The Newsletter of the Florida Mosquito Control Association Volume 6, Issue Number 5
Sept/Oct 2006

South Carolina Mosquito Control Association Annual Fall Meeting
Nov 1 – 3, 2006
Ocean Creek Resort, Myrtle Beach, SC
Visit the SCMCA website www.scmca.net for further information or contact Carolyn Morgan, SCMCA Sec./Treas. (803) 896-0655 or email: morgancl@dhec.sc.gov

Florida Mosquito Control Association
2006 Fall Conference
Nov 12 –15, 2006
Hutchinson Island Marriott Beach Resort and Marina, Stuart, FL
www.floridamosquito.org
Registration and last call for papers inside this issue of *BuzzWords*

The 2007 Annual American Mosquito Control Association Conference will be hosted by the Florida Mosquito Control Association in Orlando, FL
March 31 – April 5, 2007
The Peabody Orlando
www.mosquito.org

News from FMEL

Where did all the mosquitoes go?

The Florida Department of Agriculture and Consumer Services' mosquito reference collection has been moved to the Florida Medical Entomology Laboratory in Vero Beach. The adult specimens have been inspected and stored. The larval collection has been on loan, but will be housed at FMEL beginning November 2007.

The Florida mosquito reference and teaching collection that belonged to Dr. Richard F. Darsie, Jr., was donated to Dr. Roxanne Connelly's laboratory and is also housed at the FMEL.

Those in need of mosquito identification verification, or other information related to the collections, please contact Dr. Connelly at crr@ufl.edu or 772-778-7200. The FMEL is honored to have been selected as the recipient of these two major collections of Florida mosquito species.

2007 Advanced Mosquito Identification & Certification Course

The 2007 Advanced Mosquito Identification and Certification Course will be held March 5 – 16, 2007 at the Florida Medical Entomology Laboratory in Vero Beach, FL. Registration information and course application is posted at http://mosquito.ifas.ufl.edu under "Identification Course." Registration will be limited to 20 qualified students. As the issue of *BuzzWords* goes to press, there are 10 openings remaining.

Call for Student Papers
AMCA Student Paper Competition
2007 American Mosquito Control Association Annual Meeting
Orlando, Florida: Peabody Hotel
April 1 – 5, 2007

Hollandsworth Prize	***$1,000.00***
Honorable Mention (up to 2)	***$ 500.00***

Undergraduate and graduate student members of the AMCA are eligible to participate

Students must be enrolled in an undergraduate or graduate program at the time of submitting the title and abstract, and must be AMCA members at the time of the competition

Award will be based on written title and abstract, presentation, organization, delivery, and quality of research. For more details, or to submit a title and abstract, see www.mosquito.org - at the top menu click on "meetings" and then on the right, click on "student papers"

Or contact Dr. Roxanne Connelly at crr@ufl.edu

Deadline for submitting title and abstract is *November 15, 2006*

Call for Volunteers for 2007 AMCA Meeting in Orlando, Florida

Committee Chairs for the AMCA Meeting

If you are chairing a committee for the AMCA meeting in Orlando, Florida, please provide a list of the number of volunteers that you anticipate needing for assistance during the meeting. Include a description of the duties, how much time will be requested, and any expertise that is desirable.

Volunteers for the AMCA Meeting

If you are able to volunteer to help out during the meeting, please provide information on how much time you can devote to volunteering, what days you would be available, and any expertise that you have so that you can be assigned to areas that would make best use of your talents.

Areas where volunteers are needed:

Bag Stuffers – Thursday & Friday only

Banquet

General

**Projectionists - CURRENTLY IN HIGH DEMAND!

Registration

Room Attendants

Silent Auction Drop Off

Forward all information to George Heinlein at: gheinlein1121@hotmail.com or call George at Indian River Mosquito Control District - 772-562-2393

Please be aware that registration for the meeting is not automatically waived for volunteers. However, the registration cost for volunteers is at the AMCA member rate, whether or not the volunteer is an AMCA member. As the host organization, the FMCA is provided a limited number of complimentary registrations for volunteers by the AMCA. Should you need a complimentary registration to participate as a volunteer, please contact Kellie Etherson or Doug Carlson ethersonk@cityofgainesville.org dcarlson1@yahoo.com

Florida Mosquito Control Association, Inc.

