PHENOTYPIC RELATIONSHIPS BETWEEN IMMUNE RESPONSE, DISEASE OCCURRENCE, AND PRODUCTION IN PERIPARTURIENT HOLSTEIN DAIRY COWS

A Thesis

Presented to

The Faculty of Graduate Studies

of

The University of Guelph

by

LAURAINE WAGTER

In partial fulfilment of requirements

for the degree of

Master of Science

December, 1997

ABSTRACT

PHENOTYPIC RELATIONSHIPS BETWEEN IMMUNE RESPONSE, DISEASE OCCURRENCE, AND PRODUCTION IN PERIPARTURIENT HOLSTEIN DAIRY COWS

Lauraine Wagter
University of Guelph, 1997

Advisor:
Dr. B.A. Mallard

Peripartum immune response, disease occurrence, and production were evaluated in 136 Holstein cows and heifers from three herds. Based on antibody to ovalbumin (OVA), animals were classified using a mathematical index into high (Group 1), average (Group 2) or low (Group 3) antibody groups. Whey antibody to OVA and serum antibody to *Escherichia coli* similarly reflected the ranking of groups for serum antibody to OVA. Unstimulated and OVA-stimulated lymphocyte proliferative responses were inversely related to antibody group. Group 1 animals had the highest lymphocyte proliferative responses to concanavalin A peripartum, the greatest decrease in response at calving, and the greatest increase in response postpartum. Delayed type hypersensitivity to tuberculin did not significantly associate with antibody group. Group 3 animals had a higher projected 305-day yield for milk, fat, and protein than Groups 1 and 2; however, in two of the three herds evaluated, Group 1 animals had no clinical mastitis.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. B.A. Mallard for accepting me as her graduate student and for her never ending encouragement, advice and generosity. She has given me the necessary tools for success in my future endeavours. Chinese wisdom teaches the following: "Give a man a fish and he eats for a day. Show a man how to fish and he eats for a lifetime." I truly believe that Dr. Mallard has shown me what is necessary to successfully achieve all of my professional and personal goals in life. This is a gift that I will treasure always.

I would also like to thank the members of my advisory committee (Dr. J.C.M. Dekkers, Dr. K.E. Leslie, and Dr. B.N. Wilkie) for their encouragement, guidance and valuable suggestions throughout my research and in the preparation of this thesis. I enjoyed working with them. From them, I have gained a wealth of knowledge.

I would like to thank Marion Wilke; first, for her unending friendship and second, for her tremendous help with all the various experimental protocols detailed in this thesis.

I would also like to thank Sophia Lim for helping me complete some of the *E. coli* ELISAs detailed in this thesis. She has a great sense of humour and I really enjoyed working with her.

I would also like thank Dr. Jeanne Burton for her advice and provision of the ELISA protocol to evaluate OVA antibody. I thank her for her friendship and ability to make me laugh.

I would like to thank God for all He has given me. I believe that He has blessed me several times throughout my life and I am very grateful for that. He blessed me with an opportunity to attend graduate school and work with a very kind, patient, encouraging, talented and industrious advisor. With all my heart I thank God.

I would like to thank the love of my life, my fiancé Claude, who always told me I could do it. He has always been very supportive of all my pursuits in life. He lended a caring ear and offered advice when it was needed. With him, I look forward to all of life's challenges that lie ahead.

Sincere and warm thanks to my parents, Ellen and Gerard, for their love and support. From day one, they have been a constant source of encouragement when the going got tough. One of the many things they taught me was that success in life is sometimes preceded by hardship or failure and that no matter what, I should never give up on attempting to achieve my goals. They have taught me one of life's best lessons.

To my brother, Mark, my friend and confident growing up. I did it! Mark has always been a kindred spirit and an excellent advisor in my life. I love him very much and I value his friendship. Thanks bro!

To the rest of my family (Ann, Erica, Al, Joy, Cheryl, Tracy, and my grandparents, Alte and Gerritje Wagter) I send sincere thanks for your unending love and support.

Warmest thanks go to my friends Theresa Sedge, Catherine Bomben, Sheila Douglas, Sharon VanHemmen, Ronald & Theresa Langel, Jacqueline VandenBrink, Corinne VanKampen, Lynn Rowan, and Frs. Sean Byron and Colum Corrigan who always made me laugh and/or feel happy when I needed it most. Their friendship and love is very important to me.

I want to thank all my colleagues (Mary Jane Ireland, Shayan Sharif, Shirley Furesz, Robin Hedge, Greg Appleyard, Peter Borgs, Byram Bridle, Claudine Raymond, Sandi Bowland, Sandy Calder, Joy Prudencio, Jay Reddy, Janine Bossé, Gabrielle Ilic, Leanne DeWinter, Jutta Hammermueller, Alison Moller, Kees Plaizier, Gordon VanderVoort and Xiaguo Chang) for their assistance, encouragement, comradery, and laughter.

Further, I would like to thank all the herd managers for their cooperation and assistance in helping me conduct the research presented in this thesis: 1) Dr. Essi Evans, Michelle Carson and the staff at the Shurgain Agreserach Farm in Burford, Ontario; 2) Mr. Denis Little and the staff at the Ponsonby Research Station, Ponsonby, Ontario; 3) Mr. Gordon Dunk and Family, owners of Speedvalley Holsteins, Fergus, Ontario.

This research was supported by the Natural Sciences and Engineering Research Council of Canada and by the Ontario Ministry of Agriculture, Food, and Rural Affairs.

DECLARATION OF WORK PERFORMED

I declare that, with the exception of the items below, all the work presented in this thesis was done by me.

Hormone assays were performed and data was provided by Mary Jane Ireland. Somatic Cell Count determination was performed either by the Ontario Dairy Herd Improvement Corporation or the Mastitis Laboratory, Department of Population Medicine, University of Guelph. All complete blood cell counts were performed by the Clinical Pathology Laboratory, Department of Pathobiology, University of Guelph.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Ab	-	antibody
CBC	-	complete blood count
cfu	-	colony forming units
CMI	-	cell mediated immunity
CMIR	-	cell mediated immune response
Con A	-	concanavalin A
cpm	-	counts per minute
°C	-	degrees Celsius
CV	-	coefficient of variation
DTH	-	delayed type hypersensitivity
ELISA	-	enzyme linked immunosorbent assay
FCS	-	fetal calf serum
FITC	-	fluorescein
x *g*	-	gravitational force
h	-	hour
Ig	-	immunoglobulin
id	-	intradermal
im	-	intramuscular
L	-	litre
LOM	-	lack of measurable response
LS Means	-	least squares means
mAb	-	monoclonal antibody
M	-	molar
MS	-	mean squares
min	-	minutes
mL	-	millilitre
OD	-	optical density
OVA	-	ovalbumin
PBL	-	peripheral blood lymphocytes
PBS	-	phosphate buffered saline
PMNL	-	polymorphonuclear leukocyte
µg	-	microgram
µL	-	microlitre
ROI	-	reactive oxygen intermediates
rpm	-	revolutions per minute
rt	-	room temperature
sd	-	standard deviation
SEM	-	standard error of the mean
vol	-	volume

INTRODUCTION

Impairment of host defenses has been documented at parturition in many species, including the dairy cow (Weinberg, 1984; Clarke and Kendall, 1994; Detilleux et al. 1994; Detilleux et al., 1995). During the peripartum period of dairy cows, metabolic and physical demands are increased, and the occurrence of disease, particularly mastitis, is high (Burvenich et al., 1994). Selection for higher milk yield has resulted in a gradual increase in incidence of clinical mastitis (Harmon, 1984; Emmanuelson, 1988). Direct economic losses from clinical mastitis include costs of antibiotic therapy, milk discard, culling and death. Subclinical mastitis-related losses include decreased milk production and quality. Antibiotics have effectively treated some types of mastitis; however, consumers are concerned that they may be consuming milk products that contain antibiotic residues. Protection by vaccination against common pathogens (i.e. Gram positive pathogens) implicated in mastitis has been unsuccessful. An alternative would be to genetically select animals that are inherently resistant to a broad range of pathogens including agents of mastitis. This strategy may be possible using phenotypic candidate markers such as immune response traits that associate well with enhanced host resistance and do not compromise milk production. In mice, guinea pigs, chickens, pigs and other species, immune response phenotype varies within a population thus allowing selection for high or low immune responses that associate with certain health and production benefits (Biozzi et al., 1968; Ibanez et al., 1980; Siegel et al., 1980; Van der Zijpp et al., 1983; Mallard et al., 1992). Therefore it was hypothesized that the identification of cows

with inherently superior immune responses may lead to increased disease resistance, enhanced productivity, and reduced risks and costs associated with antibiotics and vaccines.

To determine if phenotypic variation existed in bovine peripartum immune responses and if there were associations with disease occurrence or production, 136 Holstein cows from 3 herds were evaluated over the course of one lactation. The following hypotheses were tested: 1) Dairy cows differ phenotypically, in their ability to respond immunologically, and these differences have implications with respect to resistance to disease; and 2) Variation in response phenotypes of cows can be partitioned using qualitative or quantitative procedures.

REVIEW OF LITERATURE

1.0. Relationships between host resistance and peripartum disease

Impairment of bovine host defense during the peripartum period may be associated with high concurrent disease occurrence. Impaired resistance may be due to endocrine factors associated with metabolic and physical changes occurring during gestation, parturition and lactation (Smith et al., 1973; Guidry et al., 1976; Burton et al., 1993). Infectious diseases of the peripartum period include mastitis, metritis and pneumonia. Metabolic and some reproductive diseases also predominate during this period and include retained placenta, milk fever, ketosis, and displaced abomasum. Mastitis is the most economically relevant disease. Estimated annual losses from mastitis are $35 billion (U.S) worldwide (Giraudo et al. 1997), $2 billion (U.S.) in the United States (Harmon, 1994) and $ 17 million (Can.) in Canada ($140-300 Can./cow) (Zhang et al., 1993).

The outcome of infection depends upon interaction between microbial virulence factors and innate and acquired host resistance mechanisms. Cells that mediate innate and immune mechanisms include neutrophils (PMNs), macrophages, lymphocytes and epithelial cells. These cells are present in normal bovine milk. The percentage of each cell type in a unit of volume varies depending on stage of lactation and on health of the gland. (Burvenich et al., 1994). In milk from normal glands, the macrophage is the predominant cell type, followed by PMNs, lymphocytes, and epithelial cells. Epithelial cells in milk were designated 'milk somatic cells' by Paape et al. (1963). PMNs

predominate four weeks after the cessation of lactation , followed by macrophages and lymphocytes. The first two weeks before parturition, lymphocytes predominate followed by macrophages and PMNs. During mastitis, PMNs are the predominant cell type. Defences of the mammary gland are mediated by: 1) PMNs and macrophages that phagocytize bacteria and secrete inflammatory mediators; and 2) Lymphocytes that function in antibody-mediated and cell-mediated immunity (Burvenich et al., 1994).

Mastitis is an inflammation of the mammary gland characterized by local and systemic responses (Burvenich et al., 1994). Mastitis can be clinical or subclinical, when signs are not directly observable, but somatic cell counts in milk (SCC) increase and overall production performance decreases. Mastitis is caused by a number of Gram positive and Gram negative bacteria (Appendix I, Table I) that are either major or minor pathogens. Major pathogens induce the greatest compositional changes in milk and have the greatest economic impact (Harmon, 1994). They include *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae*, *Klebsiella spp.*, and others, while minor pathogens include coagulase negative staphylococci, and *Corynebacterium bovis*. The incidence of udder infection and clinical mastitis is usually highest at parturition and during early lactation (Smith et al., 1985). Coliforms such as *E. coli and Klebsiella* are the most common major pathogens causing mastitis during this period (Appendix I, Figure 1). Because coliform mastitis is difficult to treat, natural defence mechanisms of the mammary gland have been investigated in pursuit of control procedures (Burvenich et al., 1994). Coliform mastitis may be peracute and fatal, or subclinical. Most commonly it is acute

clinical mastitis, with local and systemic signs of disease. Coliforms are Gram-negative microorganisms from the family Enterobacteriaceae and include important species from the genera *Escherichia, Klebsiella, Enterobacter, Citrobacter* and *Proteus* (Harmon, 1994; Kremer et al., 1994). The structure of the cell wall of coliform bacteria plays an important role in the virulence of the bacteria and subsequently in the pathogenesis of mastitis. The cell wall of *E. coli* has an inner cytoplasmic membrane, a peptidoglycan layer, an outer membrane that consists of two layers: a phospholipid protein layer and an outer lipopolysaccharide layer (LPS), and finally some strains possess an additional capsular polysaccharide layer. The LPS layer has three components: the O-specific polysaccharide chain, a polysaccharide core, and lipid A. Lipid A mediates the biological properties of LPS (endotoxin). Endotoxemia causes clinical signs of disease including high fever, drowsiness, appetite loss, dehydration, loss in milk production, cardiovascular failure, shock and often death (Kremer et al., 1994; Burvenich et al., 1994). Factors that contribute to susceptibility to mastitis include the complex environment (pasture, bedding, cleanliness of holding areas), management (milking practices, antibiotic therapy during lactation and dry-off) and physical trauma to the teat and/or udder (Cullor, 1995).

1.1. Innate Host Resistance Mechanisms of the Dairy Cow

1.1.1. Neutrophil Number and Function

The mature lactating dairy cow has about 200 billion mature PMNs, some of which (10^{11}

cells) are in circulating pools and others (about 68 x 10^9) in marginal storage pools adhering to blood vessel walls (Paape et al., 1963; Burvenich et al. 1994). During an infection with *S. aureus*, milk somatic cells are mainly of PMN (95%) (Daley et al., 1991) and phagocytosis is maximal when milk somatic cell counts are highest. In a review by Burvenich et al. (1994), it was concluded that during experimentally induced *E. coli* mastitis of cattle, resorption of a toxic compound from inflamed quarters was responsible for severe and systemic signs of endotoxemia and for the strong depression in milk production. PMN functions, such as their rate of diapedesis, phagocytosis and detoxification largely determine the severity of mastitis after bacterial challenge.

Peripartum impairment of PMN function has been documented. Newbould (1976) examined PMN phagocytosis of *S. aureus*, strain 321 from conception to two weeks postpartum. The number of cells actively ingesting *S.aureus* was minimal at 16 to 20 weeks post conception, increased to a maximum two weeks prepartum, rapidly declined to a minimum during the first week after parturition and gradually increased again two weeks postpartum. Saad et al. (1989) similarly observed a gradual increase in the average number of bacteria per PMN before parturition followed by a sharp decline on the first day postpartum that peaked at two weeks postpartum. Random migration of PMN, iodination, and chemiluminescence increased two weeks prior to parturition and then decreased dramatically one week after parturition (Kehrli et al., 1989b). However, Detilleux et al. (1995) demonstrated enhanced PMN phagocytosis of *S. aureus* at parturition with a reduction three weeks postpartum. This confirmed results of Kehrli et

al. (1989) in that PMN chemotaxis uptake of opsonized zymosan native chemiluminescence activity, cytochrome C reduction iodination, and antibody dependent PMN cytotoxicity were depressed from two to three weeks before parturition through to three weeks after parturition. Politis et al. (1995) also demonstrated that superoxide anion production declined significantly ($P<0.05$) following parturition relative to prepartum values. Peripartum impairment of PMN function may be relevant to peripartum disease. Prevalence of mastitis was associated with depressed PMN phagocytic ability (Heynemen et al., 1990) and PMN recruitment into the mammary gland in early lactation was associated with increased susceptibility to severe coliform mastitis (Shuster et al., 1996). Cows that developed intramammary infections had the most profound depression of PMN functions, suggesting that PMN impairment influences susceptibility to mastitis (Kehrli et al., 1989). Hill (1983) also indicated that in postpartum *E. coli* mastitis, the severity of infection depended on the ability of the cow to mobilize PMNs from blood to the mammary gland.

