COOPERATIVE REGIONAL PROJECT OUTLINE

PROJECT NUMBER: NE-112 (Revised)

OFFICIAL

TITLE: MASTITIS RESISTANCE TO ENHANCE DAIRY FOOD SAFETY

DURATION: October 1, 1997 to September 30, 2002

STATEMENT OF THE PROBLEM: Bovine mastitis is the most costly disease currently affecting dairy cattle. While significant advances have been made in controlling some types of mastitis, the complex etiology of the disease and ongoing changes in dairy practices dictate that new and more effective methods for control and treatment be developed.

JUSTIFICATION: In the United States, the dairy industry contributes in excess of 65 billion dollars per year to the national economy, and provides jobs for 1.1 million Americans. The single most costly disease of dairy cattle and a major monetary drain on the dairy industry is bovine mastitis. Mastitis is defined as an inflammation of the mammary gland that is almost always associated with bacterial infection. Mastitis affects every dairy farm and approximately 38% of the dairy cows in the United States. The National Mastitis Council estimates that this devastating disease complex costs the dairy industry more than 2 billion dollars per year or approximately $180.00 per cow. These losses are primarily due to lost milk production, increased veterinary costs, increased cow mortality, and discarded milk.

Because of the importance of maintaining milk as a safe, abundant, and economical food source, increased emphasis is being placed on methods of preventing bovine mastitis. The recent success experienced with the J5 vaccine for coliform mastitis and the cooperative efforts of NE-112 members in the evaluation of this vaccine, demonstrate the effectiveness of this group in developing procedures to control mastitis. Other areas of preventive research include studies on vaccines for staphylococcal and streptococcal mastitis and the effects of immune modulators on enhancing resistance to mastitis.

The purpose of NE-112 is to coordinate multidisciplinary research efforts in mastitis that are being conducted at various laboratories throughout the United States. The magnitude and scope of attempting to solve these problems extend far beyond the ability of any one institution. The ability to cooperate on a regional and national basis allows the integration of resources and knowledge to address this problem. Recognition of the need for a coordinated effort to study resistance of the dairy cow to mastitis resulted in the design and initiation of Regional Project NE-112. In addition to the national importance of bovine mastitis, the widespread and complex nature of the disease makes it an international problem as well. For many years, NE-112 has had an informal but very active international component. The mastitis research workers group has meet in conjunction with the NE-112 annual meeting for many years, and in recent years, the mastitis research workers topics have been included in NE-112 minutes, showing current active areas of research by NE-112 members. International visitors and collaborators are often included in these presentations. The importance of this international component has grown to the point that in this current project, we will invite international participants to become formal members of NE-112. As is the case with any new member, the prospective member will be asked to present research interests that are applicable to NE-112 and they will then be considered for membership as per the project guidelines.

RELATED CURRENT AND PREVIOUS WORK: A CRIS search revealed that the majority of the research being conducted in the United States on bovine mastitis continues to be done by members of the NE-112 Regional Project. The following is a brief review by objective of previous and related research taken from CRIS and other sources.

Objective 1: Characterize host responses or factors that affect resistance of the mammary gland to mastitis.

While the teat surface is commonly contaminated with pathogenic bacteria, intramammary infection (IMI) is relatively infrequent. This is a consequence of various defense systems collectively referred to as "resistance of the mammary gland to mastitis". Knowledge of these systems is incomplete and their interactions often unknown. Their effectiveness is subject to genetic, nutritional, physiological, and environmental influences. The research under Objective 1 of NE- 112 investigates these systems and the factors that influence their effectiveness.

The initial barrier to IMI is the teat duct. The teat duct acts as a mechanical barrier to infection. But antibacterial mechanisms are also associated with lipids or proteins in the keratin layer. Teat skin condition is also an important initial barrier to infection. Lesions on the teat surface or damage to the teat end serve as reservoirs of infection and may assist the entry of bacteria (Fox, 1992).

