ready in 20 hours. At the Auburn Lab, PCR is also used for IBV, CAV, MG/MS, Salmonella, LT, Polyoma virus, EEE, and EHD.

Detection of Salmonella by PCR.
It was reported that there is a San Antonio company performing PCR for Salmonella on feed for $25.00 per sample. The name of this company was not given, however, it was noted that there may be problems with reproducible results when testing split samples.

On a limited number of clinical samples at the Clemson Lab, PCR vs. culture has shown good correlation. PCR has also been used as a Salmonella screening tool when testing a small inoculum from meat samples. More testing is to be done.

Rapid Methods for Salmonella Testing.
At the Clemson Lab, *Salmonella enteritidis* (SE) has become a big entity for testing now since there have been problems with SE on tracebacks. There has been an effort to reduce SE in South Carolina. Currently, drag swabs are tested by the lab and if positive for SE, egg cultures are performed. Four lots of a thousand eggs each are cultured. If all four lots are negative, the producer can return to selling the eggs.

Egg Culture Method: The eggshells are sterilized then the eggs are cracked into whirlpak (20/pool). These are stomached and set aside. One ml of each pool is put into tetrathionate broth for enrichment and the process is continued. Chickens shed SE sporadically as they are stressed for example during high heat or molting. SE inside the egg is what is being looked for here, but there is always the possibility of cross contamination.

Vaccination: The vaccine for SE shows some promise. A modified live virus vaccine (administered by spray) is not approved for laying birds. Killed vaccine is approved, but the birds have to be caught individually and the vaccine administered individually. It is reported that these vaccines have a 95% chance of not being detected by conventional testing.

Environmental Culture Method: To detect Salmonella in environmental samples, do a delayed secondary enrichment. Using drag swabs, enrich in tetrathionate broth at 42 degrees C overnight and then streak onto selective plates. If these are negative, hold the tetrathionate broth at room temperature for five to seven days, then restreak and proceed as usual.

Clinical Culture Method: For clinical samples, pre-enrich in tetrathionate broth overnight and plate as usual. Hold the broth for four to five days at room temperature, and then re-streak onto XLD and BG-NB plates, incubated and observed at 24 and 48 hours for typical colonies.

Salmonella pullorum Testing.
There is some question as to the significance of serological testing for pullorum antibodies. Solvay K polyvalent *Salmonella pullorum* antigen is used in a whole blood plate test for pullorum. There are a lot of suspect reactions on this serology on the plate test for pullorum that are often culture negative. There are often false pullorum plate positives when testing show birds, for example from state and county fairs. The plate tests for pullorum have a false positive rate of 20-40%, especially when the blood is coming from brooding hens, hens on antibiotics and birds that have been agitated or stressed. If birds are being tested for exhibition at the fair, they need to be done at least two weeks prior to the fair. Plate testing is most often used to monitor "clean up" progress on culture-confirmed pullorum positive flocks.

The tube test for pullorum is preferable as serum is used and therefore is considered a more specific test. If one gets a positive on the tube test, there's usually less chance of a false positive than the whole blood plate test. Sources of tube test antigen may be Intervet or NVSL. On turkeys for NPIP testing, the tube test is performed on serum only. Weak positives usually do not culture positive.

When testing whole blood, the plate test may also pick up Group B and Group C salmonellae and other Group D salmonellae besides *S. pullorum*.

New or Improved Tests, Kits and Reagents - What's Coming?
ELISA kits for avian include IBD-XR, AI, ALV-J, MG, MS and MM. There is also an MG/MS combo in chickens and turkeys, as well as a Salmonella ELISA. ORT kits have been licensed in Europe only. One lab reported having used and liked the AI ELISA by Idexx.

ISO Certification and Routine QC Testing.
These procedures require a lot of work, including taking daily temperatures, not using expired kits, lots of paperwork, etc. Writing manuals, updating manuals, performing and documenting the quality control testing requires a full time individual devoted to QC testing. The use of commercially prepared media helps because the manufacturer does the quality control testing.

Feed Testing.
Many companies don't test for Salmonella and others are not sure of what to do with positive results.

Clostridium in Chickens: Are There Any Rapid Diagnostic Reagents or Kits?
None were mentioned. *C. perfringens* is usually associated with gut lesions or gangrenous dermatitis. Impression smears from beneath the skin or direct smears of the gut when Gram stained, will give a presumptive answer. Large Gram-positive rods on the direct stain are usually considered presumptive positive for Clostridia.

Erysipelas in Cage Layers.
This topic was not discussed.

***Haemophilus paragallinarum* - Specimen Decontamination and Culture Techniques.**
This topic was not discussed.

***Bordetella avium* - Prevalence With and Without Cryptosporidiosis.**
This topic was not discussed per se, however, cryptosporidiosis is often detected in slides of clinical material using the fluorescent antibody method. Additionally, the culture of *Bordetella avium* was discussed.

Bordetella avium Culture Method: *B. avium* is isolated from chicken's tracheas and air sacs. The API 20 NE strips are used to identify the isolate. Minimal Essential Media (MEM) plates are used to separate *B. avium* from *B. avium-like* organisms. The formulation for MEM plates was derived from the Poultry Diagnostic Research Center (PDRC) in Athens, Georgia. *B. avium* is usually isolated from tracheas and can be mucoid or flat-type colonies (smooth and rough colonies, respectively). Usually BA with yeast extract and MacConkey Agar are used in the primary isolation of the organism from clinical material.

MG Update / *M. Iowae*: Is It Still Around?
There has been a lot of MG seen in chickens and turkeys in various states. In addition, there have been PCR positives for MG in quail and broilers and in some house finches. MG has also been seen in turkeys. *M. iowae* is not being seen much now.

MG ELISA shows more suspects. If an MG ELISA is positive, the birds are cultured. In-house controls are performed for each lot using either Idexx or KPL plates. Dr. Stan Kleven at the PDRC in Athens, Georgia provides a quarterly check test set with a panel of 30 chicken sera: 10 MG positives, 10 MS positives and 10 negatives. These can be used for checking the plate test, HI's and the ELISA set (individual MG and MS kit only.) The cost is $150.00 per set and provides one-half ml per tube. With the Idexx Combo Kit (MG/MS), there are less false positive's when screening serologically than with the plate test and thus less HI's to do to confirm the screening positives. Good technique is imperative.

There have been MS outbreaks reported in Alabama and Arkansas. PCR and HI tests were positive and the isolation of mycoplasma was almost impossible. If one gets a mycoplasma isolate, send to the PDRC (Dr. Stan Kleven). Arkansas has done comparative studies of ELISA vs. HI vs. PCR vs. culture for mycoplasmas and varied antibiotic treatments. Nothing was physically wrong with the birds, yet there were positives coming up on the PCR, HI and ELISA tests. Perhaps there are MS strain variants that are especially hard to grow. Most labs are doing direct plating and broth cultures, looking for a pH change.

Are There Rapid Methods to Detect Giardia in Birds?
This topic was not discussed.

Avian Leukosis J-virus Update - PCR.
At the Auburn University Laboratory in late 1998, PCR and histopathology were performed on clinically ill birds. A high percentage of these were positive on PCR for ALV-J, many of which were broilers. An increase in positives was seen in breeders with 30-40% positive for PCR ALV-J. There was also an increase in positives from broiler-breeders where the birds showed disease after about

one month. Submissions for this have slowed down this year. The results of comparative histopathology and PCR testing of liver samples for previrus DNA, showed 70% agreement.

Influenza Antigen ELISA Kits: Are They Useful in Chickens?
It was reported that the Directigen ELISA test on a cassette is available for humans, horses, and swine. It was also reported that NVSL has done a study and found a 95% correlation between virus isolation and this test. A nasal swab is the sample and if it is positive on the ELISA, virus isolation is performed. Becton-Dickinson is the manufacturer of the kit with a single test per device. It has not been approved for use in chickens in the U.S., however, Kansas State, Minnesota and Iowa are using this test for horses and swine.

Coronavirus Update.
This topic was not discussed.

