

## ANNUAL REPORT MULTISTATE PROJECT

**Project/Activity Number:** W-102

**Project/Activity Title:** Control of Animal Parasites in Sustainable Agricultural Systems

**Period Covered:** July 1, 2000 – June 30, 2001

**Date of This Report:** September 18, 2001

**Annual Meeting Dates:** July 18-20, 2001

**Participants:** There were 15 members, advisors, and guests in attendance. The following units were represented: ARS-USDA, Beltsville, MD (Lou Gasbarre); ARS-USDA, Watkinville, GA (John Stuedemann); University of California, Riverside (Ed Platzer); University of Georgia, Athens (Ray Kaplan); University of Illinois, Urbana (Milt McAllister); Kansas State University, Manhattan (Bob Ridley); Louisiana State University, Baton Rouge (Tom Klei, Jim Miller); Virginia Polytechnic Institute and State University, Blacksburg (David Lindsay); University of Nevada, Reno (David Thawley, administrative advisor); CSREES-USDA, Washington (Bill Wagner, advisor), Rick Ciordia (former member, Pensacola, FL), Bonner Stewart (former member, Baton Rouge, LA), Joseph Vercruyse (University of Ghent, Belgium), and Mark Spire (University of Kansas, Manhattan) were guests.

**Brief summary of minutes of annual meeting:** The group spent the bulk of the meeting discussing project objectives and ongoing research within each of those objectives. Dr. David Thawley, administrative advisor, reviewed the history of the project which included a time table of activities necessary for renewal of the project and emphasized the need for the project to reflect multistate objectives and collaborative efforts. Dr. Bill Wagner, CSREES-USDA, gave an update of Federal activities including the upcoming budgets and initiated discussion on the potential of an IFAS project for the group. Next years meeting site will be at the Inn on Biltmore Estate, Asheville, NC, July 16-19, 2002. Next years officers will be Ed Platzer, Chair, Lora Ballweber, Secretary and Lou Gasbarre, member-at-large.

### **Accomplishments and Impacts:**

**Objective 1: Control of parasite diseases using biological and chemical agents and physical methods.**

#### **1A. Evaluate the anthelmintic activity of naturally occurring fungi in the laboratory and field experiments.**

- The fungus *Duddingtonia flagrans* was effective against nematode larvae in sheep feces which reduced pasture infectivity and subsequent worm burdens in tracer animals. (LA)
- A collaborative effort is continuing between GA and LA in this area of research..

#### **1B. Evaluation of the efficacy of the latest generation of endecto-parasiticides and novel anthelmintic agents.**

**1C. Determine the prevalence of anthelmintic resistance in the United States and characterize resistant parasites.**

- cDNA library was prepared from *Cylicocyclus nassatus* (Cyathostominae). Clones of some of these genes thought to be involved with anthelmintic action and resistance have been isolated and sequenced. (GA)
- Studies on B-tubulin suggest that mutations in addition to the PHE to TYR 200 are important in benzimidazole resistance in cyathostomes. (GA)
- A study was initiated in cooperation with the University of Florida, University of Kentucky, and Louisiana State University investigating the prevalence of anthelmintic resistance on large horse farms (>24 horses) in the southern United States. (GA)
- A study was initiated in cooperation with Fort Valley State University (FVSU) investigating the prevalence of anthelmintic resistance on goat farms in Georgia. Data suggests that the prevalence of ivermectin and benzimidazole resistance are very high. Levamisole resistance is less prevalent but is not uncommon. No resistance to moxidectin was detected. (GA)
- A baseline set data on the parasitism of bison has been assembled for potential use in not only bison management but could provide a basis for advancing our understanding of how anthelmintic resistance develops and is maintained. (KS)
- Results with *H. contortus* support the hypothesis that fenbendazole (FBZ) induced intestinal damage results, in part, from inhibited transport of digestive enzymes to the apical membrane. Consequently, parasite proteins that mediate apical vesicle transport processes may provide valuable targets for anthelmintics. In FBZ-resistant worms, the apical compartment was unaffected by FBZ. (WA)

**1D. Development of an animal model for cryptosporidiosis**

**Other:**

- Pyrantel tartrate had little effect on *Sarcocystis neurona* both in cell culture and in infected mice which suggest that it would not be useful for the control of Equine Protozoal Myeloencephalitis (EPM). (VA)