FINAL CALL FOR PAPERS

2006 ANNUAL FALL MEETING

Hutchinson Island Marriott Beach Resort & Marina
555 NE Ocean Boulevard, Stuart, FL 34996
772.225.3700
November 12 – 15, 2006

You are invited to submit a title for a paper to be presented at the 2006 Annual Fall Meeting of the Florida Mosquito Control Association, Inc. to be held at the Hutchinson Island Resort on Hutchinson Island in Stuart, FL, November 12 – 15, 2006. Type the title, author(s), organization(s), and address (es) exactly the way they are to appear on the program. If more than one author is listed, place an asterisk after the name of the author who is to present the paper. **Send this form to Bill Reynolds, ADAPCO, Inc., 2800 South Financial Court, Sanford, FL, 32773-8118, E-mail: wreynolds@e-adapco.com, Telephone: 800.367.0659; FAX: 866.330.9888.** Submission deadline is **October 10, 2006**.

TITLE: _____

_____

AUTHOR: (INCLUDE E-MAIL, TELEPHONE AND FAX NUMBERS OF PRESENTER)

1. _____

_____

2. _____

3. _____

ORGANIZATION:

1. _____

2. _____

3. _____

MAILING ADDRESS:

1. _____

2. _____

3. _____

REQUESTED DURATION OF PRESENTATION: ____ 10 min ____ 15 min ____ Symposium ____ Other

AUDIO/VISUAL EQUIPMENT REQUIRED: ___ Slide ___ LCD ____ Overhead _____ Other (please specify)

PAPER CATEGORY: ___ Research ___ Operations ____ Regulatory _____ Other (please specify)

Florida Mosquito Control Association
78th Annual Fall Meeting
Registration Form
Federal ID # 59-1819301
PO Box 358630, Gainesville, FL 32635-8630

The 2006 FMCA Annual Fall Meeting will begin at 1:00 p.m. on Sunday, November 12th with the FMCA Board of Director's Meeting. The general program will begin at 8:00 a.m. on Monday, November 13th and conclude at 12:00 p.m. on Wednesday, November 15th. The meeting will be held at Hutchinson Island Marriott Beach Resort & Marina, Stuart, FL.

Registration form must be faxed (352.334.2286) or mailed by November 1, 2006, for advance registration fees. There will be no refunds given after November 7, 2006. If you have any questions, please call Kellie Etherson at 352.281.3020 or email her at ethersonk@cityofgainesville.org. Please type or legibly print the following information:

Name:_____
Agency_____
Address:_____
City: _____ State: _____ Zip: _____
Phone:_____
Fax:_____Email:_____

	Advance Registration	On-Site Registration
Director	$250 _____	$350 _____
Commissioner	$250 _____	$350 _____
General Member	$225 _____	$315 _____
Student	$125 _____	$175 _____
Non-Member Director	$350 _____	$490 _____
Non-Member Commissioner	$350 _____	$490 _____
Non-Member General	$315 _____	$440 _____
Exhibitor (Comp.)	_____	
Extra Banquet Ticket	$ 65 _____	
Companion	$125 _____	
Name _____		
Dues (General Member)	$ 35 _____	
Dues (other)	$ _____	

TOTAL AMOUNT DUE: $ _____

Dues may be included and paid with the meeting registration

Please check all title boxes that apply:

- ☐ Speaker
- ☐ FMCA Past President
- ☐ Exhibitor
- ☐ Sustaining Member
- ☐ Other_____

For room reservations:

Hutchinson Island Marriott
555 NE Ocean Boulevard
Stuart, Florida 34996
1.772.225.3700/1.800.775.5936
www.marriott.com
On-line reservation code: FLAFLAA
Room Rate: $125/night

These rates are subject to state and local tax and resort fees. Please identify yourself as attending the Florida Mosquito Control Association meeting. You will not be considered tax-exempt unless you pay using a tax-exempt agency check or credit card with the agency name on it. You must have a copy of your tax exemption certificate. The cut-off date for our group rate is October 18, 2006.