Bacteriology Session Notes

Moderator, Tim Klinefelter, Iowa State University
Notes compiled by George Blackwell, CSC-AVM
with additional notes by Sara E. Rowe, Alabama Vet Diagnostic Laboratory,
transcribed and edited by George Blackwell

OSHA Visitation -- Preparing For Inspection.
Look for an inspection when there's a problem. Everyone is responsible to the Occupational Safety and Health Administration (OSHA). Check their Red Handbook. Labeling and Material Safety Data Sheet(s) (MSDS) are required by each lab for all hazardous materials. Know where they are kept Everything needs to be defined -- even agars and disinfectants. You will need a notebook for MSDSs and everyone in the workplace should be familiar with its location, how to read it and how to clean up a spill safely. Know who the Chemical Officer is for your lab.

There are special issues regarding waste disposal. For example, petri dishes of biological material need to be autoclaved and there needs to be records of all sharps disposal, etc. Check on the disposal of cystine selenite. Tissues may be picked up by contracted renderers or incinerated. Much lab waste can go to a medical waste company if properly packaged and identified.

Biosafety -- Presence And Training In Your Lab.
Many labs have Standards Operating Procedure (SOP) manuals and mandatory training for employees. Training records should be reviewed monthly and it was reported that some laboratories, such as the Kord Lab at Tennessee, hold safety meetings monthly, as well. In addition to a training officer, many labs have a safety officer. Specific safety training may be available from the National Veterinary Services Laboratory (NVSL) in Ames or through your State Public Health Association, Health Department or Fire Department. It was reported that Baker Scientific, the Eagleson Institute and others also offer training. Local military bases may have Troop Medical Clinics through which the Military Safety Officers may offer help with not only safety training, but with SOP development. The web site: *www.trainingfinder.org* was also offered for those with Internet access.

Special mention was made that laboratory eye wash stations need to be checked regularly, and that this and other safety procedures need to be addressed in the SOP's. For example, certain culture procedures must be performed under a hood. The autoclaves and biological hoods need to be understood well and training needs to be provided on their proper use.

Packaging Requirements For Shipping Specimens And Organisms.
There are NVSL training videos for shipping hazardous substances. There is also an educational CD-ROM available from SafetyPak for about $200, which allows for certification after examination. SafetyPak can be reached at 1-800-814-7484 or on the web at *www.saftpak.com*. There are training and shipping requirements available from USPS, UPS and Fed EX and check with OSHA and DOT for their regulations.

UPS will not take biohazardous materials, however, Fed EX will take some of these materials with the proper documentation. It was noted that letter campaigns have been successful with some labs to get clients to ship specimens better. Linda Cox (Kansas State) noted that *trainingfinder.org* is a useful web site. The US Post Office does not accept 6.2 category dangerous goods. If practitioners send in samples as diagnostic specimens that are leaking or otherwise not acceptable, the Post Office will call the lab.

Bioterrorism is a hot topic these days and the American Public Health Association has a veterinary section devoted to bioterrorism. The State Public Health Association may have a safety training section for biohazardous materials.

Is There Anything New To Add To Our SOP's And Protocols?
It was noted that not every lab has SOP's. The AAVLD and PROMED web sites may be useful. The Virginia Department of Agriculture has shared their SOP's with other state laboratory systems in the past and may have these on disc. All the labs need to be working on this. Tim Klinefelter (Iowa State) offered that NVSL will be putting all their SOP's on their web site. There will be a high priority on biosafety.

Fastidious Pasteurella And Pasteurella-like Organisms From Rabbits And Other Exotics.
This topic was submitted by Jim Gary (Purina Mills) who has seen offbeat pasteurella's in chickens and rabbits. Using the Biolog System, these isolates are sometimes identified as *H. parasuis* (see Quinn/Carter for the differences.) It was noted that sinus and nasal swabs are much like environmental swabs. It's not unusual to isolate a variety of bacteria including *P. trehalose*, Pasteurella-Actinobacillus-like organisms, etc. Some labs have seen similar organisms, but will usually isolate *P. multocida* or some other "true" pathogen. These others may be called out as "atypical pasteurella". Jim Gary offered that he had seen *P. haemolytica-like* organisms - some hemolytic and some nonhemolytic. Biolog Systems will call some of these isolates *P. volantium* and others *P. trehalosi*. *Mannheimia haemolytica* was the old P. *haemolytica biotype A* that was arabinose positive. *P. trehalosi* was the old *P. haemolytica biotype T* that was trehalose positive. *P. trehalosi* was seen in rabbits.

How To Differentiate *Pasteurella avium* from *P. gallinarum*.
The CDC has an "Unusual and Slow Fermenters" book that may help. John Abell at the Maryland Department of Agriculture in College Park uses gas chromatography to identify certain more fastidious bacteria. He may be able to offer suggestions. It was suggested that the addition of sterile bovine serum and/or incubation under CO2 might be useful in getting these organisms to grow.

Isolation of *Mannheimia (Pasteurella) haemolytica* And Differentiation of A & T Strains.
Most labs don't differentiate between these two; they call everything *Mannheimia haemolytica*. It was observed that there are more non-type A than type A pasteurellae isolated from lungs.

***Bordetella bronchiseptica* From Swine Lung.**
Iowa State processes a lot of these. Tim Klinefelter offered the following criteria: dextrose, citrate (+), urea (+) and susceptibility testing (gentamicin, clindamycin, neomycin resistant).

***Campylobacter fetus* Transport & Isolation: What's Available?**
Tim Klinefelter (Iowa State) uses Cooked Meat Media with Tribrissen / Amphotericin. Kathy Strelow (Wisconsin) makes a Weybridge Media that some labs are using with success. The latter is said to be good for a month. Most cultures are from abortion cases and are plated onto Blood Agar and Campy Plates, microaerophilically. Check with Connie Gates (South Dakota). There is an MIC for susceptibility. Paula Cray (Russell Research Center, USDA) has protocol for growing Campylobacter. This formulation has laked horse blood and charcoal and lasts about a month.

***H. somnus* Susceptibility.**
There is an NCCLS approved medium for fastidious organisms (Veterinary Fastidious Medium). Tim Klinefelter (Iowa State) has a Mueller-Hinton formulation for performing Kirby-Bauer testing on organisms such as *A. pleuropneumoniae* and *H. somnus*. BBL will make this formulation if there's enough volume. This formulation was tested against *H. parasuis*, however, and not found to be effective.

Johne's Disease: Anything New?
Surveys are very expensive at $15-20 per test. Linda Cox (Kansas State) is faced with having to start this and requested figures from those who are already doing these tests. Cornell is building a new lab just for Johne's testing (Dr. Sang Shin). The Kentucky Lab is doing 4000 Johne's cultures each year. Other states are going into the certification mode. It's easy to max out your equipment and media.

Serologically for Johne's ELISA, most labs are using Idexx's kit. Idexx is also working on a PCR for Johne's. North Dakota reported using a Johne's PCR which is said to compare well with culture methods, although PCR is not considered as sensitive. The ESP (Trek) System is being presented at the AAVLD in October. This system uses the detection of metabolic gases to identify the mycobacteria.

Different labs either make their own media for growing *M. pseudotuberculosis* or buy commercial media. BD Biosciences is said to have a good formulation, but it's expensive. Alice Smith (CE Kord), Connie Gates (South Dakota) and Sara Rowe (Alabama Vet Diag. Lab) all parallel tested the Remel and Becton-Dickinson prepared media and found better isolations on the Becton-Dickinson media for *M. avium subspecies paratuberculosis* on NVSL's proficiency test samples. Colonies come up two weeks faster and more colonies appear. Several labs ran parallel cultures with the check tests. Other Mycobacteria medias - 7H10 and LJ media are two that Mary Jean Bryant (Knoxville Lab) uses.

Rapid, Non-culture Identification of *E. coli 0157*.
Penn State is the *E. coli* Reference Center (Brenda Love). It was noted that some labs seem reluctant to isolate or identify *E. coli O157* because of the legal ramifications. Many labs use sorbitol positive as a screen. Look at chromogenic media. For example, there is a CHROMEagar or Rainbow Agar for 0157.

The Clemson Lab uses the VIDAS (bioMerieux) System. This semiautomated FA system can be used to identify *E. coli 0157*, Listeria and Salmonella from meat samples. The Colorado, Purdue, Penn State, South Dakota and Kentucky Labs are reported to have a PCR for *E. coli* toxins and pili. Georgia is working on providing their own. Remel has a latex kit.