Impairment of PMN function may be a cause or effect of metabolic diseases. In cows with retained placentas, superoxide production was suboptimal and PMN shape change was impaired one week after parturition (Gilbert et al., 1993). Defects in PMN function were also detected in cows with retained placenta, metritis and mastitis. Cows with metritis had lower superoxide anion production before parturition and cows with mastitis had lower chemotactic ability compared to clinically matched healthy cows (Cai et al., 1994).

Parity seems to influence the outcome of peripartum infection. Expression of the β2 integrins was reported to be lower in cows in their fourth lactation or greater (Ver Werven et al., 1997). This may compromise PMN extravasation and consequently could result in more prolonged infections (IMI) in older cows in contrast to younger cows. Gilbert et al. (1992) further indicated that cows with four or more lactations had a more pronounced impairment of PMN function when superoxide anion production, and stimulus induced shape change were investigated.

1.1.2. Macrophage Number and Function

Macrophages predominate in secretions of dry mammary glands. Macrophages are phagocytic, initiate antigen presentation, activate lymphocytes, and secrete cytokines. Macrophages stimulated by coliform bacteria or by LPS in the early stage of inflammation produce factors that stimulate PMN migration into the udder. Stimulation of macrophages induces synthesis and secretion of Tumor Necrosis Factor (TNF-α), leucotriene B4 (LKTB4), some prostaglandins (PGE), interferons (IFN), polar lipids such as Platelet Activating Factor (PAF), complement components (C'), neutral protease, and other agents that mediate local and systemic inflammation to exogenous stimuli (Kremer et al., 1990). The role of these mediators in the bovine mammary gland has not been elucidated. During mastitis, interleukin-1 (IL-1) from macrophages initiates clinical, hematological, blood-chemical and immunological changes in the host (Burvenich et al., 1994; Colditz et al., 1985; Craven, 1987; and Sandholm et al., 1986). Cell damage and

activated PMN also produce some endogenous mediators, such as PGE and LKT. The release of lysosomal granules by PMN causes local tissue damage and may amplify influx of serum components such as C' and PMN. Politis et al. (1995) have demonstrated impaired function of blood macrophages in the early postpartum period. Postpartum IL-1 and MHC class II antigen production were reported to be less than prepartum values.

1.1.3. Complement and other innate resistance mediators

Normal milk and non-lactating udder secretions contain a variety of soluble factors mediating non-specific antibacterial activity. These include C', conglutinin, lactoferrin, lysozyme and the lactoperoxidase: thiocyanate: hydroperoxide system (Kremer, 1990).

1.1.3a Complement

During inflammation, complement is mobilized from the blood into mammary secretions (Kremer, 1990). However, components of whole milk have been demonstrated to inhibit the inflammatory activity of activated complement and may not be a significant mediator of inflammation in the lactating gland (Kremer, 1990; Colditz, 1987). Alterations in complement concentration during the peripartum period are documented to occur only after parturition when complement concentration has been reported to increase (Detilleux et al., 1995).

1.1.3.b. Conglutinin

Conglutinin is a bovine serum protein that, when absorbed by erythrocyte-antibody-complement complexes, causes them to agglutinate (Stedman, 1990). Conglutinin concentrations have been investigated as a possible mediator of host resistance to infection. Conglutinin concentrations were observed to decline before parturition and reached a minimum at parturition (Detilleux et al., 1995). Decline in conglutinin concentrations may, in part, indicate reduced humoral responsiveness and increased susceptibility to disease.

1.1.3.c. Lactoferrin

Lactoferrin is the major component of the red protein fraction in milk whey (Tsuji et al., 1991) and is bacteriostatic due to its iron chelating properties in the presence of bicarbonate. Lactoferrin inhibits multiplication of bacteria by sequestering the iron that they require for growth. Lactoferrin is at low concentrations in normal milk, but in dry-cow secretions and mastitic milk lactoferrin concentration is comparatively high (Harmon et al., 1975; Kremer et al., 1990; Tsuji et al., 1991). Due to the low concentration ratio of lactoferrin to citrate, an inhibitor, lactoferrin is only effective in the non-lactating gland and mastitic lactating glands. As secretions of the non-lactating mammary gland change to colostrum, lactoferrin concentration declines and this increases the amount of iron available for bacterial growth (Kremer et al., 1990).

1.1.3.d. Lactoperoxidase: thiocyanate: hydroperoxide

Lactoperoxidase is present in normal milk and is bactericidal to *E.coli* and other Gram negative bacteria. Hydroperoxide is normally not present in milk and must be supplied by bacterial growth or by degranulation of PMN. In normal milk, activity of this system is limited by availability of hydroperoxide (Kremer, 1990).

1.1.3.e. Lysozyme

Lysozyme may contribute to lysis of coliform bacteria (Carroll et al., 1983). Concentration of this protein is low in milk and increases during mastitis. Although the concentration of lysosyme increases during infection, it is not considered to contribute significantly to protecting the mammary gland from infection (Kremer et al., 1990).

1.2. Acquired Immunity and Peripartum Disease

1.2.1. Immunoglobulin and Antibody Response

Although PMNs are the first cellular line of defense against invading micro-organisms, their function is enhanced by antibody (Nickerson, 1993). Antibodies bind to pathogenic epitopes, and also bind to PMN surface receptors, thus promoting recognition, opsonization and phagocytosis. Antibodies to mastitis causing pathogens in milk, colostrum, and serum are immunoglobulins (Igs) from the $IgG_{1\&2}$, IgA and IgM isotypes

(Burvenich et al., 1994). In all three fluids, IgG_1 is the most prominent isotype of ruminants. Immunoglobulin isotypes vary in concentration depending on the source (Appendix II, Table I). Though IgG_2 is present in lower concentrations in serum and milk it is a better opsonin than IgG_1 and is important for defense against pyogenic infections. IgG_2 has been reported to be relevant for defense against Pasteurella pneumonia in calves (Corbeil et al., 1984) and bronchiopneumonia and mastitis in dairy cows. In Danish Red cows, it has been reported that the frequency of IgG_2 deficiency among cows with pyogenic infections is much higher than among healthy animals (Nansen, 1972; Williams et al., 1975). Normally the concentration of Ig in milk is less than 1 mg/mL but this can increase to 80 mg/mL in secretions of mastitic glands (Burvenich et al., 1994). IgG_1 with serum derived complement are able to prevent growth of serum-sensitive coliform bacteria in the gland. In bovine milk a large proportion of IgA is associated with the milk fat-globule membrane. Globule membrane-bound IgA may actually increase the binding of bacteria to the fat-globule membranes, thus rendering the bacteria more difficult to phagocytize (Kremer et al., 1994). The function of IgA in the prevention of mastitis may vary depending upon the pathogen. IgA was reported to play a non-significant role in minimizing the development of *E. coli* mastitis (Kremer et al., 1994).

Several attempts have been made to develop vaccines against *S. aureus*, but without success. Vaccines have included toxoid, protein A, capsule and fibronectin in varying combinations and concentrations (reviewed by Sordillo, 1995). While these preparations

may reduce the severity and duration of mastitis, new infections are not prevented. Inclusion of capsular polysaccharide in vaccine preparation slightly reduced the rate of new infection (Watson and Schwartskoff, 1990). More recently, the combination of a crude extract of *S. aureus* exopolysaccharides and inactivated unencapsulated *S. aureus* and *Streptococcus* spp. in a vaccine decreased incidence of intramammary infections caused by *S. aureus* (Giraudo et al., 1997). Newer vaccines against environmental coliforms contain rough or R-mutants of *E. coli* or *Salmonella typhimurium*. The surface core antigens of these mutants induces formation of cross-protective antibody that provides protection against various Gram-negative diseases of animals including mastitis and calf scours. (Parker et al., 1994). These vaccines decrease incidence and severity of clinical disease but do not affect incidence of coliform infections (Sordillo, 1995).

During the peripartum period concentrations of serum IgG_1 decrease and IgG_2 increase (Detilleux et al., 1995). This phenomenon is explained by the active compartmentalization of IgG_1 into lacteal secretions during colostrogenesis. A study examining the compositional content of colostrum of heat stressed periparturient heifers, a condition that may potentially mimic the effect of stress due to gestation, parturition and lactation on host defense of the cow, indicated that heat stressed cows had a less pronounced decline in Ig two weeks prior to parturition, but had lower mean concentrations of IgA and IgG in colostrum at the first four milkings following parturition. Quantification of Ig concentration provides no information on the development of an immune response. Several investigations have evaluated Ig

concentrations; however, at present no studies have examined antibody response to mastitis pathogens or other test antigens during the peripartum period. Kollmann et al. (1993) evaluated antibody response to *Eimeria* sp. during the course of *Eimeria* infection in dairy cows followed under field conditions. Dairy cows had reduced serum IgG antibody at parturition. Nagahata et al. (1992) evaluated B-lymphocyte number and *in vitro* antibody producing activity of bovine lymphocytes in order to elucidate host defense of dairy cows during the periparturient period (10 to 14 days before parturition up to 14 days after parturition). The number of B lymphocytes did not change, but the number of cells producing antibody significantly decreased at parturition and up to three days after parturition. Further, there was wide variation in *in vitro* antibody producing activity among individual dairy cows (Nagahata et al., 1992). Variation in antibody production has formed the basis of selective breeding for high and low antibody response (Biozzi et al., 1968; Ibanez et al., 1980; Siegel et al., 1980; Van der Zijpp et al., 1983; Mallard et al., 1992). Some benefits of high antibody response, including increased resistance to extracellular pathogens and performance attributes have been reported in mice, pigs and chickens (Puel and Mouton, 1996). Burton et al. (1989) demonstrated that in dairy calves, there was significant, substantial variation of antibody response to ovalbumin (OVA) and human erythrocytes (RBC). Paternal half-sib heritability (h^2) estimates of antibody to OVA (h^2=0.48) and antibody to human RBC (h^2=0.31) were relatively high and suggested the possibility that genetic manipulation of antibody response profiles of dairy cattle may be possible.

1.2.1. Cell Mediated Immune Response (CMIR)

1.2.1.a. Cell Mediated Immune Response and Lymphocyte Proliferation

Cell mediated immunity (CMI) is generated by various sub-populations of T lymphocytes, including T helper cells (T_h), and T cytotoxic cells (T_c). Helper and cytotoxic T lymphocytes are distinguished by expression of CD4 and CD8 cluster of differentiation (CD) surface molecules, respectively. T cell populations can further be divided based on their expression of alpha-beta ($\alpha\beta$) or gamma-delta ($\gamma\delta$) receptors. T cells expressing $\gamma\delta$ T cells are found in epithelial tissue and likely function as part of the front line of defense at mucosal and skin surfaces (Hein and Mackay, 1991; Wijngaard et al., 1992). Cells expressing the $\gamma\delta$ T cell receptor constitute a greater proportion of the total lymphocyte population in cattle compared to other species such as humans and mice. During the first 1-2 weeks following birth, $\gamma\delta$ T cells constitute 60% of the total pool of lymphocytes in the blood. With increasing age these cells decline relative to $\alpha\beta$ T cells (Hein and Mackay, 1991).

Circulating $\gamma\delta$ T lymphocytes in cattle decreased on day two following the application of heat stress (Morrow-Tesch et al.,1996), a condition that may potentially mimic the effect of stress due to gestation, parturition and lactation. This was associated with an increase in the number of skin $\gamma\delta$ T cells assayed from tail head skin biopsies sampled on the same day.

In the peripartum period, CD5+ and CD8+ T cells did not change significantly from prepartum to postpartum but the number of CD4+ lymphocytes was higher after parturition (Harp et al., 1991). Pregnant women have significantly lower relative and absolute numbers of CD4+ lymphocytes throughout pregnancy returning to prepartum counts at 4-5 months after parturition (Sridama et al., 1982). This decrease in T cell numbers may account at least in part, for the reported decrease of maternal responsiveness and increased disease activity including autoimmune disorders. Local immunosuppression is likely necessary to allow for the survival of the fetus as an allograft (Sridama et al., 1982). Van Kampen et al. (1997) observed a significant decrease in the frequency of CD2+, CD4+, CD8+, CD5+, MHC class II positive, and IgM+ expression on blood lymphocytes from eight weeks prepartum to three weeks prepartum. Mastitic and healthy cows during the peripartum period were compared for their expression of these markers. Although there were no significant difference in the expression of CD2+, CD4+, CD8+, or CD5+ lymphocytes between mastitic and healthy cows during the peripartum period, the expression of CD2+ and CD4+ lymphocytes were slightly lower in mastitic cows compared to healthy cows. Further, IgM+ lymphocytes were higher for mastitic cows than healthy cows. Whether alterations in the expression of these markers are a result of disease or contribute to the pathogenesis of the disease is not known.

Blastogenesis assays are used to measure *in vitro* the ability of T or B lymphocytes to proliferate clonally to antigen or polyclonally to mitogen. Mitogens such as concanavalin

A (Con A) and phytohemagglutinin (PHA) are lectins that stimulate proliferation of T lymphocytes by binding to sugar moieties on the surface of T cells, consequently transducing an activation signal to the nucleus. Pokeweed mitogen (PWM) is a common B and T cell mitogen that is used to assess lymphocyte proliferation in both T and B cells. Periparturient suppression of *in vitro* mitogen-stimulated proliferation has been observed in several studies of dairy cows (Kehrli et al., 1989; Ishikawa, 1987; Kashiwazaki et al., 1985; Manak, 1982; Wells et al., 1977). Ishikawa (1987) reported decreased blastogenesis of Con A and PWM stimulated cells from the third trimester of pregnancy with a minimum response at parturition. Blastogenic response in both blood and milk lymphocytes to Con A , PHA, and PWM, was low during the week preceding parturition and was lowest on the day before parturition. This response increased the second week after parturition while milk proliferative response remained unchanged (Saad et al., 1989). Depression of lymphocyte proliferative response during pregnancy and the immediate postpartum period have also been demonstrated in humans (Weinberg, 1984) and sheep (Burrells et al., 1978). Only one report did not describe depressed mitogenesis of lymphocyte proliferation in cows at parturition (Detilleux et al.,1995).

There are few studies of the association between lymphocyte proliferation and peripartum disease. Kashiwazaki (1984) reported that in multiparous cows during the period when acute mastitis was prevalent, lymphocyte mitogenesis was lowest. While proliferation gradually increased to within normal values in recovery from mastitis, cows with chronic mastitis had a high proliferative response during all stages of disease. Reduced

blastogenic response of lymphocytes from mastitic cows might indicate a general impairment of lymphocyte function due to severe acute inflammation. Cows with high blood concentrations of endotoxin also had high lymphocyte proliferative responses to the Con A, PHA, PWM and LPS. Yang et al. (1988) investigated the total population of T and B lymphocytes in peripheral blood, supramammary lymph nodes and prescapular lymph nodes of cows with or without mastitis. Cows that had mastitis had fewer absolute B lymphocytes and more T lymphocytes in peripheral blood and prescapular lymph nodes compared to normal cows. Supramammary lymph nodes of mastitic cows had more T, B, and null lymphocytes compared to normal cows. Collectively, these studies suggest that low response of lymphocytes to mitogen in peripheral blood and milk during the peripartum period may coincide with the occurrence of disease in cattle.

1.2.2.b. Delayed Type Hypersensitivity

Delayed type hypersensitivity (DTH) is a localized inflammatory reaction mediated by T_{DTH} cells. These cells have been sensitized to epitopes of intracellular pathogens or contact allergens. When the host is subsequently challenged with the pathogen or contact allergen, these sensitized cells release various cytokines including interleukin-2 (IL-2) and interferon gamma (IFN-γ) that chemotactically attract monocytes (mainly macrophages) to the site of challenge. Cytokines induce monocytes to adhere to the vascular endothelium, migrate from the blood into the surrounding tissues, and become activated. Activated monocytes have an enhanced ability to phagocytose and kill

microorganisms. Delayed type hypersensitivity is usually tested on the skin, intradermally or topically. Skin thickness increases at the site of challenge as a result of inflammation and this skin-test reactivity generally peaks 2-3 days later.

