Cooperative Regional Research Project
Revision

"CONTROL OF ANIMAL PARASITES IN SUSTAINABLE AGRICULTURAL SYSTEMS"

W-102

October 1, 1999 to September 31, 2004

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PROJECT NUMBER: W-102

TITLE: **Control of Animal Parasites in Sustainable Agricultural Systems**

DURATION: October 1, 1999 - September 31, 2004

STATEMENT OF THE PROBLEM:

The Western regional research project W-102 is the only regional project dedicated to the understanding and control of livestock parasites. The objective of this revised project is to study the biology, pathogenesis and treatment of protozoan and helminth diseases of livestock. Development of control programs combining traditional chemotherapy with novel techniques of parasite control is anticipated. The reduced dependence on antiparasitic drug use will follow development of alternative parasite control measures and will be more appropriate for new, more sustainable systems of contemporary livestock production.

JUSTIFICATION:

Importance in agriculture, rural life and consumer concerns

Agriculture continues to be the dominant industry in rural communities; however, it is becoming more and more evident that stewardship of our natural resource base is not only the responsibility of farmers, but of all citizens. Consequently, society is demanding that agriculture implement environmentally sound sustainable systems of production that have low chemical usage, reduced movement of sediment and nutrients from the land, and have minimal or no off-site impacts. One component of these production systems must be parasite control. Parasites continue to be a common limiting factor in livestock production systems, decreasing the quality and quantity of livestock products and requiring periodic treatment with parasiticides. However, a thorough understanding of the pathogenesis and transmission of parasitic disease must be developed before integrated programs of parasite control can be formulated.

Extent of the problem

Parasites continue to decrease animal productivity in all livestock species and their importance is recognized by producers. The antiparasitic market is the fastest growing segment of the veterinary pharmaceutical industry (Conder, personal communication). In 1997 more equine operations, regardless of size, gave dewormers to horses than vaccinated at least one horse (CAHM, 1997). In the sheep industry, more producers indicated moderate to high levels of concern about gastrointestinal worms than any other health condition (CAHM, 1997), and in a recent veterinary services survey of cattle producers (USDA/APHIS, 1994), internal parasites were listed as the second most common cause of significant economic impact in the past 12 months.

Over the past 30 years, there has been a dramatic increase in the use of chemotherapeutic methods as a means of endoparasite control. Many of the newest generation of parasiticides offer longer residual times and increased ease of administration for the producer. While it continues to be important to evaluate the activity of new drugs against major livestock parasites it has become clear that the real utility of modern chemotherapeutic agents lies in their role as a basic component of an integrated parasite control program.

The need to look beyond conventional chemotherapy is immediate in the case of those parasitic infections where no effective treatments exist. Cryptosporidium parvum and Neospora caninum are coccidial protozoan parasites whose full economic impact is still under study. Neospora caninum has recently been recognized as a cause of abortion and neonatal paralysis in cattle. It has been estimated that abortion caused by the parasite costs the diary industry $35 million a year in California alone (Dubey and Lindsay, 1996). Cattle can be infected by two routes of transmission: 1) congenitally infected heifers may mature, become pregnant, and the organism may then cross the placenta to infect the next generation of offspring; 2) previously uninfected cows may consume N.caninum and then bear infected offspring or aborted fetuses. The relative importance of these two methods of transmission in causing reproductive losses has not been determined. Both a better understanding of the transmission of this parasite and new approaches to control are important in limiting losses to the dairy industry. Since treatment to eliminate the parasite and prevent abortion is unavailable, development of a vaccine against the parasite probably offers the most effective means of controlling cattle infections in the future. Successful vaccine production will require a thorough understanding of the host immune response to N. caninum.

Crytosporidium parvum has become increasingly important to US agriculture because of its ability to infect domestic and wild animals and humans. Outbreaks of human gastroenteritis caused by this parasite are commonly blamed on run-off from farming operations, most notably dairy operations. It is frequently assumed that young calves are the source of the parasites in the water supply. In 1997, the Science and Technology Committee of the National Beef Cattleman's Association listed C. parvum ninth in a list of production issues. Since there are no effective treatments for C. parvum at this time, future agricultural practices must be targeted to reducing the potential of farm operations for the release of C parvum and other potential human pathogens. The development of such programs depends on an accurate knowledge of the epidemiology of the parasite, and upon the development of sensitive and specific detection tools. (Fayer, 1997). Additionally, efforts to discover effective chemotherapeutic agents for C. parvum and better understand the pathogenesis of cryptosporidiosis must continue. Other coccidian parasites are of similar importance to US producers. Eimeria infections in ruminants annually produce significant losses and in poultry, treatment for this genus is universal in broiler production. This practice has led to serious levels of resistance to many of the available poultry coccidiostats.

The limitations of chemotherapy are also evident in the dramatic increase in anthelmintic resistance seen in the trichostrongylid nematode Haemonchus contortus in the south central and south eastern United States. H. contortus is a blood sucking parasite and the most important of the GI worms of small ruminants. The mild climate of the southeastern US is particularly well

suited to the life cycle of this group of nematodes. Development from the egg to the infective stage occurs on pasture and is dependent on warm weather and adequate mositure. To prevent both morbidity and mortality of animals, small ruminant producers are often forced to deworm their animals repeatedly during the grazing season. These frequent treatments can rapidly select for anthelmintic resistance (Conder and Cambell, 1995). While there are ample anecdotal reports of resistance to 1 or more drugs, the extent of resistance in the US has not been well documented. In the absence of practical alternatives, the efficacy of available anthelmintics needs to be preserved. Once the prevalence and distribution of the resistance problem are established, rational strategies of drug use can be developed and tested for efficacy in areas where resistance threatens the ability of producers to control parasite infections. Resistance to anthelmintics has also now been documented in the strongylids of horses and swine but the extent of the problem in these species is also unknown.

The increasing levels of anthelmintic resistance and efforts to reduce our dependence on traditional chemotherapeutic approaches to parasite control, provide impetus for development of novel approaches to the management of parasitism. Vaccination against gastrointestinal helminths offers an alternative approach to control in an integrated parasite control program (Smith, 1999). However, effective vaccines for nematode parasites have been very difficult to develop (at this time there is only one available—the European bovine lungworm vaccine). One of the primary reasons for this difficulty is the complexity of the immune response to gastrointestinal nematodes. To date a large number of bovine immune responses have been measured including serum antibody, serum eosinophils, cellular reactivity, and the induction of mRNA's of 6 different cytokines. None have correlated with protective immunity, although work with the economically important bovine abomasal parasite, Ostertagia ostertagi, and other animal nematodes has demonstrated the central role of cytokines in mediating immune and other responses to parasitism (Finkelman, Shea-donohue eta al, 1997; Gasbarre, 1997). Additional analysis of antihelminth immunity is required in conjunction with efforts to identify effective antigens for vaccine use.

The nematode intestine has provided a valuable source of antigens that are capable of inducing protective immunity against Haemonchus contortus and potentially other gastrointestinal nematode parasites Whole intestinal preparations, intestinal antigen complexes and individual intestinal proteins (Munn, Greenwood, et al. 1987; Jasmer, McGuire 1991; Smith 1993; Smith, Munn, et al. 1993; Jasmer, Perryman, et al. 1993) have all induced significant levels of protective immunity against H. contortus challenge infections in sheep and goats. Genes encoding some of these individual antigens have been cloned and genes encoding related proteins have been identified from other parasitic nematodes (Redmond, Knox, et al. 1997; Longbottom, Redmond, et al. 1997; Rehman, Jasmer 1998). Therefore, observations made with H. contortus in small ruminants may apply to other nematode infections. Vaccination against the equine nematode, Strongylus vulgaris, is also being studied (Klei, 1997). There are numerous questions that need to be addressed regarding vaccines based on intestinal and other antigens and their significance in control of gastrointestinal nematodes. One relates to the ability of these antigens to protect against field challenge, since most immunization trials have utilized single laboratory infections for challenge. Another is identification of recombinant proteins and presentation protocols able to induce protective immune responses that approximate those of corresponding antigens isolated from worms. A third relates to host mechanisms responsible for the protective immunity, which may provide guidance for development of antigen presentation protocols.

Successful vaccination is based on manipulation of the individual host immune response to parasites, but there is also considerable evidence that part of the natural variation seen in resistance to helminth infection in ruminants is under genetic control (Albers and Gray, 1986; Barger, 1989; Stear et al.,1990b; Gogolin-Ewens et al., 1992; Gray and Gill, 1993; Ruvuna and Taylor, 1994; Stear and Murray, 1994; Miller and Gray, 1996). It has been shown that some breeds of sheep are more resistant than others to effects of nematode infection (Altaif and Dargie, 1978; Preston and Allonby, 1978, 1979a; Baker etal., 1993, 1994; Gruner et al., 1986; Radhakrishnan et al., 1972; Courtney et al., 1985; Bahirathan et al., 1996; Miller et al., 1998). Evaluating breed resistance under field conditions and understanding the genetic mechanisms of resistance are 2 important components of this approach to parasite control. Moreover, identification of some of the genes involved in regulating resistance to helminth infection will allow early selection of genetically superior animals and may increase the rate of selection for resistance. Attempts to associate genetic markers with nematode resistance has been under investigation for a number of years (Beh and Maddox, 1996), without much success. Because nematode resistance is probably a function of multiple loci and a quantitative trait, this and investigations by others (ILRI, Kenya; AgResearch, New Zealand; and CSIRO, Australia, Roslin Institute, Scotland) are addressing a total genomic approach to finding and mapping resistance QTL. Numerous microsatellite markers have been and are continuing to be identified for gene mapping purposes. At present, an autosomal genetic linkage map of the sheep genome with over 550 markers has been published (Crawford et al., 1995) and updated (Maddox et al., 1996).

In addition to alternative techniques of parasite control based on host responses, attention is also being directed to elimination of parasite stages in the environment. It has long been recognized that chemical control, although convenient and fast acting, is only a partial and short term control procedure. In contrast, there are implications that biological control, although slow acting, may be a persistent and enduring control methodology. Biological control is highly dependent on a thorough knowledge of the control agents. For example, the nematophagous fungi (e.g. Duddingtonia flagrans) are environmentally safe and would decrease pasture contamination by trapping and killing infective nematode parasite larvae (Sterling, 1991), reducing the need for drug treatment and slowing the rate of development of drug resistance. Additionally, reduction in anthelmintic treatments will decrease drug residues in meat and meat products and also reduce chemical contamination of the environment.

Strategic management of pastures has also been shown to affect levels of infective parasitic nematode larvae (Michel, 1976). Several techniques have been utilized to reduce pasture parasite burdens including treatment of the host animal with anthelmintics at the time of peak egg shedding and then allowing the remaining larvae to die off during periods when the pastures were not grazed (Brunsdon, 1980; Donald, 1974; and Williams et al., 1986). The practice of moving cattle to crop residues following the harvesting of a non-grazed crop has been shown to offer relatively safe pastures (Michel, 1976). There are many factors influencing development of safe pastures that have not been investigated. This is particularly true of production systems that involve rotation of crop and livestock production. Little is known about the minimal time for removal of all helminth species from a particular land area or how soil and crop management practices influence the removal of certain species. Further, little is known about how long safe pastures can be maintained as safe pastures or what contamination prevention techniques are

practical for maintenance of such pastures. These issues must be examined regionally because of the enormous variation in forage and crop biology and management across the U.S.

Needs and Advantages of a cooperative approach

While chemotherapy will remain an important part of parasite control, vaccines, use of genetic variation and biocontrol offer exciting alternatives to reliance on antiparasitics alone. However, the value of any parasite control technique cannot be fully evaluated in the laboratory. Any technique of parasite control must be examined in the field within the context of the total management system. While the ultimate goal of this regional project is the development of parasite control programs in sustainable agricultural systems, there is still considerable knowledge to be gathered about the individual components of these programs and how they interact. The problem is of such magnitude that it can be addressed effectively only by a multidisciplinary approach involving scientists working on many diverse specific aspects of this problem. Progress in controlling parasitic disease of livestock can best be achieved by a comprehensive approach to research utilizing regional cooperation in the maintenance of parasite strains, exchange of samples, pooling of expertise, information and equipment and exchange of research data, while permitting individual laboratories to specialize in aspects of the master project which they are best equipped to perform. No individual station or investigator is likely to develop an effective control program. Additionally, field testing of any parasite programs must be replicated in different regions to account for the wide variation in climatic and environmental conditions in the U.S. We feel that the objectives of this project are more likely to be accomplished by pooling our efforts in the multifaceted approach proposed.

