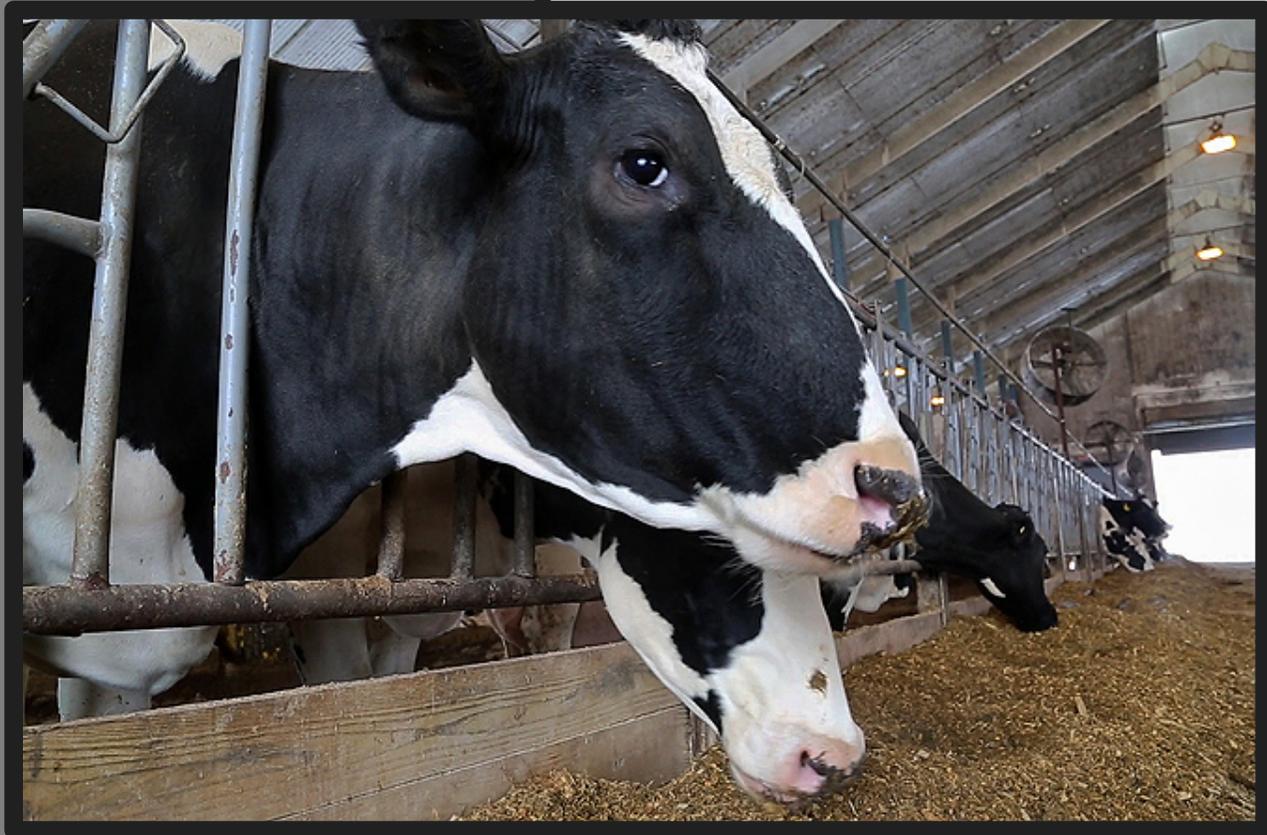


Animal Health



2017 Research Accomplishments

USDA

*Agricultural Research Service
Plains Area*

Animal Health

KANSAS

Ecology and Control of Insect Vectors

Arthropod-Borne Animal Diseases Research Unit, Manhattan, KS

Project Number: 3020-32000-007-00-D

Lead Scientist: Lee Cohnstaedt

Team Members: Dana Nayduch, Barbara Drolet, Leela Noronha, William Wilson

Female house flies acquire and accumulate more bacteria from manure than male house flies. House flies develop within, and feed upon, livestock manure, which often contains bacteria that are pathogenic to humans. ARS researchers in Manhattan, Kansas, and Kansas State University, collaborated to demonstrate that male and female house flies differentially acquire and harbor bacteria from cattle manure. Bacteria that were tested included both non-pathogenic *E. coli* and the pathogen *Salmonella* Typhimurium. Female flies obtained and accumulated more bacteria than male flies, both internally in their digestive tract and on their surface. These sex-based differences may be related to females spending more time associated with manure due to nutritional needs and egg-laying interest. This finding may help in predicting and determining risk of pathogen dissemination by house flies.