Once bacteria penetrate the teat duct and enter the mammary gland, they encounter other components of the host defense system. The cells of the immune system constitute the major intramammary barrier to infection, with the polymorphonuclear neutrophilic leucocyte (PMN) being the main cell type responsible for the elimination of intramammary bacteria. The PMN operate interdependently with other cells of the immune system and with a variety of humoral components, particularly cytokines and antibodies (Paape et al., 1991[a]). The antibacterial activity of the PMN increases during migration and surface receptor expression is unregulated (Worku et al., 1994). Migration of PMN is stimulated by chemotactic compounds elicited during the infection. The ability of the PMN from different cows to migrate in response to chemotaxins and then to phagocytose and kill bacteria is variable. Some of the factors known to affect these PMN processes are antibiotics (Paape et al., 1991[b]) and nutritional factors, e.g., selenium and copper (Hogan et al., 1992).

PMN have two killing mechanisms, one of which is oxygen dependent while the other is oxygen independent. The generation of superoxide by PMN, and the milk and tissue levels of antioxidants may also be important in host resistance. Superoxide dismutase activity increases during mammary inflammation, and heat stress lowers milk antioxidant activity (Raamesh et al., 1993). While PMN do demonstrate bactericidal activity anaerobically, their efficiency is lowered. During inflammation, oxygen tension in milk falls to extremely low or undetectable levels. Even in the healthy lactating gland, dissolved oxygen levels are substantially below those normally employed by *in vitro* assay systems (Goldberg et al., 1995). A consequence is that PMN function may be substantially down regulated during inflammation.

Other immune cells are also critical to mammary cellular defenses. The macrophage initiates the inflammatory response in the mammary gland following microbial invasion. Uptake of invading microbes by macrophages leads to the production of key signals, including C5a, IL-1, and leukotrine B4, which function as bovine PMN chemoattractants, and other cytokines that modify lymphocyte proliferation and function (Paape et al., 1991[a]). Lymphocytes modulate and effect cellular immune responses in the body, but their role in the bovine mammary gland is poorly characterized. Several studies indicate that lymphocyte blastogenesis is depressed during mammary infections (Paape et al., 1991[b]). The significance of this is unclear, although it has been postulated that it may be a factor in chronicity of *Staphylococcus aureus* infection (Park et al., 1993). A better understanding of the role of mammary lymphocytes in modulating mammary immune function is a key objective in NE-112 research (see Objective 3) because of the potential for stimulating immune function.

Immunoglobulins neutralize toxins, and opsonize and kill bacteria (Paape et al., 1991[b]). IgG_2 is the preferred antibody class for opsonization of bacteria, although IgM is also opsonic; IgM and IgG_2 are additive in their phagocytic roles. IgG_1 inhibits opsonization by either isotype (Guidry et al., 1993). IgG_1 is the predominant immunoglobulin isotype in milk and mediates phagocytosis

of bacteria by macrophages. IgA acts to neutralize toxins or block attachment of bacteria to mucosal surfaces. Microbial toxins play a part in pathogenesis of staphylococcal infections (Zavizion et al., 1995) and attachment and invasion of mammary epithelial cells by mastitis pathogens has been demonstrated (Cifrian et al. 1994; Matthews et al., 1994). Enhanced antibody responses to bacterial toxins or adhesins or against antiphagocytic capsular components may prove important to future strategies for stimulating host defense mechanisms.

Several nonspecific defense mechanism have been identified in milk. The two subjected to the greatest study have been the lactoperoxidase (LP) system and lactoferrin (LF). LF is an iron binding protein that may influence susceptibility of the non-lactating gland to gram-negative infections. LF also binds many other molecules such as immunoglobulins, DNA, and glycosaminoglycans into immune complexes, which may modify LF activity or in turn be modified by the complexing (Hurley, 1993).

Objective 2: Characterization of virulence factors of mammary gland pathogens.

Over 140 different bacterial species have been reported to cause mastitis (Watts 1988). Most cases, however, are caused by a relatively small number of organisms, most of which belong to either the streptococci, staphylococci, or Enterobacteriacae (coliforms). Work under this objective has concentrated on bacterial species that are responsible for the majority of bovine mastitis cases.