Expected benefits

It is important that the American livestock industries look toward development of integrated pest management systems for control of parasite pathogens. The development of these systems requires a thorough knowledge of the biology of the infecting organisms, the delineation of host responses that reduce parasite transmission and parasite induced pathology, the identification of potential agents for biological control, along with a better understanding of anthelmintic usage. A more complete knowledge in these areas will permit the development of sustainable agricultural systems that utilize host resistance and strategic drug treatment to reduce parasite transmission and pathology. Such integrated management systems will improve the efficiency of livestock operations by reducing costs of parasite control, lessening the potential for drug resistance in parasite populations and reducing the amount of drug usage in the food supply.

Unfortunately, transmission of the parasites is intimately correlated with local environmental conditions and local management practices. So, while general tenets of parasite-host relationships will be applicable among producers throughout the U.S., specific recommendations aimed at parasite control will have to be tailored for different geographical regions. This need to refine information to fit local conditions mandates that attempts to develop integrated pest

management systems for parasite control involve researchers from diverse geographical areas, and include experts in parasite epidemiology, immunology, genetics and molecular biology.

RELATED CURRENT AND PREVIOUS WORK:

The participants in regional research project W-102, "Integrated Methods of Parasite control for Improved Livestock Production," have published 192 peer-reviewed articles and book chapters (10 in press). In addition, 53 technical or popular articles, 10 theses/dissertations and 135 abstracts were published. This effort clearly demonstrates the continued progress shown by the cooperative efforts of the technical committee.

A search for related work was conducted in the Current Research Information System (CRIS) database. The search retrieved 102 resumes of current (termination dates of 1998 or later) Hatch, State, Industry, Animal Health and USDA Special Grant projects within the scope of the work conducted by the W-102 participants. Forty-seven (46%) were directed by W-102 participants and collaborators (6 were W-102 projects; the remainder were special grants, Hatch, etc., projects under the leadership of W-102 participants). The remaining related projects (55 or 54%) were from 23 states (including 10 of the W-102 states) and included research on parasitism in wildlife (3), general protozoal infections in domestic animals (34) and general helminth infections in domestic animals (10). Three projects involved molecular and immunological research on protozoan and helminthic disease in non-domestic animals. These latter projects represent efforts of specific individuals and/or laboratories and are not coordinated as are the W-102 efforts.

In addition to the above-mentioned projects, W-102 shares interests with other regional projects:

NC-62	"Prevention and Control of enteric Diseases of Swine"
NC-107	"Bovine Respiratory Diseases: Risk Factors, Pathogens, Diagnosis and Management"
NE-60	"Genetic Bases for resistance and Immunity to Avian Diseases"
S-145	"Nutrition and Management of Swine for Increased Reproductive Efficiency"
S-221	"Development of Profitable Beef-Forage Production Systems for the Southern Region
W-112	"Reproductive Performance in Domestic Ruminants"
NRSP-8	"National Animal Genome Research Program

These shared interests are largely evident in the types of technologies used to study, understand, and control diseases and are not manifest in the specific type of pathogen-host relationship. There is no overlap of W-102 with any of these regional projects. However, control

methodologies generated by W-102 research will contribute to the missions of the related projects. The reader is referred to "The extent of the problem" under the "Justification" section for a review of the science related to this project.

OVERVIEW OF OBJECTIVES

OBJECTIVE 1: Control of parasitic diseases using biological and chemical agents and physical methods

1A. Evaluate the anthelmintic activity of naturally occurring fungi in laboratory and field trials

IB. Evaluation of the efficacy of the latest generation of endecto-parasiciticides and novel anthelmintic agents.

1C. Determine the prevalence of anthelmintic resistance in the US and characterize resistant parasites

1D. Development of an animal model for cryptosporidiosis

OBJECTIVE 2: Define the roles of pathogenesis, immunomodulation, vaccination and genetic manipulations in parasite control

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

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

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

OBJECTIVE 3: Integration of parasite control practices into livestock production systems

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

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

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

3D. Examine the impact of anthelmintic treatment and nematode trapping fungi on transmission of equine strongyles

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

OBJECTIVES

1: Control of parasitic diseases using biological and chemical agents and physical methods

2: Define the roles of pathogenesis, immunomodulation, vaccination and genetic manipulations in parasite control

3: Integration of parasite control practices into livestock production systems

PROCEDURES:

Objective 1. Control of parasitic diseases using biological and chemical agents and physical methods. Coordinator: Healey (UT)

1A. Evaluate the anthelmintic activity of naturally occurring fungi in laboratory and field trials.

1. Efficacy of biological control measures on parasite transmission will be studied. The ability of fungi such as Verticillium, Rhopalomyces, and Catenaria to attack nematode, cestode, and trematode eggs under laboratory conditions will be determined. Selection of effective fungal species and strains will be implemented as well as improved cultivation technology to produce infective propagules for field studies. Field studies conducted by CA-Riverside will involve small pasture plots and will be done in collaboration with MD and LA. After development of successful small field tests, more extensive field testing with other members of the technical committee will be sought.

2. Another study using biological control is planned to determine how the nematode-trapping fungus Duddingtonia flagrans might be used in combination with anthelmintic treatment protocols in sheep grazing pastures predominantly contaminated with Haemonchus contortus. During a 2 to 3 year period, one group of sheep will be supplemented with feed containing fungal spores during the haemonchosis season (April through September). Another group of sheep will receive the same supplemental feed without fungal spores. Parasite infection levels will be monitored using fecal nematode egg per gram (EPG) counts and blood packed cell volumes (PCV). The pasture contamination level will be discerned either by grazing tracer lambs or enumerating infective larvae recovered from pasture herbage samples. Intervention with anthelmintic treatments will be deemed necessary at times by comparative EPG counts and PCV. It is expected that the fungal spore-fed group of sheep will have cleaner pastures and reduced reliance on anthelmintic treatment. This research will be conducted in LA and in collaboration with the Danish Center for Experimental Parasitology.

IB. Evaluation of the efficacy of the latest generation of endecto-parasiciticides and novel anthelmintic agents.

1. The latest generations of endectoparasiticide and other novel anthelmintic drugs will be screened in cattle, sheep, goats, and horses to determine better efficacy, broader spectrum, safety, zero or minimal withdrawal times, and most appropriate programming time for administration. Drugs will include the avermectins eprinomectin, moxidectin, doramectin, and ivermectin and any other new classes of anthelmintic drugs that become available. Formulations will include solutions for injection as well as topical pour-ons. Both internal and external parasites will be targeted, including nematodes of the gastrointestinal and respiratory tracts, migrating arthropods and nematodes in the extraintestinal viscera, lice, fleas, grubs, and maggots of horn and house flies. The primary intent will be to determine effectiveness of drug activity, especially in beef cattle, on fecal egg count reduction of natural occurring gastrointestinal nematodes. In addition, body weights per animal and means per group will be analyzed. Speciation of gastrointestinal nematodes will be determined by coproculture. In addition to MO, 3 geographically separated regions (MN, LS, and MS) will cooperate to provide data.

1C. Determine the prevalence of anthelmintic resistance in the US and characterize resistant parasites

1. Anthelmintic resistance in the trichostrongylid parasite Haemonchus contortus has become an urgent problem in small ruminant production throughout the world. In the United States, resistance has not been widely documented. The extent of anthelmintic resistance in H. contortus and other trichostrongyles of small ruminants will be established using both fecal egg counting techniques and a larval development assay. Fecal samples will be collected from llamas to assist with the evaluation of the extent of anthelmintic resistance that occurs in trichostrongylid parasites of llamas in the United States. Llamas are often frequently dewormed to control meningeal worm infections and anthelmintic resistance is likely to occur in their gastrointestinal parasites. Since many of the trichostrongyle parasites of llamas are shared with small ruminants, strains from llamas may infect small ruminants and increase the prevalence of resistance in those animals. This research will be conducted in VA in collaboration with MO, LA, and MS.

2. In addition to llamas, captive sylvatic ruminants and exotic bovids will be examined for anthelmintic resistance to gastrointestinal nematode populations. The larval inhibition assay (DrenchRite®, Horizon Technology, Australia) will be used to detect resistant trichostrongylid nematodes. This in vitro assay is designed to detect resistance to avermectin/milbemycin, benzimidazoles, levamisole and benzimidazole/levamisole combinations in the major gastrointestinal nematodes of sheep. The prevalence of resistance to specific target compounds in these animals will be discerned using samples anaerobically shipped to collaborating laboratories in order to avoid problems with egg development prior to the assay. Evaluation of new and existing anthelmintic compounds is necessary in order to integrate their use into strategic parasite control programs so that producers can be advised as to development of anthelmintic resistance. Documentation of the presence and extent of

anthelmintic resistance in traditional livestock, alternative livestock, captive ruminants, and exotic bovids will advance our understanding of how anthelmintic resistance develops and is maintained. This research will represent a collaborative effort between MS, KS, TX, and VA. Moreover, the larval inhibition assay will be used to test these anthelmintics in small ruminants and cattle in cooperation with extension veterinarians and animal scientists in KS, MO, MS, MN, and TX.

1D. Development of an animal model for cryptosporidiosis

1. Research will be conducted to determine the feasibility of using the non-neonatal pig (1 week to 6 months of age) as an animal model for cryptosporidiosis. Initially, experiments will be designed to provide preliminary data on various immunosuppressive regimens when administered to pigs of different age groups. Later, the non-neonatal pig model will be fully developed by conducting experiments designed to define the optimum immunosuppressive regimen, ages most susceptible to infection, minimum dosage of oocysts that will produce patent infections, and isolates of C. parvum capable of infecting the pigs. Demonstrating efficacy against C. parvum following the administration of paromomycin to infected pigs will be used to validate the model. This model can then be used for testing of novel therapies for cryptosporidiosis. This research will be conducted primarily in UT, with possible collaborations with MS and MD.

Objective 2 Define the roles of pathogenesis, immunomodulation, vaccination and genetic manipulations in parasite control. Coordinator: Jasmer (WA)

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

1. Studies in Maryland will continue to define immune responses elicited by gastrointestinal nematodes of cattle. Using the assay systems developed in cattle infected with Ostertagia ostertagi, future studies will compare responses of resistant and susceptible cattle to infection by the parasites in an effort to identify responses that are correlated with protective immunity. These studies will involve genetically selected cattle and cattle that have been immunized in a such a manner that they reduce the number of parasites developing after challenge infection. Similar studies will be performed to define protective immune responses elicited by other parasite species, such as Haemonchus placei which occupies the same organ as Ostertagia but elicits strongly protective immune responses. These studies will focus on cytokine responses in the local tissues, and also on effector cell populations at the site of infection. A number of new parameters will also be evaluated including levels of pro-inflammatory cytokines, levels of colony stimulating cytokines, and numbers of effector cell populations in the abomasal mucosa. These studies should provide more accurate markers for functional immunity in the bovine host, and will aid mapping of genes involved in immunity to GI nematodes. Through collaborative efforts to produce reagents specific for bovine cytokines efforts will be made to determine if cytokine gene expression correlates with biologically active products. The purpose of all studies in this area will be to identify protective immune

mechanisms or immune responses that directly correlate with protective immunity. The identification of immune responses directly responsible for protection will result in further studies aimed at enhancing the generation of these responses, while the identification of responses that are correlates of protective immunity would allow for a more accurate identification of resistant animals and thus facilitate the search for the protective responses.

2. Products of Ostertagia ostertagi that have immunoregulatory activity will be identified. Recent studies have shown that products of O ostertagi inhibit *in vitro* growth of T lymphocytes. Preliminary work implies that this activity may reside in a TGF-beta like moiety. It is very intriguing that a molecule of this class is also involved in the regulation of Dauer larvae formation in C. elegans. Future studies will attempt to purify the inhibitory moiety and confirm or dismiss the idea that it is a TGF-beta-like substance. Once identified the inhibitory molecule will be characterized, and the gene(s) encoding the substance will be cloned and expressed for further studies of the mechanism of immunosuppression.

Collaborative studies (LA, MD, MN) will continue to determine if Ostertagia infections also result in functional immunosuppression of their host. A growing body of evidence indicates that immune responses to certain microorganisms result in the suppression of immunity to other infections that require different immune effector mechanisms. Studies will be performed in which parasite infected and parasite free cattle will be subjected to immunization which unrelated antigens. The ability of the cattle to respond to these immunological insults will be assessed to ascertain the effect of the existing nematode infections.