Delayed type hypersensitivity has been evaluated in dairy calves (Kelley et al., 1982; Burton et al., 1989b). Kelley et al. (1982) examined DTH in thermoneutral and heat and cold stressed bull calves that were sensitized to *Mycobacterium bovis*. Heat stress reduced DTH (double skin fold thickness post id PPD injection) by 42%, 24 hours after purified protein derivative (PPD) of *M. bovis* was injected. Contact skin hypersensitivity (CS), another indicator of cell mediated immune response, to dinitrofluorobenzene (DNFB) was also reduced. Heat suppressed reactions by 38%, 24 hours after challenge. Cold suppressed skin thickness by 39%, in contrast to thermoneutrally regulated bull calves at 1-2 weeks following challenge. Among young pregnant and non-pregnant women, pregnant women were documented to be at two to three times more risk of developing tuberculosis. Among those pregnant women who had tuberculosis, their inflammatory lesions were more severe compared to non-pregnant women with tuberculosis (Rich, 1951). Visibly healthy women that recovered from primary tuberculosis had recrudescence during late pregnancy (Logg, 1944). This may indicate that DTH response is depressed during human pregnancy as a cause of exacerbated disease. Contact sensitivity to dinitrochlorobenzene (DNCB) in dairy calves was studied by Burton et al. (1989b) who demonstrated variability among calves. Paternal half-sib h^2 estimates of CS were moderate to high at 24 hours (h^2= .379±.316) and 48 hours

(h^2=.564±.369) following challenge, respectively. Variation among individuals was sufficient to respond to genetic selection if deemed beneficial (Burton et al., 1989b).

1.2.2.c. Cytokines

Cytokines are hormone-like proteins synthesized and secreted by cells of the immune system to facilitate cell activation, differentiation, and communication. In dairy cows, IL-2 plays a central role in development of specific immune responses by clonally expanding peripheral and mammary gland T cells and by establishing immune memory following mitogenic or antigenic stimulation (Sordillo, 1995). IL-2 in conjunction with other cytokines induces clonal expansion of B cells into antibody producing plasma cells and memory cells, and also promotes colony stimulating factors and IFN γ (Gillis, 1978). IL-2 expands lymphoid cell populations in the bovine mammary gland during involution and stimulates production of IgG_1 and IgG_2 in mammary tissue plasma cells (Nickerson et al. 1989). Bovine IL-2 activity is reduced one week prepartum and may be associated with increased susceptibility to mastitis (Sordillo et al., 1991).

Inteferon-gamma (IFN-γ) is another cytokine that enhances macrophage-mediated cytotoxicity against tumor cells, induces membrane bound Fc receptors for IgG on macrophages and stimulates synthesis and release of reactive oxygen metabolites from macrophages and PMNs. They appear to have an important role in regulation of acute phase responses and uncontrolled inflammatory reactions that may lead to pathological

alterations to host tissues. Postpartum dairy cows that were treated intramammarily with IFN-γ 24 hours before experimental *E.coli* challenge had fewer infected quarters and shorter durations of infection compared to placebo-treated cows. *In vitro* treatment of mammary gland PMNs with recombinant bovine IFN reversed the suppressive influence of periparturient mammary secretions on PMN function (Sordillo and Babiuk, 1991).

2.0 Relationships between peripartum immune response and peripartum metabolic and physical stress

Stress may compromise the immune function of animals in part by inducing high cortisol concentration, subnormal phagocytic function and depressed antibody response (Rogers, 1979). Stress increases adrenocorticotropic hormone secretion by the pituitary gland, and increases release of adrenal corticoids in the blood. During stress, animals have enhanced adrenal production and release of epinephrine and norepinephrin in the blood (Sconberg et al., 1993). In cattle, major stresses that increase blood cortisol concentration are castration, weaning, handling, dehorning, parturition, water deprivation, forced exercise, neonatal diarrhea, shipping, and certain conditions that cause pain (Roth, 1982). The peripartum period is a stress period unique to the dairy cow. Dairy cows go through repeated cycles of stress from the metabolic and physical demands of gestation, parturition and lactation. Many studies have demonstrated disease and impairment of innate and immune responsiveness at this time (Cai et al., 1988; Kehrli et al. 1989a&b; Gilbert et al., 1994; Kehrli et al., 1994; Detilleux et al., 1995; and Shuster et al., 1996).

Infectious diseases such as mastitis and retained placenta, as well as metabolic diseases such as milk fever, ketosis and displaced abomasum generally occur within the first two weeks of lactation. According to Goff and Horst (1997), three basic physiological functions must be maintained during the periparturient period if disease is to be avoided: 1) adaptation of the rumen to lactation diets that are high in energy density, 2) maintenance of normocalcemia and 3) maintenance of a responsive immune system. The reasons for impairment of innate and immune responsiveness may be a consequence of various nutritional and endocrine factors.

Variation in immune response profiles in the context of metabolic and physical stress may be associated with individual genetic regulation of the neuroendocrine-immune axis. Mason (1991), proposed that genetically different strains of mice and rats that vary in their immune response also vary in their stress response and consequently differ in resistance or susceptibility to disease. Lewis rats and C57/BL mice that had low stress response had a more vigorous CMIR compared to antibody-mediated immune response. These individuals were more susceptible to autoimmune disorders like experimental allergic encephalomyelitis (EAE) (MacPhee et al., 1989) and arthritis (Sternberg et al., 1989), and were resistant to pathogens requiring CMIR for their control such as *Leishmania*, *M. tuberculosis* and *M. leprae*. In highly stressed individuals, antibody-mediated immunity is proposed to be higher, and they were resistant to cell-mediated autoimmune disorders but were susceptible to pathogens requiring CMI responses for control. Accordingly, disease resistance or susceptibility in dairy cows may depend upon

metabolic and physical stresses encountered and their impact on regulation of the immune system. Individual genotypes may determine the magnitude of response to stress, resulting in either enhancement or depression of immune response and the possible culmination of disease, particularly during the peripartum period (Mallard et al., 1997b). Parturition is an acute stress, and the early postpartum period in high producing dairy cows is a time of chronic stress relating to the state of negative energy balance, when cows cannot consume enough nutrients to satisfy high volume milk production (Butler et al., 1981;Gwazdauskas et al., 1986).

2.1. Peripartum Nutrition

Parturition and the onset of lactation impose metabolic stress on the cow, causing acute deficiencies of nutrients that are necessary to support the immune system (Goff et al., 1990; Franklin et al., 1991). Towards the end of gestation, the demands for energy, protein and minerals increases, and following parturition these demands are even greater for the formation of colostrum and milk. At this time, it is not possible for the dairy cow to ingest enough feed to meet lactational demands for energy and protein. These high demands for energy and protein imposed by lactation result in a negative energy balance, and may be associated with impaired immune function (Goff and Horst, 1997). Specifically, milk fever and ketosis-fatty liver complex develop as a result of the rapid mobilization of calcium and fat respectively, from the body stores to the mammary gland. The accumulation of ketoacids in the blood during ketosis impairs lymphocyte function

(Franklin et al., 1991) and plasma concentrations of vitamin A and E decline at parturition (Goff et al., 1990). Diets that are high in energy and metabolic nutrients, such as calcium are given prepartum to ensure that dairy cows are better able to cope with the heavy metabolic and physiological demands at parturition. Supplementation of peripartum diets with vitamin A and E improves phagocytic cell and lymphocyte function, as well as Ig and IL-1β synthesis (Tjoelker et al., 1990; Daniel et al., 1991; Hogan et al., 1992; Stabel et al., 1992 and Hogan et al., 1993).

2.2. Peripartum endocrine factors

Changes in hormone concentrations during the peripartum period when physical and metabolic demands are increased, disease prevalence is high, and immune responsiveness is low, indicate that the endocrine system may play an integral role in development or prevention of disease (Goff and Horst, 1997). Hormones that have been investigated during this stress period include cortisol, growth hormone (GH), and insulin-like growth factor I (IGF-I). Cortisol is a steroid hormone secreted by the adrenal cortex and is the most potent of the naturally occurring glucocorticoids. Glucocorticoid release in response to stress is documented in many species (Blalock, 1994; Derijk and Sternberg, 1994). Cytokines released during inflammation can stimulate the eventual release of glucocorticoids, and may serve to control the magnitude of immune response (Vassilopoulou-Sellin, 1994; Spangelo and Gorospe, 1995). Growth hormone or somatotropin is a protein produced and released from the anterior pituitary that promotes

body growth, fat mobilization and inhibition of glucose utilization (Stedman, 1990). In cattle, GH is a galactopoietic hormone. The effect GH has on mobilisation of fat may explain the occurrence of ketosis-fatty liver complex during the peripartum period when GH concentrations are maximal (Goff and Horst, 1997). Injectable exogenous GH, recombinant bovine somatotropin (rbST), has been demonstrated to increase milk production (Bauman et al., 1985) and is currently approved for commercial use in the United States. Insulin-like growth factor-I or somatomedin, is a peptide synthesized in the liver. Its secretion and/or biological activity is dependent upon blood concentrations of GH (Burton et al., 1992; Davis et al., 1987). Insulin-like growth factor-I stimulates anabolic processes in bone and cartilage such as synthesis of DNA, RNA, and protein and the sulfation of mucopolysaccharides. Insulin-like growth factor-I mediates the effects of GH on the mammary gland by promoting galactopoiesis (Dehoff et al., 1988). Lymphocytes have receptors for GH and IGF-I, and can secrete GH and IGF-I. This demonstrates that immune and endocrine systems communicate bidirectionally. Chronic activation of the hypothalamic-pituitary-adrenal axis can suppress release of GH, which is immuno-enhancing (Franco et al., 1990; Burton et al., 1991). Reduction of GH may inhibit of IGF-I action on target lymphocytes. Glucocorticoids may also desensitize tissues to IGF-I, thereby diminishing its immunoregulatory effects. Due to the integration of these systems, investigation is currently focussed on the effect of stress and hormones on immunocompetence. Hoshino et al. (1991) reported an increase in GH concentration following parturition, in parallel with milk yield, that decreased as lactation progressed. Oda et al. (1989) detected a modest increase in GH concentration around

parturition but this was not enough to stimulate IGF- I release by the mammary tissues. Hoshino et al. (1991) also demonstrated a decline in serum IGF-I after parturition that remained low during early lactation and gradually increased from peak lactation until the end of lactation. Colostral GH was substantially lower than in the blood, and colostral IGF-I concentration on day one and two after parturition was higher than in plasma (Oda et al., 1989). Vega et al. (1991) attributed changes in IGF-I and GH to the decrease in metabolic demands associated with the cessation of milk production during late gestation. followed by an increase in metabolic demand associated with the onset of lactation at parturition. The demand of lactation may alter the transport of IGF-I by sequestering it from the blood. Lactogenic hormones (i.e. prolactin) prevent synthesis of IGF-I and IGF-I binding proteins during early lactation. Growth hormone concentration may enhance otherwise suppressed antibody response due to stress-released glucocorticoids (Franco et al., 1990). Basal and adrenocorticotropic hormone (ACTH) stimulated glucocorticoid concentrations one-two days postpartum, were significantly higher in cows with milk fever compared to cows without any complications following parturition (Nakao et al., 1990). Heat stressed heifers had a less pronounced decline in colostral Ig two weeks prior to parturition, but in the first four weeks of lactation mean concentrations of IgA and IgG were lower than prepartum concentrations. It may be possible to identify cows that are inherently more susceptible or resistant to mastitis through the examination of hormone concentrations and host resistance mechanisms during the peripartum period.

2.3 Milk Production

Concern has focussed on the over-emphasis of selection for enhanced milk production (Harmon et al., 1994). Because selection for high milk production has been associated with increased mastitis occurrence (Gröhn et al., 1995), the effect of production on host resistance has been evaluated. Cows selected for high milk production (pounds of milk and pounds of milk fat plus proteins) were reported to have significantly higher numbers of circulating PMN and mononuclear cells, have higher PMN resting chemiluminescence, and higher PMN directed migration than cows with average production (average pounds of milk, and pounds of milk fat plus proteins) (Detilleux et al., 1995). Significant genetic variability among daughters of sires with different production potentials was reported for total number of PMNs, PMN chemokinesis, respiratory burst assays (cytochrome C reduction, chemiluminescence, and iodination), serum concentrations of IgG_1, IgG_2, and IgM and serum hemolytic complement activity. Because selection for high milk yield did not produce unfavourable host resistance response and variation, results indicate that it may be possible to improve immunocompetence of periparturient cows and production concurrently through planned mating experiments. In contrast to the findings reported by Detilleux et al. (1995), a correlation between PMN phagocytosis in Canadian bulls and their daughters' protein production indicated a significant, negative relationship (Dürr et al., 1996). This may indicate that increasing production may compromise the ability of dairy cow PMNs to phagocytose pathogens. Because this negative relationship was only determined for PMN phagocytosis and not immune response traits, the potential to

simultaneously select cows for improved immunocompetence and production may still be possible. Relationships between production and antibody response were reported to be favourable in mice immunized with sheep red blood cells (SRBCs) and pigs immunized with hen egg white lysosyme (HEWL) (Biozzi et al., 1968; Mallard et al., 1992).

3.0. Potential for Selection to Enhance Immune Responsiveness and Disease Resistance in Dairy Cattle

Direct selection for disease resistance may be done either by selecting the most disease-resistant breeding stock under normal environmental conditions, or by challenging the breeding stock or relatives with specific pathogens (Hutt, 1959). Indirect selection is based on identification of reliable indirect markers of disease resistance (Detilleux et al., 1993). Phenotypic indicators include morphological markers (eg. eye margin pigmentation in bovine infectious keraconjunctivitis), physiological markers (eg. hemoglobin type in malaria), and innate or immune response traits (eg. PMN function, antibody response and CMI). Genotypic indicators include candidate genes (eg. major histocompatibility genes, immunoglobulin genes, and T cell receptor genes), and anonymous molecular genetic markers (eg. restriction fragment length polymorphisms, tandem repeats loci, and microsatellite loci) (Detilleux et al., 1993).

3.1 Phenotypic Markers

Experiments using immune response variation as selection criteria have been successful at directing response to be high or low (Biozzi et al., 1968; Ibanez et al., 1980; Siegel et al., 1980; Van der Zijpp et al., 1983; and Mallard et al., 1992). The continuous distribution of antibody response suggests multigenic control by several independently segregating loci (Stiffel et al., 1987; Puel and Mouton, 1996). The first selection experiment using antibody response following immunization was reported in guinea pigs assortatively mated for five generations. The immunogen used was diphtheria anatoxin and the immune responses of progeny were progressively modified in upward and downward directions (Shiebel, 1943). A similar experiment was conducted using rabbits selected for two generations based on antibody produced to *Streptococcus* sp. (Eichmann et al., 1971). A more extensive examination of antibody response variability in mice was demonstrated by Biozzi et al. (1979). Several independent selective matings were carried out with mice for antibody responsiveness to sheep red blood cells (SRBCs). SRBCs are multideterminant antigens that are strongly immunogenic in all strains of mice (Puel and Mouton, 1996). Assortative matings of mice with extreme phenotypes in upward or downward directions were repeated for successive generations until maximal divergence of the two lines was achieved (Biozzi et al., 1972). The relevance of this dichotomy pertains to the ability of mice to mount strong responses, either antibody or cell mediated either to extra- or intracellular organisms respectively. The low line (L line) was more resistant than the high line (H line) to intra-cellular organisms such as *Salmonellae*,

Yersinia, *Mycobacteria*, and *Brucellae*, when the macrophage provides the dominant defensive barrier. The H line was more resistant to extracellular microorganism including *Pneumococcus*, *Klebsiella*, *Plasmodia*, and *Trypanosoma*. The major genetic modification that explained differences between these selected lines was at the level of the macrophage. Antigen was observed to be slowly catabolized and persisted on the macrophage membrane of H line mice, whereas it was rapidly destroyed in L line macrophages. Selection of chickens based on antibody response to SRBC has also demonstrated variation and the consequent divergence of high and low lines of chickens (Siegel and Gross, 1980; Van der Zijpp et al., 1983; Pinard et al., 1992). Antibody response to SRBC and chicken erythrocytes was similarly evaluated in guinea pigs, and diverged to high and low immune response lines after successive selection for eight generations (Ibanez et al., 1980). Yorkshire pigs selected using estimated breeding values (EBVs) for both antibody and CMIR were reported to diverge into high and low immune response lines (Mallard et al., 1992). The maximum divergence of high and low responses were observed between generation 1(G_1) and 3 (G_3) with little or no response to selection after generation 4 (G_4) (Mallard et al., 1997).