A variety of virulence mechanisms have been identified that allow bacteria to enter and persist in the mammary gland and cause mastitis. These include adherence to bovine skin and mammary epithelial cells, elaboration of various toxins that destroy host tissues and immune functions, production of capsule and slime layers that protect bacteria from host defenses, and antibiotic resistance. Mammary epithelial cell culture techniques have been used to show that in the early stages of infection, *Streptococcus uberis, S. dysgalactiae,* and *S. aureus* invade mammary epithelial cells and adhere to and enter bovine epithelial and myoepithelial cells in culture (Cifrian et al., 1994; Matthews et al., 1994[a]). *Streptococcus uberis* was shown to elaborate unique proteins in culture and to possess a capsule that contributes to its virulence (Matthews et al., 1994[a]; Almeida and Oliver, 1993; Cifrian et al., 1994; Matthews et al., 1994[b]) Staphylococcal α and β toxins were shown to be synergistic and to inhibit proliferation of a bovine mammary epithelial cell line (MAC-T) (Cifrian et al., 1994). Rapid PCR identification techniques have been developed for *Streptococcus* species, staphylococci (Jayarao and Oliver, 1994), and for *Escherichiae coli.* The goal is to rapidly identify pathogens from milk samples without the need for time consuming and labor intensive culture techniques. Coagulase gene polymorphism in *S. aureus* is also being utilized to subtype and identify *S. aureus* isolates for epidemiologic studies (Aarestrup et al., 1994; Aarestrup et al., 1995; Matthews et al., 1994[a]).

Mastitis in heifers is a new important area of research. Studies have shown that as high as 90% of heifers are infected in some herds, (Nickerson, 1994). Dry cow therapy prepartum has been shown to be highly effective in eliminating these infections, with cure rates greater than 90% observed. The etiology of these infections is unknown, and it was initially suspected that infected cows could be the primary source of *S. aureus* infections in heifers. However, preliminary evidence from coagulase gene typing, RNA ribotyping, and biochemical finger printing indicate that the organisms infecting heifers probably arise from the environment.

The poor success of lactating therapy for IMI caused by organisms other than *S. agalactiae* and *S. dysgalactiae* has prompted studies on different therapy regimens and alternative therapy products. The dynamics of environmental streptococcal mastitis in Ohio for a 7-year period were summarized. Primary reasons for elimination of IMI included spontaneous cures (45.7%) and lactation antibiotics (33.2%). Of the IMI treated with lactation antibiotics, 71.5% cured (Todhunter et al., 1995). The postantibiotic effect of selected antibiotics was evaluated against mastitis pathogens, and the effect of milk on antibiotic activity against mastitis pathogens *in vitro* was determined (Owens and Ray, 1994; Owens et al., 1994).

Objective 3: Development and evaluation of techniques for modulation of host responses to mastitis pathogens.

Strategies aimed at enhancing resistance of the mammary gland to bacterial infection will have a major impact on the abatement of bovine mastitis. During the last five years, significant advances were made in several areas including the nutritional control of immunity, vaccine development, cytokine immunoregulation, and the evaluation of antimicrobial substances.

The nutritional status of dairy cattle is related directly to overall health, and proper nutrition has long been associated with the ability to fight disease. Considerable research effort has focused on the role of certain micronutrients in mammary gland immunity. Copper levels fall to their lowest levels at a time when cows are most vulnerable to mastitis (Xin et al. 1993). Copper deficiency results in lower bactericidal capabilities of bovine neutrophils and reduced production of important immunoregulatory cytokines (Torre et al., 1995). Cows supplemented with copper had lower clinical scores and reduced SCC following LPS challenge compared with unsupplemented cows. Copper status did not affect the outcome of *S. aureus* challenges. However, Cu supplementation reduced the prevalence of major pathogen IMI at calving in heifers (Harmon, 1994).

Vaccination is designed to potentiate the host's immune system toward a unique, specific antigen. Significant advances were made during the last five years in the development of effective mastitis vaccine protocols. Controlled studies have shown that an *E. coli* J5 bacterin was effective in reducing the duration of IMI and local signs of clinical mastitis in experimental challenge studies. Results from several field trials showed that although the vaccine had little impact on the prevalence of coliform infections, immunization with gram-negative core antigens decreased the incidence and severity of clinical disease (Hogan et al., 1992).

Vaccines for *S. aureus* mastitis have also been explored. A vaccine formulated of two encapsulated strains, α- and β-toxoid, and a proprietary adjuvant has been offered to a commercial company, and APHIS approval is being sought. Immunization protocols were developed that elicited antibodies to *S. aureus* capsule antigens as well. These antibodies promoted phagocytosis of encapsulated organisms.