NCCLS Update & New Antibiotics -- Efficacy Vs. Cost?
The National Committee for Clinical Laboratory Standards (NCCLS) is looking at MIC/breakpoint values in relation to blood levels in various species. It was reported that Mike Apley (Iowa State) is working on a method for MIC's. There is a need to standardize the breakpoints for veterinary Staph species as a guideline for veterinarians. Look for updates from Sensititre.

Paula Cray (Russell Research Center) is involved with the National Animal Health Monitoring System (NAHMS) study testing susceptibilities for Salmonella. She has requested that labs send her Salmonella and Campylobacter isolates from pets and food-producing animals. They will pay $10 per isolate and will send the containers back. There was nothing new from NCCLS on vet isolates of multidrug resistant *Staph. aureus* (MRSA).

Johne's Culture (Revisited).
BD Biosciences is now offering a reformulated Herrold's Egg Yolk Agar: Herrold's Egg Yolk Media with Mycobactin, Nalidixic Acid and Vancomycin (BD#222233) and a formulation without the Mycobactin (BD#222241). Clinical trials will be presented at the American Association of Veterinary Laboratory Diagnosticians (AAVLD) in Birmingham, Alabama this fall. This new formulation will be available after October 2000. Waiting time for delivery is usually four or more weeks. This custom media is already QC'd when it arrives and has a shelf life (refrigerated) of one year.

BBL (Terese Burns - Veterinary Applications Specialist) is developing an automated system to test fecal samples in approximately four week turnaround. BD Biosciences is also working on the MIDGIT or Bactec 960 to identify Johne's in a fecal sample in under four weeks.

Beta-lactamase Testing On Anaerobes: Is It Worthwhile?
This test has been found to be useful for rapid presumptive testing for *B. fragilis* which is positive in under two minutes; therefore don't use pen G. It has not been found useful for Clostridia. This is worthwhile to see if penicillin or cephalosporins can be used. On Gram negatives such as *B. fragilis* or *Fusobacterium spp.* the BD Biosciences product, Cefinase, will give a result within 30 minutes. If beta-lactamase positive, they should not use penicillin or cephalosporins in therapy. Sensititre has an anaerobic panel that the Georgia Lab uses. E tests are also used in antibiotic sensitivity testing of anaerobes.

New Gram Positive Organism Names: Are They Helpful?
API and other companies that use automated identification code books have 800 numbers for nomenclature updates, but its rumored that they're discontinuing this in favor of software/web sites. The software is said to run around $600 for the DOS-based API Labplus System. BBL markets the Crystal System for Gram positives and anaerobes. Trek markets the AP80 and AP90 at about $3 per test. Jim Gary (Purina Mills) says he prefers the Crystal System (BBL) for non-fermenters. As far as strips vs. conventional testing, it's up to the individual labs. Many labs feel that although purchased medias and test strips are more expensive, the benefits far outweigh the cost considerations. This is especially true in a clinical setting where the weight of having coded statistical data lends more authority to the identification. This may take some of the art out of microbiology, but many clinician's seem to like it. The down side may be the curse of too much information and having to report out everything down to bacterial speciation.

It was noted that the term "Low Likelihood" means only that there haven't been very many organisms put into the database, not that the identification is shaky. The more field organisms we send to the companies compiling the databases, the better the database.

Nomenclature Update & Convenient References and Web sites.
Some useful web sites include:

Bacterial nomenclature: www.dsmz.de/bactnom/bactname.htm
Bacterial names with standing in nomenclature: www.bacterio.cict.fr/
International Journal of Systematic Bacteriology: http://ijs.sgmjournals.org/contens-by-date.o.shtml

Also, companies such as Becton-Dickinson (*www.bd.com* or 800-666-6433); organizations such as the AAVLD, ASM, and AVM; as well as the USDA, state departments of agriculture, veterinary schools, and universities can also be helpful. Check the web site listings in this publication.

Automated Identification Systems: Do They Work For Animals?
The consensus was that the automated systems currently on the market do not work well for animals. The automated systems are all human based and they need more veterinary isolates sent to these companies to broaden their database.

Interfacing With Sensititre -- Computer Specifications.
Roxie Maddux (MSU Brethitt Vet Center) reported having trouble with the QC strains MIC'ing on Sensititre vs. what they should be. The consensus here was that breakpoints are more useful to most veterinarians than MIC's. Small animal people can use human labs to give MIC results. Paula Cray (Russell Research Center) has some human vs. veterinary data.

Other Topics.
Other topics discussed included the following:

Jim Gary (Purina Mills) has an egg yolk formulation from Remel for Clostridia that he likes. *C. perfringens* shows a differential white halo. He also recommended the CDC BA plate and PEA, which inhibits Proteus.

Order charts from "Anaerobe Systems" through the Heartland Chapter.

Strep. equi has been reported out of joints of some horses. This begs the question: Is this organism (perhaps a vaccine strain) causing problems?

There has been EEE in Kansas thought to be a vaccine strain.

Fish Microbiology Contacts: Dr. Michael Mauel at the Tifton Lab in Georgia and Jane Semier at the Brethitt Lab in Hopkinsville, Kentucky.

It was reported that laboratories that perform Leptospira MAT serology need a new source of Lepto enrichment for maintaining the Leptospira cultures. BD Biosciences said they would check on this.

Dr. Lloyd Lauerman's PCR Book is still available through the AAVLD. Write to Dr. Arthur A. Bickford, UC Davis, P.O. Box 1522, Turlock, California 95381. Phone: 209-634-5837.

Virology Session Notes

Moderated by John Landgraf, National Veterinary Services Laboratory
Notes compiled by Laura Clark, Breathitt Veterinary Center, Murray State University
and Woody Frazier, Florida Department of Agriculture
Edited by Rob Poston, Louisiana Veterinary Medical Diagnostic Lab, LSU School of Veterinary Medicine

Swine Influenza Virus H3N2.
This new influenza variant was first discovered two years ago in swineherd outbreaks in Texas, North Carolina, Minnesota, and Iowa. Two antigenically distinct H3N2 viral reassortments have been recovered from infected animals, one a double reassortment containing genes from human and swine influenza viruses, the other a triple reassortment with genes from human, swine, and avian viruses. Since their discovery, H3N2 variants have become established within US swine populations. For further information on H3N2 subtypes and their prevalence, please see Zhou, NN, *et al*, *J Virol* 1999 Oct; 73(10): 8851-6, Genetic reassortment of avian, swine, and human influenza A viruses in American pigs, and Webby RJ, *et al*, *J Virol* 2000 Sep; 74(18): 8243-51, Evolution of swine H3N2 influenza viruses in the United States.

Recent lab efforts have uncovered other new influenza strains. The variant H1N2 has been recovered on several farms in Wisconsin, and is now seen in other states and in Canada. Karasin *et al* (2000) describe influenza H4N6 in a herd of pigs in Canada. This variant was associated with migratory waterfowl, which are increasingly viewed as a significant reservoir for influenza viruses. For further information, please see Karasin AI, *et al*, *J Virol* 2000 Oct; 74(19): 9322-7, Isolation and characterization of H4N6 avian influenza viruses from pigs with pneumonia in Canada.

Influenza H3N2 is isolated with the Madin-Darby Canine Kidney (MDCK) established cell line, primary Swine Kidney (SK) cell culture, or in embryonated eggs. Gene Erickson of the Rollins Animal Disease Lab in Raleigh, North Carolina has developed a procedure to culture influenza virus with MDCK cells. (This procedure was published in the *AVM 1999-2000 Newsletter*). NVSL uses SK cells maintained in medium with 8% fetal bovine serum (FBS). Once the cells have grown to confluency, they are washed three times with 10µg/ml trypsin. The cells are inoculated, then are rinsed and re-fed with serum-free medium. This same procedure also is used effectively to culture Equine Influenza Virus from clinical samples. MDCK cells reportedly yield high hemagglutination (HA) titers, but low titers relative to infectivity. In embryonated eggs, influenza virus typically grows to high infectious titers.