**Objective 2. Define the roles of pathogenesis, immunomodulation, vaccination, and genetic manipulation in parasite control.**

**2A. Define immune mechanisms that lead to host immunity or pathology in parasitic infections.**

- We have genotyped over 300 F2 progeny obtained from the matings of two commercial meat-type broiler chickens and showed a significant association of several markers with coccidiosis disease resistance associated traits. (USDA-Beltsville)

- We have developed techniques to show how the mucosal immune system of neonatal pigs changes with age. These techniques can now be used to determine how neonatal pigs exposed to *T. gondii*, or other infections, respond at local and systemic sites and be evaluated for the local induction of cytokine gene expression, e.g. for interferon-gamma production, and activation of lymphoid cells. (USDA-Beltsville)
- We have cloned and prepared good quantities of a novel biotherapeutic, the expressed recombinant porcine cytokine interleukin-12. This cytokine has proven to be effective in certain in vitro tests and is now being tested for its efficacy on pig mucosal immune system development. (USDA-Beltsville)
- We have demonstrated that the cytokines interleukin-4 (IL-4) and IL-13 work through the IL-4 receptor to cause intracellular Stat6 activation that turns on protective immunity to a variety of gastrointestinal worm parasites. Use of recombinant IL-4 and IL-13 can induce worm expulsion from the intestine. (USDA-Beltsville)
- Identified the host immune mechanism that mediates protection against avian coccidiosis. Genes for 2 chicken immune cell secretory products, interleukin-2 (IL-2) and IL-15 were cloned. Their recombinant proteins were produced for functional studies that demonstrated enhanced host cell-mediated immunity and increased disease resistance against coccidiosis. (USDA-Beltsville)
- We demonstrated that a larval stage of *Ostertagia* produces material that suppresses the normal growth of bovine T lymphocytes that occurs after stimulation of the T cell antigen receptor and down regulates signal transduction to the cell nucleus, resulting in a stoppage of the normal progression of receptors for growth factors. (USDA-Beltsville)
- We successfully produced a cDNA expression library from *O. ostertagi* and identified one antigen/peptide that is immunoreactive with host antibodies from infected animals and that appears developmentally regulated. Investigations are proceeding in determining the relationship of this protein to in vivo development of the parasite. (USDA-Beltsville)
- We cloned and expressed the bovine IL18 gene and developed competitive PCR for measurement of expression of bovine IL18. We have also expanded measurement of cytokine gene expression in genetically parasite-resistant and susceptible cattle to include measurement of IL13, TGF-beta, and TNF-alpha. (USDA-Beltsville)
- Showed that naturally acquired GI nematode infections affect the ability of the animals to generate certain kinds of immune responses. Cattle grazed under different levels of parasite control in a standard production system showed