3. Previous studies have demonstrated that ponies immunized with irradiated L3 of the nematode S.vulgaris are protected from challenge infections whereas those immunized with worm extracts in RIBI adjuvant show signs of increased pathology as compared to uninfected controls (Monahan et al., 1994). These observations suggest that different immune responses are induced by these two different immunization regimes and that these two responses are associated with either protection or pathology. Cytokine gene expression will be measured in ponies immunized by these two methods during a challenge infection and these profiles compared to uninfected controls. Cytokine gene expression in cells collected from blood and cecal lymph nodes will be measured using an RT-QPCR method previously described (Swiderski et al.,1998). Cells will be collected from ponies at day -4, 4, 9 and 14 following challenge infections of 1000 L3. Surgical methods previously described will be used to collect cecal lymph nodes (Swiderski et al., 1998). Lymphocytes will also be cultured with parasite antigen, separated into CD4+ and CD8+ cells by magnetic bead separation and cytokine gene measured. Cytokine gene expression to be measured includes, IL-2,IL-4, IFN gamma, IL-10 and IL-5 (LA).

4. Recent studies demonstrate that resistance to the gastrointestinal worms N. brasiliensis, Heligmosomoides polygyrus, Strongyloidies venezuelensis, and Trichinella spiralis are regulated by activation of the intracellular enzyme, the STAT-6 molecule, that binds to the common alpha chain of the IL-4/IL-13 receptor. This indicates a common mechanism of control of phylogenetically and physiologically different nematodes that infect the mammalian gut. Elucidation of the mechanism of IL-4 versus IL-13-induced immune responses to gastrointestinal

parasites, and their relationship to the interleukin receptor-activated STAT-6 molecule that regulates IL-4 and IL-13 gene activation will be a key event in the understanding of resistance to gastrointestinal parasites. Parasite infection models provide insight into the design of more complicated and costly studies of responses of swine to parasitic infections. Human and mouse agonists and antagonists of the IL-4/IL-13 receptor will be evaluated on pig lymphoid and epithelial cells isolated from the intestines in order to determine their application to control of infection. Development of cytokine measurement techniques in swine infected with Ascaris suum and Trichuris suis will be evaluated to provide recommendations for control of these important economic and the emerging zoonotic aspects of these infections. Recent data indicates that these infections may have a broader role in foodborne infections. It is now clear that prior infection with Trichuris suis enables Campylobacter pylori to establish more vigorously in the intestine with resultant effects on animal health and on foodborne transmission of this human pathogen.

5. Studies will be undertaken to clone, express and evaluate cryptic gut-associated proteins derived from Ascaris suum that can be used to attenuate host infection. Attention will be directed toward proteins that may have more universal applicability in reducing parasitosis from other common nematodes parasites of swine. IL-4 will be cloned and expressed to develop quantities of the biologically active molecule to test its therapeutic and adjuvant activities against gastrointestinal nematodes. Combinational treatment with common nematode antigens and IL-4/IL-13 receptor agonists will be evaluated for its potential to activate protective mechanism common to the STAT-6-dependent resistance that exists in murine models of resistance to infection. Swine IL-12, and other important swine cytokines will also be cloned and expressed to test for use as immune modulators to enhance resistance to economically important protozoan parasites and to evaluate their use as activators of innate immunity. Potentially these compounds could be used to reduce early weaning and transportation-induced losses in production costs in neonatal swine.

6. Immunity to the protozoan parasite, Neospora caninum, will be examined in several systems. The cell mediated immune response to N. caninum in dogs will be determined with lymphocyte proliferation assays using Alamar blue (Zhi-Jun et al., 1998). Flow cytometry will be used to examine specific T and B cell populations in infected dogs. Humoral responses will be determined using an Immunofluorescent Antibody (IFA) test. Immunodominant antigens will be identified by western immunoblotting techniques. Sheep will be used as a model for N. caninum induced abortions. Pregnant ewes will be infected at various times in gestation and the fetal outcome evaluated (VA).

7. Studies have been initiated to define immune responses in naïve and immunized cattle after infection with Eimeria (MD, MN) or Neospora (MD, VA). Initial studies are focused on the identification of the recognition sites of parasite antigens by the host immune system, and upon the characterization of lymphoid populations stimulated. Competitive PCR techniques for at least 13 different bovine cytokines are available, and these assays are being used to discern important shifts in cytokine gene expression. Once important cell populations and/or cytokines responses are identified, attempts will be made to employ a number of immunomodulators, including recombinant cytokines, to enhance desirable immune responses.

8. Studies will continue to define the immune responses to Cryptosporidium infections in neonatal calves. Susceptible and resistant calves will be challenged with the parasite to assess immune responses such as cytokine profiles, cellular reactivity, changes in lymphocyte subpopulations, and effector cell numbers in the mucosa. In addition the effect of exogenous cytokines and gamma-Interferon inducing agents on immunity to the parasite will be determined

9. Current efforts to produce colostrum for immunotherapy of cryptosporidiosis in young calves will be expanded. Research plans will build on recent experiments wherein gene gun immunization of periparturient cows with plasmid DNA encoding C. parvum antigens elicited anti-C. parvum antibodies in colostrum. Studies will include using other plasmid expression vectors, genes for other C. parvum antigens, and use of liposomes or cytokines to enhance immune responses. The colostrum will be tested in an adult mouse model and in calves against C. parvum infection. Some calves will be tested for response to immunization by oral inoculation with irradiated C. parvum oocysts.

10. Efforts will continue to identify protective antigens of Eimeria species in chickens and to develop a comprehensive understanding of host protective immunity. Molecular probes, anti-cytokine antibodies, and sensitive molecular techniques to evaluate cytokine production in chickens with coccidiosis will be developed and used in conjunction with strategies to express avian cytokines. Develop an effective mucosal immunization strategy against coccidiosis using viral and bacterial vectors as well as naked DNA.

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

1. Monoclonal Antibodies (Mabs) against the sporozoite and oocyst stages of Cryptosporidium parvum have been produced. To identify antigens from other stages in the host, a panel of Mabs against the intermediate stages (meronts) of this parasite will be generated (UT). Briefly, neonatal BALB/c mice (1 week of age) will be experimentally infected with C. parvum via oral inoculation with infective oocysts. Intestinal infections will be resolved as the mice become older. At approximately 2 to 3 months of age, mice will be challenged by intraperitoneal injections with antigens prepared from intermediate stages (meronts) of the parasite. Meronts will be produced by infecting 1-day-old white Leghorn chickens. At 3 or 4 days of age, the chickens will be killed and meronts collected from the terminal ilea. Mice will be subsequently challenged at 2 to 3 month intervals using antigens prepared from sonicated oocysts. Five to 7 days prior to killing, mice will be challenged a final time by intraperitoneal injection of either chicken-derived meront antigens or sonicated oocysts. A standard fusion protocol will then be followed. Resultant hybridomas will be screened with the ELISA and an indirect immunofluorescence assay (IFA) for production of Mabs against C. parvum meronts. The IFA will be used to observe Mabs specific for meronts growing in bovine fallopian tube epithelial (BFTE) cell culture. These monoclonal antibodies will be used to monitor in vitro cultures for screening of antiparasite inhibitors and may identify antigens that have potential value for immune protection against C. parvum infections. (UT).

2. Due to the initial success of field trials using a gel formulation of irradiated Eimeria oocysts to protect against coccidiosis, we plan to expand our vaccination trialsusing lower doses of oocysts to achieve protection. Recombinant Eimeria antigens associated with metabolic stages of intracellular parasites will be produced based on reverse transcriptase and PCR reaction of mRNA to identify targets for protective immunity. Cooperative agreements will be developed to test metabolic proteins as targets for drug treatment to prevent coccidiosis.

3. Studies on vaccination with recombinant Eimeria antigens using direct DNA injection of plasmid DNA will be expanded by comparing different expression plasmids and the use of liposomes and cytokines to enhance responses. Through an understanding of the mechanism of tissue gene expression, testing of live delivery vectors that may replicate antigen processing and expression will be attempted.

4. The importance of cytokines in response to vaccination and pathology will be examined, particularly against foodborne parasites like Toxoplasma gondii. Expression of recombinant IL-12 will be evaluated for its effect on protection and its potential as an adjuvant in combination with vaccination against toxoplasmosis. Detailed studies to define immunity to T. gondii will focus on effective vaccines; efforts will be aimed at identifying unique antigens that stimulate protective anti-parasite responses. Parallel efforts will be focused on use of DNA vaccination to stimulate protective immune responses against this foodborne parasite.

5. Collaborative efforts will identify antigens of Haemonchus contortus for use in vaccine trials and the geographic distribution of antigens Multiple genes encoding intestinal antigens from H. contortus have been cloned. These include GA1, cysteine protease and metallopeptidase proteins. Recombinant proteins encoded by these antigens will be expressed in bacteria as glutathione transferase (GST) fusion proteins, isolated by conventional methods and tested in immunization trials (WA). Moreover, antibody, nucleic acid probes and PCR primers exist to determine geographic conservation of gut antigens that are vaccine candidates of this parasite. Field isolates of H. contortus from geographically disparate worm populations will be evaluated for conservation of these antigens (LA, TX, VA. WA).

6. Whole intestine dissected from adult worms (WA), or H11-HgalGP (Moredun Research Institute) will be used to immunize kids or lambs, respectively. Immunized animals will be field challenged (kids in MO; lambs in LA) on pasture during known periods of high parasite transmission. Immunized and challenged animals will be compared with respective control groups to determine whether immunization methods and antigens, shown to induce protection against laboratory infections, will have efficacy against natural challenge infections. Recent work has indicated that adjuvants other than those currently being used should be evaluated for sustaining the antigenic activity of the antigens. These studies will be conducted in MO, LA and WA with additional collaboration with the Moredun Research Institute in Scotland(D Smith).

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

1. Microsatellite genomic markers for sheep will be used to identify alleles that may account for differences in resistance to H. contortus infections among sheep breeds (LA,TX,UT). Gulf coast sheep (Native sheep) are significantly more resistant to H. contortus than Suffolk sheep. Approximately 400 F2 lambs (from large half-sib families) will be phenotyped for H. contortus infection level based on fecal nematode egg count data. DNA from lambs classified as resistant (20% lowest infection level) and susceptible (20% highest infection level) will be compared and screened across a panel of 150 microsatellite markers that are spaced 20-30 cM apart on the ovine gene map. We aim to map Quantitative Trait Loci (QTL) fixed for alternative alleles in the two breed groups which explain the breed difference. We will also test the hypothesis that major QTLs are not fixed, but are segregating within one or the other breed. Analyses will be performed by regression based interval mapping methods. In addition, a similar, but smaller resource population consisting of Native and Rambouillet sheep at Texas A&M University (TX) will be similarly assessed. Other collaborations on this study are with Utah State University, the University of Georgia (A McGraw), and the Roslin Institute in Scotland (C Haley).

2. The feasibility of selection for resistance to H. contortus infection as a management tool in profitable wool breeds will be investigated by crossbreeding experiments using resistant St. Croix sheep. In addition, individual variation will be assessed in a University flock as a prelude to an intrabreed selection program aimed at increasing parasite resistance (VA). Positive results on these projects will provide resources for mechanistic investigations in collaboration with LA. Experiments will also be conducted to characterize parasite resistance among goat breeds using fecal egg count analysis and hematologic parameters.

3. Cooperative studies (MD, UT) have been initiated to begin linkage mapping of genes involved in resistance to GI nematodes in cattle. Phenotypic data (currently third generation) will be combined with linkage mapping to identify genomic areas associated with enhanced or diminished resistance to the parasites. A number of polymorphic probes which give moderately good coverage of the genome have been identified, and the statistical model for data analyses based on the population structure has been developed. Offspring of selected breedings of cattle will continue to be tested for their parasite resistance phenotype. Past work has indicated that several commonly used measures of parasite burdens are inaccurate, or are applicable to the less pathogenic genera infecting cattle. New parameters are being continually tested for their accuracy and precision in both detecting parasite numbers in the host, and for measuring host immunity to the parasites.