Diagnostic reagents are available from NVSL and commercial sources. As performed by NVSL, Hemagglutination Inhibition (HI) tests for influenza virus antibody using Tom Turkey red blood cells (RBC). Rooster RBCs also serve in HI, but reportedly are not as sensitive. Subtype-specific monoclonal antibodies are available from NVSL. Centaur markets the Directigen FluA ELISA test kit, manufactured by Becton-Dickinson, that detects influenza A viral antigen in swabs from human specimens. This kit has been shown to be particularly useful in revealing influenza A in equine or swine sources. The timing of specimen collection is crucial for the successful recovery of virus, which appears in detectable levels only in the first five days of infection, while the host is febrile. Specimen handling precautions, necessary to preserve infectious virus for culture, are not necessary when using the Directigen kit, which renders a test result in only 15 minutes.

All influenza viruses are recognized for their zoonotic potential to become infectious for humans. Infectious clinical material or cultured virus should be handled in a biosafety level two (BL-2) environment.

For further information on swine influenza H3N2 and other variants, or on lab techniques to isolate influenza virus, please contact John Landgraf. For further information on the Directigen FluA ELISA test kit, please contact Michael Street of Centaur (800/236-6180).

Porcine Circovirus (PCV).
One of the two known biotypes of PCV is recognized to cause Post-weaning Multisystemic Wasting Syndrome (PMWS), pneumonitis, and possibly other health problems in swine. Porcine Circovirus also appears in co-infections with other pig viruses, such as Porcine Reproductive Respiratory Syndrome (PRRS) virus, causing a compounded exacerbated illness.

Laboratory techniques and reagents to detect PCV increasingly have become available since the virus was first recognized as a serious health threat to young pigs. A direct fluorescent antibody (DFA) conjugate to PCV, manufactured by American BioResearch, currently is available from VMRD. With DFA, Circovirus is often found in the lymph nodes of infected pigs. The MSU Breathitt Veterinary Center in Hopkinsville, Kentucky reportedly detected Circovirus in pig lung by DFA. With a glucosamine treatment, Steve Bolin of the Respiratory Diseases Livestock Research Unit, National Animal Disease Center, USDA, Ames, Iowa, was said to have successfully cultured PCV on PK-15 cells from buffy coat, tonsil, and liver specimens. According to Tischer *et al* (1987), a 50-fold increase in PCV titer can be realized by treating infected cell cultures with 300 mM glucosamine. Tischer *et al* conclude that "glucosamine and DEAE-dextran initiate PCV replication by enabling the PCV genome to get entry to the cell nucleus that normally can be achieved only by inclusion in the daughter nuclei at the end of mitosis." For information on culturing Circovirus from porcine clinical material, please refer to the *AVM 1998-99 Newsletter,* the *AVM 1999-00 Newsletter*, or contact John Landgraf or John Black. For information on glucosamine treatment for Circovirus culture, please see Tischer, L, *et al*, *Arch Virol* 1987; 96(1-2): 39-57, Replication of porcine circovirus: Induction by glucosamine and cell cycle dependence.

Without a truly sensitive laboratory host cell line, the culture of PCV is technically challenging. The PK-15 cell line, the current choice for culture attempts and reagent production, is readily found with PCV contamination, which is believed to come from the trypsin used in cell line maintenance. PCV has been recovered from commercially prepared trypsin. NVSL maintains a parent PK-15 cell line that reportedly is free of Circovirus. Porcine cell lines should be screened every six months to check for possible Circovirus contamination. For information on screening procedures, please contact John Black.

An interesting growth dynamic seems to occur in cell lines persistently infected with PCV. John Black observed that a persistently infected PT-1 cell line undergoes a decrease of infected cells following several passages; with time, the infected cell count seems to rebound.

The benefit of PCV serology testing is unknown; most pigs are found with antibody to Circovirus by indirect fluorescent antibody (IFA) testing. For a report on the development of an ELISA assay for PCV antibody in pig serum, please see Walker IW, *et al*, *J Vet Diagn Invest* 2000 Sep; 12(5):400-5, Development and application of a competitive enzyme-linked immunosorbent assay for the detection of serum antibodies to porcine circovirus type 2.

Porcine Picornaviruses.
In the US, eleven serotypes (types 1-11) of porcine picornaviruses are recognized. As enteroviruses, they are transmitted by the oral-fecal route, and have been associated with mild outbreaks of swine polioencephalomyelitis. Enteroviruses are now believed to be a primary cause of mild, self-limiting vesicular disease in swine. Porcine picornaviruses also have been implicated in reproductive problems, and were ranked with porcine parvovirus (PPV) among the now anachronistic grouping of SMEDI agents (stillborn, mummification, embryonic death, and infertility).

As described by Howard Dunne in *Diseases of Swine*, pig enteroviruses are readily isolated in many porcine cell lines, such as the PK-15 line, from feces, brain, or tonsil of infected pigs. John Black reported on a picornavirus isolated from commercial swine serum. NVSL has the capability to perform serological testing for antibody to porcine picornavirus types 1-8.

Porcine picornaviruses seem to be only a minor concern to pig health in the US. They have greater significance in swine outside of the US, thus are viewed more as foreign infectious agents of animal disease. For information on porcine enteroviruses, please see Dunne, HW, ed., *Diseases of Swine*, 4th ed, Iowa State University Press, 1975, p.353. For a recent discussion of swine enteroviruses, please see Straw, BE, *et al*, eds. *Diseases of Swine*, 8th ed., Iowa State University Press, 1999. For further information on the detection of porcine picornaviruses, please contact John Black or John Landgraf.

From a porcine vesicular condition, NVSL has recovered a possible enteroviral isolate distinct from the eleven known domestic porcine types. The isolate seems to resemble a coxsackie virus, a group of picornaviruses known to produce vesicular or papular rash in humans. Preliminary characterization reveals that the isolate readily grows in a wide range of pH conditions.

Porcine Reproductive & Respiratory Syndrome (PRRS).
Veterinary diagnostic facilities in Minnesota have identified the European strain of PRRS virus in four domestic herds. From the first affected herd, an isolate was recovered by culture on the MA-104 cell line. PRRS virus was detected in subsequent herds with polymerase chain reaction (PCR) techniques. Preliminary attempts to propagate the isolate reportedly failed in porcine alveolar macrophage (PAM) cell culture, which is known to grow only certain field strains of PRRS virus. NVSL intends to investigate the growth characteristics of this isolate in the MaRC cell line.

Property rights seem to be interfering with research in the detection and diagnosis of PRRS virus, and perhaps other emerging infectious pathologic agents of animals. New viral isolates and cell lines of potential commercial value are being patented in greater frequency, regardless whether they are ever profitably used in commercial ventures. The materials become proprietary and cost-prohibitive for non-profit research and diagnostic interests. This trend has permanently disrupted the informal network that had once existed between facilities to share materials and resources.

Herpes Cytomegaloviruses of Swine and Horses.
Porcine cytomegalovirus is responsible for inclusion body rhinitis, clinically evident as nasal discharge in baby pigs. Infection typically is diagnosed by histopathology with the demonstration of herpes inclusion bodies in cells of the mucus membrane of the nasal septa. Reportedly, a porcine fallopian tube cell line has been used to culture porcine cytomegalovirus from clinical material.

Equine herpes cytomegalovirus, or Equine Herpesvirus type 2 (EHV-2) is commonly thought to establish a mild subclinical infection in immunologically naive animals. It has been found in young horses with mild rhinitis and pharyngitis, a condition colloquially known as the “two-year old snots.”

Most horses are found to have antibody against EHV-2, so serodiagnostic attempts are believed to have little value.

Bovine Adenoviruses (BAdV).
The significance of adenoviruses in cattle disease is uncertain, but some references note the possibility for mild respiratory illness. Bovine adenoviruses most likely appear in asymptomatic or subclinical infections. Ten to twelve serotypes of bovine adenoviruses are recognized. NVSL performs IFA testing for antibody to BAdV types 1 and 5. NVSL also has the capacity to culture BAdV, and reportedly obtains occasional isolates. BAdV culture attempts can be spoiled by the presence of BAdV antibody in bovine serum cell culture medium.