Polymerase Chain Reaction: Detecting the invisible presence of biological weapons and future benefits for mosquito-borne disease surveillance

With the advent of the recent scares of anthrax in mail and subsequent closing of post-offices and other buildings, the technique of polymerase chain reaction (PCR) has gained public attention. Mosquito-borne disease specialists routinely use PCR to detect small amounts of viruses like West Nile virus and St. Louis encephalitis virus. One might ask how PCR can detect biological reagents when only small amounts are available. A good analogy for how is that PCR allows you to look for that needle in a haystack. If, for example, one spore of anthrax or a single West Nile virus is in a sample, PCR is sensitive enough to detect them. Here I will discuss PCR and provide an example of how this extremely sensitive method is employed.

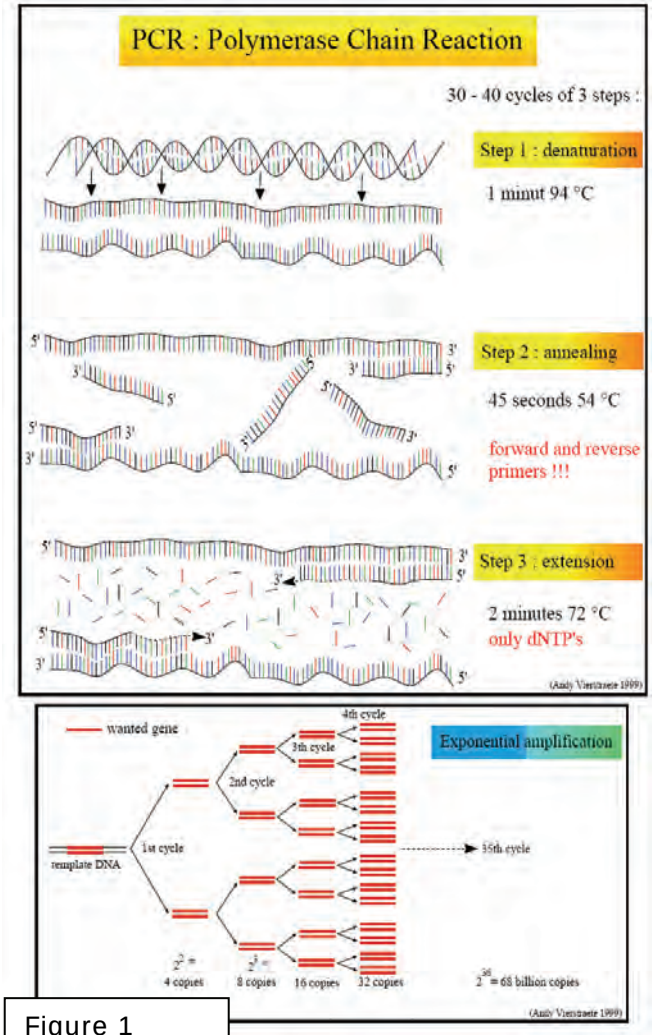


Figure 1

The PCR technique was invented in 1985 by Kary B. Mullis (Saiki et al,. 1985; Mullis and Faloona, 1987), a scientist working at a biotechnology firm called Cetus Corporation. His discovery allowed scientists to make a million copies of a scarce sample of DNA and revolutionized the diagnosis of genetic defects, the detection of the AIDS virus in human cells, and criminology, by allowing a link to be made between a specific person and trace samples of blood or hair left at a crime scene. This technique has also been used to detect trace amounts of biological signatures of viruses, spores and bacteria.

Almost any DNA sample can be used in a PCR reaction, including DNA samples over 7000 years old. Additionally, PCR may be sensitive enough to detect and amplify trace amounts of the DNA target, corresponding to one molecule. Unfortunately, when very small amounts of DNA are used, contamination of the PCR reaction can become a major problem. Contamination may come from a variety of sources, including the researcher who is performing the experiment, the tubes that are being used to set up the reaction and even the enzymes and buffers used in the reaction itself. To reduce the likelihood of contaminating sequences interfering with the desired amplification, a PCR experiment should have between 0.1 and 1 microgram of genomic DNA (note 1000 micrograms equal 1 gram). How many copies of the target DNA does this amount to? Approximately 144,000 molecules of your target sequence.