Selective breeding of cattle to enhance resistance to mastitis using somatic cell count (SCC) is currently under evaluation. Current industry trends favour a low somatic cell count in milk secretions. A SCC that is too low may be detrimental to innate mechanisms of resistance to mastitis and therefore must be used with caution. Estimates of genetic correlation between SCC and mastitis vary, but values are mainly positive (r=

0.81; Madsen, 1989; r=0.3, Weller et al., 1996). SCC is now considered the primary trait used to evaluate susceptibility to mastitis and enables indirect selection for resistance to mastitis (Shook, 1994; Dekkers et al., 1998). Selection based on occurrence of clinical mastitis is unreliable because it is not routinely recorded, it has complex aetiology, and observations on the occurrence and severity of mastitis are usually subjectively evaluated by producers. Several records on SCC are available through dairy herd improvement corporations that provide a substantial database from which to determine estimated breeding values for SCC. SCC and its logarithmic transformation, somatic cell score (SCS), have higher heritability (h^2=ranging between 0.10-0.12) (Emmanuelson et al., 1988; Banos and Shook, 1990; Boettcher et al., 1992) than clinical mastitis (h^2=0.03) (Emmanuelson, 1988; Madsen, 1989). However, low heritability estimates of SCS, in contrast to some production traits, indicate that SCS is influenced to a greater degree by environmental factors. Low heritabilities suggest that SCS and mastitis will respond more slowly to genetic improvement than milk yield (Shook, 1993; Boettcher et al., 1992). Research conducted in Ontario by Dekkers and Burnside (1994) evaluating estimated transmitting abilitiies (ETAs) for SCS indicated that daughters of the poorest sires had double the average SCC (transformed from SCS) of daughters of the best sires and that sires whose daughters had a higher SCS tend to have more clinical mastitis problems. This research indicated that, although adding SCS to genetic selection will reduce genetic progress for production by <2 percent, it will also slow down the current genetic deterioration of resistance to mastitis which accompanies selection for increased production. Inclusion of SCS in the selection index would be beneficial because costs for

treatment of mastitis and other related expenses would decrease, increasing the revenue per cow per year by 0.3 to 1.0 percent, despite a slight decrease in milk sales. Although there is some benefit to using SCS as a selection tool, it is not as highly heritable as some indicators of immune response. Antibody response to ovalbumin (OVA) in dairy calves was reported by Burton et al. (1989) to be moderately heritable (h^2=0.48). In contrast to SCS, antibody response to OVA may be more promising as a selection tool for improved inherent disease resistance (Burton et al., 1989).

Dekkers et al. (1996a) recently developed a sire index called the total economic value index (TEV) that includes traits weighted according to their economic importance. Among these traits are production, herd life, and udder health. Production accounts for 64% of the TEV, herd life for 26%, and udder health, which includes SCS, accounts for 10%. Although production is the most economically important, some emphasis is now placed on resistance to mastitis by evaluating SCS. As soon as more highly heritable indicators of immune response are determined, additional information about udder health could be added to the TEV.

3.2 Genotypic Markers

Genotypic markers of selection that may be associated with resistance or susceptibility to disease include the major histocompatibility complex (MHC), immunoglobulin (Ig) genes, and T cell receptor (TcR) genes (Lewin, 1989). Selection based on genotypes that

are beneficial or deterimental to animal traits of interest is called marker assisted selection (MAS)(Schmutz et al., 1992; Soller, 1994). Genetic study of mastitis currently includes techniques developed in the field of molecular, quantitative and statistical genetics. Quantitative traits such as milk yield, immune response, and SCC are influenced by a large number of unknown genes. These may include a few genes with major effects. Knowledge of major genes can be useful for genetic improvement programs (Shook, 1989; Detilleux et al., 1994). Variation at the molecular level that allows for selection of various genotypes is called polymorphism. Genes suspected of involvement in the expression phenotype are called candidate genes. One example of major genes relating to disease are those within the MHC (Detilleux et al., 1994). These genes may, in part, contribute to development of mastitis because of their involvement in regulation of immune responses (Schukken et al., 1994; Mallard et al., 1995). Although no actual selection studies of dairy cattle have been conducted using candidate markers of immune response phenotype, several studies have reported associations of immune response genes such as MHC with immune response phenotype or disease (Lewin, 1989; Mallard et al., 1992; Dietz et al., 1997).

3.2.1 Associations between immune response genes and disease resistance or susceptibility

Genes that govern host resistance regulate both innate and acquired immune responses. Examples of innate host response genes are those that encode nonspecific opsonins,

receptors (such as CD18) and enzymes that are involved in involved in phagocytosis. Genes that control the specificity of immune response include MHC, Ig and TcR genes (Kelm et al., 1997). Genes have also been identified that govern specific responses to certain diseases. For instance, the Nramp gene, which is highly conserved among species, is thought to regulate host resistance to intracellular-pathogenic diseases such as brucellosis in cattle and salmonellosis in chickens (Malo et al., 1994). With respect to the occurrence of mastitis, much research has focussed on association with genes that reside in the MHC.

In cattle, MHC genes encode Bovine Leukocyte Antigens (BoLA) and are located on the short arm of bovine chromosome 23 (Fries et al., 1986). The MHC system is divided into three regions that contain genes encoding class I, class II and class III molecules. Class I molecules function as restriction elements for CD8+ cytotoxic T lymphocytes (CTL) and are important mediators of antiviral and antitumour effector responses. Class II molecules function as restriction elements for CD4+ helper T cells that largely mediate effector function through the release of cytokines. Class III genes encode complement. BoLA class II has three subregions (DQ, DR, and DY) with two DQA, two DQB, one DRA, and three DRB genes. Restriction Fragment Length Polymorphism (RFLP) analysis of the BoLA class II genes has revealed 30 DQ and 31 DRB RFLP-defined haplotypes (Sigurdardóttir et al. 1988). Of the three DRB genes, the third DRB gene (DRβ3) is functionally expressed and is highly polymorphic. Exon 2 of the DRβ3 locus encodes the binding cleft of the BoLA class II DRβ3 molecule expressed on antigen

presenting cells.

Cows expressing class I BoLA-A molecules such as CA42 on lymphocytes of dairy cows that were challenged with *S. aureus* had an increased susceptibility to *S. aureus* infection (Schukken et al., 1994). Another class I allele, w7, was also associated with an increased risk of infection (Lie, 1985).

Mejdell et al. (1994) studied 492 Norwegian bulls progeny tested for clinical mastitis and ketosis and typed for class I BoLA-A alleles. Bull progeny serotyped for the expression of allele A2 were associated with relative resistance to mastitis. Solbu et al. (1982) also detected an association between allele w2 and mastitis resistance. Association of BoLA class I haplotypes with resistance or susceptibility to mastitis (measured as the likelihood of isolating bacteria from milk) were further demonstrated by AArestrup et al. (1995). Allele A10(w50) was associated with the increased likelihood of isolating bacteria, while A19(A6) was associated with decreased likelihood of isolating major pathogenic bacteria and coagulase negative *staphylococci* (CNS). The A16 allele was associated with decreased likelihood, and the A31(A30) allele had an increased likelihood of isolating *S. aureus*. Solbu et al. (1982) also found an association between BoLA w16 and mastitis susceptibility.

Resistance to *S. aureus* mastitis was reported to be enhanced in dairy cows expressing the BoLA class II DR_B restriction fragment polymorphism (RFLP) (Schmutz et al.,1992).

Susceptibility to mastitis in Swedish red and white cattle was associated with the BoLA DQ^{1A} allele. Clinical mastitis was 8.0-8.3% more common in daughters of sires possessing the BoLA DQ^{1A} allele (Lundén et al., 1990). Allele BoLA-DRB3.2*23 was reported to be significantly associated with occurrence of severe mastitis (Sharif et al., 1997). Alternatively, other investigators have reported a beneficial effect of the BoLA-DRB3.2*23 allele, because it is associated with resistance to persistent lymphocytosis caused by bovine leukosis virus (BLV)(Xu et al., 1993; Sulimova et al., 1995; and Zanotti et al., 1996). If MHC is used as a selection tool to enhance disease resistance, it should be used with caution because selection of an allele that is associated with resistance to one disease may unfavourably result in increased susceptibility to another disease.

3.2.2. Associations between host resistance genes and host resistance response phenotypes of cattle

Genes that clearly associate with immune responsiveness and ultimately affect the outcome of disease could be important for development of a sire selection index that identifies cattle with enhanced immune responsiveness and improved inherent disease resistance.

Certain class I BoLA-A alleles have been associated with SCC (AArestrup et al., 1995) . Alleles A11 and A12 were associated with decreased SCC, while A21 and A26 were

associated with increased SCC. High antibody response to human serum albumin (HSA) in Norwegian Red bulls was associated with class I BoLA allele w16 and w6 (Lie, 1979; Lie et al., 1986). Low antibody response was associated with allele w2. Antibody response to the synthetic antigen (T,G)-A--L was highest for bulls that had w8 and w20 alleles. Half sisters of these bulls that also had high antibody to HSA, had the class I allele w16 and were susceptible to mastitis. In contrast, cows with low antibody to HSA were associated with allele w2 and were resistant to mastitis. (Solbu et al., 1982). Additional evidence of an association between w16 and mastitis susceptibility was reported by Larsen et al. (1985). Mastitis susceptibility was associated with the M allele of the M blood group that in turn, is highly correlated with the presence of w16 (Lewin, 1989). Class I BoLA-A alleles such as w15 (w8) and w16 were associated with greater circulating mononuclear cell and total leukocyte numbers, and alleles w27(w10), w11, and w20a were associated with lower numbers of these circulating cells (Weigel et al., 1991). Allele w16 was associated with greater antibody-independent PMN cytotoxicity, unstimulated lymphocyte proliferation, serum conglutinin activity and lowered dependent PMN cytotoxicity. Allele w19(w6) was associated with decreased conglutinin activity and decreased PMN iodinations. Increased antibody-dependent PMN cytotoxicity was observed for animals bearing allele w14(w8) and decreased PMN iodination, serum conglutinin and unstimulated lymphocyte blastogenesis were observed in individual carrying w20a or EU28D (Weigel et al., 1991).

Cows expressing the BoLA DRB3.2 genotype had increased serum IgM, increased

complement, and decreased mononuclear cell numbers (Dietz et al., 1997). Allele DRB3.2*16 was positively associated with a higher EBV for mastitis (Kelm et al.,1997). Allele DRB3.2*8 was positively associated with an increased EBVs for clinical mastitis, IgG_2^b and the CD18 allele. Alleles DRβ3.2*11, DRβ3.2*23 and IgG_2^a and the recessive bovine leukocyte adhesion deficiency (BLAD) allele were associated with decreased clinical mastitis (Kelm et al., 1997). A positive genetic association was found between allele DRβ3.2*24 and EBV for mastitis caused by a major pathogen and between allele DRβ3.2*3 and mastitis caused by a minor pathogen (Kelm et al., 1997). Variation in lymphocyte proliferative response to OVA was reported to be linked to bovine MHC class II immune response genes (Glass et al., 1990).

Numerous associations between MHC alleles and immune response or disease have been reported. However, it is not yet clear how these associations may be best utilized to improve the health of dairy cows. MHC genes are just a few of many genes regulating immune response. It may be more relevant to select directly based on immune response phenotype, as has been reported for pigs (Mallard et al., 1992; Wilkie et al., 1997).

GENERAL METHODS

Experimental Design

Identifying variation in immune response traits during the peripartum period, and any association with disease or production traits is the first step toward breeding dairy cows with superior health attributes. To evaluate phenotypic variation in peripartum antibody and cell-mediated immune responses of dairy cows, a total of 136 Holstein dairy animals (88 cows and 49 heifers) from 2 research herds (Herd 1, n=32, 6 heifers and 26 cows; Herd 2, n=67; 34 heifers and 33 cows) and 1 commercial herd (Herd 3, n=37, 8 heifers and 29 cows) were examined weekly from dry-off (approximately eight weeks prepartum; wk-8) to six weeks postpartum (wk 6) (Appendix III, Fig. 1). To stimulate production of antibody during the peripartum period, all cows and heifers received intramuscular (im) injections of a mastitis endotoxemia preventive vaccine, an Rc mutant of *Escherichia coli* O111:B4 (Rhône Mérieux *Escherichia coli* J5, Rhône Mérieux, Lenexa, KS) with the manufacturer's adjuvant. In addition, cows were simultaneously administered ovalbumin antigen (OVA, Type VII, Sigma Chemical Co., St. Louis, MO) approximately 8 weeks (4 mg) and 3 weeks (2 mg) prior to predicted calving dates. At parturition (wk 0), cows received an additional immunization of the OVA dissolved in phosphate buffered saline (PBS - 0.1 M, pH 7.4) (2 mg, im). Peripheral blood was sampled via tail venipuncture at weeks -8, -3, 0, 3, 6, and 9 relative to parturition, and centrifuged to monitor serum $IgG_{1\&2}$ concentration, and antibody to OVA and J5 *E. coli*. Colostrum and milk samples

were also collected to measure antibody to OVA and $IgG_{1\&2}$ concentration in whey. Colostrum was collected at the first milking following parturition. Milk samples were stripped from all quarters approximately 2-4 hr after morning milking. Colostrum and milk samples were stored frozen without preservative at -20°C until time of whey separation and Ig quantification.

To evaluate delayed type hypersensitivity (DTH) as a measure of cell-mediated immune (CMI) response a subset (n=36) of cows from research Herd 2 (Ponsonby Research Station, Elora, Ontario; n=15 cows and 21 heifers) were given a 1.5 mg/mL intradermal injection of the Bacillus Calmette Guerin (BCG; Connaught, Mississauga, Ontario) vaccine in the left caudal tail fold at wk 1 postpartum. At wk 3 postpartum, animals that received the BCG vaccine were given a 0.1 mL (250 US Tuberculin Units) intradermal injection of the purified protein derivative (PPD) of *Mycobacterium tuberculosis* and 0.1 mL of the control (PBS), in the right caudal tail fold. These sites were located proximally to one another, about 4 cm apart. Injection sites in the left and right caudal folds were located approximately the same distance from the base of the tail head (10 cm) and across from one another (Appendix III, Figure 2). Double skinfold thickness was measured at 48 and 72 hours using Harpenden Skin Calipers (John Bull, England).

As a measure of peripartum lymphocyte proliferation, lymphocytes were harvested from whole blood at weeks -3, 0, 3, and 6 relative to parturition and cultured with OVA antigen (5 μg/mL) and the T-cell mitogen concanavalin A (Con A; 5 μg/mL).

Production Data

Production data were obtained through monthly reports from the Ontario Dairy Herd Improvement Corporation (Ontario DHIC). All monthly milk samples were tested for SCC and compositional content (fat%, protein%) by the Central Milk Testing Laboratory, Guelph, Ontario. In addition, milk samples from cows in research Herd 1 (Shurgain Research Farm, Burford, Ontario; n=26 cows and 7 heifers) were tested weekly by Ontario DHI. Projected 305 day production parameters for milk, fat, and protein were used as measures to compare production between cows from the three herds investigated. Three hundred and five day (305-day) projections were calculated based on the last test day before the end of lactation and on at least 100 days in milk (DIM). This allowed comparisons between animals at different stages of lactation when the data was collected and between animals with varying lactation lengths.