- decreased T cell responses to mitogens and to immunization with a T cell antigen, and in the case of some classes of antibody, mildly elevated humoral immune responses. (USDA-Beltsville)
- We demonstrated that oral inoculation of calves with *N. caninum* oocysts resulted in infection of the calves, and in rapid and strong local and systemic humoral and cellular immune responses. These results indicate that immunologically-based control programs for Neospora infections of cattle may be practical, and further identify regions of the immune system that should be targeted in such programs. (USDA-Beltsville)
  - Phenotyping of F<sub>2</sub> lambs continued to result in a relatively large range of FECs and PCVs indicating that nematode resistance was segregating. *Haemonchus contortus* infection in naturally resistant Native sheep appears to have an immunological basis which involves CD4<sup>+</sup> T lymphocytes. (LA)
  - We have examined the roles of inducible and endothelial nitric oxide synthase in resistance to sporocyst induced *S. neurona* infections in genetically defined mice. We found that mice lacking the gene for inducible nitric oxide synthase (iNOS) and mice lacking the gene for endothelial nitric oxide synthase (eNOS) were resistant to clinical infection and had no lesions in their brains at necropsy. In contrast, clinical disease and deaths occurred in mice with no gene for interferon- $\gamma$  (IFN- $\gamma$ ). Microscopic lesions were seen in the brains of IFN- $\gamma$ -lacking mice. Results of this study indicate that iNOS and eNOS are not major mediators of resistance to *S. neurona* infections. Results of this study suggests that IFN- $\gamma$ -mediated immunity to *S. neurona* may be mediated by non-nitric oxide dependent mechanisms. (VA)
  - *Neospora hughesi* is a recently described apicomplexan parasite that has been associated with several cases of equine protozoal myeloencephalitis. The biology of this new parasite is just beginning to be defined. Towards this understanding, we report important differences between the nucleotide and deduced amino acid sequences of the dense granule proteins GRA6 and GRA7 of *N. hughesi* and *Neospora caninum*. This information can be used to differentiate the two species and contribute to further understanding of the prevalence and biology of *N. hughesi*. The newly defined proteins of *N. hughesi* are referred to as NhGRA6 and NhGRA7 in keeping with the protocol for naming homologous proteins of the Apicomplexa. Genes of the two dense granule proteins of *N. hughesi* (isolate Nh-A1) and four different isolates of *N. caninum* were isolated via PCR and their DNA sequences were determined. Computer analysis indicated that the two gene sequences were identical among all four *N. caninum* isolates. However, the gene for NhGRA6 was found to be 96 nucleotides longer at the 3' end than that of NcGRA6, resulting in a protein product that is 32 amino acids larger than NcGRA6. Two tandem repeat sequences were identified at the 3' end of the NhGRA6 gene. These repeat sequences contributed to the lengthening of the carboxy terminus of NhGRA6 in comparison with that of NcGRA6. The larger

size of NhGRA6 was further confirmed by Western blot analysis in which NcGRA6 monospecific antibodies recognised a protein of approximately 42 kDa in *N. hughesi* whole tachyzoite preparation but a protein of 37 kDa in *N. caninum* whole tachyzoite preparation. Analysis of GRA7 gene sequences indicated a 6% and 14.8% difference at nucleotide and amino acid sequence level, respectively, between NcGRA7 and NhGRA7. Despite the same number of residues in the deduced amino acid sequences of all the GRA7 proteins, Western blot analysis indicated a difference in the migration pattern of NhGRA7 in comparison with NcGRA7. Results of our study indicate that diagnostic tests based on differences in dense granule sequences and antigenicity may have potential to differentiate between *N. hughesi* and *N. caninum*. Such diagnostic tests would be valuable tools to aid in our understanding of the epidemiology of these parasites. Additionally, dense granule proteins are immunogenic and they may have potential as use in recombinant vaccines against neosporosis. (VA)

- We have expressed *N. caninum* dense granule 1 (NcGRA7) and surface reactive sequence 2 (SRS2) in the RB51 vaccine strain of *Brucella abortus*. We are currently evaluating these candidate vaccines in a gerbil model of neosporosis. We hope to vaccinate cattle against both abortion-causing agents using this approach. (VA)
- Additional work on genetic variation in resistance to ovine gastrointestinal nematodes was performed using the Katahdin sheep breed. Katahdin sheep were created in the United States by crossbreeding Virgin Island white hair sheep with several European breeds. Since the Virgin Island sheep (also known as St. Croix) sheep have shown increased levels of resistance to *Haemonchus* and other trichostrongylid nematodes, it has been assumed by producers that Katahdin sheep will also be resistant. A study was conducted comparing worm burdens in naturally infected Katahdin (5) and Barbados Blackbelly (4) lambs. Barbados Blackbelly sheep are also hair sheep from the West Indies considered to have increased parasite resistance. Katahdin lambs had significantly higher worm burdens than Barbados Blackbelly lambs suggesting that in crossbreeding to produce the Katahdin sheep, which has more desirable carcass characteristics, some of the parasite resistance of the original hair sheep foundation of the breed has been lost. (VA)
- Abomasal globule leucocyte numbers were also counted in the 2 sheep breeds and in 24 naturally infected goat kids. Globule leucocytes have been associated with development of immunity to nematode parasitism in sheep. Abomasal tissue samples were collected for globule leucocyte analysis by fluorescence microscopy. Numbers of globule leucocytes were not different in the 2 sheep breeds but lambs had significantly higher globule leucocyte counts than goat kids. These results are supportive of a difference between sheep and goats in their globule leucocyte response to parasitism but indicate that globule leucocyte production is not prominent in the cellular response of goat kids to GI parasitism. (VA)