Several studies have attempted to enhance bovine mammary gland defenses with recombinant bovine cytokines. *In vitro* and *in vivo* studies indicate that IL-2 enhances the functional capabilities of mammary gland mononuclear cell populations. Exposure to IL-2 markedly enhanced responses of local mononuclear cell populations to suboptimal mitogen concentrations that normally do not elicit a proliferative response (Torre et al., 1992). Lymphocyte populations isolated from lactating bovine mammary tissues also had increased cytotoxic and bactericidal activities following *in vitro* culture with IL-2 (Shafer-Weaver, 1996). Others have shown that IL-2 may be an effective adjuvant with mastitis vaccine protocols (Pighetti and Sordillo, 1995). Administration of IL-2 in late lactation animals was effective in accelerating involution and stimulating local antibody production (Nickerson et al., 1993). However, intramammary IL-2 was ineffective as an adjunct to standard antibiotic dry cow therapy. When administered in doses that far exceed the documented maximum tolerable dose, IL-2 plus antimicrobials did not improve cure rates and was associated with abortions at 3-5 d post-treatment (Hogan et al., 1995). Experimental results clearly indicate the ability of other recombinant cytokines to modify host defenses during instances when the immune system is compromised or during the pathogenesis of mastitis causing bacteria. Several cytokines (TNF, IL-1, and IL-6) are released during the early stages of coliform mastitis. Elevated levels of these cytokines were correlated to acute inflammatory reactions in the mammary gland (Shuster et al., 1993) and mortality associated with endotoxic mastitis (Sordillo and Peel, 1992). Subsequent studies showed that mammary gland macrophage populations have an enhanced ability to produce TNF-α during the periparturient period, and that prophylactic treatment with IFN-α may modify the production of TNF-α (Pighetti and Sordillo, 1994).

The possibility of using other novel immunomodulators to modify the pathophysiology of mastitis also was studied. Intravenous infusion of sodium salicylate was antipyretic during intramammary inflammation, but did not substantially reduce inflammation (Morkoc et al., 1993). Recombinant human IL-1 receptor antagonist was evaluated *in vivo* as a therapy for endotoxic mastitis in cows. Although the biological activity of IL-1 in mastitic whey was blocked, treatment with IL-1 receptor antagonist did not have any clinical benefit (Shuster and Kerli, 1995).

New approaches for the use of antimicrobial substances in the prevention and treatment of mastitis was researched extensively during the last five years. Work on non-antibiotic methods of controlling mastitis included an evaluation of a nisin-based teat dip formulation. Ambicin N also was evaluated in the treatment of *S. aureus* infections (Sears et al., 1992).

Antibiotic combinations and treatment regimens also were assessed for efficacy against IMI. Systemic oxytetracycline in combination with intramammary antimicrobials did not improve cure rates for *S. aureus* IMI over the dry period (Erskine et al., 1994). Intramuscular ceftiofur was not effective in eliminating *S. agalactiae* compared with intramammary penicillin-novobiocin (Erskine et al., 1995). Pirlimycin "blitz" treatment of two herds with a high percentage of chronic *S. aureus* cows resulted in low cure rates post-treatment (Timms, 1995). Intramammary cephapirin and amoxicillin were ineffective in treatment of mild clinical mastitis caused by environmental pathogens.

Objective 4: Characterize the effect of mastitis and mastitis control practices on dairy food safety.

Current methods for the control and prevention of bovine mastitis have the potential for creating human health hazards both from antibiotics used to treat mastitis and from chemicals used to sanitize teats and equipment. There is a potential for pathogenic bacteria present in infected mammary glands to contaminate milk and meat, resulting in human illness. The heightened public awareness concerning human health issues gives added emphasis to these potential areas of concern.

In recent years, dairy foods have been implicated in several outbreaks of illness in children and adults. Pathogens that have been implicated in human illness that have been isolated from the mammary gland include *Listeria monocytogenes*, *Salmonella* species, *E. coli*, and *Campylobacter* species. No clear link between such outbreaks and bovine mastitis have been made, and insufficient information is currently available to determine the potential risk. There is a need for studies to evaluate appropriate measures that reduce or eliminate organisms and chemical contamination from the food supply.