Dose-dependent fate of the pathogenic bacterium *Salmonella* Typhimurium in house flies. *Salmonella* Typhimurium is a pathogenic bacterium often associated with food borne illness, and previously found in wild-caught house flies. Adult house flies acquire *Salmonella* bacteria when they feed on contaminated substrates such as animal dung and garbage. The survival and persistence of bacteria within the fly gut directly impacts transmission potential. ARS researchers in Manhattan, Kansas, described for the first time, a dose-dependent effect on the survival of the *Salmonella* Typhimurium in house flies where, surprisingly, lower doses of bacteria proliferated to a greater extent within the fly than higher doses.

Additionally, irrespective of dose, *S. Typhimurium* both persisted and proliferated in house flies, making them important reservoirs and potential vectors of this pathogen. This finding can help better understand the risk flies pose in harboring and transmitting bacterial pathogens such as *Salmonella* to livestock and humans.



Adult house fly feeding on droplet containing *Salmonella* bacteria

Bacterial dose and fly sex both affect pathogen excretion from house flies. House flies ingest, harbor and excrete pathogenic bacteria, transmitting (or vectoring) them in the process. How the amount, or “dose”, of bacteria a fly ingests, as well as how the sex of the fly impacts bacterial excretion, has not been studied. ARS researchers in Manhattan, Kansas, demonstrated for the first time that both fly sex and bacteria does impact excretion of the pathogen *Salmonella Typhimurium* from house flies. Male house flies fed a low dose of bacteria shed the highest proportion of infected droplets, while males fed a high dose shed the most bacterial cells per droplet. Interestingly, both male and female house flies fed a dose of bacteria ten times lower, shed a higher percentage of the amount that had been ingested, compared to the high dose treatment. Collectively, these results show that male flies may be more important as vectors for this pathogen than females, irrespective of the ingested dose. Further, both male and female flies that ingest low doses of bacteria could serve as more substantial long-term disseminators of bacteria in the environment, thereby causing a greater risk to animal and human health. Both bacterial abundance and fly sex may influence excretion of bacteria from flies, and should be considered when assessing the risk of house fly transmission of pathogens.

Double knock-down: Using RNA-interference (RNAi) to suppress and then restore gene function in the biting midge. *Culicoides* biting midges transmit pathogenic viruses to livestock and deer. Understanding the interactions between these insect vectors and the viruses they transmit is the first step in developing methods for blocking virus

transmission. Because very few molecular tools for studying midges exist, understanding virus-vector interactions can be difficult. ARS researchers in Manhattan, Kansas, and Kansas State University showed for the first time that the molecular tool

RNA-interference (RNAi) can be used both to knock down, and subsequently to restore, the activation of genes in midges. This type of technology will allow



scientists to experimentally manipulate midges, which can help in understanding the genetic components that underlie the midge’s ability to transmit viruses.