The University of Georgia Veterinary Diagnostic Lab at Tifton discovered an agent resembling adenovirus in bovine endothelium. Viral inclusions were demonstrated in the endothelium of the uterus; electron microscopy revealed viral particles in the cytoplasm of affected cells. A preliminary identification of adenovirus was made based on the size of apparently non-enveloped particles in the cytoplasm of infected cells, and on an initially suspicious reaction on PCR for Adenovirus (Atadenovirus). Further analysis showed that the agent was Bovine Herpesvirus type 4 (BHV-4). Ten cows were found with evidence of virus, which was associated with severe metritis 7-21 days post parturition. The significance of this finding is being investigated. For further information, please contact Charles "Sandy" Baldwin, or see Czaplicki G and Thiry E, *Prev Vet Med* 1998 Jan; 33(1-4):235-40. An association exists between bovine herpesvirus-4 seropositivity and abortion in cows.

Cache Valley Bunyavirus in Cattle.
Cache Valley Virus (CVV) is a mosquito-transmitted Bunyavirus noted to cause arthrogryposis (unnaturally flexible joints) and hydranencephaly (head enlarged by cerebral fluid blockage) in new-borne lambs following infection of the pregnant ewe. Although focal outbreaks have occurred during lambing season in the US, CVV is thought to have only minor disease significance in domestic sheep. CVV is not recovered from affected lambs, but has been found in mosquitoes. Reportedly, CVV has been isolated from a horse with vesicular disease, and from an ostrich in Florida; the significance of these cases remains unknown. Persons residing in arbovirus-endemic areas are commonly found with CVV antibody, suggesting the possibility for human asymptomatic infection. CVV is cultured in Vero cells; its isolation usually is incidental, typically not connected to a health problem.

John Black reported on the culture of CVV from a trial lot of commercial-source FBS. The presence of CVV and other viruses in commercial cell culture serum indicates a need to irradiate animal-sourced products to improve lab safety and reduce potential biohazards. Also, the recovery of CVV from a bovine source warrants searching for the virus in association with cattle disease. The closely related Bunyavirus, Akabane, adds credence to the possibility that CVV has some yet undiscovered disease role in US cattle.

Akabane virus is found in Southeast Asia, Australia, and Africa, where it is an endemic problem of cattle, sheep, and goats. The bovine fetus becomes infected with Akabane virus when an infected mosquito has bitten the pregnant cow. If the viral infection is heavy and early during gestation, abortion results. Infections later in gestation lead to hydranencephalic or crippled calves. Akabane virus has been cultured from the placenta or fetal brain and muscle tissue using cell culture or suckling mice, but reportedly is recovered only from absolutely fresh material. The virus is difficult to find in calves once born. Serodiagnosis of both Akabane and CVV includes detecting viral antibody in fetal or pre-suckle neonatal serum, or by demonstrating seroconversion in paired

maternal sera. For further information about Akabane virus, please see Murphy, FA, *et al*, *Veterinary Virology*, 3rd ed., Academic Press, New York.

BVD ELISA.
Marge Muenzenberger of Kansas State University reported that their lab successfully screens serum samples for BVD virus using the BVD antigen ELISA test kit from Syracuse Bioanalytical. (Please see the *AVM 1999-2000 Newsletter* for additional information on this test kit.) Immunohistochemical staining on ear notch biopsies for BVD virus is used by some facilities to detect persistently infected animals, which when positive are found ladened with virus.

Among the various available immune probes to detect BVD viral antigen, polyclonal antibody in an IFA format gives the strongest signal. A DFA reagent made from polyclonal antiserum is satisfactory for detecting BVD viral infected cells. Monoclonal antibody to BVD virus fails to provide a strong signal in an IFA format. For further information on the fluorescent detection of BVD viral antigen, please contact John Black.

Viral culture is a popular, sensitive method to screen animals for BVD viral persistence, provided that the cell line used in the attempt remains free of non-cytopathic BVD viral contamination, often derived from commercial FBS. The cell line should be documented to be free of BVD virus by periodic screening for BVD viral antigen with DFA or IFA methods.

The FBS used in cell line maintenance also should be demonstrably free of detectable BVD antibody, which interferes with isolation attempts. Although fetal serum theoretically lacks antibody, immunoglobulin content can still be demonstrated in many FBS lots. Commercial-source FBS typically is pooled, and may contain trace amounts of BVD antibody below levels detectable by quality assurance testing. When screening FBS for BVD antibody, the FBS lot should be negative at a 1:2 final dilution in a serum neutralization (SN) test. John Black recommended a low immunoglobulin-G (IgG) content of FBS to maintain cell lines for BVD viral isolation attempts. The low IgG FBS lot also should to be irradiated to assure that it is free of BVD viral contamination. All sources of raw bovine biological products potentially contain infectious BVD virus. Steve Wessman reports that NVSL rigorously screens serum lots for BVD antibody before irradiation, and accepts less than 20% of all tested lots. Once a good lot is found, it is irradiated before use.

Serological responses to BVD virus type 1 vs. type 2 are distinguishable by SN; the homologous reaction typically displays a four-fold higher titer than heterologous reactants. NVSL uses PCR methods for BVD viral typing on isolates or clinical material.

Rabbit Calicivirus.
This virus is the causative agent of Rabbit Hemorrhagic Disease (RHD), which is considered a foreign animal disease in North America. It has been seen in Spain in the fall of 1999. This past year, the virus made an abrupt appearance in the US, causing the death of 26 of 27 animals in an Iowa rabbitry. At this time, it is unknown how the virus entered the US. Additional outbreaks have been encountered in Mexico.

RHD virus apparently fails to grow in lab cell lines. The virus is identified by immunohistochemistry, however other putative rabbit caliciviruses may cross-react with RHD viral diagnostic immune probes. RHD virus apparently affects only European lines of rabbits, such as those commonly used in laboratory research. Wild, native US rabbit species seem to be unaffected by the virus. For diagnosis, clinical material from suspected cases is sent to the Foreign Animal Disease Diagnostic Laboratory, P.O. Box 848, Greenport, Long Island, New York 11944-0848, phone

516/343-2500 x256. For further information on the occurrence of RHD in the US, please contact John Landgraf.

West Nile Virus (WNV).
WNV has been found in the US from New England to North Carolina, and is showing signs of further spread. The virus typically is recovered from migrating crows and jays, but has been seen in over 70 different species of birds, and in over 30 cases involving horses. WNV is an arthropod-borne, zoonotic flavivirus, and is considered a BL-3 pathogen, requiring strict biological containment and handling of infectious materials.

A network of state public health laboratories is doing most WNV surveillance, however certain veterinary testing facilities (i.e., NVSL, Louisiana Veterinary Medical Diagnostic Lab, Virginia Dept. of Agriculture) participate in surveillance efforts. Labs receive samples from dead crows or horses, then attempt culture of WNV using the Vero cell line and perform PCR on clinical specimens and culture products. For information on the PCR technique in detection of WNV, please contact Donna Johnson at 515/663-7551, e-mail Donna_J_Johnson@aphis.usda.gov.

WNV serology is being performed in Florida using HI methods. Some cross-reaction is encountered between WNV and St. Louis Encephalitis (SLE) virus, another flavivirus, but homologous reactions in serological tests yield four to eight fold higher titers than heterologous reactions. Wild ducks and geese seem to readily develop WNV antibody during infection. In many states, conventional arboviral sero-surveillance efforts include testing sentinel chickens, which reportedly do not respond well to WNV. However, according to Senne *et al*, seven-week-old chickens developed WNV-specific antibody, detectable at five days post inoculation (DPI) by the plaque reduction neutralization test, and seven DPI by IFA. For further information, please see, Senne DA, *et al*, *Avian Dis* 2000 Jul-Sep; 44(3): 642-9, Pathogenicity of West Nile Virus in chickens.

Facilities participating in the search for WNV were said to be having difficulty obtaining diagnostic reagents for WNV from the federal Centers for Disease Control and Prevention (CDC). The AAVLD may address this problem, and is ready to suggest a species-neutral serology format, such as a competitive assay, which does not require validation for each animal species. Commercial diagnostic reagents for WNV are becoming available, such as from BioReliance (14920 Broschart Road, Rockville, MD 20850-3349, ph 301/738-1000, fax: 301/783-1036) For information on these products, please contact Dr. Bob Peters, e-mail BPeters@bioreliance.com.