Basically, PCR is the repetitive copying of a section of double-stranded DNA in a short period of time. Two target specific oligonucleotide primers that flank the DNA target are used. One primer binds to the bottom strand (antisense strand) while the other primer binds to the top strand (sense strand) of the two-stranded DNA molecule.

The PCR reaction consists of three stages each performed at a different temperature: Denaturation, Annealing, and Extension (Fig. 1). The target DNA is first extracted from the trace sample into a test tube. Then the test tube is heated to a high temperature causing the separation of the two strands of the target DNA molecule. This step is called Denaturation. Next the oligonucleotide primers, along with other components that are needed to complete the actual PCR reaction, are added to the test tube containing the denatured DNA. The two target strands are allowed to cool in the presence of the primers. The primers are specific for and bind to opposite ends of the specific target DNA segment of interest at a specific temperature that is related to the length and sequence of the primer. The binding of the primers is called

Annealing. An enzyme, DNA polymerase, which functions like a crane that lifts cement blocks into the frame of a building, is added to the test tube. The DNA polymerase binds to the free ends of the primers and adds free nucleotides, similar to the cement blocks, in a sequence specific manner, and makes new DNA strands. This step is called Extension and is carried out at a higher temperature than the annealing temperature. Successive rounds of new DNA strand generation follows by continuing to increase and decrease the temperature for more rounds of denaturation, annealing and extension. At the end of each round the number of DNA molecules doubles so that with 30 -40 rounds you have many millions of copies of the target DNA. Because of the specific nature of the PCR design, a positive PCR experiment should generate a product of a specific size. Depending on what answers were being sought using PCR, the presence or absence of a band could be the difference between exposure to a biological weapon or not. Unfortunately, because of the sensitivity of the PCR reaction, there are many steps where contaminants can be introduced, confounding the findings and making analysis inconclusive.

Certainly PCR has revolutionized our ability to detect microorganisms like many mosquito-borne pathogens. It has also revolutionized the detection of biological weapons.

Biological weapons, also called the third weapon of mass destruction, have been around since the Middle Ages when soldiers catapulted the bodies of dead smallpox victims over fortress walls in the hope of infecting their enemies. Biological agents, for the most part, are easily manufactured, transported and dispensed. Of concern is the lag time between a biological attack and the appearance of the symptoms in those exposed to the biological agent. Because many biological agents are contagious, this lag time could provide time for an infected person to spread the disease. To address this growing concern many laboratories are working together to increase our capabilities to detect and respond to an attack by biological weapons. Using state of the art bio-detector technology, a portable PCR machine that is fully automated for real time sample collection has been developed. Because of its small size and efficiency, this PCR machine permits identification of biological organisms within minutes even when concentrations are low.

One can see that the bio-detector technology and portable PCR machines hold great promise for adding to our capabilities for assessing the risk due to mosquito-borne pathogens. Imagine the day when we will have such equipment to monitor our sentinels in real time for the presence of mosquito-borne pathogens.

Chelsea T. Smartt, Ph.D. Assistant Professor
UF/IFAS/Florida Medical Entomology Laboratory
Department of Entomology and Nematology

References

Mullis, K.B. and Faloona, F.A. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol.* 155:335-50.

Reese, R.J. 2004. Analysis of Genes and Genomes. John Wiley &Sons, Ltc. England.

Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A. and Arnheim, N. 1985. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*. 230(4732):1350-4.

Sentinel Chicken Surveillance: Some Pitfalls in Analyzing the Data

Florida mosquito control organizations, the Florida Department of Health, and the Florida Department of Agriculture and Consumer Services rely on the information provided by Florida's sentinel chicken surveillance programs. The data from the sentinel flocks are perhaps our best line of defense against West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) transmission in Florida.