4. Immune based mechanisms that contribute to differences in resistance demonstrated by different sheep breeds will be evaluated by several approaches. Dexamethasone will be used in attempts to abrogate resistance (LA). CD4+ T lymphocytes will also be depleted in resistant lambs in attempts to abrogate resistance (LA, WA). Humoral and T lymphocyte responses, including mucosal and lymph node responses from the abomasum, will be compared between suppressed/depleted and control lambs. Samples of abomasum will be evaluated by quantitative histological analysis of mast cells, globule leukocytes, B cells and intracellular cytokines (LA, VA).

5. Determination of the genes which control T. gondii resistance will require testing many more SLA inbred pigs, as well as testing outbred swine. As candidate genes involved in resistance are identified, studies will be needed to determine their applicability in both inbred and outbred populations of pigs. Currently most pigs which are resistant have fewer parasites in their tissues. For foodborne protozoan parasite infections it remains to be determined whether genetic resistance must be complete, i.e., whether no parasite can be left in the tissue or whether significant decreases in parasite burden are effective. An alternate approach to controlling this infection could involve combination of genetic control with vaccination. Because the swine industry has been so effective in controlling parasitic infections in their modern facilities research in MD has expanded into detailed analyses of the developing immune system of the neonatal pig. In collaboration with PIG USA studies are underway to assess mucosal immunity in neonatal pigs. These data are being collected on pigs from defined genetic lines so that future studies can be performed to correlate parameters associated with overall disease resistance with the genetic alleles that regulate mucosal immunity and the related cytokine responses.

6. Due to increasing drug-resistance of Eimeria parasites, development of alternative control strategies toward coccidiosis control is critically needed. Although the nature of host genes which are involved in the control of coccidiosis is not known, many chicken DNA markers which are available now enable us to explore a marker-assisted genetic improvement strategy for poultry disease control. Researchers at the Avian Disease and Oncology Laboratory, East Lansing, MI, are collaborating on an effort to use chicken genome markers to map different commercial lines of poultry. A new program will be initiated to identify microsatellite markers which are associated with coccidiosis disease resistance in commercial broiler chickens. Lines of poultry are simultaneously being evaluated for their genotypes, using the microsatellite markers, and for their resistance to several important poultry diseases, including Marek's disease and coccidiosis. These studies should lead to the identification of DNA markers associated with disease resistance and should enable producers to establish a marker-assisted genetic improvement strategy for their core breeding lines. These DNA marker-based gene improvements in chickens will lessen the production losses due to parasites both by developing parasite resistant stock and by avoiding costs associated with use of drugs to control these infections. Overall, consumer confidence will be enhanced.

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

1. Although easy to perform, current diagnostic tests for cryptosporidiosis are based on positive binding to parasite oocysts by antibodies which are non-specific. Future work will build on the identification of C. parvum specific antigens. Preparation of specific sera and confirmation of reactivity with recombinant proteins will facilitate the development of C. parvum-specific monoclonal antibodies for detection of C. parvum oocysts in environmental samples.

2. Experimentation will attempt to develop novel nucleic-acid based molecular diagnostic assays for the identification of Cryptosporidium species, genotypes of C. parvum, and other emerging agriculture-mediated pathogens (MD). The value of LDS isozymes of C. parvum as targets for therapeutic intervention or for detection of viable oocysts in a simple dip stick test

will be examined. These assays will be evaluated using portable, analytical thermal cycler platforms. The feasibility of multilabeled, fluorogenic PCR probes for simultaneous one-tube, real-time identification of several pathogenic microorganisms will also be evaluated. Both inexpensive, high-throughput sample preparation methods and automated sample processing platforms will be tested for use in providing nucleic acids suitable for molecular diagnostic assays. Testing will be conducted to determine if these techniques are applicable to the detection of parasitic organisms in the environment. Studies will also be conducted to examine the utility and cost-effectiveness of these assays for use by various agricultural, food safety, and environmental-quality agencies and laboratories.

3. Ostertagia ostertagi is the most economically important parasite of cattle in the US. Because of the particular biology of this host-parasite system, there are no effective means to adequately estimate the number of Ostertagia ostertagi without killing the host. Recently studies in MD demonstrated that Ostertagia differ from all other trichostrongyle nematodes by the addition of a repeat sequence in a highly conserved region of ribosomal DNA. Using PCR technology it is possible to identify DNA from Ostertagia eggs, and to develop a semi-quantitative assay that detects Ostertagia eggs in the feces. This assay will be used to determine if the number of Ostertagia eggs in feces is representative of the number of adult Ostertagia in cattle (MD). As a corollary, attempts will be made to identify the number of eggs that correlate with economic loss. This latter aspect is very complex, and will involve the input of a number of the co-operating institutions (GA, KS, LA, MD, MO, MN, TX, VA).

OBJECTIVE 3: Integration of parasite control practices into livestock production systems. Coordinator: Stromberg (MN)

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

1. These studies will determine the impact of various soil and crop management strategies on the cleansing of parasitic larvae from contaminated pastures. A long-term experiment has been underway to determine the rate of soil carbon restoration on highly degraded, previously cultivated land using coastal bermuda grass pasture, hayed and unharvested management systems. The area was free of parasitic larvae prior to grass establishment and an effort has been made to prevent contamination of the area by judicious pre-grazing anthelmintic treatment of cattle. Parasites present will be identified and quantified in an effort to associate grazing intensity, pasture fertilizer source (poultry litter and inorganic), with the pasture parasite burden. Pasture parasite burdens will be determined using parasite-free tracer calves. The next phase of this experiment will include overseeding the bermuda grass with tall fescue in order to move toward almost year-around grazing. Characterization of the pasture parasite burden will continue in the second phase. Another experiment is being planned to investigate the impact of endophyte-infected tall fescue on subsequent cropping systems. As a part of this study, which will be executed on parasite-contaminated pastures, the impact of various soil and crop management systems (including conventional and minimum tillage, with and without cover cropping) on pasture larval survival will be evaluated (ARS-GA, GA).

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

1. Another group of studies will evaluate how combinations of chemotherapeutic treatment and animal/pasture management strategies impact species shifts and pasture larval contamination. These studies will determine the impact of managed intensive rotational grazing and the use of anthelmintics on the productivity of stocker beef cattle, cows and their calves, dairy replacement heifers and lactating dairy cows as well as the maintenance of grass (forage) availability and gastrointestinal parasitism. Most if these will be multi year studies using replicates and comparison to conventional methods. These studies will be conducted at multiple sites (ARS-GA, ARS-MD, MO, MN, TX)

2. Studies will continue on a Pennsylvania dairy farm that has been practicing intensive rotational grazing for the past 13 years. Previous work had demonstrated that the parasite control procedures initiated at the onset of the grazing program were insufficient to control economic losses due to GI nematodes. Using an intensive strategic anthelmintic program, the producer's pastures were returned to an infection level where little economic impact due to the parasites could be demonstrated (LA, MD). Future studies will use this well-characterized working dairy to determine the effect of different management and drug programs on the control of GI nematode parasites. Because this producer is an active member of the Northeast Grazing and Research Center, the Northeast Pasture Consortium, and a number of grazing discussion groups, the results of these studies will be directly conveyed to producers through the auspices of these organizations (LA, MD, MN).

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

1. Sheep and goat farms using a variety of anthelmintic parasite control programs will be tested for anthelmintic resistance in LA, MN, MS, TX using a larval development assay. The prevalence of resistance and its association with management practices in both sheep and goat flocks will be evaluated and resistant parasite genera will be identified.

2. Strain differences in O. dentatum in swine will be compared experimentally by allowing development to the infective stage under four different environmental conditions. These infective L3 will be used to infect pigs and eggs will be harvested as the infections become patent and then weeks later into the patent period. This will be repeated using the early and late patency eggs. The determination of the pre-patent period for all 8 strains as well as the original will be compared. Preliminary data suggests that different prepatent periods will result. Morphological and DNA characteristics will be used in evaluating these differences. These strains will then be used in studies to determine anthelmintic resistance in LA.

3D. Examine the impact of anthelmintic treatment and nematode trapping fungi on transmission of equine strongyles

1. The efficacy of daily feeding of Duddingtonia flagrans following anthelmintic treatment on the reduction of strongyle parasite numbers in ponies maintained on pasture in LA.

Mixed breed ponies 1 to 3 years of age, with naturally acquired infections of strongyles will be used. These will be housed on six pastures of approximately 1 acre each. Each pasture will house 3 ponies. Three pastures will be included in each treatment group. Ponies in one treatment group will receive fungal treatment and the control will not. Prior to turnout (day 0) all ponies will be treated with ivermectin at the recommended dose of 0.2 mg/kg of body weight. Fungal treatment will begin five weeks after treatment (day 35) a time prior to the reappearance of strongyle eggs in the feces. This will allow time for ponies to become accustomed to being fed fungus on a daily basis. It will also insure that the pastures are not contaminated with strongyle L3. Necropsies and worm counts will be conducted on ponies 10 weeks after the initiation of the fungal feeding (day 105) as previously described (Monahan et al., 1998). This period will allow for eggs to reappear, contaminate the pasture and provide for a significant exposure of ponies to pasture L3 during the peak transmission season.

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

1. The efficacy of on-farm treatment technologies for destroying C parvum oocysts will be examined as well as the role of environmental factors affecting transport of oocysts to surface waters, and the use of grass buffer strips as a mechanism to minimize/eliminate oocyst transport. On-farm composting of manure to temperatures over 35°C may be an effective method of reducing or eliminating oocyst viability. Pilot composting experiments in resulted in a 50% reduction in oocyst viability. Additional experiments are needed to optimize the time-temperature relationship and the effect of bulking agents on oocyst mortality It is frequently postulated that contamination of surface waters is due to surface runoff of oocysts from land-applied manure. However, environmental factors affecting the rates/extent of oocyst runoff are poorly understood. Major factors likely to control runoff are soil texture, soil slope, soil moisture, vegetation cover, and rainfall duration/intensity. The effects of these variables will be systematically investigated in order to formulate manure management strategies which eliminate or minimize the potential for oocyst transport. Grass buffer strips have been proposed as a means for reducing runoff of sediments, nutrients, and protozoan parasites/bacterial pathogens. Previous research has demonstrated the efficacy of buffer strips in removing sediments from runoff water. There is conflicting data regarding the efficacy of buffer strips in removing bacterial pathogens; there is no data with respect to protozoan parasites. To the extent that buffer strips may be implemented as a mechanism for reducing sediments and nutrients in runoff, it is important to determine their efficacy with respect to removal of pathogens/parasites from runoff waters (MD).

2. A herd of breeding cows that previously suffered an outbreak of abortion due to neosporosis will be studied over the course of 2 or more years. Pregnancy and calving rates will be determined and compared to serologic status of animals. Heifers born to seropositive cows will be included in the study, until they have calved themselves. The results of avidity ELISA testing, performed in Dr. Camilla Björkman's laboratory in Uppsala, Sweden, will be compared over time to help refine test parameters used to determine duration of infection. Standard production parameters will be compared between seropositive and seronegative animals. These data should allow development of management and culling strategies based upon economic impact (IL).

EXPECTED OUTCOMES:

Completion of the objectives described in this proposal will not only increase our understanding of host parasite relationships but bring us closer to applying this knowledge to both traditional and innovative methods of parasite control. Knowledge of the extent of anthelmintic resistance and evaluation of new products will allow cooperating members of this proposal to recommend the most efficient and economic use of antiparasitics in diverse regions of the U.S. This information will be presented to producer and practitioner groups around the country by committee members. Members of the regional project have been actively involved in outreach or continuing education programs in the past and the committee is now evaluating the feasibility of establishing a site on the world wide web (WWW) that would provide interested individuals and groups with "state of the art" information on parasite control. We believe that this form of technology transfer should reach our target audience in an effective manner.

Although it is unlikely that vaccines for helminth and protozoan parasites will be commercially available in the next 5 years, continuing the collaborative research begun in the previous project will substantially increase the probability that important commercial vaccines will be developed in the near, and not the distant, future. Similarly, other innovative strategies like the use of nematophagous fungi will receive important testing during the 5 years covered by the next project and the relevance of these techniques to modern animal management will be evaluated under field conditions.