Disease Data

Occurrence of infectious and metabolic diseases were investigated throughout the study period. All disease events were recorded by the herd manager. If an animal had two or more episodes of the same disease event, it was recorded as one event for the study period.

Antibody Quantification by Enzyme Linked Immunosorbent Assay (ELISA)

Antibody to OVA

Serum was separated from coagulated peripheral blood by centrifugation (700 *x g*, 15 min) and stored frozen (-20°C) until time of assay. Milk samples were centrifuged twice (11000 *x g*, 15 min) to separate fat from whey. Whey was stored frozen at -20°C. Antibody to OVA was detected by ELISA according to the procedure described by Burton, et al., 1993. Dynatech Immulon II flat bottom 96-well polystyrene plates (Fisher Scientific, Don Mills, Ont.) were coated with a 3.11 x 10^{-5} M solution of OVA (OVA, Type VII, Sigma Chemical Co., St. Louis MO) dissolved in carbonate-bicarbonate coating buffer (pH 9.6). Plates were incubated (4°C, 48h), then washed with PBS and .05% Tween 20 (Fisher Scientific, Don Mills, Ontario) wash buffer, (pH 7.4) using a EL403 plate washer (Biotek, Mandel Scientific, Guelph, Ontario). Plates were then blocked with a PBS-3% Tween 20 solution and incubated (rt, 1h). Plates were washed and diluted test sera (1/50 and 1/200) or milk whey (Neat, 1/10, 1/100 and 1/400) and controls were added using the quadrant system described by Wright (1987). After blocking, sera samples were added in duplicate, and milk whey samples were added in quadruplicate. Plates were incubated (rt, 2h). Subsequently, alkaline phosphatase conjugate rabbit anti-bovine IgG (whole molecule) (Sigma Chemical Co., St. Louis, MO) was dissolved in wash buffer, added to the plates and incubated (rt, 2h). P-Nitrophenyl Phosphate Disodium tablets (pNPP) (Sigma, St. Louis, MO) were dissolved in a 10% diethanolamine substrate buffer, (pH 9.8). Plates were washed with wash

buffer, pNPP was added to the plates and then incubated (rt, 30 min). Plates were read on a EL311 automatic ELISA plate reader (BIO-TEK Instruments, Highland Park, Vermont) and the optical density (OD) was recorded at 405 and 630 nanometres (nm) when the positive control reached OD≥.999. The 630 filter was used as a reference filter to correct for fingerprints and irregularities in the plastic of the plates. The mean of the number of replicates added to each plate was corrected to an OD = 1.0 by multiplying by the inverse of the mean of the positive controls. Corrected means of each dilution were then added together to give an additive OD value.

Negative and positive controls included a pooled sample of pre-immunization sera and a pooled sample of sera from cows 14 days post secondary immunization, respectively. Sera from 20 animals was tested by ELISA to determine antibody at 4 dilutions (1/50, 1/200, 1/800, and 1/3200). The dilutions 1/50 and 1/200 provided responses with minimal prozone that corresponded to anticipated antibody curve kinetics based on the immunization schedule, and allowed a clear differentiation between positive and negative controls. For a small subpopulation of cows these dilutions exhibited some prozone effects, the dilutions were added together to provide an index of antibody. Similarly, in order to determine the optimal sample dilutions that would be used to quantify antibody in milk whey, milk from two cows was serially diluted (neat, 1/2, 1/4...1/512) to determine the dilution that had minimal prozone, and allowed optimal differentiation of responses of positive and negative control sera. Acceptable dilutions included Neat, 1/10, 1/100 and 1/400. These dilutions were added together to give and index of whey

antibody.

Antibody to E.coli J5

Lyophilized *E.coli J5* (American Type Culture Collection, Rockville, Maryland, USA) was grown in 5mL Tryptic Soy Broth (TSB) for 2 days to obtain log phase growth. This culture was then transferred to a 1 L flask of sterile TSB and sealed aseptically. The culture was incubated (37° C, 12 hrs, 200 rpm) on an INNOVA platform shaker (New Brunswick Scientific, Edison, New Jersey). A 1mL sample of cells was diluted logarithmically and plated on blood agar to determine the colony forming unit count (cfu). The number of cfu was 1.13×10^9. Live cells were then pelleted by centrifugation (5000 g, 15 min). Cells were washed in PBS and pelleted by centrifugation 3 times (first wash, 5000 x g, 15 min; second and third washes, 7500 x g, 15 min) Cells were suspended in PBS at a final volume of 1 L. The culture was then heat-killed by boiling for 2 hours. The final preparation was diluted until an absorbance reading=1.0 at 540 nm was obtained. The *E.coli* J5 was stored frozen (-20°C) until time of assay.

Serum was separated from coagulated peripheral blood by centrifugation (700 *x g*, 15 min) and stored frozen (-20°C) until time of assay. According to the method described by Rhône-Mérieux Animal Health (Lenexa, KS: 1994 personal communication), heat-killed *Escherichia coli* strain J5 (ATCC, Rockville, MD) was coated at a concentration of 6.25×10^7 cfu per mL onto Dynatech Immulon II polystyrene 96 well flat bottom plates

overnight at 4°C. After washing with wash buffer (PBS plus .05% Tween 20), 1% gelatin was added to block non-specific binding and plates were incubated (rt, 1h). Plates were washed and four replicates of test serum (dilutions of 1/1000, 1/1500, 1/2000 and 1/2500) were added using a modified quadrant system (Wright, 1987). One column with PBS-.05%Tween 20 was used as a blank, one column of fetal calf serum (FCS, Bockneck Laboratories, Can Sera, Rexdale, Ontario, Canada) was used as a negative control and one column each of the negative and positive controls prepared from pooled pre- and post immunization sera were plated, respectively. Test sera were incubated (rt, 2h), and then the plates were washed with PBS-.05% Tween 20. Horseradish peroxidase conjugate goat anti-bovine IgG whole molecule in PBS (1/4000) (The Binding Site, Birmingham, England) was added and the plates were incubated (rt, 1h). After subsequent washing with PBS-.05% Tween 20, the substrate, 2,2'-azino-di-(4-ethyl-benzthiazoline sulphonate-6) (ABTS) was added and plates were incubated (rt, 30 min). Plates were then read on an EL311 automatic ELISA plate reader (BIO-TEK Instruments, Highland Park, Vermont) and the OD was recorded at 405 nm and 490 nm. The mean OD of the four sample replicates were corrected for each plate by multiplying by the inverse of the mean of the positive controls. Based on the immunization protocol and phenotypic observation of curve kinetics of all dilutions tested, the 1/1000 dilution consistently allowed for differentiation between positive and negative controls, exhibiting minimal prozone effect. Therefore 1/1000 was the dilution of choice for comparison between animals.

The same pooled positive sera used in the OVA ELISA was tested to ensure a differentiation between pre-immune negative sera and post secondary immunization sera. This positive control was determined to be suitable for this assay because an OD of 1.0 was reached at a dilution of 1/200 while the negative sera had an OD that was <0.100. Negative control sera in this assay was prepared by absorbing boiled whole cell *E.coli* J5 in pooled non-vaccinated sera. FCS was also used as a negative control.

Quantification of Immunoglobulin $G_{1\&2}$ by Radial Immunodiffusion (RID)

Quantification of $IgG_{1\&2}$ concentration in sera

Radial immunodiffusion (RID) was used according to a modified method described by Mallard et al. (1992) to determine the concentrations of $IgG_{1\&2}$ in serum at weeks 0, 3, and 6 relative to parturition. Immunodiffusion medium was prepared by dissolving 2% Seakem agarose (FMC Bioproducts, Mandel Scientific, Guelph, Canada) and 2% Polyethylene Glycol 8000 (Carbowax 8000, Fisher Scientific, Fairlawn, NJ) in PBS. Rabbit anti-bovine isotype specific $IgG_{1\&2}$ (VMRD, Pullman, WA) was suspended in the immunodiffusion agarose at a concentration of 33% (vol/vol) for IgG_1 and 30% (vol/vol) for IgG_2. Immunodiffusion medium was held in a liquid state and poured into 5 mL immunodiffusion plates. Agarose was allowed to solidify and then three rows of wells, 6 wells per row, were punched with a 3mm glass pipette tip. Standard concentrations of IgG_1 (1800 mg/100mL) and IgG_2 (1600 mg/100mL) as controls (VMRD, Pullman, WA) were diluted (neat, 1/2, 1/4, 1/8, 1/16, 1/32) and five microlitres of these standard serial

dilutions were added to the top row of each plate. Five μL of each test sample was added to the two bottom rows of each plate. Plates were incubated (rt, 20 h) in a humidified chamber. Afterwards, ring diameters were measured using a calibrated grid held over a fluorescent light source. Ring diameters from standards were used to make a standard curve for each plate determined by linear regression. By plotting ring diameter on the x axis and the log of the concentration (mg/100mL) on the y axis, the concentration of Ig could be determined.

Quantification of $IgG_{1\&2}$ concentration in whey

In order to determine Ig concentration in colostrum, immunodiffusion medium was prepared by dissolving 2% Seakem agarose (FMC Bioproducts, Mandel Scientific, Guelph, Canada) and 2% Polyethylene Glycol 4000 (Carbowax 3350, Fisher Scientific, Fairlawn, NJ) in PBS. Rabbit anti-bovine isotype specific $IgG_{1\&2}$ (VMRD, Pullman, WA) was suspended in the immunodiffusion agarose at a concentration of 33% (vol/vol) for IgG_1 and 30% (vol/vol) for IgG_2. The procedure for the preparation of RID medium and plates for colostral whey samples was essentially the same as described for sera except that polyethylene glycol 3350 was used instead of 8000 to improve ring clarity. Colostrum samples were centrifuged twice (11000 *x g*, 15 min) to separate fat from whey prior to plate application.

To determine Ig concentration in milk, immunodiffusion medium was prepared by

dissolving 2% Seakem agarose and 2% Polyethylene Glycol 4000 (Carbowax 3350, Fisher Scientific, Fairlawn, NJ) in PBS. Rabbit anti-bovine isotype specific IgG_1 (VMRD, Pullman, WA.) was suspended in the immunodiffusion agarose at a concentration of 12.5% (vol/vol). Milk samples were centrifuged twice (11000 *x g*, 15 min) to separate fat from whey. The procedure for the preparation of RID medium and plates for milk whey samples is essentially the same as that described for colostrum except that the concentration of goat-antibovine sera suspended in the immunodiffusion media was 33% for IgG_1 and 30 % for IgG_2. Whey from wk 3 was tested for both $IgG_{1\&2}$ subclasses. At wk 6 however, IgG_1 only was tested in whey because very low concentrations of IgG_2 exist in normal milk.

Examination of the Cell- Mediated Immune Response (CMIR)

Delayed Type Hypersensitivity

A preliminary study was conducted to determine if the Herd 2 was previously exposed to *Mycobacterium tuberculosis* or other cross reactive antigens from *Mycobacterium paratuberculosis*. Five cows and six heifers were injected intradermally with 0.1 cc of the PPD of *M. tuberculosis* (Connaught, Mississauga) and a control dose of 0.1 cc PBS (pH 7.4) in the right caudal tail fold located proximally to one another (approx. 4 cm apart) PPD was injected in a designated area above the PBS site (Appendix III, Fig. 2). Both injection sites were 10 cm from the base of the tail head. Prior to injection, injection sites were encircled with a coloured marker and a pre-test and pre-control

thickness measurement was taken in triplicate, using Harpenden skin calipers (John Bull, England). This measurement was identified as the time=zero hours measurement. After 24 and 48 hours, skin thickness measurements were taken to assess the percent increase in skin thickness of control and test sites. It was determined that the herd had not previously been exposed to the *M. tuberculosis* antigen because 95% of all the animals tested had very little or no increase in skin thickness at the injection sites (i.e a 0-7% increase in skin thickness) and that the BCG/PPD test system would be suitable to measure DTH responses in this herd.

Two animals from the Herd 2 were selected to determine the optimal time point following the injection of PPD that would yield a maximal response and ensure that actual DTH responses were induced. Animals were evaluated at 0, 6, 12, 24, 48 and 72 hours post PPD challenge. Measurements taken at 6 to 12 hours were used to ensure that the response to antigen was not characteristic of an antibody-mediated reaction. In cattle, the maximal response to PPD is normally observed around 72 hours (Radostits et al, 1990). Preliminary results indicated that the response was optimal at 48 hours, therefore both time points were evaluated for comparison between animals.

Prior to immunization using PPD, and a PBS control, a pre-test and pre-control (at time= 0 hours) skin thickness measurement was obtained in triplicate from each of the 36 animals evaluated. Forty eight and 72 hours after secondary challenge, these measurements were taken again. The amount of skin thickness increase at 48 and 72

hours expressed as a percent increase in skin thickness was calculated as follows:

% increase in skin thickness = (((A-B)/B)-(C-D)/D)))X100

where A=mean test thickness (at time=48, 72 hours),

B=mean of pre-test thickness (at time=0 hours),

C=mean of control thickness (at time=48, 72 hours),

D=mean of pre-control thickness (at time=0 hours).

Cows could be classified according to their percent increase in skin thickness as either non-responsive or low responders (less than one sd below the mean), moderate responders (between one sd below and one sd above the mean), or high responders (more than one sd above the mean).

Lymphocyte Proliferative Response

Lymphocyte proliferation assays were performed according to the procedure of Chang, et al. (1993). Peripheral whole blood was centrifuged (850 *x g*, 15 min) and buffy coats were diluted in phosphate buffered saline (PBS 0.1M, pH 7.4). Peripheral blood lymphocytes (PBL) were separated by density gradient centrifugation (1000 *x g*, 30 min) of buffy coats using aqueous Histopaque 1.077 (Sigma Chemical Co. St. Louis, MO.) Cell pellets were washed by centrifugation in PBS (400 *x g*, 7 min) and suspended in a volume of culture medium (Rosewell Park Memorial Institute; RPMI- 1640, and 100 I.U. penicillin-streptomycin, prepared by Central Media Laboratory; Ontario Veterinary

College, University of Guelph, Guelph, Ontario.) and 10% FCS and brought to a final concentration of 2.0 x 10^6 cells/mL in culture medium. To determine specific clonal proliferative responses to antigen, a stock solution (50 µg/mL) of OVA (Sigma Chemical Co., St. Louis MO) dissolved in RPMI - 1640 was prepared and stored in small aliquots at -70°C. Five µg/mL of OVA was added to 6 replicates of test lymphocytes in 96 well flat-bottom plates (Nunc, Fisher Scientific, Don Mills, Ontario). Medium was added to 6 well replicates of cells as non-stimulated controls and this represented background or unstimulated cell proliferation. As a general indicator of lymphocyte proliferation, Con A mitogen similarly prepared from stock solution (50 µg/mL) and diluted (5 µg/mL) was added to 6 replicates of cells on a separate plate containing an additional 6 wells as medium controls. Following 24 h of incubation with OVA or Con A(37°C, 6% CO_2) cells received an 18 h 'pulse' incubation with 0.5 µCi methyl tritiated thymidine per well (ICN Biochemical, Canada Ltd. Montreal, PQ). Plates were frozen until cells were harvested using a plate harvesting system (LKB Wallac, Turku, Finland) onto fiberglass filter mats (LKB Wallac, Turku, Finland). Radioactivity was recorded as counts per minute (cpm) of test minus non-stimulated controls of retained radioactivity measured by a beta plate liquid scintillation counter (LKB Wallac,Turku, Finland). OVA antigen preparations were tested using the above described method at a concentration of 5 µg/mL, 10 µg/mL, and 20 µg/mL. Although lymphocyte proliferative responses did not differ significantly between the tested concentrations, 5 µg/mL was selected to induce PBL proliferation in subsequent assays. To determine the concentration of the mitogen able to induce optimal PBL proliferation, Con A concentrations were tested at 2µg/mL, 5µg/mL

and 10μg/mL. Five μg/mL yielded maximal lymphocyte proliferative responses and was therefore selected as the concentration applied in further investigations.