In this summary we urge those involved in sentinel chicken surveillance to appreciate the potential pitfalls of the way sentinel chicken surveillance is conducted, particularly during

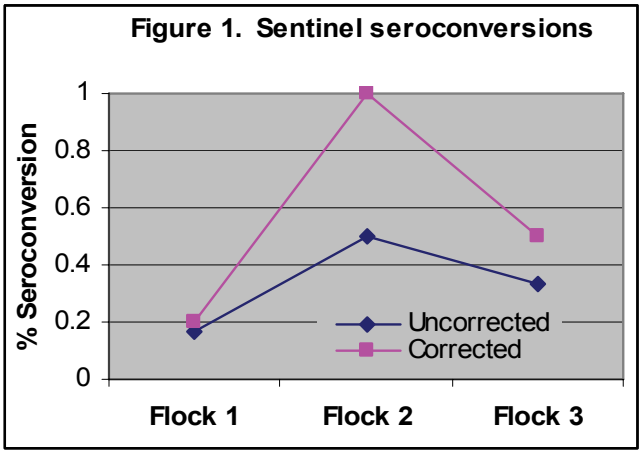
times of high transmission. The pitfalls could result in false interpretations of the surveillance data.

The most important point to realize is that our sentinel chickens provide information based on seroconversions to eastern equine encephalitis virus (EEEV), SLEV, and WNV. Chickens produce antibody ca. 14 days after infection. A seropositive chicken was therefore infected ca. 2 weeks prior to the detection of antibody. During this two week antibody "incubation" period, the chicken is essentially out of the information loop. Therefore, these antibody-positive sentinel chickens are positive for 2 weeks prior to antibody detection, but do not provide any useful surveillance data. The positive chicken is effectively dead as far as its sentinel surveillance usefulness once it is infected and before it produces detectable levels of antibody. This fact must be understood to thoroughly appreciate the sequence of events associated with sentinel chicken surveillance. Furthermore, an understanding of these events may help jurisdictions reduce the number of sentinel chicken sera they analyzed and help to reduce the cost of these surveillance programs.

Let's assess the impact of this delay in antibody production on sentinel chicken surveillance programs. Consider a flock of 10 sentinel chickens that are bled once a week. During week 1, two of the chickens are reported as antibody-positive and they are replaced with two uninfected chickens. During week 2, two additional sentinel chickens are reported as antibody-positive. One might believe the seroconversion rate for both weeks was the same – 20% (2/10 positive sentinels). This, however, is incorrect because during the second week there were only 8 susceptible chickens in the flock since two are replacements that could not show detectable antibody. Therefore, the actual seroconversion rate for the second week is 2/8 or 25%. Failure to recognize the fact that sentinel chickens require ca. 2 weeks to seroconvert, and once they do are no longer effective sentinels, can seriously underestimate the actual weekly seroconversion rate, because the denominator used to calculate the rate is reduced directly by previous seroconversions.

Let's consider another example. One seropositive sentinel chicken is reported in a flock of six birds. This positive sentinel is removed from the flock and replaced with a seronegative sentinel. It is important to remember that even if this new sentinel is bitten by an infected mosquito on the first night it is placed into the flock, it will not be detected as positive for two weeks. Therefore, it is not possible to detect transmission to this chicken for two weeks and it is not necessary to even bleed this bird until the second week after it is placed into the flock. Furthermore, in this example there are really only five susceptible sentinels in this flock following infection of the first bird. Therefore, the number of birds tested during the second week is five, not six. During the second week of sampling, an additional seroconversion will result in a seroconversion rate of 1/5 = 20% and **not** 1/6 = 17%. Likewise, if two additional sentinels from this flock seroconvert during week 3, the total number of susceptible chickens in the flock is three. The single sentinel added during Week 1 and the two sentinels added during Week 2 have not been in the field long enough to seroconvert. Therefore, the seroconversion rate for this flock during Week 3 is 2/3 = 67% **and not** 2/6 = 33%. It should be evident that it is essential to accurately track the true number of susceptible birds in each and every sentinel flock. Failure to track the number of susceptible sentinel chickens in a flock will significantly change the denominator used to calculate the flock-specific seroconversion rate, and may have a profound effect on data interpretation, especially when there are substantial seroconversions.

