## United States Department of Agriculture

Cooperative State
Research, Education, and
Extension Service

2002 No.3

## Vaccine against horn flies could curb economic losses

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he horn fly is a major insect pest in many parts of the world. Adult flies of both sexes take blood frequently throughout the day from cattle and other livestock, causing losses in meat and milk production and also damaging hides by transmitting a nematode skin parasite.

The economic impact of losses from the horn fly in North America approaches \$1 billion a year.

Control of the horn fly is difficult because of its widespread tolerance to most kinds of insecticides and its ability to develop resistance rapidly to new products. With support from USDA's National Research Initiative (NRI), researchers at Auburn University are taking a novel approach to offer an economically and biologically feasible alternative.

The researchers are developing a vaccine that, if successful, will prevent blood feeding on cattle and other hosts by adult horn flies, and thereby deprive these insects of the nourishment they need for maintenance and reproduction.

To identify possible targets for vaccine development, the researchers first collected large quantities of horn fly saliva for biochemical analysis (see photo).

## HORN FLY SALIVA, Using saliva accumulated fr

Using saliva accumulated from approximately 2,500 flies, the researchers identified a major factor that inhibits thrombin, a key enzyme important in stimulating coagulation.

By inhibiting thrombin, the horn fly salivary factor slows blood clotting to keep it in a liquid state for easy consumption. Because of its specific mode of

TO COLLECT HORN FLY SALIVA,
THE RESEARCHERS TETHER AN
ADULT FLY TO A WOODEN STICK
AND INSERT ITS PROBOSCIS INTO
A SMALL GLASS TUBE CONTAINING
MINERAL OIL. FOLLOWING INJECTION WITH SEROTONIN, THE FLY
SALIVATES INTO THE OIL. THE
INSET SHOWS THE TIP OF A HORN
FLY'S PROBOSCIS.



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action, this protein molecule was designated as "thrombostasin."

If this horn fly factor were to be introduced into livestock as a vaccine, it could potentially stimulate a cow's immune system and protect cattle and other livestock from further feeding by the horn flies.

Purification of thrombostasin from horn fly saliva and analysis of its molecular structure led to the cloning of its cDNA from a salivary gland library.

The flies used for this part of the study came from a continuous colony maintained by the USDA-Agricultural Research Service (ARS) Knipling-Bushland Livestock Laboratory in Kerrville, Texas. Using standard laboratory procedures, a recombinant form of thrombostasin is now being produced in enough quantity to evaluate its potential as a vaccine candidate.

Analysis of its molecular structure indicates that thrombostasin is a unique protein encoded initially as a "pre-protein," which means that it is produced first as a long molecule that is subsequently processed to the shorter, active form found in saliva.

This characteristic is unlike antithrombin proteins described in the saliva of other blood-feeding insects such as mosquitoes and black flies. It indicates a greater level of complexity in the biochemical mechanisms and evolution of blood feeding by medically important insects and ticks than was previously understood.

Thrombostasin is the first molecule of its kind described from the Stomoxyinae, an important group of flies found around the world, which includes several other pests of livestock.

## **IMPACT**

Anti-feeding vaccines for blood-feeding flies are attractive for several reasons – ease of administration, specificity for a particular target species, longevity of effect, and environmental acceptability.

Success in identifying, purifying, and subsequently producing a recombinant protein such as thrombostasin for vaccine study demonstrates the possibility of using crucial salivary factors as antigens.

This approach is particularly appealing for controlling insects such as horn flies and related pests of livestock that do not have a wide array of anti-clotting factors in their saliva.

Studies are currently under way using a rabbit model to determine if vaccination can stimulate the formation of antibodies specifically to thrombostasin and if blood feeding by horn flies on an immunized animal limits the amount of blood they ingest. If these are successful, a final trial in cattle is planned.





The research reported in this factsheet was sponsored by the Entomology & Nematology Program of the Biology and Management of Pest and Beneficial Organisms Division of the National Research Initiative Competitive Grants Program. To be placed on the mailing list for this publication or to receive additional information, please contact the NRI (202-401-5022 or NRICGP@reeusda.gov). The factsheet also is accessible via the NRI section of the Cooperative State Research, Education, and Extension Service website (http://www.reeusda.gov/nri).

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