Antibiotics in milk pose a potential human health hazard to allergic individuals, and research areas that emphasize prevention rather than treatment can help reduce this risk. Surveillance methods to detect antibiotic residues in milk and meat are currently being evaluated to improve their specificity and enhance the ease of their use on the farm. Improved accuracy is needed in this area to both prevent unnecessary waste of saleable milk and protect the public from exposure to antibiotic residues.

Food safety is an important issue to consumers, producers, and government agencies. The increased public and political interest that this area is receiving indicates its potential impact. Very little information is available concerning the relationship between bovine mastitis and these human and food safety issues; therefore, a need exists to answer these questions and determine what steps, if any, need to be taken to insure that mastitis control and treatment procedures do not adversely impact public safety.

While this proposed new objective includes some new areas of research to the project, it is important to note that some research topics have been in progress for several years that would fall under this new objective. Ongoing research topics that would be covered by this new

objective include the study of residues in milk and the evaluation of residue detection assays, the identification of mastitis pathogens that may also cause human disease, and the determination of antibiotic resistance patterns of mastitis pathogens.

Areas of research pertaining to this new objective that are planned or in progress include the effects of BST on clinical mastitis and the relationship to antibiotic use (Louisiana, Michigan), rapid detection methods for *E. coli* 0157:H7 and *L. monocytogenes* (Vermont, Tennessee), human and animal safety concerns with persistent teat dips (Iowa), antibiotic residues in milk (California, Maryland), environmental impact of mastitis pathogens (California), impact of raw milk consumption on human health (California), and mastitis organisms associated with human food-borne illness (California).

OBJECTIVES:

Objective 1: Characterize host responses or factors that affect resistance of the mammary gland to mastitis.

Objective 2: Characterization of virulence factors of mammary gland pathogens.

Objective 3: Develop and evaluate techniques for modulation of host responses to mastitis pathogens.

Objective 4: Characterize the effect of mastitis and mastitis control practices on dairy food safety.

PROCEDURES: BY OBJECTIVE

NE-112 members will continue to share experimental protocols, reagents, and mastitis pathogen isolates. Prominent examples of joint planning and coordination by the cooperating stations are as follows: 1) development and implementation of intramammary experimental challenge protocols for both contagious and non-contagious pathogens, and models such as toxin infusion, 2) methods of identification of mastitis pathogens, most notably coagulase-negative staphylococci and coliform bacteria, 3) the exchange of reagents (i.e., common monoclonal and polyclonal antibodies as well as various bovine immunoglobulin isotypes, cell surface proteins, and bacterial toxins), and 4) biotechnology reagents that include various cytokines and genetic probes.

Objective 1: Characterize host responses or factors which affect resistance of the mammary gland to mastitis.

Investigators at NADC Ames, Iowa and Connecticut will use immunoassay techniques to develop assays for detection of cytokines in milk and mammary secretions. These assays will be shared with other NE-112 members to help determine the role of cytokines in the immune response to mastitis. Studies are planned at Ohio to determine the role of vitamin E in the bovine immune response to mastitis. At Kentucky, the role of antioxidants such as vitamin E, ceruloplasmin, and superoxide dismutase in heat stress related mastitis will be studied. The immunoglobulin response to iron regulated outer membrane proteins in gram-negative bacteria will also be investigated at Ohio. At Connecticut and USDA-Beltville, tissue culture techniques and immunoassay procedures will be used to study the chemotactic properties of mastitic and normal milk and the role of bacterial adherence in mastitis. Biochemical techniques will be used to evaluate lactoferrin synthesis and the consequence of lactoferrin interaction with other macromolecules on mammary immunity at Illinois. Also at Illinois, the effects of transport of immunoglobulins into colostrum will be studied. Genetic variation and its effect on the periparturient immune system will be studied at NADC. In related studies, the incidence of mastitis in heterozygous daughters of bulls with an autosomal recessive lethal defect will be determined. At Vermont, studies are planned to determine the effect of fatty acids on the

growth of mastitis pathogens. NE-112 members at Louisiana and Ohio will collaborate by providing isolates of selected mastitis pathogens for evaluation. This combined group of isolates will also be used at Vermont to evaluate the growth response of mastitis pathogens in a "casein-based" media with and without plasmin.

Objective 2: Characterization of virulence factors of mammary gland pathogens.