Orbivirus Pathogenesis, Epidemiology, and Control Measures

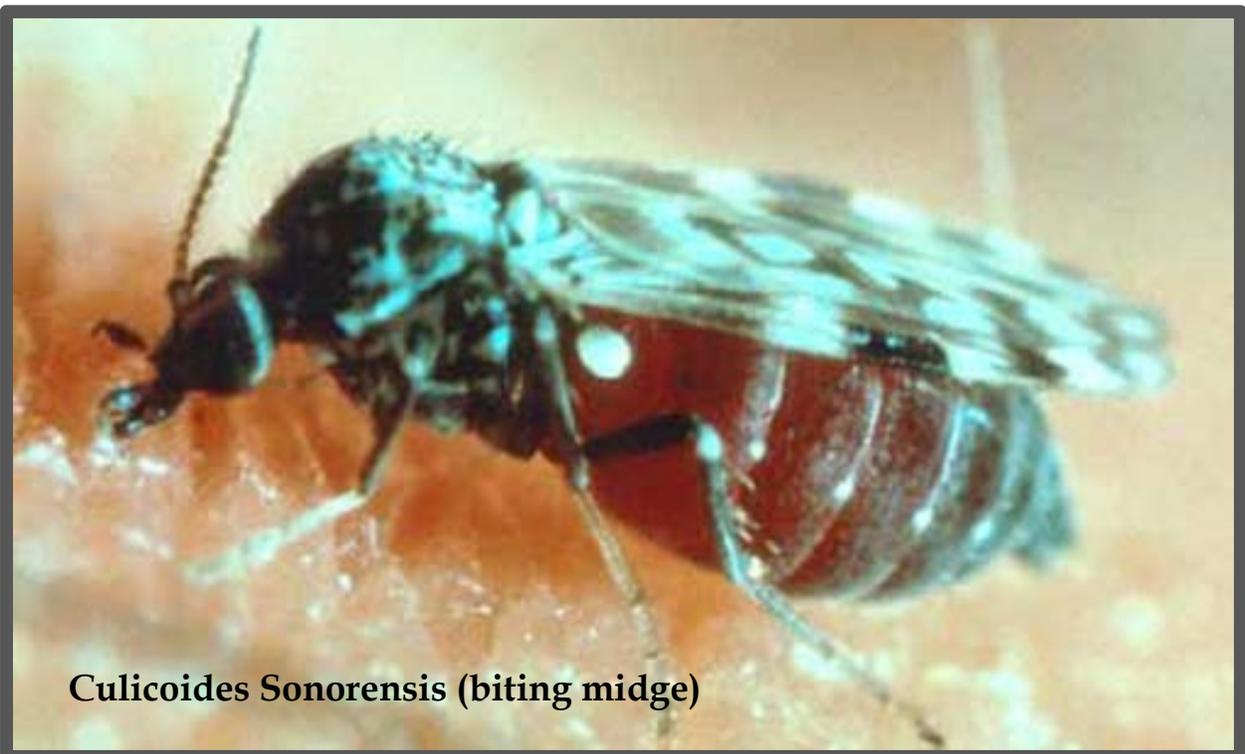
Arthropod-Borne Animal Diseases Research, Manhattan, KS

Project Number: 3020-32000-010-00-D

Lead Scientist: Barbara Drolet

Team Members: Dana Nayduch, William Wilson, Leela Noronha

Dynamics of epizootic hemorrhagic disease virus serotype 2 infection within the vector, *Culicoides sonorensis*. *C. sonorensis* biting midge is a confirmed vector of epizootic hemorrhagic disease virus (EHDV), which causes mortality in white-tailed deer, and more increasingly in cattle. Currently, of the seven EHDV serotypes, only three are present in the USA, and very few studies have focused on the infection time course of these serotypes within the midge. The objective of the research was to characterize EHDV-serotype 2 infection within the midge by measuring vector infection prevalence, virus dissemination, and viral load over the course of infection. ARS researchers in Manhattan, Kansas, fed midges a blood meal containing EHDV-2 and sampled at specific time points post-feeding. Midges were processed using virus isolation techniques to calculate infectious virus quantities, using molecular techniques to calculate the number of viral RNA genomes, and using immunohistochemistry to determine if and when the virus moved from the gut of the midge to the salivary glands where it could be transmitted the next time the midge fed. Virus was found to infect the insect's midgut, escape the midgut, amplify, and be disseminated to other organs by way of the hemolymph; a fluid in the insect analogous to blood. Virus reached the salivary glands as soon as 5 days after feeding. This study was the first to examine EHDV-2 infection



Culicoides Sonorensis (biting midge)

dissemination, infection prevalence, and viral titer simultaneously within *C. sonorensis* over the course of infection. Our data suggests that *C. sonorensis* midges are likely to be able to transmit EHDV-2 only 5 days after ingesting a blood meal from an infected animal.

