Report on Visit to the National Tick Collection, Statesboro, Georgia

I had the privilege of visiting the **Institute of Arthropodology and Parasitology, the National Tick Museum,** and Dr James Oliver and his colleagues Dr Lorenz Beati, and Dr Craig Banks on March 22, 2006. Dr Lauri Byerley, Krishna Shah, and Michelle Sellers of the Jemsek Research Team also attended. Dr. Oliver is a renowned tick researcher and has published numerous studies on ticks and the infections they carry, especially in the southern US. The collection is located at Georgia Southern University in Statesboro, Georgia and is owned by the Smithsonian. The museum is planning on creating a virtual library of all tick articles.

The information below has been edited from a visit summary written by Krishna Shah, MS.

Information from our discussion

A recent project for the Institute is collecting 24,000 (!) live *Ixodes scapularis* (black-legged ticks) for Pfizer to test for a new tick repellent. To collect this many Institute personnel are doing tick drags on an island off the Georgia coastline.

Dr Craig Banks mentioned the edge effect where the ticks are concentrated. The ticks congregate in the grassy area just on the edge of a road where they are likely to pick up hosts or blood meals. On the island where they are collecting the ticks are confined by the road on one side and the ocean on the other side. They are so numerous they are visible as dark clumps on the sea oats along the road at the level that gives them accessibility to deer or humans passing by. The edge effect is seen in any place where grassy areas join woods. The ticks aren't necessarily found just in the woods.

Ticks sometimes play possum on grass stalks. They will lie on top of grass or leaves or oat stalks without any movement. If you blow on them, they will extend their legs and move toward the source of the CO2. The ticks are attracted to carbon dioxide which is the reason it is used to make tick traps.

Transmission ability of ticks is defined by:
- Ability of Vector to pick up the bacteria
- Ability to maintain infection across molting
- Ability to transmit infection to a host

Sample size for survey of ticks in a particular area is not known. No standard number is set in the entomology field to get an accurate survey of an area. It is suggested that a large population of ticks should be collected.

Reservoir host species have a higher prevalence than the vector species of the bacteria. A focal point of concentration seen in an infection area usually corresponds to the territory range of the reservoir host. For example, mice have a small territory and can infect the ticks in that area. Deer can move from one focal point to another spreading the bacteria in a larger area. Birds, however, have a large territory range and can spread infections over large areas. Ticks can survive falls of a bird in mid-flight. Therefore, birds can spread Borrelia over their flight path.

Ticks usually don't switch hosts once they are attached. Unless the host dies they prefer to stay where the blood meal is. Ticks can survive the winter because they go underground until warmer weather. However, a sudden frost can kill them off. Or, they may overwinter on a deer or other suitable animal.

Natural predators of ticks are: Ox pecker bird (in Africa), Fire ants, Rodents, Guinea hens

In addition to *I. scapularis*, *I. afenis* and *I. minor* are most often infected with Borrelia.

Five fossils of ticks have been found to date. They are very similar to the structures of ticks now.

The Dermacenter tick does not transmit *Borrelia burgdorferi* but can transmit bacteria that causes relapsing fever.

Overview of traits of *I. scapularis* and *A. americanum*:
***Ixodes scapularis* (Blacklegged tick [deer])**

- Loves the cool weather so the moisture from the ocean is ideal for them. They hide out when the sun comes up.
- They prefer 98-100% humidity.
- The adult scapularis is active over winter but not during the summer because of the heat.
- The larva and nymphs love moisture and stay in the ground. In the south one needs to trap lizards and skinks to collect them.
- Can't pick up by flagging in the south because of their nature to stay closer to the cooler ground.
- No difference in host selection from north to south except for availability as they are dependent on what host is available.
- Since higher number of lizards in the south and scapularis like the cool moisture of the ground, a higher number of *I scapularis* ticks are found on the lizards in the south.
- The nymph's quest higher up in the north because there aren't as many hosts available closer to the ground.
- Can collect larva/nymph's by dragging if one person goes ahead kicking over leaves and another following with a flag.
- Imbeds into the hosts and usually leaves a mark—mouthpart is longer.
- Is not a hardy tick in the lab—doesn't have a long life.
- January/February is the peak month to get ticks off deer. If the tick has gone through the winter, it is weathered and will be sturdier.