Studies are planned on the binding of various mastitis pathogens to leukocytes using the recently developed β-2 intergrin model (NADC). Several NE-112 members (Louisiana, Ohio, California, Kentucky) will collaborate with the NADC group in this effort by supplying isolates of various mastitis pathogens. At Beltville and Tennessee, factors affecting bacterial adherence to mammary epithelial cells including bacterial cell surface components, epithelial cell surface components, toxins, and antibiotics will be studied using the mammary epithelial cell model recently developed through NE-112 collaborative studies.

At Vermont, the lysostaphin gene has been cloned and modified for epithelial cells. The expression of the gene and secretion of gene products will be studied. Also at Vermont, adenovirus and retrovirus vectors are being studied and developed as a means for gene introduction into bovine mammary epithelial cells.

Studies will be conducted at Tennessee and Vermont on rapid non-culture methods of identifying mastitis pathogens. PCR fingerprinting for staphylococci and streptococci will be refined, as will ribosomal RNA ribotyping and phage typing for identification of staphylococci (Vermont and Louisiana). In all such studies, bacterial strains and isolates will continue to be shared freely among units (Texas, Kentucky, Louisiana, Washington, and California). The etiology of heifer mastitis will continue to be investigated at Washington, Louisiana, Vermont, Tennessee, Virginia and Kentucky. The effects of fly control on heifer mastitis will be investigated and the optimum time prepartum for treatment determined. Fomites and reservoirs of *S. aureus* mastitis in heifers will be characterized and management strategies developed to block transmission. Strains of *S. aureus* from heifers will be identified using recently developed techniques in PCR fingerprinting and ribotyping. Isolates from flies and other environmental sites will also be identified to determine the source of heifer isolates (Tennessee and Louisiana). At Tennessee, the immunogenicity of capsular polysaccharide purified from *S. aureus* will be determined and a method of capsular serotyping developed.

The outer membrane protein of gram-negative bacteria cultured in synthetic media will be studied and the dynamics of naturally occurring IMI will be investigated (Ohio). Information from these studies will be added to the growing body of knowledge concerning environmental mastitis and its control with the J5 coliform vaccine.

A major problem in the therapy of bovine mastitis with antibiotics is the poor response of *S. aureus* mastitis to therapy during lactation. Recent studies by NE-112 members indicate that extended duration therapy enhances cure rate. Studies are planned at Louisiana to determine the optimum duration of therapy, which antibiotics are most effective, and if therapy results can be enhanced by previous vaccination or other forms of immune modulation.

Objective 3: Develop and evaluate techniques for modulating host responses to mastitis pathogens.

The incidence of mastitis increases when defense mechanisms of the mammary gland are impaired. If immunomodulators can be used to augment immune functions at critical periods during the production of milk, then the incidence of mastitis should be reduced. Information obtained from Objective 1 over the last five years will be useful when investigating new methods of modulating the mammary gland immune system. Areas of continued study will be the role of micronutrients in host defense, vaccine development, devising novel immunoregulatory strategies,

and evaluation of effective antimicrobial therapies. Members of the NE-112 project will work in collaboration when designing experiments to address each of these areas.

Selenium is probably the best characterized micronutrient with regard to immunoregulatory effects. Many studies by NE-112 members have documented the benefits of dietary selenium supplementation for the control of bovine mastitis. Increased neutrophil influx and bactericidal ability associated with adequate selenium nutrition is critical to the resolution of an infection within the bovine mammary gland. Future studies will examine how inadequate selenium nutrition may contribute to mammary endothelial cell dysfunction. Collaborative studies between Ohio and Pennsylvania will examine how alterations in mammary endothelial cells may influence neutrophil extravasation into sites of tissue damage.

Studies of the effects of copper inadequacy on host defense and immune function will continue as well. Researchers at Kentucky will evaluate different copper sources (inorganic vs. proteinate) in repletion studies. Copper adequate or inadequate heifers will be used to determine the effects on antibody responses to *E. coli* J5 vaccination. Collaboration between Kentucky and Pennsylvania will continue to evaluate the impact of copper inadequacy on the cytokine response of the mammary and clinical severity after local challenge with endotoxin or *E. coli.*

Vaccines currently available for contagious pathogens do not appear to eliminate chronic mastitis or consistently reduce the incidence of new infections. However, considerable progress has been made over the last several years in the development of an effective vaccine against coliform mastitis. Several stations will work to improve the J5 *E. coli* vaccine. California will attempt to improve the commercially available J5 *E. coli* antigen while researchers at Ohio will evaluate new subunit coliform vaccines by experimental challenge and natural exposure trials. The work on new and improved vaccine antigens will be complementary to the development of novel antigen delivery systems. The potential of using poly (D, L, lactate-co-glycoside) microspheres to deliver a variety of mastitis vaccine antigens is being developed at USDA-Maryland. Other stations are exploring the use of cytokines, micronutrients, and other immunomodulators as potential mastitis vaccine adjuvants (California, Ohio, Pennsylvania).