Rift Valley Fever Pathogenesis, Epidemiology, and Control Measures

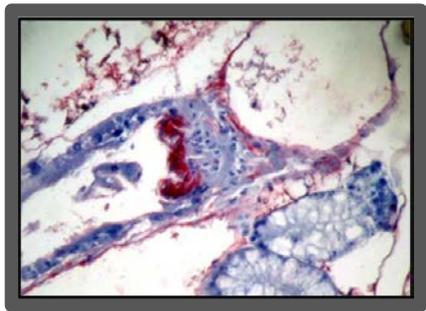
Arthropod-Borne Animal Diseases Research Unit, Manhattan, KS

Project Number: 3020-32000-009-00-D

Lead Scientist: William Wilson

Team Members: Barbara Drolet, Leela Noronha

Development of a subunit vaccine for Rift Valley fever (RVF). RVFV is an important



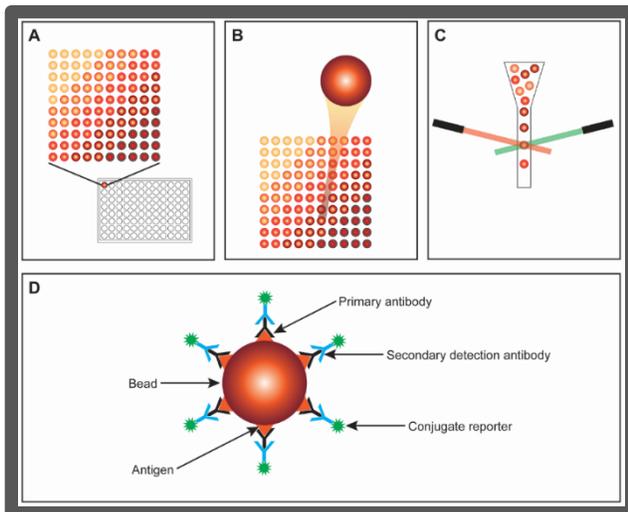
RVFV in esophagus of mosquito

animal and human mosquito-transmitted pathogen in Africa that could be introduced into the United States. ARS researchers in Manhattan, Kansas, and collaborators at Kansas State University developed an efficacious, safe to produce and use, subunit RVFV livestock vaccine. This candidate vaccine has been patented and was licensed to a commercial company for



development (APHIS license pending). The subunit vaccine provides a new tool to prevent RVFV disease outbreaks that is compatible with a differentiating infected from vaccinated animals (DIVA) approach using new diagnostic tools also developed by this team.

Multiplex detection of antibodies to Rift Valley Fever (RVF) viral proteins. ARS



researchers in Manhattan, Kansas, and Kansas State University collaborators previously developed a differentiating infected from vaccinated (DIVA) control approach for the transboundary zoonotic mosquito-transmitted virus causing periodic outbreaks of RVF in Africa. This is a devastating disease in Africa with the potential of introduction into non-endemic countries. To utilize a DIVA control strategy effectively, a companion diagnostic test is needed. Therefore, the team has developed a multiplex serological test that can simultaneously

detect antibody responses to multiple antigenic proteins. Thus, in a single assay, the test can determine and confirm if an animal has been infected or vaccinated.

NEBRASKA

Genomic Intervention Strategies to Prevent and/or Treat Respiratory Diseases of Ruminants

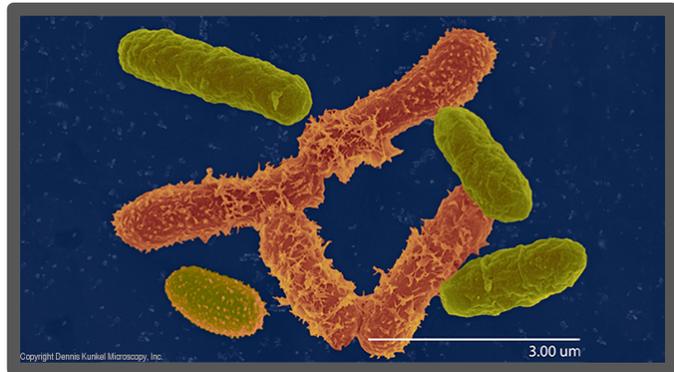
Genetics, Breeding, and Animal Health Research Unit, Clay Center, NE