Table 1. Matrix of Cooperative and Interactive Linkages in W-102

PROCEDURES	RESEARCH CENTERS														
	ARS-B	ARS-W	CA-R	GA	IL	KS	LA	MN	MO	MS	MT	TX	UT	VA	WA
OBJECTIVE 1: Parasite control using biological and chemical agents and physical methods															
Biological Agents			X				X								
Chemical Agents						X	X	X	X	X				X	
Cryptosporidium Model													X		
OBJECTIVE 2: Pathogenesis, immuno-modulation, vaccination and genetic manipulation															
Pathogenesis							X				X			X	
Parasite Antigens							X				X	X		X	X
Genetic Manipulation	X						X					X	X	X	X
Diagnosis	X														
OBJECTIVE 3: Integration of parasite control practices into livestock production systems															
Soil and crop management practices		X		X											
Integrated anthelmintic/pasture managementsystems	X	X						X	X			X			
Impact of management on anthelmintic resistance							X	X		X		X			
Impact of anthelmintics and nematode trapping fungi							X								
Impact of management practices on transmission of protozoa	X				X										

ARS-B –ARS Beltsville ARS-W – ARS-Watkinsville CA-R – University of California, Riverside

ORGANIZATION

Regional Technical Committee: The Technical Committee shall consist of the Administrative Advisor (non-voting), CSRS representative (non-voting), a technical representative from each participating SAES appointed by the director, and a technical representative of each cooperating USDA research laboratory named by the appropriate administrator. The responsibility of the Technical Committee shall be to coordinate research activities of the participants and to carry out such other functions as outlined in the Manual for Cooperative Regional Research SEA-CR/OD-1082.

Officers: These shall consist of a Chair, a Secretary, and a Member-at-Large. The Secretary will assume the office of the Chair and the Member-at-Large will assume the office of secretary.

Executive Committee: this subcommittee, consisting of the Chair, Secretary, Member-at-Large, and the Administrative Advisor will act as directed by, and for, the Technical Committee between meetings.

The time and place of the annual meetings will be decided by vote of the members after consultation with the Administrative Advisor or by the Executive Committee when so directed.

Control of Animal Parasites in Sustainable Agricultural Systems

SIGNATURES:

Advisor's Signature — 1/10/1999
Date

CHAIR, REGIONAL ASSOCIATION OF DIRECTORS — 3/24/99
DATE

Administrator, Cooperative State Research Service — Date

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ATTACHMENTS:

Project Leaders
Resources
Critical Review
Publications
Appendix D

Project Leaders

State, Laboratory	Principal Leader	Specialization
ARS-Beltsville, MD. Parasite Immunology Laboratory	L.C. Gasbarre	Immunoparasitology
ARS-Watkinsville, GA, So. Piedmont Con. Res. Center	J.A. Stuedemann	Animal Nutrition and Managment
California, Riverside SAES	E.G. Platzer	Physiology/Biochemistry
Georgia (SAES)	R. Kaplan	Epizootiology/Molecular Biology
Illinois (SAES)	M McAllister	Epizootiology/Pathology
Kansas (SAES)	R.K. Ridley	Epizootiology/Chemotherapy
Louisiana (SAES)	J. Miller	Genetics, Immunology
Minnesota (SAES)	B.E. Stromberg	Epizootiology/Immunology
Missouri (SAES) SAES	R.W. Corwin	Epizootiology/Chemotherapy
Mississippi (SAES)	L. Ballweber	Epizootiology/Immunology
Montana (SAES)	C.A. Speer	Immunololgy
Texas (SAES)	T.M. Craig	Epizootiology/Genetics
Utah (SAES)	M.C. Healy	Immunology/Chemotherapy
Virginia (SAES)	A.M. Zajac	Epizootiology/Chemotherapy
Washington (SAES)	D.P. Jasmer	Immunology/Molecular Biology

RESOURCES

	OBJECTIVE			RESOURCES	
PARTICIPANT	1	2	3	SY (% R, E, T)*	PY TY
ARS-Beltsville					
Louis C. Gasbarre		X	X	0.4 (100, 0, 0)	0.0 0.3
Joan Lunney		X		0.3 (100, 0, 0)	0.0 0.3
Dante Zarlenga		X	X	0.3 (100, 0, 0)	0.0 0.3
Ronald Fayer		X	X	0.3 (100, 0, 0)	0.0 0.3
Joseph Urban		X		0.3 (100, 0, 0)	0.0 0.3
Hyun Lillehoj		X		0.3 (100, 0, 0)	0.0 0.3
Mark Jenkins		X	X	0.3 (100, 0, 0)	0.0 0.3
James Higgins			X	0.3 (100, 0, 0)	0.0 0.3
ARS-Watkinsville					
John A. Stuedemann			X	0.10 (100, 0, 0)	0.0 0.10
Dinku M. Endale			X	0.05 (100, 0, 0)	0.0 0.05
Dwight H. Fisher			X	0.05 (100, 0, 0)	0.0 0.05
Alan J. Franzluebbers			X	0.05 (100, 0, 0)	0.0 0.05
Harry H. Schomberg			X	0.05 (100, 0, 0)	0.0 0.05
Dwight H. Seman			X	0.05 (100, 0, 0)	0.0 0.05
Bonner T. Stewart			X	0.05 (100, 0, 0)	0.0 0.05
H. Ciordia			X	0.10 (100, 0, 0)	0.0 0.10
California, Riverside					
Edward G. Platzer		X		0.35 (69, 0, 31)	0.0 0.0
Georgia					
Ray M. Kaplan			X	0.10 (50, 0, 50)	0.0 0.0
Illinois					
Milton McAllister			X	0.05 (50, 0, 50)	0.0 0.05
Kansas					
Robert K. Ridley	X			0.1 (30, 20, 50)	0.0 0.0
Heather Trumble	X			0.0	0.0 0.8
Louisiana					
James E. Miller	X	X		0.5 (30, 5, 0)	0.0 0.0
Thomas R. Klei		X	X	0.2 (20, 2, 0)	0.0 0.0
T. Bonner Stewart			X	0.1 (20, 2, 0)	0.0 0.0
John B. Malone	X		X	0.1 (20, 2, 0)	0.0 0.0
Maria T. Pena	X	X	X	0.0	0.8 0.0

RESOURCES (Cont'd)

	OBJECTIVE			RESOURCES	
PARTICIPANT	1	2	3	SY (% R, E, T)*	PY TY
Louisiana (Cont'd)					
Edmond Kabagambe	X	X	X	0.0	0.2 0.0
Sharon R. Barras	X	X	X	0.0	0.0 0.8
Melanie R. Chapman	X	X	X	0.0	0.0 0.5
Minnesota					
Bert E. Stromberg	X	X	X	0.1 (50, 5, 45)	0.0 0.0
Gary Averbeck	X	X	X	0.0	0.0 0.1
Missouri					
Robert Corwin	X		X	0.5 (40, 20, 40)	0.0 0.0
Mississippi					
L.R. Ballweber	X			0.5 (70, 15, 15)	0.0 0.5
Montana					
C. A. Speer	X			0.2 (90, 0, 10)	0.0 0.0
Mark Jutila	X			0.0	0.0 0.2
Eric Wilson	X			0.0	0.0 0.2
Utah					
Mark C. Healy	X	X		0.32 (10, 10, 0)	0.0 0.5
Shiguang Yang	X	X		0.0 0.0 0.5	
Texas					
T. M. Craig	X	X	X	0.1 (15, 35, 50)	0.2 0.1
W. S. Ramsey		X		0.1 (15, 35, 50)	0.0 0.1
S.K. Davis		X		0.1 (15, 35, 50)	0.0 0.1
Virginia					
Anne M. Zajac	X	X		0.25 (60, 20, 20)	0.0 0.1
David S. Lindsay		X		0.25 (70, 10, 20)	0.0 0.0
Washington					
Douglas P. Jasmer		X		0.2 (75, 0, 25)	0.1 0.25
TOTALS				7.12	1.3 7.7

*Percent appointment in research (R), extension (E), teaching (T)

CRITICAL REVIEW

WORK ACCOMPLISHED DURING PREVIOUS PROJECT

Progress was made in each of the three areas identified in the project proposal: 1) Determine patterns of parasite transmission, evaluate the impact of management practices, and develop improved diagnostic techniques 2) Identify and characterize parasite antigens and the host immune responses they elicit at the molecular and cellular levels 3) Determine the efficacy of modern chemotherapeutic agents including the prevalence of resistance and optimal treatment regimes.

Objective 1. Determine patterns of parasite transmission, evaluate the impact of management practices and develop improved methods of diagnosis.

Determine transmission dynamics to aid in the design of strategic control programs.

New information on the transmission of trichostrongylid parasites of cattle under several management programs was gathered in several regions with widely different patterns of climate and forage growth. In Georgia (in collaboration with researchers in Louisiana), a long-term experiment was conducted to determine the rate of soil-carbon restoration of highly degraded, cultivated land using Coastal bermuda grass. Harvest management systems included grazing at high or low grazing pressure, hayed and unharvested. As a part of this study, it was to be determined whether land under cultivation over an extended period of time could be maintained parasite-free with judicious cattle management. After four years, there were no differences among pasture fertilization or grazing pressure treatments, and the pastures remained 'essentially free' of nematode parasites. Steer average daily gain (ADG), across all treatments and all years, was outstanding. Reasons for this excellent ADG could be attributed to relatively good pasture conditions and that pastures were 'essentially free' of nematode parasites. Recently, a survey of the presence of dung beetles was initiated, and at least 14 species were identified. Results suggested that dung beetle numbers were higher in the high than the low grazing pressure treatments with an indication that numbers were highest in areas that were fertilized with broiler litter compared to nitrogen fertilized paddocks. The concept of establishing and maintaining essentially parasite-free pasture areas warrants greater emphasis. In Mississippi, the influence of anthelmintic treatments (fenbendazole vs. ivermectin) on performance of stockers grazing fungus-infected or fungus-free tall fescue was evaluated over 3 years. There was no difference in performance among treatment groups.

In Minnesota, studies on nematode larvae development and survival revealed that relatively few larvae were recovered from herbage surrounding dry fecal pats compared to wet ones. There was a direct correlation between the volume of the fecal pat and the number of larvae recovered from the herbage and most were recovered close to the fecal pat. Larvae migrated about the same distance form the pats irrespective of the volume of the pat. The contributions of cows and calves to pasture contamination was evaluated. Based on amount of feces deposited and FECs (fecal egg counts), calves exceeded the contribution by cows about half way through the grazing season indicating the importance of starting the grazing season on clean pasture. Strategically

dewormed cow-calf herds had much lower infection levels than non-dewormed herds. A survey of parasites of dairy cows determined the prevalence of helminths in 2 and 3 year old dairy cows. There were very few parasites identified.

In Virginia, the effect of gastrointestinal parasitism was compared in a traditional continuous grazing program compare to a low input sustainable program combining crop and pasture grazing. Little difference was seen in fecal egg counts, but cattle in the low input program consistently gained more weight.

A 3-year project was initiated in Missouri to study relationships between forage supply, stocking rate, gain per acre, and average daily gain of yearling cattle on either continuously stocked or rotationally stocked pasture. To date, there was little difference between herds representing each pasture type as regards FEC and larvae type, and most were Ostertagia and Cooperia spp. The mode of administration of highly efficacious dewormers was compared to determine relative persistence based on FEC of trichostrongylid species. More specific identification of these parasites was obtained by coproculture from treated and control animals. Persistence of deworming with formulations of anthelmintics given once only at the beginning of summer grazing to cows in a cow-calf herd were compared to a natural cycle of trichostrongylid parasitism of nontreated cow-calf herds kept on separate pastures. Persistence was demonstrated for treated cows based on depressed FEC and especially for the untreated calves of treated cows having much lower FEC than calves with nontreated dams.

In small ruminant studies, the effect of pasture rotation in Minnesota on the level of parasitism in lambs was evaluated. Four replicates were grazed continuously and compared to 4 replicates grazed rotationally. There was no significant differences in parasitism, as followed by FECs. The rotationally grazed lambs FECs increased more rapidly than those grazed continuously, however the continuously grazed lambs peaked at a slightly higher FEC.

Researchers in 3 southeastern states examined variation in breed and individual resistance to Haemonchus contortus in small ruminants under both natural and experimental conditions. A 3-year breeding program was conducted to evaluate direct breed effects, maternal breed effects, and heterosis for Haemonchus contortus infection between Suffolk and Gulf Coast Native breeds of sheep in Louisiana. For each year and overall, fecal egg count (FEC) and blood packed cell volume (PCV) data, collected from birth through 7 months of age, were higher and lower, respectively, for Suffolk lambs compared to Native lambs, and data for F_1 lambs were intermediate. Weight gain was also intermediate for F_1 lambs. It was consistently demonstrated that the Gulf Coast Native breed was significantly more resistant to nematode infection than the Suffolk breed, and that F1 lambs were intermediate. Heterosis analyses showed that F_1 lambs were more like Native lambs regarding resistance, indicating that resistance was a dominant trait, and weight gains did not favor either breed. A terminal cross between Suffolk and Gulf Coast Native sheep may offer increased resistance to Haemonchus contortus infection, thus reducing chemical control costs.