The role of cytokines and other endogenous factors in the pathophysiology and prevention of mastitis will be the focus of several stations. Platelet activating factor (PAF), a potent inflammatory phospholipid, can augment macrophage activation states. Studies are underway at Pennsylvania to determine how PAF can modify the production of TNF-α from mammary gland macrophage populations during the pathogenesis of acute coliform mastitis. NADC plans on producing recombinant soluble bovine proteins to block inflammation during acute coliform mastitis as well. The adhesion molecule ligand bovine ICAM-3 and soluble TNF-α receptors will be cloned, sequenced, and expressed. These recombinant proteins will be characterized and evaluated for their role in mammary gland inflammation. In addition, studies will be initiated to investigate how rations can minimize plasminogen activation in bovine mammary glands (Vermont).

Several stations will investigate methods that will potentiate the efficacy of antimicrobial therapies, reduce food safety concerns, and enhance profitability for dairy producers. Studies on therapy of clinical mastitis are planned at several sites (Michigan, Illinois, Virginia, and New York). Studies to evaluate the efficacy and cost-effectiveness of antibiotic therapy for clinical mastitis will be conducted at Illinois. Michigan will evaluate the safety of using the antimicrobial protein Nisin as an intramammary infusion. The efficacy and cost effectiveness of novel teat dips will be evaluated as well. Iowa and Louisiana plan to develop and/or evaluate barrier dips for prevention of mastitis during the dry period. The efficacy of a thixotropic agent as a germicidal postmilking teat dip also will be evaluated (Iowa). In order to facilitate the development of other innovative teat dip products, collaborative studies at Vermont and Washington will be conducted to evaluate relationships between skin and teat end condition with susceptibility to IMI. Studies on the incidence and control of mycoplasma mastitis are planned at New York and California.

Objective 4: Characterize the effect of mastitis and mastitis control practices on dairy food safety.

Studies are planned at Vermont and Tennessee to develop rapid detection methods for *E. coli* O157:H7 and *L. monocytogenes*. Human and animal safety concerns related to persistent teat dips will be investigated in Iowa; persistent barrier teat dips will be studied to determine if they can be used as a replacement for dry cow therapy thus reducing antibiotic usage and residue potential. The human health concerns associated with antibiotic residue in milk will continue to be investigated in California. Also in California, studies on the epidemiology of *E. coli* O157:H7 are planned. Isolates of *E. coli* will be supplied from collaborating units in Louisiana, Ohio, Tennessee, and Kentucky. These studies will help determine if the O157:H7 strain of *E. coli* is a factor in bovine mastitis. Also, the effect of verotoxins on the mammary gland immune response will be investigated. The impact of raw milk consumption on human health will be studied in California, and the potential role of mastitis organisms in human illness investigated.

Expected Outcomes

The successful completion of the objectives outline in this project will provide vital new information to enhance control and prevention programs for bovine mastitis. Because mastitis is a very complex disease system caused by may different bacterial species, it is necessary to address both control and prevention aspects to build an effective mastitis program.

Development of new vaccines for the different types of bovine mastitis is a major area of work. Vaccines for both *S. aureus* and streptococcal mastitis would provide a major breakthrough for control of these difficult infections. Similar vaccine success with the J5 coliform vaccine has already had a major impact on coliform mastitis, with a return of $57/cow/lactation. It is currently estimated that 25% of the US dairy herd is now being vaccinated with gram-negative core antigen vaccines with an expected efficacy of 70%. Other studies on cytokines and immune modulators could result in major changes in both therapy and vaccine protocols, and in vaccine and treatment delivery systems.

Determination of the etiology of heifer mastitis will provide important information for control of new infections in younger animals. Determining the optimum time to treat heifer mastitis will result in increased cure rate for these infections.