Project Number: 3040-32000-034-00-D

Lead Scientist: Carol Chitko-McKown

Team Members: Michael Clawson, Michael Heaton, Gregory Harhay, Aspen Workman

Development and publication of a test that identifies strains of Mannheimia haemolytica that cause bovine respiratory disease. *M. haemolytica* is the principle bacterial species that causes respiratory disease in cattle. However, not all strains associate equally with disease or antibiotic resistance. In collaboration between ARS researchers in Clay Center, Nebraska, and the University of Nebraska - Lincoln, a test was developed that distinguishes between strains predominantly associated with bovine respiratory disease and antibiotic resistance, and those that associate more as commensals. Available for use without restriction, the test can assist in the detection and control of pathogenic *M. haemolytica* strains in cattle.



Genetic and Genomic Approaches to Improve Swine Reproductive Efficiency

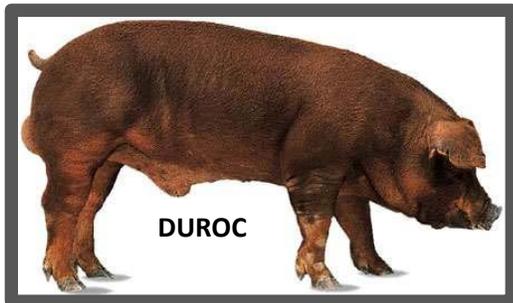
Reproduction Research Unit, Clay Center, NE

Project Number: 3040-31000-094-00-D

Lead Scientist: Gary Rohrer

Team Members: Danny Nonneman, Brittney Keel

Genetic factors associated with changes in feeding behavior during heat stress were identified. Annual production losses due to extreme heat in the U.S. swine industry are approximately \$300 million, with \$200 million incurred in the finishing phase of production. Knowledge of genetic factors associated with tolerance to extreme temperatures in swine is devoid. Therefore, ARS researchers in Clay Center, Nebraska, conducted studies to monitor feeding behavior in growing pigs and evaluate how each



pig altered their feeding pattern in response to increased ambient temperatures. Results indicate that gilts are less tolerant to high temperatures than barrows, while Duroc-sired pigs are the most resilient to warm temperatures and Landrace-sired pigs the most severely affected. A genome-wide association study identified several regions associated with changes in feeding behavior, and

indicate that genes known to detect stimuli (chemical, taste, etc.) may be causing these genetic differences. These results indicate that selection for pigs resilient to heat stress is possible and the genetic markers identified may be useful to the swine industry.

Genetic sequence variants have been used to identify causal variation and map complex traits. One of the key aims of livestock genetics and genomics research is to discover the genetic variants underlying economically important traits such as reproductive performance, feed efficiency, disease resistance/susceptibility, and product quality. ARS researchers in Clay Center, Nebraska, sequenced the genomes of 72 influential sires and dams of the U.S. Meat Animal Research Center swine herd. They identified approximately 22 million variants and have submitted them to public databases. By utilizing the swine genome annotation, researchers found that only ~139,000 of these variants were expected to alter or disrupt the protein coded by a gene and/or to regulate protein production. These variants are most likely to have a significant effect on phenotypic variation. Five hundred sixty-five variants were classified as high-impact loss-of-function (LOF) mutations. The LOF variants, as well as functional variants within reproductive quantitative trait locus regions, were submitted for inclusion in a commercially-available genotyping microarray.

Integrated Management of Stable Flies

Agroecosystem Management Research Unit, Lincoln, NE

Project Number: 3042-32000-010-00-D

Lead Scientist: David Taylor

Team Members: Kristina Friesen, Junwei Zhu

Long-lasting biting fly repellent from coconut oil. ARS researchers in Lincoln, Nebraska, discovered that hydrolyzed medium chain fatty acids from coconut oil protect cattle from biting insects, including stable flies and horn flies, for up to 4 days. The compounds repel stable flies and kill horn flies. US and international patents are pending and a commercial product is under development. This is the first natural repellent with extended longevity. In addition to



repelling biting flies, these compounds deter blood feeding of other insects including mosquitoes, ticks, and bed bugs as effectively as currently available insect repellants. Treatment costs are less than \$0.10 per animal, significantly less than currently available options for livestock producers.