## **2B. Identify and test parasite antigens that can serve as targets for control of parasitic infections**

### **2C. Identify genetic differences in responses to gastrointestinal nematodes both within and between breeds.**

- We have begun genotyping a population of cattle consisting 12 generations of over 300 animals, which have been completely characterized in terms of resistance to gastrointestinal nematode infection. Characterization of host genes that encode important production traits such as disease resistance requires diverse by defined cattle populations and facilities for verification in different environments and under different management programs of laboratory results. This validation will be accomplished through collaborative research efforts in the US with W-102 member institutions in Minnesota and Louisiana, Non-member institutions in Texas, Arkansas, Pennsylvania, and Missouri, and internationally through the LABEX program between EMBRAPA of Brazil and USDA. With these collaborators we have initiated studies aimed at both the discovery and subsequent validation of ruminant genes that control resistance to both endo- and ecto-parasites. (USDA-Beltsville)

### **2D. Identify new or improved methods of diagnosis of parasitic infections.**

- Human trichinellosis originating from swine has been on the decline in recent years in the United States and in countries of the European Union, but infections emanating from sylvatic (wildlife) genotypes and hosts remains a major source of human infection in these countries. However, the absence of distinguishing morphological characters of *Trichinella* prevents proper diagnosis. We developed a simple and single PCR test in association with Dr. C.M.O. Kapel from the Royal Veterinary and Agricultural University in Denmark that is capable of differentiating all well-defined genotypes of *Trichinella* as well as distinguishing single larvae. This work will greatly facilitate the elucidation of parasites within this genus for epidemiological studies as well as permit species diagnosis from host biopsies for identifying the sources of human infections. (USDA-Beltsville)
- Toxoplasmosis is one of the most prevalent parasitic diseases in the humans and agricultural animals; recent evidence indicates that pig products are a major source of *Toxoplasma gondii* infection in the U.S. ARS Scientists at Beltsville, MD, with funding from the National Pork Producers Council, have developed a sensitive molecular, TaqMan assay for the *T. gondii* parasite in pig or other animal tissues. This assay can now be used to determine whether pork products are contaminated with this parasite as well as to assess whether vaccine or biotherapeutic treatments reduce the *T. gondii* burden in live pigs. This new test will be an important epidemiologic tool for USDA scientists and regulators, and

- will help them develop new strategies to prevent or control this foodborne infection. (USDA-Beltsville)
- Identifying drinking water that is contaminated with viable *Cryptosporidium parvum* parasites is critical to ensuring the health of the American public. ARS scientists developed a molecular technique to rapidly detect this parasite in a water sample. This test strongly correlated detection with infectivity of the organism. This test can provide a rapid assessment of contamination of water with *Cryptosporidium*. (USDA-Beltsville)
  - Identification of field isolates of *Cryptosporidium* is essential to determining the source and potential infectivity for humans and animals. With collaborators, molecular techniques have been developed and used to identify new genotypes and new species of *Cryptosporidium*. Three new genotypes and one new species were identified. These findings add to our data base on the genetic diversity of these organisms providing tools for identifying waterborne and foodborne parasites. (USDA-Beltsville)
  - Species of a new and emerging group of waterborne protozoan parasites causing severe diarrhea in humans have been identified in cattle and pigs. Studies have been initiated by ARS scientists to determine the prevalence of these organisms and which species are infective for both livestock and humans. One of the species in humans, *Enterocytozoon intestinalis*, was found not to be infectious for calves. This finding eliminates cattle as a suspected source of environmental contamination with this parasite. (USDA-Beltsville)
  - Prompt and accurate detection of the protozoan parasite *Cryptosporidium parvum* is necessary to insure safe water supplies and healthy livestock. ARS Scientists, as well as collaborators at the Centers for Disease Control, developed a real time, fluorogenic-probe-based PCR assay for detection of the parasite. The assay detected *Cryptosporidium* parasites in spiked source and finished water samples, as well as in ground water samples from West Virginia. The assay can potentially provide a new and improved detection capability for the water industry and community water treatment plants that will protect public health. (USDA-Beltsville)
  - *Ostertagia ostertagi* remains the most economically important parasite in temperate regions of the world, but to date there is no accurate way to quantify the level of infection in individual animals. We have been developing a DNA based assay to diagnose and quantitate *O. ostertagi* infections in cattle from fecal eggs. In the past year we have continued testing the assay on cattle grazing infected pastures to validate the use of the assay under field conditions. Once validated the assay will be used to identify those individual members of the herd which account for the majority of parasite transmission, thus allowing for more targeted parasite control programs. (USDA-Beltsville)