Canine Distemper Virus (CDV) in Cats.
As reported in the *AVM 1999-2000 Newsletter*, CDV has been recovered from an outbreak of distemper in African lions. John Black reports finding CDV antibody in the serum of domestic cats, which evidently are susceptible to asymptomatic infection by CDV. Domestic cats have been hypothesized to be a reservoir species for CDV. In cases of dog distemper of undetermined source, Black suggests searching the environment for infectious cats. The finding of CDV antibody in cats suggests a theoretical epizootiological model for CDV in domestic pets: Cats obtain CDV from a recently vaccinated dog in the household; the vaccinal strain of CDV establishes an asymptomatic infection in cats, but possibly reverts to virulence for dogs through cat-passage. CDV is then shed by the cat and transmitted to another susceptible, unvaccinated dog. Because of the implications of such a theory, further investigation into the host-agent relationship of CDV in domestic cats probably is warranted.

Increasingly, paramyxoviruses are found able to establish cross-species infection. Once CDV enters a divergent host species, it seems to acquire changed properties relative to its infectivity, and perhaps

other phenotypic traits. For example, although the porpoise morbillivirus is genetically similar to CDV, it does not seem to adapt and grow in lab hosts or exhibit cell tropisms with the same characteristics as native CDV.

Enteric Virus Test Kits.
Pathasure Enteritis ELISA Kit, available from Vetoquinol Diagnostics, Quebec, Canada (450/586-2252 or 450/586-4649) detects Rotavirus, Coronavirus, and E. coli K-99 in a 96-well ELISA plate format with breakaway strips. Currently, a USDA permit is required to obtain the kit. When applying for a kit importation permit, the applicant must attest that the kit contains no infectious animal, plant, or human cells or products, and that the kit will be used for research purposes only. For information on importation permits, please contact USDA Veterinary Services at 301/734-7760 or 301/734-7885, Fax 301/734-8910.

Syracuse Bioanalytical, Inc. of Ithaca, New York, reportedly is developing a kit to detect Rota and Coronavirus in fecal specimen. For technical or purchase information, please call 607/226-0609.

Abbott Lab's latex agglutination (LA) test kit to detect rotavirus in fecal specimens has been sold to Alexon-Trend of Ramsey, Minnesota, and probably will be produced by their latex kit subsidiary Seradyn Laboratory Products, 7998 Georgetown Road Suite 1000, Indianapolis, Indiana 46266, phone 800/428-4072, fax 317/610-3888. Abbott's TESTPACK ***ROTAVIRUS*** *ELISA* assay is no longer available.

Meridian Diagnostics of Cincinnati, OH (800/543-1980) markets the Premier Rotaclone, a breakaway well ELISA test kit to detect Rotavirus antigen in patient fecal samples. Although this kit is approved for human use only, it works well in detecting group A rotavirus enteritis of animals. For experiences using Rotaclone, please contact Rob Poston at 225/LSU-9777.

New Kits.
Centaur - Improvements have been made to the ELISA test kit for EIA antibody. This kit is approved for regulatory testing, and is currently available. For information on this and other Centaur products, please contact Mike Street at 800/236-6180.

IDEXX - An ELISA test kit is now available to test bovine serum for antibody against *Neospora caninum*. IDEXX has a direct ELISA test kit to detect specific antibody in swine serum against *Mycoplasma hyopneumoniae*, and is developing a swine influenza virus H1N2 antibody test kit, which should be available by the spring of 2001. The improved DNA probe kit to detect Johne's Disease bacilli in fecal specimens reportedly performs with the sensitivity and specificity of culture, but renders a test result in two days, compared to 18-24 weeks on culture. For information on these kits and other IDEXX products, please contact John Lawrence or Russ Shoberg at 800/551-0998.

VMRD - Substrate slides are available to test for antibody to *Neospora caninum* with the IFA format. Monoclonal antibodies to distinguish BVD virus Type 1 and Type 2 will soon be available. For information on these and other VMRD products, please contact Miladin Kostovic at 800/222-8673.

Check Test for Vesicular Stomatitis Virus (VSV).
NVSL will soon be distributing check-tests for certification to perform VSV antibody testing. Any interested state animal testing laboratory with appropriate facilities can participate in the VSV regulatory and surveillance program. No fee will be assessed to labs taking the check-test. For additional information, please contact Ken Eernisse of NVSL.

New Experiences with Diagnostic PCR.
John Landgraf noted the development of PCR techniques to detect *Mycoplasma spp.* at NVSL. Lanqing Li of the CS Roberts State Veterinary Diagnostic Lab at Auburn, Alabama stated that the polymerase chain reaction (PCR) and reverse transcription PCR (RT-PCR) are powerful techniques in molecular biology, widely used today for the detection and characterization of many human and animal pathogens. The Auburn facilities uses PCR and RT-PCR for routine diagnostics, including the detection of Eastern Equine Encephalomyelitis (EEE) virus, Infectious Bronchitis Virus (IBV), Bluetongue Virus (BTV), and Epizootic Hemorrhagic Disease (EHD) virus. PCR testing is used to detect *Mycoplasma spp.* of avian and mammalian sources, Chicken infectious anemia virus, Avian leukosis virus subgroup J (ALV-J) provirus, Polyomavirus, Johne's Disease bacilli (*Mycobacterium paratuberculosis*) and *Clostridium perfringens*. Their facilities run about 6000 PCR tests annually. Restriction fragment length polymorphism (RFLP) assay is used for the differentiation of IBV biotypes, and to speciate Mycoplasma. For further information about PCR techniques in veterinary diagnostics, please contact Lanqing Li.

Advances in Cell Culture and Virus Propagation.
A convenient method of temporary cell line storage is being employed at NVSL. Following trypsin-dispersal of the cell monolayer, the cells are refrigerated. Cells stored this way are useful to seed new flasks for up to one week after trypsinization. The effect of this method on the susceptibility of the cell culture to viral infection has not yet been determined.

For the culture of lab-adapted bovine virus, and in viral culture attempts from bovine clinical sources, the BT cell line seems to maintain maximum susceptibility for about twenty passages or splittings; after this point, the susceptibility of BT cells for virus infection seems to drop off.

For the culture of Encephalomyocarditis (EMC) virus from clinical specimens, the BHK cell line was reported to be a sensitive lab host.

To preserve infectivity of stocked Bovine Respiratory Syncytial Virus (BRSV) a 45% sucrose solution in phosphate buffered saline was recommended as a cryogenic medium.

SOPs, QA/QC, and Protocols.
As part of a sound QA/QC program, technical protocols ought to be reviewed on an annual basis. A quarterly schedule should be established for the maintenance of lab equipment and instruments, such as micropipette and thermometer calibration, and the defrosting or deicing of freezers. Conjugates should be checked for function and stability on a routine basis. The NVSL suggests a five-year standard shelf life for reagent antisera and conjugates. Testing protocols are available from NVSL for a $2.00 charge. For further information on NVSL resources in support of laboratory QA/QC efforts, please contact John Landgraf or Gary Gustafson.

Bovine Spongiform Encephalopathy (BSE).
John Landgraf discussed a case involving spongiform encephalopathy in a flock of sheep in Vermont. Following importation of the sheep from the Netherlands, authorities discovered that the sheep had been previously exposed to BSE. The affected flock was eradicated after a quarantine period. The condition of these sheep was said to resemble atypical scrapie. BSE and scrapie are diagnosed histologically, with assistance by immunohistochemistry techniques. The US still is considered BSE-free.

Virus taxonomy.
For recent taxonometric changes to viral nomenclature, members are referred to the web site of The International Committee on Taxonomy of Viruses, *http://www.ncbi.nlm.nih.gov/ICTV/*

The Future of Veterinary Diagnostic Laboratories

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Top 10 Forecasts from *Outlook 2000*

To mark the year 2000, the editors of THE FUTURIST magazine have selected their annual "top 10" forecasts for the future. These forecasts, included in a 16-page Outlook 2000 report, came from leading scientists, researchers, and scholars whose views of the future have been published in THE FUTURIST during the last year. The **Top 10** forecasts (*in italics*), chosen on the basis of their interest, significance, and plausibility, are as follows:

1. *The number of centenarians worldwide will increase from 135,000 today to 2.2 million people by 2050.* This is certainly good news for those of us who will be approaching the centenary mark by that date.

2. *By 2010, biomonitoring devices that resemble wristwatches will provide wearers with up-to-the minute data about their health status*. Actually, something like this is already occurring with the use of similar devices to monitor blood sugar or oxygen saturation.