TEXAS

Improved Practices to Conserve Air Quality, Maintain Animal Productivity, and Enhance Use of Manure and Soil Nutrients of Cattle Production Systems for the Southern Great Plains

Livestock Nutrient Management Research Unit, Bushland, TX

Project Number: 3090-31630-005-00D

Lead Scientist: Richard Todd

Team Members: Heidi Waldrip, David Parker

Comprehensive review of the science of nitrous oxide in beef feedyards. Beef feedyards are a source of the greenhouse gas nitrous oxide, but measured emissions are inconsistent and vary widely because of differences in cattle management, weather, and challenges with measurement methods. Therefore, a comprehensive literature review conducted by ARS researchers in Bushland, Texas, State College, Pennsylvania, and Texas A&M AgriLife Research in Amarillo, Texas, identified inconsistencies in measured emissions, and evaluated factors related to nitrous oxide losses. Numerous knowledge gaps were identified and recommended for future research. This work was a featured article in the Journal of Environmental Quality.

Greenhouse gas emissions from manure more accurately and quickly measured.

Nitrous oxide is a greenhouse gas emitted from cattle manure and soils. Nitrous oxide emissions have traditionally been measured using chambers that cover the emitting surface and rely on several gas samples collected over a 30 to 60 minute period.



However, such methods provide poor time resolution, thus, ARS researchers in Bushland, Texas, and Texas A&M AgriLife Research,



Amarillo, Texas, developed an improved method that relies on a real-time, continuous nitrous oxide analyzer to accurately quantify nitrous oxide emissions from manure and soil in only 60 seconds. The improved method resulted in faster and more accurate measurement of greenhouse gas emissions from manure that revealed new dynamics in nitrous oxide emissions.

Rainfall amount increases nitrous oxide emissions from open-lot beef cattle feedlots.

Nitrous oxide is a greenhouse gas emitted from livestock manure, but little is known of factors effecting emissions. ARS researchers in Bushland, Texas, and Texas A&M AgriLife Research, Amarillo, Texas, studied how rainfall affects nitrous oxide emissions from beef cattle feedlot manure. Nitrous oxide emissions were monitored after applying simulated rainfall to dry manure at five different rainfall amounts between 0 and 2 inches. Nitrous oxide emissions were elevated for 45 days after rainfall. These results indicate that improved drainage from feedlot pens will help to reduce nitrous oxide emissions from livestock manure.

Better analytic tools improve manure management.

Analytical techniques to study manure are usually based on methods developed for soils, but these may be inadequate or inappropriate. Therefore, ARS researchers in Bushland, Texas, and New Orleans, Louisiana, and the University of Minnesota, collaborated on an invited review paper that highlighted advanced and novel analytical techniques for manure. Spectroscopic methods, modified extraction techniques, and other techniques have vastly improved the characterization of organic matter, nitrogen, and phosphorus in manure.

These techniques will help develop improved manure management practices that minimize environmental risk while improving manure fertilizer value.



Simple and accurate methods estimate nitrous oxide emissions from beef feedyards.

Cattle production contributes to emission of the greenhouse gas nitrous oxide, but studies have shown that feedyard nitrous oxide emission rates are highly variable. ARS researchers in Bushland, Texas, and Texas A&M AgriLife in Amarillo, Texas, looked at how regional weather and manure characteristics affect nitrous oxide emission rates.



Using field study data, mathematical models were developed to predict nitrous oxide losses based on easily measured manure properties. These included concentrations of nitrate and soluble carbon, water content, organic matter stability, and temperature. Emission estimates made with the new models agreed well with emission rates measured at feedyards. These models can help determine how changing climate and management affect the environmental footprint of beef production.