The new objective on food safety will address possible human health concerns and help ensure that mastitis control and prevention methods do not impact public health.

The members of NE-112 have a long standing record of productivity and dissemination of data through a variety of avenues. In addition to numerous peer review journal articles, this group publishes proceeding, popular press articles, book chapters, and audio visual publication. All of the members of NE-112 are closely associated with cooperative extension programs. Many have joint extension appointments and all participate with their local extension personnel in various informational programs designed to disseminate information directly to producers. Previous examples of such efforts are illustrated by the 58 popular press articles and 2 audio visual tapes produced by NE-112 members in recent years. Future plans include similar publications. In addition, the NE-112 and Mastitis Research workers are in the process of establishing a home page on the internet to increase our availability to the public. This document will be presented at this web site along with timely information and new developments in mastitis control and prevention. The unique blend of basic and applied research that make up this project ensure that major advancements will be made in this important area, and that this information will be quickly made available to the dairy producers of the nation.

ORGANIZATION

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Washington	L. K. Fox*

The issue addressed in this project is a problem that is present throughout the breadth of the nation, and participants were selected from outside the region on the basis of common interest and special competence in the various research areas applicable to the project. The organization of this project will be in accordance with that set forth in the *Manual for Cooperative Regional Research*. The project will be administered by a technical committee consisting of the project leader from each of the participating stations. An executive committee will consist of the Chairman, Vice-Chairman, Secretary, and the Administrative Advisor. The officers will serve one year after which the Vice-Chairman automatically becomes chairman and the Secretary becomes Vice-Chairman. This executive committee will conduct business between meetings. Meetings will be called once a year by the Administrative Advisor. At these meetings, research accomplishments will be reviewed and recommendations made for coordination. Annual reports of research data from each station will be called for by the chairman. These reports will be compiled and sent to each participant prior to the annual meeting. The responsibility for regional summaries and publications will be assigned at the meetings.

SIGNATURES:

Regional Project Title:

Administrative Advisor — Date: 6-12-97

Chair, Regional Association of Directors — Date: 8/11/97

Administrator
Cooperative State Research Service — Date: 8/25/97

References Cited

Related Current and Previous Work

Objective 1

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Objective 3:

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ATTACHMENTS:
PROJECT LEADERS:

Name	State	Area of Specialization and Department
J. S. Cullor	California	Immunology-Veterinary Pathology
L. M. Sordillo	Pennsylvania	Immunology
T. J. Yang	Connecticut	Immunology-Pathology
D. E. Morin	Illinois	Mastitis Management-Vet. Clinical Medicine
M. E. Kehrli	Iowa-NADC	Immunology
L. L. Timms	Iowa	Dairy Extension-Animal Science
R. J. Harmon	Kentucky	Lactational Physiologist-Dairy Science
S. C. Nickerson	Louisiana	Histology-Agricultural Experiment Station
R. J. Erskine	Michigan	Mastitis Management-Vet. Clinical Medicine
R. N. Gonzales	New York	Microbio-EPI
K. L. Smith	Ohio	Physiology of Lactation-Dairy Science
A. J. Guidry	Beltsville, MD	Immunology-IDRH
S. P. Oliver	Tennessee	Lactation Physiology-Dairy Science
J. W. Pankey	Vermont	Mastitis Management-Animal Science
L. K. Fox	Washington	Immunology-Vet. Clin. Med. & Surgery
J. R. Roberson	Virginia	Mastitis Management

RESOURCES (1995):	SY	PY	TY
California	0.50	1.50	2.00
Connecticut(S)	0.20	0.50	0.20
Illinois	0.20	0.80	0.50
Iowa	1.00	0.00	1.00
Iowa State	0.10	0.00	0.00
Kentucky	1.00	0.80	1.45
Louisiana	0.90	1.00	0.30
Michigan	0.75	0.10	0.50
New York(C)	0.60	0.50	0.50
Ohio	1.05	0.00	2.00
Pennsylvania	1.36	2.00	1.00
Beltsville, MD	2.00	1.00	2.00
Tennessee	0.30	1.00	0.75
Vermont	1.65	0.70	2.75
Virginia	0.50	0.00	0.05
Washington	1.60	1.00	1.00
Totals	13.71	10.90	16.02