To further evaluate breed resistance to nematode infection in Louisiana, two hair sheep breeds (St. Croix and Katahdin) were also included in this program as a comparison to the wool breeds for level of resistance. Results indicated that Gulf Coast Native and St. Croix sheep were similar

in resistance to infection, and Katahdin sheep were almost as susceptible as Suffolk sheep. A 2-year crossbreeding program (Rambouillet x Gulf Coast Native) was conducted to introduce the callipyge gene into Gulf Coast Native sheep and to evaluate nematode infection between F_1 callipyge carrier, F_1 callipyge non-carrier, and Gulf Coast Native lambs. FEC and PCV (Packed Cell Volume) were monitored, from birth through seven months of age, and results indicated that F_1 lambs, whether callipyge carrier or not, acquired heavier nematode burdens than Gulf Coast Native lambs. Therefore, terminal cross callipyge Gulf Coast Native crossbred lambs did not retain nematode resistance. In collaboration with the Louisiana researchers, studies were initiated to evaluate genetic selection for host resistance to gastrointestinal parasites (primarily Haemonchus contortus) in Gulf Coast Native and Rambouillet sheep under grazing conditions in Texas. Comparisons were made under pasture conditions by FECs, serum protein and PCV. The Rambouillets had higher FECs and lower serum protein and PCV throughout the grazing season. The offspring of reciprocal crosses were extremely variable and the majority of animals had values between those of the parent populations, but favoring the Gulf Coast Native indicating that the trait is dominant. Crosses of the F_1 generation (F_2) showing either resistance or susceptibility to infection were selected for genetic studies.

In Virginia, studies were initiated to examine the relative resistance of sheep and goat breeds. At necropsy after natural challenge, kids of the Myotonic and Pygmy breeds had significantly lower worm burdens than Spanish and Nubian kids. Haemonchus and Trichostrongylus were the predominant genera. Studies on resistance within sheep breed were initiated using ewes and lambs that were experimentally infected with Haemonchus contortus. In lambs, PCV and FECs are correlated and repeatable. In ewes, PCV appears to be a more repeatable measure of Haemonchus infection than the FEC.

Effects of breed on parasite transmission were also examined in cattle. A 1-year study was conducted in Louisiana to determine level of nematode infection in cows and their nursing calves, and replacement heifers, and to compare the Angus to the Brangus breed. Calves had the highest infection levels followed by heifers and cows, respectively. Angus cows and calves had significantly higher infection levels than Brangus cows and calves, but no differences were seen in heifers.

A miniature horse was diagnosed as having an unusual invasion of the spinal cord with Angiostrongylus cantonensis, a lung parasite of rats. An epidemiological investigation showed that the wild rat population was highly infected with Angiostrongylus cantonensis, and snails (Physa spp.) indigenous to the local area were shown to be capable of harboring immature stages of the parasite. Grazing domestic animals may become infected by consuming infected snails.

In swine research, observations on pre-patent period differences among Oesophagostomum spp. in pigs led to initiation of studies to determine if different environmental conditions are a factor. Pigs experimentally infected with Metastrongylus spp. had reduced weight gain and feed efficiency. The potential for non-synchronic infections of freeze -resistant and domestic species of Trichinella within the same host was determined. At present, the parasite in infected meat can only be inactivated by freezing or cooking; however, there exists in nature a freeze-resistant species. The capability for the two putative species of Trichinella to non-synchronically infect the same host and thus potentiate gene flow between the species and introduce the freeze-

resistant genotype into the domestic cycle was demonstrated. Results from this work may directly affect recommendations on the safe storage of uncooked pork products.

A study was conducted on the transmission of Fasciola hepatica in goats. Testing snails for infection, using tracer sheep, and FEC determinations indicated that few snails were infected, transmission occurred only in September and October, and animals were infected all year, respectively. The snail Lymnaea modicella was present during most of the year and occasionally in high population densities. Laboratory cultures of this snail appeared to be highly susceptible to infection. Lymnaeids collected from other sites, during the fall months, were found infected, and thermal sites may be considered year-round sources for fluke transmission. The gastrointestinal parasite burdens in bison were examined. The ad lib treatment with fenbendazole as a feed additive was successful in reducing FECs of nematodes in yearling bison. The prevalence of Fascioloides magna in captive elk indicated approximately 20% of the ranches were fecal positive, and suggested the potential for transmission to domestic livestock.

To examine risk factors associated with Toxoplasma gondii infection, tachyzoites of Toxoplasma were added to raw goat's milk and maintained at 4□C. Organisms were able to survive for several days suggesting that this may route of infection may occur naturally.

Determine the efficacy of biological control measures on parasite transmission

Investigators in California and Louisiana collaborated to investigate aspects of biological control using the fungus Verticillium chlamydosporium. Under *in vitro* conditions, the fungus produced hyphae that enveloped and consumed the contents of Ascaridia galli and Parascaris equorum ova, but rarely invaded ova of Trichuris suis. These results were encouraging, but may limit the usefulness for biological control in the field. Future studies on invasion under soil conditions need to be done. A 1-year study was conducted to determine the effect of feeding horses Duddingtonia flagrans spores, and results showed that the development of cyathostome infective third stage larvae on pasture was reduced at all times of the year, suggesting that this form of biological control warrants further experimentation.

Determine the significance and transmission patterns of parasites in alternate livestock, game ranched animals and wildlife sympatric with livestock.

The potential transmission of psoroptic scabies and babesiosis (via common tick vectors) between cattle, deer, and bighorn sheep was studied. Mites found in bighorn sheep were found on sympatric deer in New Mexico, but not in California. Morphometric analysis of these mites did not reveal any differences that could be used to suggest that taxonomic differences exist between mites isolated from different hosts. However, antigenic comparisons and protein biochemical differences suggested that there were significant differences between these isolates. Babesiosis was detected in several populations of wild ungulates, but this same pathogen did not have equivalent prevalence in sympatric cattle herds. Initial findings suggested that the distribution of tick vectors (Dermacentor and Ixodes spp.) strongly influenced the distribution of infections. It was demonstrated that Babesia, Psoroptes mites, and ixodid ticks were not

transmitted between cattle and wild ungulates. Research was conducted by investigators in Colorado , Texas and Washington and California.

Transplacental transmission of toxoplasmosis and the dynamics of anti-Toxoplasma gondii antibodies in the dam and her cria were studied by researchers in Iowa and Maryland. Llamas, either experimentally infected or naturally infected developed high anti-Toxoplasma antibody titers. Crias born to these dams were normal and showed no signs of toxoplasmosis. Pre-suckle cria serum anti-Toxoplasma antibody titers were not detectable, whereas post-suckle titers were elevated. It appears that toxoplasmosis is not transmitted transplacentally and anti-Toxoplasma antibodies are only passed via colostrum, which contained high anti-Toxoplasma antibody titers. Sarcocytis was identified in tissues from llamas and alpacas imported from South America. No sarcocysts were found in tissues of llamas born and raised in North America.

Bison herds were studied to determine the epidemiology of nematode infection and the influence of management on production. One herd was and one herd was not vaccinated, dewormed or given feed supplement. Infection levels in the better managed herd were lower (predominantly Haemonchus, Cooperia, and Ostertagia) than that in the poorer managed herd (predominantly Trichostrongylus, Cooperia, and Ostertagia). Both visual and palpated condition scores were higher for the better managed herd. An additional study on the seasonal transmission pattern of nematode parasites of bison grazing the Konza prairie of Kansas indicated Cooperia oncophora was the predominant species overall, Trichostrongylus spp. was the predominant genus in the summer, and Ostertagia demonstrated a southern summer inhibition pattern.

A study utilizing domesticated sheep as tracer hosts was conducted to evaluate the transmission pattern of Camelostrongylus mentulates and Trichostrongylus colubriformis on the Edwards plateau of Texas. The seasonality of transmission was precipitation limited, and both major parasite species were being transmitted during the cool season (Oct through April. Camelostrongylus underwent summer arrest. The transmission pattern for both appeared to follow the pattern for similar species in small ruminants.

The significance and transmission patterns of parasites was determined in emus in Mississippi. Strongyle and Capillaria spp. eggs predominated. Deletrocephalus spp. eggs were present indicating cross-transmission of the parasite from rheas may be possible. Coccidial organisms were not present. Very few parasites were found during necropsies and include: Cyathostoma spp., Trichostrongylus tenuis, and Libyostrongylus douglassi. Found that the prevalence of Cryptosporidium parvum and Giardia spp. in white-tailed deer from Mississippi and Virginia were less than 10%. Analyses indicated the probability of infection with Cryptosporidium parvum decreased with increasing age of animal. White-tailed deer do shed cysts and oocysts of both parasites in the environment and must be considered potential sources of contamination. Work in Maryland confirmed that only fawns of white-tailed deer were infected with Cryptosporidium parvum. Other wildlife including fish, reptiles and amphibians could not be artificially infected. Numerous chemical disinfectants (bleach, ammonia, formaldehyde, ethylene oxide, bromomethane, and ozone) and temperature (boiling and freezing) were tested against Cryptosporidium parvum oocysts, and only boiling was effective in killing the organism. These results will help scientists plan new strategies to decrease Cryptosporidium parvum contamination of the water supply.

Develop and evaluate improved immunological and molecular diagnostic methods

Experiments were conducted to compare methods enumerating cyathostome larvae encysted in the mucosa. Digestion methods were as accurate as those that use transmural illumination. Repeated digestion of the tissues did not increase yields of these stages, and may have decreased yields of early or hypobiotic L_3

The quantitative fecal sedimentation technique was compared to the number of mature Fasciola hepatica present in calves, and there was a high correlation between the two. A quantitative Western blot was developed that detects a 26-28 KD coproantigen of Fasciola hepatica.

A study was conducted to determine the reliability of FECs. Repeatability of a FEC in cattle was approximately 0.6. Using this figure, an accurate measure of a herd FEC value requires that 15-20 individuals be sampled. To ascertain an individual's "true" FEC value, a maximum of 3 repeated samples should be taken. Researchers developed DNA probes and PCR assays for the diagnosis of parasitic infections of cattle. These rapidly differentiate and quantify the numerous types of parasites within the host by identifying eggs to genus. A method based on differential enzymatic amplification of parasite genomic DNA was developed to diagnose and quantitate Ostertagia eggs in feces of infected animals. Analyses have confirmed that serum pepsinogen levels are reasonable indicators of Ostertagia ostertagi burdens, but that total FECs are not accurate indicators of resistance. The application of these techniques by diagnostic laboratories will permit veterinarians to avoid costly and time consuming drug treatments for animals infected with nonpathogenic parasites. Parasite gastrointestinal nematode FECs in cattle are characterized by an "overdispered" distribution, indicating that most animals regulate the egg output of the parasite or become resistant to infection, but a small number of cattle remain highly susceptible, and are responsible for the majority of parasite transmission, and thus should be the target of control procedures.

A genetically engineered serology test for bovine and swine cysticercosis was developed. The test is based on genetically engineered parasite proteins useful in the serodiagnosis of infection prior to meat products reaching the consumer. When fully developed, this test will assist inspectors in providing safer meat products to American consumers. A PCR based method for the differentiation of species in the genus Trichinella was also developed. This has formed the basis of several epidemiological studies looking at the prominent Trichinella types in North American wildlife as well as the arctic regions of Canada and shown that the domestic Trichinella is not the dominant species in sylvatic hosts, and that Trichinella from wild game obtained from arctic regions of Canada cannot be inactivated by freezing as previously recommended.

The identification and characterization of native antigens of Neospora caninum allowed development of tests useful for detecting infection in mice and cattle, and possibly other domestic species.

In vitro cultivation of Babesia and Theileria species for the preparation of suitable DNA markers to differentiate among the various isolates is ongoing. Developed probes to differentiate bovine

and wildlife Babesia and Theileria and these have improved diagnosis and study of bovine and wild ruminant theilerioses. This study will aid in determining which species of parasites are shared among which species of hosts and how transmission occurs from one host species to another.

Objective 2. Identify and characterize parasite antigens and the host immune responses they elicit at the molecular and cellular levels.