Cattle Fever Tick Control and Eradication

Livestock Arthropod Pest Research Unit, Kerrville, TX

Project Number: 3094-32000-039-00D

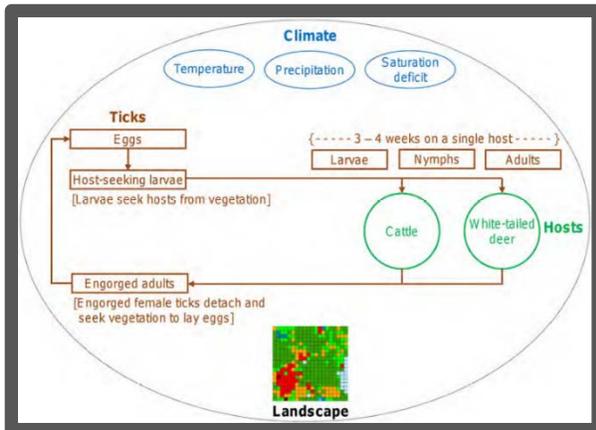
Lead Scientist: Adalberto Perez de Leon

Team Members: Kimberly Lohmeyer, Robert Miller, Pia Olafson, Allan Showler, Donald Thomas, John Goolsby, Felicito Guerrero, Weste Osbrink

Integrated control of ticks. ARS researchers in Kerrville, Texas, and Edinburg, Texas, in collaboration with APHIS-VS, the Puerto Rico Department of Agriculture, and local livestock producers, completed field research for integrated control of the southern cattle fever tick in Puerto Rico. The combined use of safer acaricides, and vaccination against the cattle fever tick, prevented outbreaks of bovine babesiosis in the dairy and beef cattle farms where the research was done. The livestock industry of Puerto Rico is interested in adopting integrated tick management practices based on these research outcomes.



Ecologic modeling. ARS researchers in Kerrville, Texas, and Edinburg, Texas, published



research describing the development of a model to assess the effect of interactions between white-tailed deer, climate variation, and habitat diversity on the efficacy of methods used by the Cattle Fever Tick Eradication Program to eliminate tick outbreaks in south Texas. The model also considered the livestock-wildlife interface, because in some areas cattle and deer share the ecosystem. Results of the model simulations identified aspects of the tick life cycle associated with infestations in deer

that could be targeted to enhance prevention, and the management of cattle fever tick outbreaks in the U.S.

Nilgai lure. Research to develop methods to treat nilgai antelope against cattle fever tick infestations was conducted by ARS researchers in Edinburg, Texas, and in Kerrville, Texas. Nilgai is an exotic wildlife species originally from the Indian subcontinent that was introduced to south Texas, which is related to cattle. A lure could attract nilgai to sites for non-invasive treatment against cattle fever



ticks. Research outcomes were published describing the results of field tests with experimental lures. Offal was the most attractive of the three lures tested; a way to attract nilgai to a specific location provides the opportunity to test non-invasive methods to control cattle fever tick infestations.

Genomics of Livestock Pests

Livestock Arthropod Pest Research Unit, Kerrville, TX

Project Number: 3094-32000-036-00-D

Lead Scientist: Felicito Guerrero

Team Members: Kevin Temeyer, Robert Miller, Adalberto Perez De Leon, John Goolsby

Publication and release of the cattle tick genome sequence. The genome of an organism contains the information necessary to allow that organism to develop and survive over its lifetime. The genome of the cattle tick, *Rhipicephalus microplus*, is huge and complex to sequence, containing over twice the amount of DNA as the human genome. ARS researchers in Kerrville, Texas, in collaboration with researchers at Murdoch University's Centre for Comparative Genomics, Murdoch, Australia, have released and published the genome sequence for this cattle tick. This dataset contains sequences from genes involved in evasion of bovine host immune response, pesticide resistance, maintenance of pathogens, and feeding, among others. This new comprehensive sequence information is facilitating tick vaccine research and pesticide resistance monitoring.