Flow Cytometric Assay for the detection of CD Surface Molecules on Lymphocytes either not stimulated or stimulated with Con A or OVA

To determine which lymphocyte subsets were present after stimulation with either Con A or OVA, cells were stained with monoclonal antibodies recognizing 5 cell surface markers according to the method described by Van Kampen and Mallard (1997). The monoclonal antibodies used in this study were kindly provided by Dr. Jan Naessens of ILRAD (Institute for Animal Health, Compton, Berkshire) and included antibodies to the following cell surface markers: CD2+ (IL-A43), CD4+ (IL-A11), CD8+ (IL-A105), WCI (IL-A29), and IgM (IL-A30). A subset of animals (n=10) from research Herd 2 (Ponsonby, Elora, Ontario; n=7) and the commercial herd (Speedvalley Holsteins, Fergus, Ontario; n=3) were evaluated for expression of these lymphocyte cell surface markers at weeks -3, 0, 3, and 6 relative to parturition. Lymphocytes were prepared and cultured as previously described for lymphocyte proliferation assays, however, each 96 well plate was divided into quadrants each with 24 wells. Twenty four replicates each of Con A stimulated, OVA stimulated (at 5μg/mL and 20μg/mL) and non-stimulated controls were cultured for 42 hours (the same total duration used in the lymphocyte proliferation assays). After 42 hours, cells were harvested by pipette, washed with PBS and transferred to 10 mL glass test tubes. Cells were centrifuged (400 x *g*, 10 min), and

supernatants were poured off and cells were resuspended in 250 μL PBS + 0.1M Azide. Immunostaining was performed in 96-well round-bottom plates (Corning, New York, NY). Fifty μL of cells and 50 μL of diluted primary antibody were added to each well and incubated (20 min, rt). After incubation, 100 μL of PBS + 0.1M sodium azide (Fisher Scientific, Fairlawn, NJ) was added to each well to wash the cells. Cells were suspended by mixing on a shaker and centrifuged (400 *x g*, 6 min). Supernatants were then removed using an aspirator. This washing procedure was performed twice. Fifty μL of FITC-conjugated goat anti-mouse IgG(H+L) (Cedarlane Laboratories. Hornby. Ontario) was then added to the cells and cells were incubated (rt, 20 min). After incubation, plates were washed twice as described above. Cells were fixed in 1% paraformaldehyde and transferred into 3 mL polystyrene tubes (Becton Dickinson. Lincoln Park, NJ) containing 300 μL of 1% paraformaldehyde. Tubes were covered with parafilm and refrigerated until time of assay.

A FACS Scan flow cytometer (Becton Dickinson. Lincoln Park, NJ) was used to acquire all lymphocyte subset data. LYSIS II software (Becton Dickinson. Lincoln Park. NJ) was used for fluorescence data analyses. Lymphocytes were gated from other populations based on their forward and side scatter characteristics. Five FITC histograms were plotted for each cow. time point and culture condition observed. Histograms representing fluorescence of cells expressing CD2 (pan T cell), CD4 (helper T cells), CD8 (cytotoxic/suppressor T cells), WCI ($\gamma\delta$ T cells), and IgM (B cells) cell surface markers were examined. The region of background fluorescence was established with the

negative control marker, M1. Everything to the right of this marker was considered positive (Appendix III, Fig. 3).

Complete Blood Cell Counts

Complete Differential Blood Cell Counts were determined by the Clinical Pathology Laboratory at the Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada. Counts included the percent and number erythrocytes, banded neutrophils, segmented neutrophils, lymphocytes, monocytes, basophils, eosinophils, as well as total leukocytes.

Somatic Cell Counts in Milk

Weekly somatic cell counts (SCC) of the Shurgain herd were obtained using the weekly sampling service offered by Ontario DHI. Weekly samples of cows in the Ponsonby and Dunk herds sampled 1-4 hours after morning milking were tested for SCC by the Mastitis Laboratory at the Ontario Veterinary College, University of Guelph, Guelph, Ontario Canada.. Monthly somatic cell counts were obtained from Ontario DHI records for all three herds.

Classification of Cows Based on Antibody

Biological Classification Using Antibody Curves

Serum antibody to OVA from the first herd investigated were graphed individually for each cow at weeks -8, -3, 0, 3, and 6 to examine antibody profiles. Evaluation of these curves during the peripartum period through to peak lactation indicated that enough variation existed to rank animals according to antibody to OVA. Cows that showed consistently above average antibody to OVA were categorized as high or Group 1. Cows that had average antibody up until parturition and thereafter showed a reduction in antibody following tertiary immunization were classified into Group 2. Cows that had average antibody until three weeks prepartum and showed a progressive reduction in antibody following secondary immunization were classified into Group 3.

Subsequent investigations of immune responses between cows in the other herds studied revealed similarities. However, subtle differences in the amplitude and direction of antibody curves, in relation to the immunization schedule, indicated that the data was continuous in nature. Thus, the groups determined for Herd 1 wouldn't necessarily apply to all herds. It was clear then, that individual profiles of antibody to OVA were on a continuum, and any classification method implemented would benefit from a quantitative approach to readily and appropriately partition phenotypic variation between cows.

Serum antibody to OVA was evaluated over time intervals, rather than discrete points in time. Individual animal antibody curves from week -8 to week 6 relative to parturition (week 0) were dissected into components reflecting the response to antigen following immunizations. The primary component was defined as the change in antibody to OVA from week -8 to week -3 relative to parturition following primary immunization at week -8 (Primary= OD value at week-3 minus OD value at week -8). The secondary component was defined as the change in antibody to OVA from week -3 to parturition following secondary immunization at week -3 (Secondary= OD value at week 0 minus OD value at week -3). The tertiary component was defined as the change in antibody to OVA from parturition to week 3 following tertiary immunization at parturition (Tertiary=OD at week 3 minus OD at week 0). The quaternary component was defined as the change in antibody to OVA from week 3 to week 6 (Quaternary= OD value at week 6 minus OD value at week 3). The quaternary component was included to observe the change in antibody between the end of the immediate postpartum period (wk 3) and peak lactation. These components were added together to give a total index of antibody to OVA between wk -8 and wk +6 relative to parturition as follows:

y_{total}= primary + secondary + tertiary + quaternary

where,

y= total antibody;

primary, secondary, tertiary, and quaternary components are as previously defined; primary, secondary, tertiary, and quaternary components when positive, have an equal weight of 1.

Animals that exhibited a reduction in antibody following secondary or tertiary immunizations were weighted with a coefficient of 1.5. Only secondary and tertiary components were weighted in this manner, because this is the period when lowered host resistance mechanisms are thought to contribute to increased occurrence of disease. The coefficients for weighting negative secondary and tertiary components were optimized using the original biological assessment for grouping animals. Several iterations were run to determine a coefficient that reflected the biological assessment of grouping animals based on the magnitude and direction of response to immunization.

The mean of y_{total} was determined and animals that exceeded one standard deviation above the mean were classified into the high antibody group (Group 1; n=18). Animals that were one standard deviation below the mean were classified into the low antibody group (Group 3; n=23). Animals with a y_{total} that ranged between one standard deviation below and above the mean were classified into the average antibody group (Group 2; n=95).

Statistical Methods

Least squares analysis of variance (ANOVA) and corrected means (least square means, LS Means) were generated using the General Linear Models (GLM) Procedure of the Statistical Analysis System (Helwig and Council, 1982). A model was constructed for the following dependent variables: antibody to OVA in sera and whey, antibody to *E.coli* in sera, concentration of $IgG_{1\&2}$ in serum and whey, background lymphocyte proliferation and lymphocyte proliferation following culture with Con A or OVA, DTH, SCS and production variables. Sources of variation included in the model for each dependent variable are summarized in Table 1. Data that did not show a normal distribution, as indicated by the univariate procedure of SAS (Helwig and Council, 1982), were transformed to natural logarithms. The Proc CORR procedure of SAS was used to generate Pearson product moment correlation coefficients between immune response parameters and production variables. Results were considered to be statistically significant if the p-value was ≤ 0.05 and trends were reported at a p-value ≤ 0.10.

Models indicated are base models. Some parameters were excluded if non-significant in order to generate LS Means.

Model 1: **Antibody to OVA in serum and whey, Ig in serum and whey and *E. coli* in serum**

$$y_{ijklmn} = \mu + herd_i + season\text{-}yr_j + cow(group*parity)_{klm}$$

$$+ week_n + group_k + parity_l + (group*parity)_{kl} + (group*week)_{kn} + e_{ijklmno}$$

where, $y_{ijklmno}$ = observed response of cow m in group k and parity l for each sample week of each cow,

μ = the population mean,

$herd_i$ = fixed effect of herd (i=1,2,3),

$season\text{-}yr_j$ = fixed season-year effect (j= Spring 1994, Summer 1994, Fall 1994, Winter 1994/1995, Spring 1995, Summer 1995, Fall 1995, Winter 1995/96),

$group_k$ = fixed effect of group based on antibody to OVA (k=1,2,3),

$parity_l$ = fixed effect of parity (l=1,2, or >3),

$(group*parity)_{kl}$ = fixed effect of group*parity interaction,

$cow(group*parity)_{klm}$ = random effect of cow-grouped within group*parity term,

$week_n$ = fixed effect of sample week (n= -8,-3, 0, 3, 6, 9),

$(group*week)_{kn}$ = fixed effect of group by week interaction term;

$e_{ijklmno}$ = random or residual error term.

When parity was not significant, the cow term was edited to reflect the appropriate nested variable.

Model 2 **Cell Mediated Immune Responses and Lymphocyte Proliferation**

$$y_{ijklmnop} = \mu + herd_i + season\text{-}yr_j + cow(group*parity)_{klm} + week_n + group_k + parity_l + (group*parity)_{kl} + (group*week)_{kn} + replicate_o + b(cov)_{ijklmno} + e_{ijklmnop}$$

where all variables are as described for model 1 except.

$y_{ijklmnop}$ = observed response of cow m in group k and parity l for each replicate o at each sample week .

$replicate_o$ = fixed effect of replicate (o=1,2,3,4,5,6).

and $b(cov)_{ijklmno}$ = regression coefficient of $y_{ijklmnop}$ on resting cell proliferation for the klm^{th} cow

The model for DTH was:

$$y_{ij} = \mu + group_i + e_{ij};$$

where. μ= the population mean.

$group_i$ = fixed effect of group based on antibody to OVA (i=1,2,3),

e_{ij} = random or residual error term.

Parity was not included in this model because it was not significant, and when included with group, did not provide enough degrees of freedom to run the analysis of variance.

Tests of hypothesis of group or parity were tested against the MS random error term for

cow. Type III Sums of Squares corrected for all other variables within the model were used account for the variation in immune responses.

CHAPTER I

Periparturient Antibody Profiles of Holstein Cows: An Initial Immunobiological Assessment

Abstract

To evaluate phenotypic variation in peripartum immune responsiveness of dairy cattle, 33 Holstein cows and heifers were immunized with ovalbumin (OVA) and *Escherichia coli* J5 at weeks -8 and -3 prior to parturition. At parturition (week 0), cows received an additional immunization of OVA. Blood was collected at weeks -8, -3, 0, 3 and 6 relative to parturition to measure serum immunoglobulin (Ig) concentration, and antibody to OVA and *E.coli*. Colostrum and milk were also collected postpartum to measure Ig and antibody to OVA. All animals had measurable antibody to OVA following primary immunization, but some cows had a reduction in antibody following second and/or third immunizations. Antibody to OVA was used to classify animals into three groups recognizing animals with sustained antibody to OVA before and after parturition (Group 1), animals that had a reduction in antibody following immunization at parturition (Group 2), and animals that had a reduction in antibody following immunizations at week -3 and at parturition (Group 3).

Keywords: bovine, peripartum period, disease resistance, antibody

1.0 Introduction

During the peripartum period, depression of neutrophil number and function (Kehrli et al., 1989b; Gilbert et al., 1993; Cai et al., 1994; Detilleux et al., 1995), lymphocyte proliferation (Ishikawa, 1985; Saad et al., 1989; and Kehrli et al., 1989a), complement (Detilleux et al., 1995), conglutinin concentration (Detilleux et al., 1995) and serum IgG_1 concentration (Ishikawa, 1987; and Detilleux et al., 1995) have been observed. However, variation in antibody response during the peripartum period has not been systematically evaluated. Alterations in host response during the peripartum period may be related to the physiological changes that occur as a result of gestation, parturition, and early lactation. Alteration in host response may associate with resistance or susceptibility to disease. The objectives of this study were threefold: 1) to investigate antibody response during the peripartum period; 2) to classify animals based on variation of antibody; and, 3) to determine if antibody was associated with the occurrence of disease.

2.0 Materials and Methods

2.1. Animals and Treatments

Thirty three Holstein cows and heifers were examined from approximately eight weeks prepartum (week -8), based on predicted calving dates to six weeks postpartum (week 6). Twenty-six animals were multiparous cows and seven were primiparous heifers. Cows

received an intramuscular (im) injection of a mastitis endotoxemia preventive vaccine with the manufacturer's adjuvant (Rhône Mérieux *E. coli* J5, Rhône Mérieux, Lenexa, KS) along with the antigen OVA (Type VII, Sigma Chemical Co., St. Louis, MO), at weeks -8 (4 mg OVA) and -3 (2 mg OVA). At parturition (week 0), animals received an additional immunization of OVA without adjuvant dissolved in phosphate buffered saline (PBS - 0.1 M, pH 7.4) (2 mg, im). Ovalbumin was chosen as an inert soluble antigen to which these animals had likely not been previously exposed. *E. coli* J5 was used as a complex, insoluble, biologically relevant antigen to which most dairy cows were likely to have been previously exposed. Profiles of antibody to OVA were used to classify animals into three groups recognizing animals with sustained measurable antibody before and after parturition (Group 1), animals that had a reduction in antibody following immunization at parturition (Group 2), and animals that had a reduction in antibody following immunizations at week -3 and at parturition (Group 3)(Fig. 1A).

2.2. Blood and Milk Sampling Schedule

Blood was collected by tail venipuncture at week -8, and weekly from weeks -3 to 6 relative to parturition. Serum was used to monitor immunoglobulin $G_{1\&2}$ concentrations, and determine antibody to OVA and *E. coli* J5. Colostrum and milk were collected to determine antibody to OVA and to monitor IgG_1 (weeks 0, 3, 6) and IgG_2 (weeks 0 and 3) concentration. Colostrum was collected at the first milking following parturition. Milk was obtained from all quarters approximately 2-4 hr after morning milking. Colostrum and milk

samples were frozen without preservative at -20°C until the time of whey separation and analysis.

2.3. Anti-OVA Enzyme Linked Immunosorbent assay (ELISA)

As described in the General Methods section, page 42.

2.4. Anti-E. coli J5 ELISA

As described in the General Methods section, page 44.

2.5. Radial Immunodiffusion Assay

As described in the General Methods section, page 46.

2.6. Disease Occurrence

As described in the General Methods section, page 41.

