Poster 38: Cattle Fever Tick, *Rhipicephalus annulatus* (Acari: Ixodidae), and the Quest for Discovery of Its Natural Enemies in the Balkan Region

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Rhipicephalus annulatus Say (Ixodida: Ixodidae), the Cattle Fever Tick (CFT), is a hard tick native to Mediterranean region with several hosts such as cattle and white tailed deer. It transmits two lethal diseases, piroplasmosis and babesiosis. It is an invasive species in US, with a significant impact on cattle production especially in Texas. Extensive use of acaricides resulted in resistance. Special attention is given to the Balkan region for discovery of natural enemies since molecular analysis of CFT showed that the Texas population is similar to those of Bulgaria and Romania. Biological control of CFT is the main objective of this study. There are few examples of successful classical biological control of livestock pests such as blood-feeding bush flies, *Musca vetustissima* Walker, and buffalo flies, *Haematobia irritans* (L.) (Diptera: Muscidae) (Waterhouse, 1974). Attempts for possible biological control have been focused on the use of known parasitic wasps of the genera *Ixodiphagous* (Hymenoptera: Encyrtidae). There are seven recognized species: *Ixodiphagus texanus* Howard, *Ixodiphagus hookeri* (Howard), *Ixodiphagus mysorensis* Mani, *Ixodiphagus hirtus* Nikolskaya, *Ixodiphagus theilerae* (Fielder), *Ixodiphagus biroi* Erdos, and *Ixodiphagus sagarensis* (Geevarghese). These parasitic wasps parasitize ticks belonging to the genera *Ornithodoros*, *Amblyomma*, *Dermacentor*, *Hyalomma*, *Haemaphysalis*, *Ixodes*, and *Rhipicephalus*. These parasitic wasps seem to have 1-2 generations per year (Hu *et al*., 1998).

Nematodes have also been investigated as biological control agents for ticks (Samish and Glazer, 1991; Samish and Rehacek, 1999; Samish *et al*., 2004). Most of the research has involved use of commercially available entomopathogenic nematodes of the families Heterorhabditidae and Steinernematidae. The only free-living stage of the nematode, the third/infective juvenile, actively locates and enters the host via natural openings, and then

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releases symbiotic bacteria that kill the host within 24–72 h. The nematodes then multiply within the host cadaver, and by 6–18 days post infection, thousands of infective juveniles are released into the environment. However, currently available entomopathogenic nematodes such as *Steinernama carpocapse*, can affect and kill CFT, but do not reproduce (Samish *et al*., 2004, Molina-Ochoa *et al*., 2009).

Extensive travelling and cooperation with various institutions and small family farms which are not using any or have very restricted use of acaricide products in Greece, Turkey, Albania and Bulgaria resulted in the creation of a wide network of potential tick exposure sites which we hope to result in location and development of specialized parasitoids or entomopathogenic nematodes for biological control of CFT.

Materials and Methods: In each of three locations in Greece and Bulgaria, two, six to nine months old calves were used for exposure of *Rhipicephalus annulatus* to its natural enemies. Two thousand laboratory reared tick larvae were transferred to the back of the calves which were kept in a cattle chute. They were given enough time to seek the location of their preference on the calves where they would chose to have their blood meal. The calves were then released from the chute into a large pen or into nature for feeding and the exposure of ticks to their possible natural enemies for 25 days. After this they would be returned to a smaller, approximately 5 m x 5 m pen in which the ground would be cleaned beforehand to be able to see the falling engorged ticks easier. Claves would be fed in this pen and the ground of the pen would be twice checked for the presence of fallen, engorged ticks. These were collected and transferred to a vial covered with a piece of cotton for air exchange. All the vials were kept in an incubator at 27°C, 80% RH and 12 hours light and 12 hours darkness for emergence of possible parasitoids or entomopathogens.

Soils samples from resting places of cattle, sheep and goats from various regions of the above mentioned countries were collected. Wax moth, *Galleria mellonella* (Fabricius) (Lepidoptera: Pyralidae), larvae were exposed these soils to detect nematodes which could attack the ticks feeding on the cattle, sheep and goat.

Tick eggs were exposed to possible egg parasitoids and this was documented by using a Brinno camera taking pictures every 1 sec. The same method was used to find out whether any predators would feed on seeking larvae.

Reared parasitoids or nematodes then will be used for host specificity tests to determine host specificity and whether their uses for biological control of cattle fever tick could be recommended.

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