Identify parasite antigens and stages that induce protective immune responses

Collaborative research in Louisiana, Washington, Texas and Maryland identified antigens of the nematode Haemonchus contortus and investigated the value of these antigens as vaccine candidates. Gut surface proteins (GA1) of H contortus were examined as vaccine candidates. The gene sequence encoding GA1 proteins indicated they could be relevant to design recombinant antigens. Both p46 and p52 are released from the gut membrane and both were detected by immunoblot in gut membrane fractions using anti-recombinant GA1 antibody, but only p46 was detected in E/S products of *in vitro* cultured adult worms. These results confirm that p52 is also expressed as a membrane protein and that gut membrane proteins are released from worms, but that intact release from worms may be a variable characteristic of nematode gut surface proteins. E/S proteases from USA and Kenya isolates of adult Haemonchus contortus were identified as predominately cysteine proteases, while some activity from metallo and aspartic proteases were also detected, and these may be useful as vaccine candidates. The variation observed in characterization of these proteases indicated that none were found that occur in all isolates of Haemonchus contortus investigated.

Additionally, it was found that antibody and CD4 T lymphocytes contribute to worm expulsion after immunization of goats with gut antigens of Haemonchus contortus. Infection established CD4 T lymphocytes in abomasal lymph nodes, but the lymphocytes produced less IgG against gut antigens than ALN lymphocytes in gut-antigen immunized/challenged kids. Expression of 6 secreted or membrane antigen genes were evaluated in the gut of Haemonchus contortus during the parasitic life cycle and among geographic isolates of this worm. The genes for these proteins are predominately expressed in the parasitic stage of life and were detected in all geographic isolates tested. In addition, cathepsin B-like cysteine proteases from nematode gut represent potential targets for parasite control by immunity and chemotherapy. Comparative analysis implicated significant phylogenetic and functional diversity in these proteins that may be relevant to these topics of control. Cellular changes in this parasite from lambs treated with fenbendazole included disruption of apical vesicle transport of secretory vesicles in Haemonchus contortus gut, inhibition of erythrocyte digestion and gross disintegration of the anterior region of the gut. These dramatic changes were associated with expression of sensitive forms of type I and II beta-tubulin genes in gut. Protection against these effects in benzimidazole/ivermectin resistant worms was associated most strongly, but not exclusively, with phe200-->tyr substitution in the gut type I beta tubulin. The likely expression of these genes throughout the parasitic phase of the life cycle suggests that immunity induced by these antigens will be

detrimental to all parasitic stages. Findings with geographic isolates, and other comparisons with results from the Moredun group, indicated that the gut proteins investigated are highly conserved geographically, which is reassuring for vaccine research.

A long-term program to evaluate vaccination of sheep against Haemonchus contortus under field conditions was initiated. Studies were conducted using H. contortus crude gut antigens, and specific ConAH11 and HgalGP gut antigens in both mature ewes and lambs. Under natural challenge conditions, protection is not as good as protection observed under experimental, one-time, challenge infections as observed in studies by others. The limited protection observed was after 2-3 boosters. Serum anti- Haemonchus contortus antibody levels were elevated after vaccination. Gut antigens may not be of that much value under field conditions. Studies will continue to evaluate different adjuvant systems in hopes of improving protection.

In Mississippi, candidate antigens were investigated for vaccination against the bovine helminth, Haemonchus placei. A detergent extract was prepared from homogenized intestinal tissue of adult H. placei. Monoclonal antibodies were generated against the polyvalent extract. Immunohistochemistry verified the intestinal location of the antigen in H. placei and several other ruminant gastrointestinal nematodes. Showed that antigens from the intestine of H. placei resulted in significantly lower FECs in immunized animals and significant increases in serum IgG1 and IgG2 titers but no reduction in the total number of nematodes. However, more immature and fewer adult females were present in immunized calves. Initiated a study investigating the immunoprotective nature of antigens derived from the intestine of Haemonchus placei in cattle challenged with Ostertagia ostertagi.

Work was carried out in Mississippi and Texas on antigens of the bovine fluke, Fasciola hepatica. A dot-ELISA technique for diagnosing liver fluke infections in llamas was also developed using E/S from sheep derived F. hepatica. A 12 KDa protein was isolated from adult Fasciola hepatica. Thioredoxin was found in numerous tissues and several stages of the life cycle. Further work on the 12 Kda thioredoxin protein of F. hepatica was done. Technical problems have slowed progress in identifying localization of the protein at the cellular level. However, areas of greatest activity were elucidated. Stage and organ specific identification of activity was determined.

Identification of antigens of protozoan coccidian organisms was carried out by researchers in Maryland and Washington. Vaccination protocols using gamma-irradiated attenuated oocysts from 3 Eimeria spp. were developed for protecting chickens against infection. This work is now being expanded to floor pen trials to reproduce field conditions in the poultry industry. The gamma-irradiation method was adapted for investigating genes and gene products associated with metabolizing intracellular Eimeria that may serve as targets for protective immunity and for incorporating into antigen delivery vectors. Using genetic engineering methods, a recombinant Eimeria sporozoite antigen was produced which effectively immunized chickens. Large-scale purification of the yeast-derived recombinant protein is being undertaken for conducting floor pen vaccination studies. INF-g was identified as a protective agent and the gene for chicken INF-g was cloned. Partial protection against coccidiosis was obtained by immunizing chickens with DNA encoding parasite proteins. Microsatellite markers were evaluated to identify alleles associated with coccidiosis resistance traits in commercial broiler meat-type chickens . Initial

screening found some alleles associated with oocyst production and 11 markers were statistically significant. Stable chicken hybridomas were developed which secreted monoclonal antibodies blocking parasite invasion of lymphocytes. These results are important in developing novel immunologic control of coccidiosis in chickens

Neospora caninum infection and disease were induced into immunocompetent Balb/C mice, and protection to systemic Neospora infection was induced using whole tachyzoite antigen and Freund's adjuvant. The immunization protocol induced both a humoral (seroconversion) and cell-mediated immune response. A difference in pre-pregnancy and post-pregnancy infection effects in Balb/C mice infected with Neospora tachyzoites was demonstrated. Post-pregnancy infection resulted in an increased number of fetal resorptions. With pre-pregnancy infection, however, the number of resorptions did not differ between groups but the number of fetuses/dam was significantly less. The pre-pregnancy infection reflected a reduced rate of pregnancy and suggested early embryonic death or conception failure. These findings will allow us to test the efficacy of immunization against Neospora-induced reproductive loss in the mouse model. Specific methods of antemortem diagnosis of Neospora infection in cows were investigated. The maternal antibody response was evaluated using Neospora caninum tachyzoite antigens with sera from cows with confirmed Neospora-induced abortion and 13 major proteins were observed. Although the immunoblot banding pattern varied from cow to cow, four proteins were detected in all cows with confirmed Neospora abortion. Monoclonal antibody 4A4-2, which binds to the surface of live tachyzoites, recognized only the 63 kDa protein. Sera from cattle experimentally infected with Toxoplasma gondii, Sarcocystis cruzi, Sarcocystis. hominis or Sarcocystis. hirsuta did not inhibit 4A4-2 binding to Neospora caninum proteins.

Define mechanisms of protective immunity

Mechanisms of immunity to helminth parasites were investigated in Louisiana and Maryland. Studies were initiated to investigate the host immune response to nematode infection in horses. Ponies vaccinated with Strongylus vulgaris irradiated L3 and parasite naive ponies both showed a Type 2 cytokine response following experimental challenge, however, the response was earlier in the vaccinated ponies suggesting that this type of response is involved in the protective immunity. These studies provide valuable information in helping to better understand how the host responds to infection and may lead to improved methods of combating nematode parasites.

A long-term study was initiated to study the relative resistance of cattle to nematode infection. Pasture exposure of cattle selected for high or low resistance indicated 3 phenotypes: innate resistance, acquired resistance, and susceptible, existing at approximately a 1:2:1 ratio respectively. Protective immunity against the different parasite genera arose at different rates. Cattle became resistant to reinfection by Nematodirus very early after exposure, followed by Cooperia, and finally, much later, resistance to Ostertagia. Assays and reagents to study cytokine (IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, TGF, TNF, and IFN-g) expression in cattle were developed. To determine the cellular and molecular basis of immunity, lymphoid tissues draining the site of infection in cattle genetically selected for high and low resistance were examined. Lymphoid architecture was disrupted as the number of parasites increased and a distinct hyperplasia of lymphocytes was observed, with decreases in T cell percentages, and a concomitant increase in B cells. This shift appears to favor the transcription of IL-4 and IL-10,

and decrease the transcription on IL-2 and perhaps INF-g. This pattern of response is considered to be the classic protective immune response to intestinal nematode infections, yet was apparently ineffective for Ostertagia. This discrepancy is an area of current research emphasis. RNase protection assays and competitive PCR'S were developed to study both qualitatively and quantitatively, levels of interleukin RNA induction for these cytokines. The bovine cytokine IL-12 and IL-15 genes were identified, cloned and expressed in experimental quantities. These cytokines are believed to play important roles in regulating the animal"s capability to immunologically respond to parasite infections. This work allowed initiation of studies on the capability of these cytokines to function both prophylactically and indirectly as adjuvants in attenuating bovine infections resulting from parasites as well as other pathogens. These studies provide important tools for the development of techniques that use the host immune system to control parasite-induced economic losses, thus lessening our reliance on heavy anthelmintic usage.

A study was conducted to examine endocrine consequences of parasite-induced (Haemonchus contortus) stress in ruminants. Serum cortisol levels correlated directly with inoculation level indicating a role of the steroid in host response to the disease. It was also shown that Ostertagia induced stereotypic anti-nematode responses, but these responses did not protect the cattle from a challenge infection. Conversely, Haemonchus induces much less apparent responses, but the responses did protect from reinfection. Resistance to a specific species of GI nematode can be both associated and non-associated with resistance to other parasite species.

Molecular probes were developed for several swine cytokines (IL-2, IL-10, IL-12, and IL-15) so that local responses to parasitic infections could be sensitively assayed. Such probes will enable researchers to quantitate changes in levels of cytokines during the disease process and to identify exactly which swine cytokine, and associated helper T-cell subset, enables pigs to mount a protective immune response against the infection. When combined with studies aimed at developing monoclonal antibodies against these cytokine proteins, such reagents will enable researchers to test these novel immunomodulators for their effect on disease progression and for their potential ability to act as immune adjuvants. A thiol and metalloprotease from Trichuris suis was characterized and cloned. These enzymes may be involved in parasite feeding behavior or migration in the host intestine, and are immunogenic and protect swine from infection. A direct link between infection of swine with Trichuris suis and enhanced bacterial-induced necrotic proliferative colitis was shown. Anthelmintic drug and vaccine prophylaxis were effective in reducing the propensity for secondary bacterial disease. Immunological studies were conducted which demonstrated that exogenous IL-12 completely protected neonatal mice from experimental infection with Cryptosporidium parvum. These studies will set the stage for development of novel biopharmaceuticals to control this waterborne infection. A cDNA library was constructed from Ascaris suum intestinal cells to identify genes encoding cryptic proteins as broad spectrum vaccine candidates.

In collaborative research between Maryland and Minnesota, immunity to bovine coccidiosis was investigated. Young, coccidia-naive calves maintained on lasalocid and inoculated with Eimeria bovis were resistant after the ionophore was removed. It appears that these calves had enough exposure to infection when the coccidia were controlled by the ionophore, to elicit a protective immune response. An ELISA test was developed for Eimeria bovis using oocyst antigen to

study the immune response to infection in calves. Local and peripheral cell responses are being evaluated for surface markers and extraction of RNA.

Investigators in Maryland, Utah and Washington investigated mechanisms of immunity to the important protozoan parasite, Cryptosporidium parvum. Infections of neonatal calves resulted in a rapid and marked increase in the induction of INF-g. Studies on identification of T lymphocytes in intestinal mucosa that are associated with acute Cryptosporidium parvum disease in neonatal calves, suggested that a T-cell mediated response involving CD8+ T lymphocytes and a subset of CD4+ cells occurred in gut mucosa during infection.

In immunosuppressed mice using intravenous inoculations, it was determined that a somatic migratory route can be used by Cryptosporidium parvum in susceptible immunosuppressed adult C57BL/6N mice. DHEA treatment of Cryptosporidium parvum infected mice reduced both fecal oocyst shedding and parasite colonization of the ilea. Up-regulation of the immune system by exogenous DHEA may be useful in the treatment and palliation of cryptosporidiosis.