3. *Exercise will promote mental well-being as well as a healthier body, helping people fight chronic pain, depression, chemical dependence, and even schizophrenia.* This is certainly not a new topic but one that is being supported more and more by research findings.

4. *Tiny electronic microchips implanted in a person's forearm could transmit messages to a computer that controls the heating and light systems of intelligent buildings*. Such devices in the form of a wearable badge are already available and can be used to locate a person within a building as well as alter the environmental settings within whatever room the person is in based on their personal profile. This includes not only lighting and temperature, but music and even video displays of scenery or art. Bill Gates is reputed to have such a system installed in his home.

5. *The twenty-first century could see widespread infertility and falling birthrates.* There are already many developed countries that intentionally are either near or even below the level of birthrate needed to maintain their populations at current levels. Generally, as a country becomes more developed, there is a concomitant drop in birthrates.

6. *Farmers will become genetic engineers, growing vaccines as well as food.* Farmers have already become genetic engineers as evidenced by the recent furor over the production of Genetically Modified Organisms (GMOs), both plants and animals, by the US, which is being used as a non-tariff trade barrier by the European Union. Several start-up companies are already working on commercial ventures to produce vaccines and other biologicals such as insulin.

7. *The worldwide consumption of meat will double by 2050.* Generally, as countries become more developed, their per capita consumption of meat as a protein source rises proportionately. This means that the market's need for animal protein will demand the continued growth of the livestock and poultry industries.

8. *Ninety percent of the world's 6,000 languages could go extinct by 2100.* The world will be a smaller, and in some ways less rich, place. You may be startled to be flying in a foreign country and hear the pilot of the plane conversing with the control tower in English. All air traffic control worldwide uses English as the primary language. However, another side to this coin is that the client base is becoming more diverse and you are more likely than ever to have more than one client for whom English is a second language.

9. *Water scarcity could threaten 1 billion people by 2025.* This will be a major counterbalancing force to the need to expand meat production. Not only will water scarcity be an issue but water quality will be an issue that will also require the expertise of laboratorians to maintain.

10. *Human population will level off by 2035, while pet populations will increase dramatically.* This prediction has been corroborated by the findings of the American Veterinary Medical Association as well. More pets means more demand for testing services as well as a willingness to pay for perceived value.

Veterinary diagnostic laboratories will be affected, directly or indirectly, by many of these predicted changes. More specific changes can be grouped, loosely, into External Changes and Internal Changes. Examples of External Changes include changes in client characteristics, the global marketplace, food safety expectations, antibiotic resistance monitoring and the growing threat of bioterrorism. Internal changes for the laboratory may include growing quality assurance expectations, increased lab specialization, the addition of value-added services, implementation of new methodologies and impact of the revolution in information systems technologies.

External Changes

The characteristics of the laboratory's client base will change to reflect more advanced training and specialization among private veterinarians. The percentage of vets that go on to do a specialty internship or residency after graduation from veterinary school is growing. When these vets become clients of the laboratory their expectations for the amount, quality and specialization of the testing they want will be higher than their predecessors.

Both current and future veterinarians, as well as producers and pet owners, are becoming more electronically sophisticated and computer literate. In fact, many veterinary schools now require incoming students to be equipped with a computer as part of their standards for education. These clients will expect more contact with the laboratory through their computers (web pages, e-mail) and will expect laboratories to be able to utilize the power of computer technology to enhance the testing services offered.

The demographics of veterinarians are changing rapidly also. Many veterinary schools now report that their entering classes of veterinary students are over 80% female. One only has to read any of the multitude of popular works that discuss the differences in the way the sexes communicate to realize that this change in the gender balance among veterinarians will have ramifications for how laboratories will communicate with clients. Finally, more of the private veterinary clinics the lab deals with are likely to become larger, multi-doctor, private or corporate practices that will be looking for business economies of scale, including discounted rates for high volume testing services.

Another external force affecting labs will be the transition to a global marketplace that is taking place. The last negotiated General Agreement on Tariffs and Trade (GATT) established the World Trade Organization (WTO, *http://www.wto.org*) as a new arbiter of international trade practices. The WTO has designated the Organization International des Epizooites (OIE) to establish standards for the testing and movement of animals and animal products in international commerce. Although the OIE was established in 1924, this designation by the WTO has thrust it into a prominent role.

The OIE has adopted a list of monitored animal diseases divided into List A, those with the potential to inflict grave economic devastation, and List B, those with lesser but still important economic impact. It publishes a Manual of Standards for Diagnostic Tests and Vaccines, which defines the criteria for testing for the List A and List B diseases. Further, the OIE recently adopted a Standard for Management and Technical Requirements for Laboratories Conducting Tests for Infectious Animal Diseases, which is a major subset of the ISO 17025 standard for laboratory accreditation. More information on the OIE and its published standards is available from their web site at *http://www.oie.int*.

The trade rules established by the GATT are based on the "Golden Rule" of doing unto others as you would have them do unto you. Specifically, they embody the following principles:

> *Transparency* -- all aspects of trade, policies and mechanisms, must be documented and available for inspection by trading partners.
>
> *Equivalency* -- trading partners do not have to have the exact same infrastructure or processes, as long as they can show that they are equivalent in outcome.
>
> *Scientifically Based* -- trade barriers must be based on documented, scientifically based and verifiable reasons.
>
> *Infrastructure* -- a country must demonstrate that it has the adequate infrastructure for surveillance and monitoring of animal diseases including adequate laboratory capacity and quality as well as the regulatory infrastructure necessary to deal with animal diseases.

These principles will require laboratories to have documented, scientifically based procedures in place if their test results are to be accepted for certifying the health of animals in trade, both nationally and internationally.

The issue of Food Safety is currently a hot topic that promises to expand even further. Traditionally, food safety has encompassed both pathogens and chemical contaminants in food products and, to a lesser extent, the animals themselves. However, there is a new effort to expand the focus of food safety practices to include so-called "pre-harvest" food safety, which a euphemism for placing more stringent requirements in place at the farm level. Already, there are large packing companies in the swine industry that will only accept animals from farms that are certified under the National Pork Producer Council's Quality Assurance program. It is realistic to expect producers to be forced into pre-slaughter testing for common food safety pathogens and residues. This pre-harvest testing will need to have very fast turnaround times and be highly specific to prevent unnecessary interruption of the food supply. Newer techniques such as the Polymerase Chain Reaction (PCR) and immunomagnetic separation will be used to meet these needs.

Antibiotic resistance monitoring is another topic on the front burner for both veterinary and human medicine. A Federal Draft Action Plan has been proposed that contains more than 80 "action items" dealing with aspects of the development of antibiotic resistance in humans as well as food and companion animals. Ramifications for laboratories may include increased requests for monitoring therapeutic drug levels in companion animals as well as alterations in antibiotic susceptibility testing panels. In addition, the Food and Drug Administration (FDA) has proposed a "New Animal Drug Approval Framework Document" which drastically alters the process to be applied in consideration of applications for bringing new animal drugs to market as well as the likelihood of keeping an approved animal antibiotic on the market. This framework includes the assessment of the importance of each antibiotic for use in human medicine first, if it is a drug of last resort in human medicine, it will not be available for use in animals. If it is not an antibiotic of last resort, but is still one of the big guns among antibiotics, the new process will require post-approval monitoring of the development of antibiotic resistance to the drug and the drug will be pulled from the veterinary market if resistance rises above a designated action level. This type of monitoring will be carried out in the veterinary diagnostic labs with the data being made available to the FDA.

In the emerging arena of bioterrorism, the Federal Bureau of Investigation (FBI) has been designated by the President as the lead agency for investigation of all suspected incidents. Although initially only bioterrorism targeted directly at the human population was considered, realization has dawned that bioterrorism directed against the livestock and poultry industries would also have devastating consequences for the US economy. This pushes veterinary diagnostic labs into a prominent role for detecting such incidents quickly. The agents frequently mentioned as likely bioterrorist weapons, *Bacillus anthracis*, *Yersinia pestis*, *Clostridium botulinum*, are in fact much more familiar to veterinary labs than to human labs and there is therefore a movement underway to add the expertise of veterinary labs to the national surveillance system for bioterrorism. In addition, it is possible that a strictly animal disease agent such as one of the potentially devastating foreign animal diseases (Foot and Mouth, Classical Swine Fever) could be the agent used, in which case the veterinary labs would again be the logical first points of contact for detection. A National Laboratory Network for surveillance is being established utilizing both human and veterinary diagnostic labs. The veterinary effort is being coordinated through the LSU College of Veterinary Medicine and will initially enroll 20-25 veterinary labs across the US. The first goals of this network will be to establish standardized protocols for detection of the agents of concern as well as a proficiency testing program. The 2001 meeting of the AVMA will feature a symposium on bioterrorism issues.