Figure 1 shows the difference in seroconversion rates in three sentinel flocks during a two week period based on the seroconversion reports shown in Table 1. Note the profound effects in the seroconversion rate for Flock 2 where 3/6 birds seroconverted during Week 1. Therefore, the number of susceptible sentinels in Flock 2 during Week 2 will be three, **not** 6. Also note that by carefully tracking the seropositive



chickens and by not analyzing sera that have no chance of being antibody-positive, the number of

test samples, and cost of the surveillance program can be reduced. In this example, only 12 birds need to be tested during Week 2, not all 18. Again, it is important to track the seroconversion status of individual chickens and to remember that there will be a 2-week seroconversion lag for infected birds. As an extreme example, consider the possibility that an entire flock of six sentinels is infected on the same night. These chickens will remain in the flock for at least two more weeks until they seroconvert. The replacement birds will not provide data for another two weeks, even if they are infected on the first night they are placed into the field. This flock will not provide any meaningful surveillance data for at least a month following the first round of transmission highlighting the importance of early-season transmission data in forecasting potential epidemic situations.

It is, of course, always interesting to review real-life situations. Pinellas County sentinel chicken surveillance during 2005 indicated the possibility of significant levels of WNV transmission in the southern part of the county. The number of sentinel chicken seroconversions reported in Pinellas County for the weeks of 7/5, 7/11, 7/25, and 8/1/2005 were 3, 7, 12, and 7 respectively. The overall seroconversion rate for Pinellas' 56 birds was 5, 12.5, 21, and 12.5% respectively. However, if the total number of sentinels is corrected for the presence of susceptible chickens, then the **real** seroconversion rates are 5, 13, 26, and 19%, respectively. This effect is even more pronounced if we focus only on the three flocks in southern Pinellas County where WNV transmission was focused.

The important message here is to keep track of the weekly status of sentinel chickens. Remember, there is a two week lag between the time the chicken is infected and the time that antibody becomes detectable. One way to reduce the impact of this lag, especially during periods of high transmission, is to routinely replace all of the chickens in flocks located in areas of high transmission and to hold the exposed birds for two weeks to assess seroconversion rates. In this way you would maintain the full complement of susceptible sentinels each week, despite the extensive levels of transmission. Instances when this extreme level of sentinel chicken surveillance is necessary would be extremely rare. We know that early season (June and July) arboviral transmission to sentinel chickens is far more indicative of potential epidemic transmission than is late season arboviral transmission to sentinel chickens. Having a full component of susceptible sentinels in the field during these two months is critical to forecasting arboviral epidemics. The weekly, or biweekly, cycling of selected flocks would provide accurate transmission information for each week.

We have urged districts to consider using an ARDS strategy in selected areas of their counties to accurately assess the spatio-temporal distribution of high levels of early season arboviral transmission (Tabachnick et. al. 2005. Buzzwords 5(2): 6). The ARDS surveillance strategy, implemented only during very selected times in very selected places, reduces the negative impact of keeping chickens that have seroconverted and are non-susceptible as part of the exposed flocks.

Just some food for thought.

Walter J. Tabachnick,
Director/Professor and Jonathan Day,
Professor
Florida Medical Entomology Laboratory
University of Florida/IFAS

	Flock Size	No. seroconverting	% serconversion	corrected total flock size	corrected seroconversion
Week1					
Flock 1	6	1	0.16666667		
Flock 2	6	3	0.5		
Flock 3	6	2	0.33333333		
Week 2					
Flock 1	6	1	0.16666667	5	0.2
Flock 2	6	3	0.5	3	1
Flock 3	6	2	0.33333333	4	0.5

Deadline for submissions to be included in the November/December 2006 issue of *Buzz Words* is December 5, 2006. Please send articles and change of address information to:

Dr. Roxanne Connelly, Editor, FMEL
200 9th Street S.E., Vero Beach, FL 32962 or buzzwords@ifas.ufl.edu