2.7. Milk Somatic Cell Count

Milk (AM/PM composite sample) was collected weekly by the herd milker during milking

to determine somatic cell count (SCC). Only SCC that coincided with the day of blood sample collection for each week were reported. SCC, an indicator of subclinical mastitis, was transformed to somatic cell score (SCS) for analysis. SCS is the natural logarithm of SCC in cells/μL and is calculated as follows (Shook, 1993):

$$SCS=[\log_e(SCC/100) \div \log_e(2)] + 3$$

2.8. Statistical Methods

Type III least squares analysis of variance (ANOVA) and corrected means (least square means, LS Means) were generated using the General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS; Helwig and Council, 1982). The statistical models used included fixed effects of antibody groups (1,2,3), cow nested within antibody group, and week relative to parturition (weeks -8, -3, 0, 3, and 6). In preliminary analysis, the effect of parity was not significant and was therefore removed from all subsequent models. A model was constructed for the following dependent variables: antibody to OVA in sera and whey, antibody to *E. coli* J5 in sera, and the concentration of $IgG_{1\&2}$ in serum and whey. Sources of variation included in the model for each dependent variable are summarized in Table 1. Data that were not normally distributed, as indicated by the univariate procedure of SAS, were transformed to natural logarithms (whey antibody to OVA, serum antibody to *E. coli*, serum and whey IgG_2). Pearson product moment correlation coefficients between immune response variables were generated using the correlations procedure of SAS (Proc

CORR). Results were considered to be statistically significant if the P-value was ≤.05 and trends were reported at P-values ≤.10.

3.0. Results

3.1. Antibody to OVA

3.1.1. Antibody in serum

Serum antibody to OVA varied significantly over the peripartum period and individuals could readily be classified into three antibody groups: the high antibody group (Group 1, n=12; 6 heifers, 6 cows) versus animals that exhibited a reduction in antibody to OVA following immunization either postpartum (Group 2, n=12 cows) or pre- and postpartum (Group 3, n=9; 8 cows, 1 heifer). Approximately 1/3 (Group 1) of the animals had consistently above average serum antibody to OVA following immunization at weeks -8, -3, and 0 relative to parturition. The remaining animals had OD values measuring antibody to OVA that were close to the population mean or lower than the population mean and did not respond following immunization at week -3 or parturition (Fig. 1A). All cows, including those of Group 3, had serum antibody greater than background (week -8) at week -3 and therefore were considered low responders rather than non-responders. The statistical model (ANOVA) accounted for 94.19% of the total variation in serum antibody to OVA over the peripartum period. Effects of cow (P≤.0001), antibody group (P≤.0001), week (P≤.0001), and the interaction between antibody group and week (P≤.0001), contributed significantly

to the variation in serum antibody to OVA (Table 1).

3.1.1. Antibody in Whey

Cow ($P \leq .0001$), week ($P \leq .0001$), and antibody group ($P \leq .0001$) contributed significantly to the variation in antibody in whey (Table 1). There was also a tendency for the interaction between antibody group and week ($P \leq .09$) to account for variation in whey antibody to OVA. Population LS Means of whey antibody to OVA declined significantly following parturition, such that at week 0 the OD value was 1.456 compared to 0.645 ($P \leq .004$) at week 3 and $0.366 \pm .20$ ($P \leq .0001$) at week 6 (Fig. 1B). At weeks 3 and 6, Group 1 animals were significantly ($P \leq .05$) higher than Group 3 cows.

3.2. Antibody to E. coli J5

Cow ($P \leq .0001$), week ($P \leq .0001$), and antibody group (.0001) all contributed significantly to variation in antibody to *E. coli* J5. OD values of pre-immunization sera (week -8) indicated that these animals had minimal measurable *E. coli* J5 antibody (population mean of OD = .296; n=33) compared to post-vaccination antibody at week -3 (.739) and week 0 (.789). Antibody to *E. coli* J5 was positively correlated with antibody to OVA (r^2=.59, $P \leq .0001$).

3.3. IgG_1 & IgG_2 in serum, colostrum, and milk

Antibody group significantly contributed to the variation of serum IgG_2 ($P \leq .0001$) only. Group 3 animals had a significantly ($P \leq 0.05$) higher serum IgG_2 concentration than Groups 1 and 2 at parturition. Antibody to OVA was negatively and significantly correlated with serum IgG_2 ($r=-0.23$; $P \leq 0.05$).

3.4. Disease Occurrence

Of the 33 animals evaluated, 54.5% were considered healthy during this study. Of the diseased animals, seven animals had mastitis (21.21%), seven had ketosis (21.21%) and three animals had other diseases (9.09%). Animals in Group 1 that had above average antibody to OVA, had the lowest percent occurrence of disease overall (17%) and actually had no clinical mastitis (Fig. 2). According to a t test of significance, disease occurrence overall was not significantly different between antibody groups, but mastitis occurrence for Group 1 animals was significantly ($P<0.05$) lower than for Group 3 animals.

3.5. Somatic Cell Score (SCS)

At parturition, LS Means of SCS was lowest for Group 2 animals (SCS=3.2) compared to Group 1 (SCS=4.36) and Group 3 (SCS=4.98) cows. At weeks 2,3,4, and 6 after

parturition, all groups differed significantly from one another, and, Group 1 animals consistently had the lowest SCS while Group 3 animals consistently had the highest SCS.

4.0. Discussion

Host defenses have been documented to be impaired at parturition in many species, including the dairy cow (Weinberg, 1984; Clarke and Kendall, 1994; Detilleux et al. 1994; Detilleux et al., 1995). Further, it has been suggested that normal physiological conditions, specifically hormone fluctuations that occurr concurrently with the progression of pregnancy and in response to the stress of parturition, may contribute to lower peripartum immune and innate host-resistance mechanisms. Previous investigations have reported suboptimal polymorphonuclear (PMN) cell function, serum Ig concentrations, and mitogen-driven lymphocyte proliferation during the peripartum period in dairy cows. Antibody before and after parturition has not been investigated.

It is unlikely that these animals had been previously exposed to OVA. Thus, antibody to OVA was utilized to partition animals into three antibody groups: animals with sustained antibody to OVA before and after parturition (Group 1), animals that had a reduction in antibody following immunization at parturition (Group 2), and animals that had a reduction in antibody following immunizations pre-and postpartum (Group 3). Variation in antibody to *E. coli* J5, a biologically relevant antigen, was more difficult to partition. Pre-immunization *E. coli* antibody was significantly lower compared to post

immunization antibody in this herd. This indicates that the *E. coli* J5 antigen would be useful for classifying animals in the herd evaluated according to their antibody but does not indicate that another herd will respond in the same way. Pre-immunization antibody may be higher in other herds where Gram negative bacteria are frequently encountered.

Nagahata et al. (1992), examined B lymphocyte populations in order to evaluate host defense in dairy cows during the periparturient period. This study found no significant changes in the number of B lymphocytes of cows from two weeks before until two weeks after parturition. However, they did report a significant decrease in antibody producing cells immediately after parturition. The authors suggested this indicated a decrease in B lymphocyte function during the immediate postpartum period. This is consistent with the low peripartum antibody seen in some animals in the present study.

Although it has been reported that serum antibodies decline at parturition and colostral antibodies increase due to the sequestration of immunoglobulins into the mammary gland (Detilleux et al., 1995), this study suggests that lower antibody in serum does not necessarily relate to Ig transport. For instance, Group 1 cows, that had the highest serum antibody, also tended to have higher whey antibodies to OVA postpartum, when compared to animals of Groups 2 and 3. Initially, it was questioned whether low serum antibody may be associated with higher antibody in the colostrum or milk. This data indicates that animals with high serum antibody also supply high concentrations of antibody to the mammary gland.

Although more study is required to evaluate the consistency and breadth of antibody before and after parturition in subsequent lactations, this study has demonstrated significant individual variation during the peripartum period, and confirms that not all cows have depressed antibody. In swine, animals with inherently high and low immune response phenotypes can also be identified in a population (Mallard et al., 1992). In light of previously reported heritability (h^2) estimates of bovine antibody response (Burton et al., 1989), data from this study may suggest that Group 1 animals could be inherently better able to produce antibody, in spite of the metabolic and physical demands of the peripartum period. Estimates of h^2 of peripartum antibody have not been calculated, as does h^2 of response to other test antigens and pathogens during the peripartum period. However, animals with high antibody to OVA did have the lowest occurrence of peripartum disease, particularly mastitis (0% occurrence), and significantly lower SCS scores following parturition than animals that had a reduction in antibody to OVA following secondary and tertiary immunizations. Thus, antibody should be evaluated further as a potential marker of peripartum disease resistance.

Table 1. Analysis of variance of antibody response to ovalbumin (OVA) and *E. coli* J5, and the concentration of immunoglobulin $G_{1\&2}$ in serum and whey

			Source of Variation			
Dependent Variable	R^{2a} (%)	C.V.[b] (%)	Cow (Group)[c]	Week	Group[d]	Group* Week
Antibody Response						
Serum anti-OVA	88.31	21.66	.0001	.0001	.0001	.0001
Whey anti-OVA	74.97	-127.86[e]	.0001	.0001	.0001	.09
Serum anti-*E. coli*	78.29	-60.42	.0001	.0001	.0001	ns[f]
Immunoglobulin concentration						
Serum IgG_1	64.91	6.97	ns	.0001	ns	ns
Serum IgG_2	67.19	4.22	ns	.0001	.0001	0.004
Whey IgG_1	87.16	18.92	ns	.0001	ns	ns
Whey IgG_2	95.11	14.15	ns	.0001	ns	ns

a R^2 = coefficient of determination
b C.V. = coefficient of variation
c Cow(Group) = Cow nested within group
d Group= variation due to antibody group of animals classified with high, average, or low antibody to OVA
e negative C.V. are from log-transformed data
f ns= not significant

Fig. 1. LS Means of antibody to OVA in A) serum and B) whey by antibody group following immunization at weeks -8, -3, and 0 as measured by enzyme linked immunosorbent assay (ELISA). Group 1 = high antibody ; Group 2 = reduction in antibody following immunization postpartum (week 0); Group 3 = reduction in antibody following secondary and tertiary immunizations pre- and postpartum; PM = population mean. Animal classification is based on serum antibody to OVA. Significant differences between animals in the three groups are indicated by different letters above error bars ($P \leq .05$).

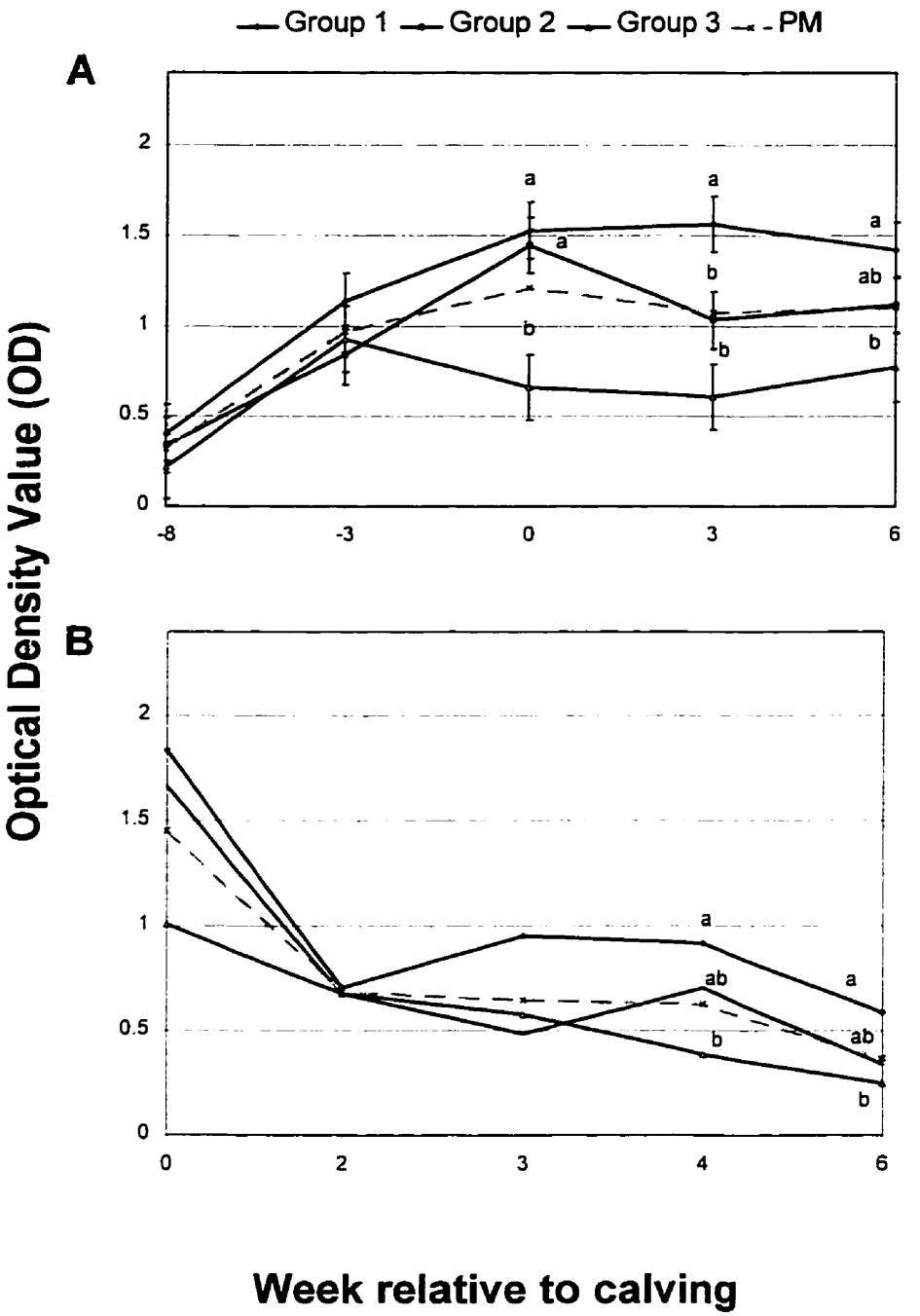
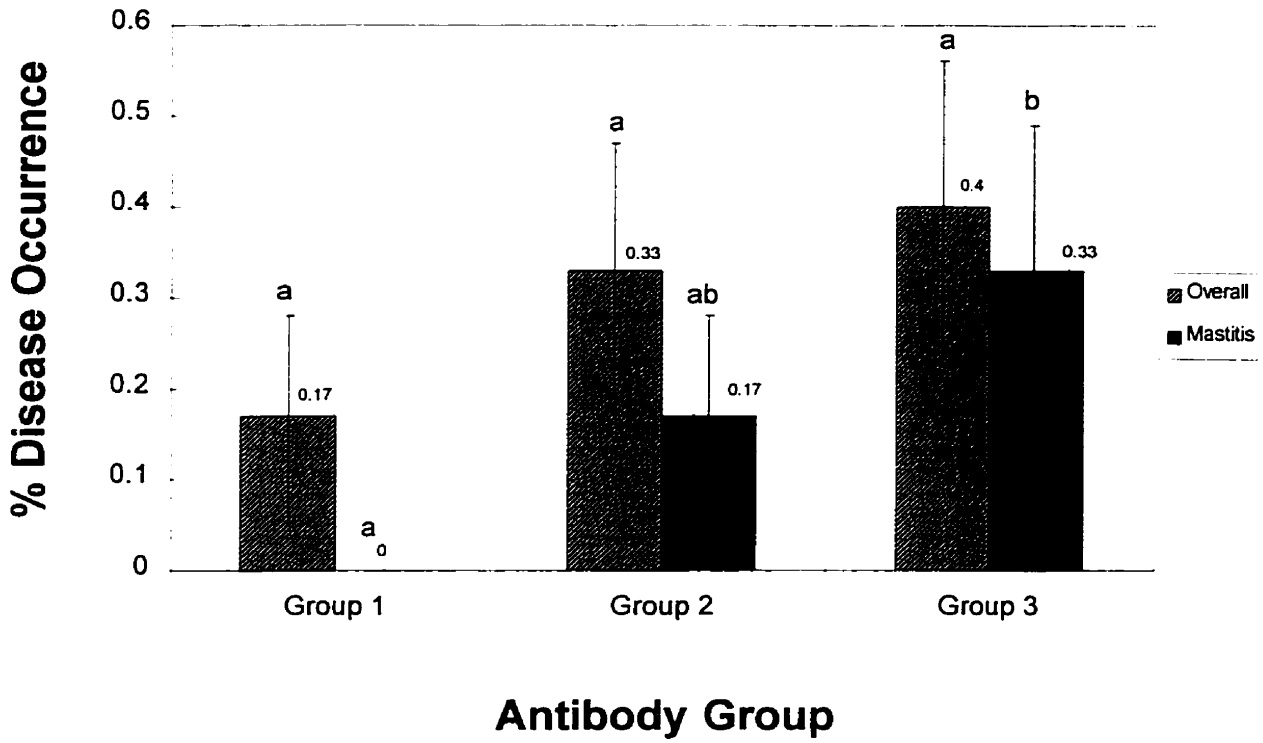

Group 1
Group 2
Group 3
PM
A
B
Optical Density Value (OD)
Week relative to calving
2
1.5
1
0.5
0
-8
-3
0
3
6
4
a
ab
b

Fig. 2. Percent (%) overall disease and mastitis occurrence by antibody group. Group 1 = high antibody; Group 2 = reduction in antibody following immunization postpartum (week 0); Group 3 = reduction in antibody following immunization pre- and postpartum. Animal classification is based on serum antibody to ovalbumin (OVA). Numbers above bars represent precise percent disease occurrence in each group. Standard error was calculated based on the proportion of animals having (p) or not having (q) disease, and the sample size (N) within each antibody group. $SEM=\sqrt{(pq/N)}$. Significant differences between animals in the three groups are indicated by different letters above error bars ($P \leq .05$).