***Amblyomma americanum* (Lone Star tick)**

- Love the sun. Come out of hiding to soak in the sun when it comes up.
- They prefer 90% humidity.
- Are very active and move fast.
- They are a very aggressive species and will attach to any host that is available to feed.
- Can easily be picked up by flagging.
- Doesn't leave a mark at the bite. Their mouthparts are shorter.

***Borrelia burgdorferi* (Bb)**
There are 250 strains of Bb in the south alone. Only some of the strains are pathogenic. It is suggested that the plasmids make them pathogenic and so it is important to find out which of the plasmids make a particular strain pathogenic. This will be harder to show in vitro as well as in vivo. Roughly 20% of the 250 strains can be grown in BSK-II media.

Two new species of Borrelia, MI8 and MI2 were found recently. They do not fit into the *B. andersoni, B. lonestari or B. burgdorferi* species. They have been isolated from rodents and are under the *B. burgdorferi* senu lato group.

Borrelia burgdorferi sensu stricto and *Borrelia basseti* are closely related. Basseti can cause Lyme disease. The B31 antigens will not pick up basseti species and they do not culture well. Basseti was found in central Europe.

The Georgia health officials also claim that there is no "Bb in GA". Dr Oliver has been working for the last 15 years to show Bb is in the south. Statistically, Lyme disease can be made less prevalent in the US by narrowing the definition of "Lyme Disease". The number of people who will fit the definition of "true Lyme disease" will become smaller and smaller with stricter definitions.

Dr Oliver believes that the presence of the 31 kDa (OspA) and 22-25 kDa (OspC) protein bands is evidence of Lyme disease. Also the 41 kDa (flagallin) protein band is indicative of Borrelia infection but does not tell you whether it is *B bergdorferi*. These three proteins alone, however, do not meet the CDC criteria.

Definition of *endemic*: an infection is present from year to year. This would qualify North Carolina as an endemic region for *Borrelia burgdorferi*. Definition of rare is subjective based on the persons who are responsible for setting up the criteria. It is best to avoid the use of this word in epidemiology.

Dr Oliver states that one of the reasons the Western Blot serology is negative is due to the exclusive use of B31 strain antigens. This will make getting positive results in the south harder. He believes that the Borrelia species has been around much longer in the south and has had a chance to differentiate. So a cocktail of species infect people down here and they will be harder to identify with antigens just from the B31 strain. In the North the organism has not differentiated as much. People may have asymptomatic infections with Bb.

Dr Oliver collaborates with Dr Kerry Clark (FL) who found Bb in lizards as well as other animals. Dr Oliver has done culture work for Bb but isn't as familiar with molecular biology. He will be working with Clark on the molecular biology techniques to study Bb.

Borrelia is a tissue parasite so cannot be found readily in the blood. The ear area of mice and deer are usually good places to look for Borrelia.

There are no known toxins released by the bacteria to date.

Borrelia lonestari

Dr. Alan Barbour holds a patent for lonestari primer set and PCR method to detect lonestari. It has not yet been definitively shown to cause disease or to be the cause of 'southern Lyme disease."

Babesia

Dr Clark has found Babesia DNA in humans. *I. scapularis* is transmitting Babesia. Classical Babesia is microti and divergens. One patient with Babesia has died. He had a splenectomy which may be a contributing factor to the death.

Procedures performed

Nested PCR done for Borrelia. First set of primers: 16S rRNA (Universal primers). This identifies whether Borrelia is present. These primers are for a highly conserved gene of Borrelias. The second set of primers is for specific species of Borrelia. Their DNA extraction procedure will isolate DNA from cysts and vegetative forms. Nested PCR can have contamination problems so have to be careful from DNA isolation step to the PCR reaction.

Other activities

We toured the tick collections and looked at several from other countries under the microscope. The collection also has almost every study and article that has been published about ticks or the organisms they carry.

The Assistant Curator, Dr. Beati, would like *I scapularis* ticks from our region for her genetic studies. Please contact info@tic-nc.org if you think you will be able to find specimens and would be willing to submit them.

Edited by Marcia E. Herman-Giddens

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