Management of Flies Associated with Livestock

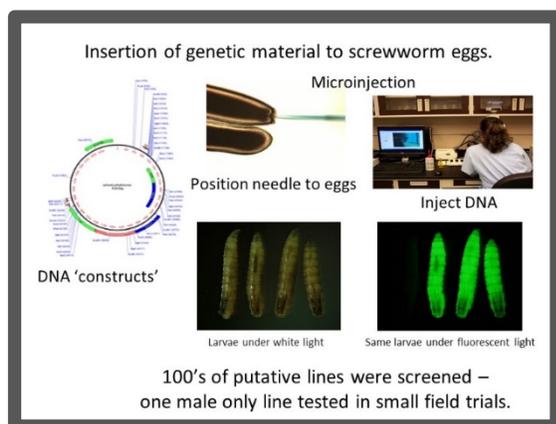
Livestock Arthropod Pest Research, Kerrville, TX

Project Number: 3094-32000-038-00-D

Lead Scientist: Kevin Temeyer

Team Members: Kimberly Lohmeyer, Pia Olafson, Allan Showler, Weste Osbrink, Steven Skoda, Pamela Phillips, Adalberto Perez De Leon

Construction of transgenic male-only screwworm strain.



ARS researchers in Kerrville, Texas, in collaboration with researchers at North Carolina State University, the Animal and Plant Health Inspection Service (APHIS), and the Panama-U.S. Commission for the Eradication and Prevention of Cattle Screwworm (COPEG), completed bioengineering construction of a transgenic male-only strain of screwworms ready for production and distribution, coordinating a critical path to development. The genetically engineered male-only strains were transferred to Methods and Development

section of COPEG for further evaluation in field trials scheduled for the coming year. Production use of male-only strains is expected to reduce production costs and biological waste by approximately 50%. Demonstration of successful performance compared to non-engineered strains will enable use of the male-only strains for full production, sterilization, and release, providing huge savings in expense and generation of waste.

Effects of p-anisaldehyde on horn fly repellency, mortality, and reproduction. The horn fly, *Haematobia irritans irritans* (L.), is an economically important blood-feeder that mainly attacks cattle worldwide. As resistance to conventional insecticides increases, alternative control tactics are being investigated. p-Anisaldehyde occurs in many plants and it is bioactive against some arthropods. ARS researchers in Kerrville, Texas, developed a series of bioassays that are effective for assessing a range of horn fly responses to chemicals. In our study, p-anisaldehyde was lethal to horn fly eggs at 0.00001% and possibly less. Mixed into cow manure, 5,000 - 20,000 ppm p-anisaldehyde reduced horn fly larvae by 85.4% - 100%. p-Anisaldehyde caused some immobilization of adult horn flies when exposed by direct contact with spray droplets and by volatiles. Adult mortality was 90% - 100% in response to 5% - 10% concentrations by 30 min, and the concentration at which 50% of the horn flies died and the concentration at which 90% of the flies died. Complete horn fly mortality was achieved by exposure to volatiles from 0.75% p-anisaldehyde by 3 h in an enclosed space; exposure to volatiles is more lethal to adult horn flies than droplets. Although horn flies were not repelled, the compound completely deterred feeding from cotton pads soaked in bovine blood. Exposure to sublethal concentrations of p-anisaldehyde did not affect horn fly egg production and hatching. These early findings about p-anisaldehyde indicate that horn fly eggs are very vulnerable to p-anisaldehyde, hence, delivery approaches might now be developed such that horn fly eggs can be exposed to the compound. Adult horn flies are also susceptible, making p-anisaldehyde a potential organic tactic for controlling horn fly infestations.



Identification of screwworm ovipositional attractants.

ARS researchers in Lincoln, Nebraska, and Kerrville, Texas, completed experiments utilizing secondary screwworm that succeeded in identification of four volatile ovipositional attractants. Replication of these results for primary screwworm is expected to improve production efficiency by increasing the average number of

eggs successfully produced for inoculation of the larval medium used for screwworm production. This is particularly important for the male-only strain as it will reduce the quantity of fertile females needed and reduce the cost of their production.

Association of salivary acetylcholinesterase with arthropod disease vector capacity. ARS researchers in Kerrville, Texas, previously reported that acetylcholinesterase in tick saliva was a likely modulator of host immune response to parasite or pathogen presence. Additional studies found that mosquito and sand fly saliva also contain measurable acetylcholinesterase activity, unlike saliva from horn flies, stable flies, or house flies, suggesting a strong link between salivary acetylcholinesterase and ability to vector pathogens. This finding suggests that salivary acetylcholinesterase may be an important factor in disease transmission, presenting a new paradigm and identifying a novel target for studies to understand and prevent disease transmission by arthropod vectors.