- Differentiation of common gastrointestinal nematodes of cattle from fecal eggs requires labor-intensive procedures by personnel experienced in morphometric analysis. We have developed a simple molecular test to differentiate 5 distinct parasite genera infecting cattle. This test will permit identifying hosts carrying the most pathogenic parasite species and reduce unnecessary drug treatment of those animals harboring non-pathogenic parasites and in so doing reduce costs to the producer and to the consumer, as well as reduce the potential for drug residues in processed meats. (USDA-Beltsville)

### **Objective 3. Integration of parasite control practices into livestock production systems.**

#### **3A. Examine effect of soil and crop management practices on parasite transmission.**

- There are no registered compounds available for treatment and control of tapeworms in laying hens. The only current option for tapeworm control is interruption of the transmission cycle by suppression of the intermediate host(s). Our work has identified two principal vector insects, *Dermestes maculatus* and *Musca domestica*. To facilitate development of management systems, we have determined the developmental rate and heat units for *Choanotaenia infundulium*. (CA)

#### **3B. Evaluate efficacy of integrated anthelmintic/pasture management programs in parasite control.**

- Anthelmintic treatment of cattle prior to movement to parasite-free pastures has resulted in the pastures and cattle remaining parasite free after eight years of grazing. (USDA-Watkinsville, GA and GA)
- The growing movement towards rotational grazing programs for small dairy farmers raises the possibility of increased problems caused by GI nematodes. We have been working with a private dairy farmer in Pennsylvania and ARS scientists at University Park, PA to alleviate problems due to these parasites. In the past year we have been monitoring the herd while following a program that minimizes anthelmintic treatments, but still tries to minimize parasite transmission. Our results have shown that different parasite species have different optimal transmission periods. Anthelmintic programs designed to control the most common species may fail to control less abundant species, which can then become major problems for animal productivity. Development of adequate control programs must be carefully designed to impact all parasite species and not just the most evident genera. Because of changing consumer preferences there is a rapidly growing number of small farmers interested in organic meat and milk production. One of the most important concerns of these producers is adequate parasite control in the absence of anthelmintic drugs. We have initiated studies with 2 different organic farmers aimed at using host genetics to control parasite transmission. These studies will provide organic meat and milk producers with

means to lessen the economic impact of parasites on their production systems.  
(USDA-Beltsville)

### **3C. Evaluate the impact of management systems on the development of anthelmintic resistance.**

#### **3E. Impact of management practices on transmission of protozoan parasites.**

- Understanding the relative importance of oocyst infection versus reactivation of latent tissue cyst stages that lead to congenital infection of the fetus and abortion is critical to developing control measures against neosporosis. A serological study was conducted in a southeastern U.S. dairy herd that experienced, over a 4-month period, an *Neospora caninum* -associated abortion storm where standard diagnostic assays ruled out other common abortifacient organisms. A subset of fetuses showed *Neospora caninum* in brain as indicated by immunohistochemistry; serological investigation using a variety of diagnostic assays showed that aborting cows had higher anti- *N. caninum* titers than non-aborting cows. Furthermore, application of an avidity assay showed that aborting cows experienced recent *N. caninum* exposure which is consistent with a point source, possibly oocyst infection. This is the first description of serology being useful for identifying potential source of *N. caninum* infection to a dairy herd and suggests that abortion storms may result from non-congenital infection by the parasite and that measures to prevent infection by oocysts are warranted. (USDA-Beltsville)
- In an outbreak of bovine abortion caused by the protozoal pathogen, *Neospora caninum*, the infection was demonstrated to have been transmitted to cows via a point source such as contaminated feed or water. Cows that had been infected with *Neospora* prior to the abortion outbreak had developed immunity that prevented them from suffering abortion following re-exposure. (IL)

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