Internal Changes

While Quality Control (QC) and Quality Assurance (QA) have long been an integral part of any reputable laboratory's standard operations, a movement is underway towards expanding these concepts, particularly QA. The new scope of QA will encompass not just the testing itself but also facilities, equipment, the working environment, staffing and training, Standard Operating Procedures, record keeping and result validation. This leads to a consideration of how veterinary labs will be recognized or accredited as to the quality of their work in the future. Currently, there is a smorgasbord of proficiency test panels available from the USDA's National Veterinary Services Laboratory (NVSL, primarily regulatory serology), the American Association of Veterinary Laboratory Diagnosticians (AAVLD, bacteriology and toxicology) and the Veterinary Laboratory Association (VLA, clinical chemistry, bacteriology, pathology, parasitology) among others. Still, significant gaps exist such that there are no independently verified proficiency panels for many tests and there are many tests being run that have never been validated. Only the AAVLD currently offers an "accreditation" program for state/university labs. The term "accreditation" is used in a very specific manner on the international scene, to denote a determination of competence on a test by test basis whereas AAVLD Accreditation has been based on QA of general laboratory operations, not

specific test competency. As external pressure increases to move towards international "accreditation" standards (all USDA labs are pursuing such internationally recognized accreditation), state labs will also need to move in that direction for their work to be accepted for national and international trade. The accepted international standard for general laboratory accreditation is set by the International Standards Organization (ISO) and is captured in their ISO 17025 document. A laboratory can only be accredited as ISO 17025 compliant by an internationally recognized accreditor of which there are only 2 in the US, one of which, the American Association for Laboratory Accreditation (A2LA), is moving into the area of diagnostic lab accreditation and will be the ISO 17025 accreditor for USDA's labs. The AAVLD does not anticipate becoming an ISO accreditor but rather may function as a resource of expertise for A2LA in their development of diagnostic lab accreditation specifics. However, the AAVLD will likely continue its "accreditation" program, which will be based closely on the recent OIE standard mentioned above. AAVLD accreditation started out as an effort to provide labs with an assessment of their strengths and weaknesses and to provide them with documentation that could be used to secure additional resources to improve the quality of their operations. The AAVLD believes this need will continue to exist.

The continuing addition of new test modalities (e.g. PCR, RFLP) and even new diseases to test for (e.g. West Nile Fever) will only reinforce the trend towards testing specialization. No laboratory can afford to "do it all" and with the availability of rapid transit of specimens and return of results, the additional delay in obtaining test results incurred by shipping specimens to outside labs has already decreased to less than 24 hours and may shrink even more. These developments will promote regional or even national cooperative testing arrangements.

Another internal change that is coming is what is referred to in the business world as "value-added services". This refers to taking a raw product and adding value to them, in the case of laboratory test results this may be in the form of providing pre-testing sampling guidelines or of adding interpretations or analyses to the raw data to make that data more useful to the client. This type of approach will require more integration of epidemiology into the laboratory to assist in herd or flock-level assessments of disease status. Instead of merely providing a table of positive/negative or titer values, an analysis or interpretation of those results, which incorporates knowledge of the sensitivity, specificity, and positive and negative predictive value of the test used will add value to the product. Labs may go even further through the use of analytical programs (e.g. Monte Carlo simulations, Baysian analysis, risk analysis software) to aid the client in making decisions based on the test results or to counsel them on additional testing strategies. Some labs have already begun to incorporate Geographic Information System (GIS) software into their arsenal of services, which facilitates both visualization of the geographic distribution of disease as well as temporal changes in that distribution.

As has already been referred to, there are multiple new methodologies, particularly in the area of molecular biology, that are moving into the mainstream of routine laboratory testing. The inventor of the Polymerase Chain Reaction (PCR) method recently was awarded the Nobel Prize for this powerful tool which permits the in vitro replication of billions of copies of a single strand of DNA or RNA, a technique which does not rely on the viability of the organism or, to a great extent, on the quality of the sample, two parameters that have long plagued the ability of the lab to derive useful and accurate results from the types of samples frequently submitted to the lab. Once sufficient copies of the organism's DNA or RNA are in hand, techniques such as Restriction Fragment Length Polymorphism (RFLP), which uses enzymes known to cleave nucleic acids at defined points along with Pulsed Field Gel Electrophoresis (PFGE), which uses an electric current to separate the fragments based on their charges and Monoclonal Antibodies (Mabs), which target known sequences of amino acids are used to obtain "fingerprints" of an organism that serve as a unique identifier down

to the species and even strain level. Besides being used to detect disease organisms, an interesting new application of these molecular diagnostic techniques is the recent adoption by horse breed registries of DNA typing to replace blood typing for registering purebred foals. A multidisciplinary team of engineering and genetic scientists at the University of Michigan has created a miniature "laboratory on a chip" that automatically analyzes DNA samples and reports the results electronically. The first working prototype of this machine is only the size of a nickel.

Another recently developed technique that will grow in application is the use of Immunomagnetic Beads, small plastic spheres with a metal core that are coated with a Mab targeted at a surface molecule of a particular organism. These beads are mixed with a specimen and then a magnet is used to separate the beads from the specimen mixture, pulling along with them the targeted organism if present to produce a concentrated residue of the organism for further testing. This simple technique can greatly improve the identification of an organism in a sample, even when it is only present in small numbers, by segregating it from other organisms in the sample that would mask it.

Recently, a team of Swiss researchers reported that a microfabricated silicon cantilever could be made to flex upon binding DNA. Immobilized on each 1 Fμm thick by 50 Fμm long cantilever were about 1010 molecules of a short segment of single strand DNA. Binding the complementary strand of DNA to form double strand DNA molecules caused the cantilever to bend. "We have found a way to get DNA to do the work for us, so we don't need batteries, motors, or the like to operate tiny machines," said James Gimzewski, a winner of the 1997 Feynman Prize in Nanotechnology. The National Aeronautics and Space Administration and the National Cancer Institute have a joint effort underway to develop “nano-explorers” based on this principle that could detect, diagnose and even treat diseases from inside the body. This technology could become an adjunct method of antibody or antigen detection as well.

The last internal change to be considered involves the information revolution that is currently underway. The future in hardware systems appears to be an effort to liberate the operator from the desk-bound computer hardware of today. One effort is the development of “wearable” computers. These devices are already commercially available, although the wearable models typically are not as powerful as the desktop PC in terms of processor speed or storage. They are already available in speeds up to 400 MHz however and use a small, clip on screen that extends in front of one eye which, because of its placement close to the eye, gives the appearance of a full-sized screen. Continued improvements in software, particularly voice-recognition software, will soon make manual data entry obsolete as well. The other hardware trend is the Personal Digital Assistant (PDA). The PDA has already reached a stage of development that includes color screens and database software that can be synchronized with a larger database through the desktop PC. Data entry on a PDA is currently via its touch sensitive screen but voice-recognition software may eventually be deployed on these miniature marvels as well. The PDA has quickly become the standard for carrying reference information and recording patient data among the human medical profession. It could easily be adapted as a portable tool to record test results on in the laboratory as well.

As was mentioned above, voice-recognition software has steadily improved and promises to break the ties to the keyboard that are a current constraint. Also, the use of an Internet portal for moving laboratory information both into and out of the lab could be of immense benefit by permitting the submitter to do most of the data entry for the submission, subject to review by the lab, which would relieve the data input bottleneck and to retrieve their results, resulting in substantial postage savings for the lab as well. Another significant trend in information handling will be a changeover from just delivering raw results to providing more interpretation of the data. This may involve the use of

standardized, statistical algorithms based on the sensitivity and specificity of a test to give clients better predictive values for the results or the incorporation of Geographic Information System software which will allow the client to visualize the spatial and temporal distribution of one to many rounds of testing.

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