Genetics of resistance to gastrointestinal nematode infections

Studies were initiated to determine the mechanism(s) of resistance in Gulf Coast Native sheep. CD4+ T-cell ablation experiments were conducted, and ablation had no apparent effect on resistance between depleted and non-depleted animals, even though FACScan analysis showed a significant reduction in the circulating $CD4^+$ T-cell population. Therefore, immune-mediation may not be important

Determined that FECs in cattle were influenced by host genetics (heritability = 0.2-0.3), and that certain bulls produce susceptible calves at rates 20-25 times than that of other bulls. Similarly, the ability to respond to parasite antigens as measured by circulating anti-parasite antibody responses was strongly controlled by host genetics (heritability = 0.8-0.9). Serum antibody responses did not correlate with FECs indicating that although both are under genetic control, the resulting values were influenced by different genes or sets of genes. Reagents were developed to serologically identify bovine major histocompatibility complex (BoLA) class I and class II determinates. These reagents were used to produce a small herd of BoLA class I and class II identical animals. Bulls with demonstrated propensity for producing high and low EPG calves were then used to produce parasite "resistant" and "susceptible" lines. These cattle are being used to define the immunological mechanisms which render cattle immune or susceptible to infection with gastrointestinal nematodes. Studies were initiated to identify phenotypic and genetic markers for resistance/susceptibility using the bovine linkage map.

Basic immunogenetic studies demonstrated a relationship between host genetics and the efficacy of recombinant antigen immunization in chickens. Immunological studies demonstrated that TNF and INF-g production differs in genetically defined chickens which showed different levels of coccidia disease susceptibility. These results enhance our understanding of genetic control of immune response to coccidia and will be useful for chicken breeding program for poultry industry. Swine were identified which are genetically resistant to Trichinella spiralis infections.

At least two genes encode this disease resistance, one of which was mapped to the swine leukocyte antigen complex. Extensive immune analyses have yet to identify the mechanism for this resistance. Initial studies with Toxoplasma gondii infections found genetically resistant pigs. Studies were initiated to evaluate regulation of this resistance. These experiments incorporate immunologic analyses using bioassay and mRNA assays. An irradiated oocyst vaccine was effective in reducing parasite burdens, but not completely. More extensive mapping studies are planned to help breeders identify genetically resistant stock and to prevent transmission of these foodborne diseases.

Objective 3. Determine the efficacy of modern chemotherapeutic agents, including the prevalence of resistance and optimal treatment regimens.

Prevalence of resistance to currently used anthelmintics

Researchers in Virginia, Mississippi, Texas and Missouri have worked collaboratively to establish regional levels of anthelmintic resistance in small ruminants and examine strategies for effective anthelmintic treatment in the face of resistance In Missouri, a mail survey to address management practices including perception of parasite problems was developed for goat producers. An initial distribution to a small number of producers indicated that most had moderate to severe parasite problems and anthelmintic resistance was present. Goat nematodes (primarily Haemonchus contortus) resistant to fenbendazole, ivermectin, and levamisole were described in Virginia. Stability of resistance was monitored and susceptibility of Haemonchus contortus to levamisole returned after discontinuing its use but resistance came back quickly. Resistance to macrolides and benzimidazoles was still present. The Drenchrite larval development assay system was able to evaluate the level of resistance to all three major classes of anthelmintics, and was able to detect small highly resistant worm populations that might be overlooked by traditional means in sheep. The test was also validated for use in detecting resistance to Ostertagia ostertagi and Cooperia punctata in cattle.

Short term approaches to anthelmintic resistance problems were evaluated. The use of benzimidazoles daily for 3 days rather than as a single dose increased efficacy as judged by FEC reductions. Even more useful was 2 drugs of less than optimal activity from separate action groups used concurrently. This approach was useful in reducing FEC to acceptable levels. Morantel administered at daily low levels compared to monthly treatment with ivermectin indicated no differences between groups. Split treatments of benzimidazole anthelmintic at 12 to 24 hour intervals showed an increase activity if the minimal split dosage is adequate, and that morantel at an enhanced dose has minimal effects in controlling levels of Haemonchus in a flock where levamisole resistance was minimal.

Evaluate efficacies of new chemotherapeutic agents

Valuable information on the efficacy of a new macrolide product, doramectin, under local grazing conditions was collected by research in several states. In Georgia, treatment with injectable doramectin or ivermectin reduced EPG and increased calf weight gain, but no weight

gain differences were seen between the treatments. Therefore, both anthelmintics appeared to be equally effective in controlling nematode parasites which resulted in equivalent production. Treatment with ivermectin sustained release boluses was essentially 100% effective in controlling nematode infections and treated calves significantly outgained control calves. Efficacy of doramectin against naturally acquired Strongyloides papillosus infections in calves was 100%. In Mississippi, doramectin pour-on was evaluated in a fall calving herd on weight gain of calves. The cumulative nematode FEC of pour-on treated cows and calves was significantly reduced, and body weight of pour-on treated calves at trial termination was significantly greater. Efficacy of doramectin pour-on was also tested against artificially induced nematode infections. Treatment resulted in >95% reductions in numbers of trichostrongyles present at necropsy. The duration of activity of doramectin on helminths in ewes and lambs was evaluated in Minnesota. The FECs of the doramectin treated group dropped to 0 by day 7 and remained low through day 35. Studies conducted on Texas ranches comparing the value of various anthelmintics in cow calf operations had variable results. The overall conclusion was that timely treatment was usually an economically sound decision.

The efficacy of new products against parasites of horses and swine was also investigated. Moxidectin pour-on and ivermectin were very effective against mature and migrating Ascaris suum. The efficacy of a new macrocyclic lactone against internal parasites of horses was similar to that of ivermectin and moxidectin. The efficacy of pyrantel-pamoate against cyathostomes was reduced in horses where it had been indicated that they had developed resistance to pyrantel pamoate and oxibendazole. Efficacy of moxidectin gel was very high against all mature and most immature nematode parasites in horses, and less effective against Gastrophilis intestinalis. There was some suggested activity against encysted cyanthostome larvae. The relevance of transglutamase as a potential target of immunity or chemotherapy was investigated by *in vitro* culture/enzyme inhibitor experiments. It was shown that this enzyme was essential for the molt of L3 Strongylus spp. to the L4 stage.

In collaborative work in Minnesota and Virginia, the popular organic compound, diatomaceous earth, was found to have little effect upon nematode infections of cattle or sheep. A botanical was tested in ewes by FEC reduction, and there was no evidence of anthelmintic activity.

The antimalarial drug artemisinin and two highly active derivatives, artemether and arteether, were found to be ineffective against the protozoan parasite Cryptosporidium parvum. It was demonstrated that Cryptosporidium parvum can complete its entire life cycle (from sporozoite to infective oocyst) in a primary culture of bovine Fallopian tube epithelial (BFTE) cells. Cultivation in BFTE cells will enable investigators to further study interactions between the parasite and the host cell and provide a reliable system for evaluating potentially efficacious compounds. Monoclonal antibodies were developed against the sporozoite and oocyst stages of Cryptosporidium parvum. These antibodies will be used to facilitate the enumeration of Cryptosporidium parvum type I meronts in the BFTE cell culture system. A structural interaction between a human serine protease inhibitor (serpin), alpha-1-antitrypsin, and components on the surface of Cryptosporidium parvum sporozoites was demonstrated, and showed that cryptosporidial serine protease activity is functionally active during excystation. A potent anticryptosporidial effect for serpin compounds *in vitro* and *in vivo* was also described. The serine protease component of Cryptosporidium parvum appears to be directly involved in

the excystation process and inhibitors of this class of enzymes are strong antagonists of excystation. The demonstrated *in vitro* and *in vivo* efficacy of protease inhibitors, in combination with the synergistic activity they have with other anticryptosporidial compounds such as the aminoglycoside paromomycin, suggests a potential for chemotherapeutic intervention as anticoccidial agents. The immunosuppressed [orally administered FK-506 (Tacrolimus)] non-neonatal pig was shown to be a good animal model for human cryptosporidiosis. Paromomycin was determined to be therapeutically effective against Cryptosporidium parvum as determined by reductions in fecal oocyst shedding, parasite colonization, and villus atrophy in the ilea and terminal ilea of immunosuppressed adult C57BL/6N mice. An immunosuppressed adult mouse model was developed for Cryptosporidium infections for the evaluation of treatments and water quality monitoring. This model was used to test a number of new propriety compounds for efficacy. Syringomycin-E, an antifungal agent and phytotoxic antibiotic that is produced by the plant bacterium Pseudomonas syringae, was effective against the early developmental stages of Cryptosporidium parvum in Madin-Darby bovine kidney (MDBK) cell culture, and warrants evaluation *in vivo*.

The bioenergetics of nematode infective larvae was studied, which may benefit development of control measures. The insect-parasitic nematode Steinernema carpocapsae was used as a model for Haemonchus contortus, as the behavior of both are similar and Steinernema carpocapsae was more readily available. Because arginine phosphate was found in larvae of both species, characterization of arginine kinase activity was investigated. Arginine kinase was partially characterized in the infective larvae of Haemonchus contortus. The specific activity was lower but the affinity for substrates and cofactors was similar to that reported for Steinernema carpocapsae. Arginine kinase was partially characterized from infective larvae of other GI nematodes, and large differences were seen among different parasites, and different life cycle stages. Therefore, arginine kinase activity probably is not a target for control. A cryopreservation procedure was developed for the metacestodes of Mesocestoides corti and a Mesocestoides sp. isolate of canine origin. These studies will provide a means to cryopreserve metacestodes for future reference specimens for *in vitro* culture.

Efficacy of chemotherapeutic agents against helminth parasites of nontraditional livestock species

The efficacy of ivermectin against gastrointestinal helminths in llamas was evaluated. Llamas were treated with bovine injectable and pour-on formulations. The injectable formulation was highly effective in removing helminths and the pour-on formulation was not. Blood levels of ivermectin were detected in more injected animals than pour-on animals. Topical treatment of llamas with eprinomectin substantially reduced helminth infection levels, which also reduced, but did not eliminate, infestation with Chorioptes. Daily feeding of llamas with pyrantel pamoate prevented establishment of experimental infections with Paralapostrongylus tenuis. These findings are useful to producers and veterinarians in the selection of appropriate anthelmintics for use against common gastrointestinal nematodes of camelids and for the prevention of meningeal worm (Paralapostrongylus tenuis). Collaboration was with investigators in Texas.

DEGREE TO WHICH OBJECTIVES HAVE BEEN ACCOMPLISHED

Progress in accomplishing both objectives 1 and 2 has been continual and steady with many completed investigations and others that are ongoing. The project has investigated many aspects of epidemiology, management, breed resistance, ecology, strategic deworming, rotational grazing schemes, diagnosis, host-parasite immune related interactions, and vaccine development involving a wide range of both livestock and wildlife species and parasite species. Also, work done with laboratory animals and *in vitro* culture systems have produced information on the host-parasite relationship that will be useful in many animal species. This new information has provided an expanded base for parasitologists, veterinarians and producers to better manage a broad range of parasites and the problems they cause. Collaboration between station investigators on numerous studies brought appropriate expertise and resources together to stimulate mutual interests and accomplish, without which progress would have been much slower.

Progress in accomplishing objective 3 is for the most part directly related to industry directions and support. Because no new classes of anthelmintics have been developed, industry has reduced their work on efficacy evaluation studies. During the early part of the project period, industry did support some strategic deworming and production work, which has since dissipated. Laboratory and *in vitro* culture work, which are and can be supported by mostly small grants continue to produce valuable information that are and may be useful under field conditions. One major thrust that was envisioned for the project, prevalence of anthelmintic resistance across regions, was attempted by some, but because of the lack of funding could not be fully instituted.

INCOMPLETE WORK OR AREAS NEEDING FURTHER INVESTIGATION

Because of drug resistance and food residue and environmental concerns, it is imperative that investigations continue to find alternatives to the use of chemicals in livestock production. These alternatives are not intended to replace chemical control, but augment their use in integrated control programs. To accomplish this endeavors, work needs to be continued on the epidemiology of parasitic infections and the host-parasite interface. Work started on this project that needs further investigation include: establishing a genetic/immunologic basis for resistance of various hosts to parasitic infection, development of vaccine and biological control methods, expanding investigations on the prevalence of drug resistance, and integration of established control methods with those newly developed which will become sustainable. The objectives for this revised petition address these areas.

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