% Disease Occurrence
0.6
0.5
0.4
0.3
0.2
0.1
0
a
0.17
a
0
a
0.33
ab
0.17
a
0.4
b
0.33
Overall
Mastitis
Group 1
Group 2
Group 3
Antibody Group

CHAPTER II

A Quantitative Approach to Classifying Holstein Dairy Cows Based on Antibody and the Relationship Between Antibody and Peripartum Disease Occurrence

Abstract

A quantitative approach was developed to partition phenotypic variation of peripartum antibody profiles of Holstein cows and to determine associations with peripartum mastitis. Using a mathematical index, 136 cows and heifers from three herds were classified into high (Group 1), average (Group 2) or low (Group 3) antibody groups. Antibody group for antibody to ovalbumin (OVA) in whey and antibody to *Escherichia coli* in serum ranked similarly for antibody to OVA in serum. Differences in serum and whey IgG_1 concentrations between antibody groups were not significant. Serum IgG_2 concentration varied between group, herd and across time. Whey IgG_2 did not differ significantly between antibody groups within herd. Occurrence of mastitis was negligible for Group 1 animals in Herds 1 and 3. In contrast, Group 1 animals from Herd 2, had the greatest occurrence of mastitis while Group 3 had the lowest. Though not significant, milk somatic cell score (SCS), was lowest for Group 1 animals in Herd 1 and lowest for Group 3 animals in Herd 2, thus following the distribution frequency of clinical mastitis in those herds. Somatic cell score for animals in Herd 3, did not differ significantly between antibody groups and did not correspond with the distribution of clinical mastitis.

Keywords: antibody, variation, disease, mastitis, peripartum period

1.0 Introduction

Gestation, parturition, and lactation affect nonspecific and specific host defense mechanisms of periparturient dairy cattle (Kehrli et al., 1989a&b; Nagahata et al., 1992; Saad et al., 1994; Detilleux et al., 1995; Dietz et al., 1997; Mallard et al., 1997). Prevalence of disease is high during the periparturient period is high compared to other stages of lactation, coincident with impaired immune and/or innate host resistance mechanisms either before or immediately after parturition

In the pre- or postpartum periods, neutrophil function (Detilleux et al., 1995; Kehrli et al., 1989b; Gilbert et al., 1993a; and Cai et al., 1988), complement activity (Detilleux et al., 1995), conglutinin concentration (Detilleux et al., 1995), and somatic cell count (Shuster et al., 1996) suggest impaired innate resistance in dairy cows. Concentration of serum IgG_1 declined while serum IgG_2 concentration increased at parturition, an effect due to selective transfer of IgG_1 to lacteal secretions (Detilleux et al., 1995; Ishikawa, 1987). Antibody dependent cell cytotoxicity (Kehrli, 1989a), number of antibody plaque forming cells (Nagahata et al., 1992) and lymphocyte proliferation to mitogen and antigen (Ishikawa, 1985; Saad et al., 1989; and Kehrli et al., 1989a) have been reported to be subnormal in peripartum cows.

Altered innate and immune responsiveness during the periparturient period has been associated with susceptibility to disease, particularly mastitis (Cai et al. 1994; and

Gröhn et al. 1993). Cai et al. (1994) indicated that periparturient cows with mastitis had lower neutrophil chemotaxis and cows with metritis had lower superoxide anion production compared to healthy controls. Selection for high and low antibody has been associated with altered disease resistance in a number of species (Biozzi et al., 1968; Siegel et al., 1980; Ibanez et al., 1980; Van der Zijpp et al., 1983; Mallard et al., 1992 and 1998). These studies suggest that it may be possible to identify cows with high or low immune response status during the peripartum period and that this classification could be associated with health and performance. Indeed, there may be sufficient phenotypic variation to classify cows based on peripartum antibody profiles (Mallard et al., 1997; Ch. V). However, classification was based on a biological assessment of individual antibody patterns following immunization; a method that does not provide for classifying cows across a number of herds or within a large population. These results indicate that a standardized approach to partition phenotypic variation among cows within the population is therefore required.

The objective of this study was to confirm the existence of high and low antibody profiles among individuals across three herds and to devise a method for quantitatively classifying cows into groups based on antibody to standardized immunization protocols. Relationships were evaluated between antibody, immunoglobulin concentration, milk somatic cell score, and disease occurrence with respect to antibody group.

2.0 Materials & Methods

2.1. Animals and Treatments

Antibody was evaluated in 136 Holstein dairy cows and heifers from 2 research herds (Herd 1, n=32, 6 heifers and 26 cows; Herd 2, n=67; 34 heifers and 33 cows) and 1 commercial herd (Herd 3, n=37, 8 heifers and 29 cows) from eight weeks prepartum (week -8) based on predicted parturition dates to six weeks postpartum (week 6). Forty nine animals were primiparous heifers, 47 animals were in their second lactation and 41 were multiparous cows (>2 lactations). Antibody was evaluated as previously described (Mallard et al., 1997; Ch. V). Animals received an intramuscular (im) injection of ovalbumin (OVA; Type VII, Sigma Chemical Co., St. Louis, MO) and a mastitis endotoxemia preventive vaccine with the manufacturer's adjuvant (Rhône Mérieux *E. coli* J5, Rhône Mérieux, Lenexa, KS) at weeks -8 (4 mg) and -3 (2 mg). At parturition (week 0), animals received an additional immunization of OVA in phosphate buffered saline (PBS - 0.1 M, pH 7.4) (2 mg, im). OVA was chosen as an inert test antigen to which these animals had not likely been previously exposed. *E. coli* J5 was used because dairy cows could be expected to have been previously exposed to *E. coli*, a complex antigen having biological relevance.

2.2. Blood and Milk Sampling Schedule

Blood was collected by caudal tail venipuncture at approximately week -8 relative to parturition, and weekly from weeks -3 to 6 relative to parturition. Samples were used to monitor serum immunoglobulin $G_{1\&2}$ and serum antibody to OVA and *E. coli* J5. Colostrum and milk samples were collected to monitor whey $IgG_{1\&2}$ and antibody to OVA in whey. Colostrum was collected at the first milking following parturition. Milk samples were stripped from all quarters approximately 2-4 hr after morning milking. Colostrum and milk samples were stored frozen without preservative at -20°C until time of whey separation and immunoglobulin quantification.

2.3. ELISA for OVA Antibody Detection In Serum and Whey

Antibody to OVA was detected by ELISA, and quantified based on optical density measurements according to a procedure previously described (Mallard et al., 1997; Ch. V). Sera samples (weeks -8, -3, 0, 3, and 6) diluted 1/50 and 1/200 were assayed in duplicate. Whey samples (weeks 0,2,3,4, and 6) diluted 1/10, 1/100, 1/400 and undiluted were assayed in quadruplicate.

2.4. ELISA for E. coli J5 Antibody Detection In Serum

Antibody to *E. coli* J5 was measured according to the method previously described

(Mallard et al., 1997; Ch. V). Serum samples (weeks -8,-3, 0, 3, and 6) diluted 1/1000 were assayed in quadruplicate.

2.5. Radial Immunodiffusion Assay

Radial immunodiffusion was used according to the method described by Mallard et al. (1992) to determine the concentrations of serum $IgG_{1\&2}$ at weeks 0, 3, and 6 and whey IgG_1 at weeks 0, 3, and 6 and whey IgG_2 at weeks 0 and 3.

2.6. Milk Somatic Cell Count

Milk (AM/PM composite sample) was collected weekly to determine somatic cell count (SCC), an indicator or subclinical mastitis. Only SCC that coincided with blood sample collection for each week were used in evaluation. SCC were transformed to somatic cell score (SCS) for analysis. SCS is the natural logarithm of SCC in cells/mL and is calculated as follows:

$$SCS=[\log_e(SCC/100)\div\log_e(2)] + 3 \quad \text{(Shook, 1993)}$$

2.7. Mastitis Occurrence

Occurrence of clinical mastitis was recorded throughout the study period by herd

managers. Two or more events of mastitis it were recorded as one event for the study period (Martin et al., 1993). Incidence of mastitis occurrence was calculated by dividing the number of animals within an antibody group that had at least one disease event by all the animals in that antibody group, and multiplying this number by 100. Mastitis occurrence was evaluated for associations with antibody group within each herd, using odds-ratio (OR) (Martin and Meek, 1987). Odds-ratios in this study were calculated on a within herd basis, as the ratio between the rate of mastitis in one antibody group versus the rate of mastitis in the rest of the herd (i.e. the other two groups). Odds-ratio is the approximate relative risk when the rate of disease in the population is relatively infrequent (<5%) (Martin and Meek, 1987). Odds ratios values were tested for significance using the chi-square test (Martin and Meek, 1987).

2.8. Quantitative Classification of Animals Based on Antibody to OVA

Serum antibody to OVA was evaluated over time intervals, rather than discrete points in time. Individual animal antibody curves from week -8 to week 6 relative to parturition (week 0) were dissected into components reflecting the response to antigen following immunizations. The primary component was defined as the change in antibody to OVA from week -8 to week -3 relative to parturition following primary immunization at week -8 (Primary= OD value at week -3 minus OD value at week -8). The secondary component was defined as the change in antibody to OVA from week -3 to parturition following secondary immunization at week -3 (Secondary= OD value at week 0 minus

OD value at week -3). The tertiary component was defined as the change in antibody to OVA from parturition to week 3 following tertiary immunization at parturition (Tertiary=OD at week 3 minus OD at week 0). The quaternary component was defined as the change in antibody to OVA from week 3 to week 6 (Quaternary= OD value at week 6 minus OD value at week 3). The quaternary component was included to observe the change in antibody between the end of the immediate postpartum period (wk 3) and peak lactation. These components were added together to give a total index of antibody to OVA between wk -8 and wk +6 relative to parturition as follows:

y_{total}= primary + secondary + tertiary + quaternary

where,

y= total antibody;

primary, secondary, tertiary, and quaternary components are as previously defined;

primary, secondary, tertiary, and quaternary components when positive, have an equal weight of 1.

Animals that exhibited a reduction in antibody following secondary or tertiary immunizations were weighted with a coefficient of 1.5. Only secondary and tertiary components were weighted in this manner, because this is the period when lowered host resistance mechanisms are thought to contribute to increased occurrence of disease. The coefficients for weighting negative secondary and tertiary components were optimized using the original biological assessment for grouping animals. Several iterations were

run to determine a coefficient that reflected the biological assessment of grouping animals based on the magnitude and direction of response to immunization.

The mean of y_{total} was determined and animals that exceeded one standard deviation above the mean were classified into the high antibody group (Group 1; n=18). Animals that were one standard deviation below the mean were classified into the low antibody group (Group 3; n=23). Animals with a y_{total} that ranged between one standard deviation below and above the mean were classified into the average antibody group (Group 2; n=95).

2.9. Statistical Methods

Type III least squares analysis of variance (ANOVA) and corrected means (least square means, LS Means) were generated using the General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS; Helwig and Council, 1982) to evaluate the effects of herd, season-year, cow, antibody group, parity, week, and their interaction terms on antibody to OVA and *E. coli*, and immunoglobulin concentration (Table 1). Tests of hypothesis of main effects were tested against the MS for cow. Sources of variation that were not significant were removed from the model for calculation of LS Means. Data that did not show a normal distribution (*E. coli* antibody, serum IgG_2 and whey IgG_2) as indicated by the univariate procedure of SAS (SAS, 1982), were transformed to natural logarithms. LS means were converted back to original units from $\log_e$

transformed data. Consequently, standard errors of means are not shown. The Proc CORR procedure of SAS was used to generate Pearson product moment correlation coefficients between immune response parameters. Results were considered to be statistically significant if the p-value was <0.05 and trends were reported at the p-value <0.10.

3.0. Results

3.1. Serum Antibody to OVA

Cow, antibody group, week, and the interaction between antibody group and week contributed to the variation (P<0.0001) in serum antibody to OVA (Table 1). Herd did not significantly contribute to the variation in serum antibody to OVA. As expected, the rank of antibody to OVA was Group 1>Group 2>Group 3 except at week -8 prior to immunization and significant differences were noted between all groups at weeks -3, 0, 3, and 6. Population LS means significantly (P<0.0001) increased from pre-immunization (week -8) to week -3 (post primary immunization) in all antibody groups and OVA antibody varied significantly across time at weeks -3, 0, 3, and 6. (Fig. 1).

3.2. Whey Antibody to OVA

Herd contributed significantly (P<0.01) to variation in antibody to OVA and therefore,

herds were further analyzed separately. Cow, antibody group, and week all significantly contributed to the variation in antibody to OVA in whey ($P<0.0001$); however, there was no significant contribution of the interaction term antibody group and week to the variation in response. For all herds, antibody to OVA in whey by antibody group, ranked similarly to the antibody observed for serum, such that Group 1 > Group 2 > Group 3. This was consistent for colostral and milk whey from parturition until week 6 of lactation (Fig. 2A,B, and C). Least squares means of antibody to OVA in whey for all herds declined significantly from parturition to peak lactation. Correlation analysis between antibody to OVA in sera with antibody to OVA in whey, indicated a positive and significant relationship for Herd 1 ($r=0.45$; $P<0.0001$), Herd 2 ($r=0.28$; $P<0.001$) and Herd 3 ($r=0.45$; $P<0.001$) respectively.

Antibody to E. coli J5 in sera

Herd contributed significantly ($P<0.003$) to variation in antibody to *E. coli* J5 and therefore, herds were further analyzed separately (Table 1).

3.3.1. Herd 1

Cow and week each significantly ($P<0.0001$) contributed to the variation in antibody to *E. coli* J5. OD values measuring anti-OVA were significantly different between antibody groups from week -3 to week 6, and the rank of LS Means of antibody to *E. coli* by

antibody group was Group 1>Group 2>Group 3 (Fig. 3A). Least squares means of antibody to *E. coli* J5 varied during the peripartum period (week -3 to week +3) and up to peak lactation (week +6) and were significantly higher (P<0.0001) than pre-immunization antibody at week -8 for all animals, regardless of group (OD value = 0.275) (Fig. 3A). Correlation analysis, comparing antibody to *E. coli* J5 with antibody to OVA in sera, indicated a positive and significant relationship (r=0.56; P<0.0001). The correlation between serum anti-OVA and *E. coli* for Group 1, 2, and 3 was 0.66(P<0.001), 0.59 (P<0.0001) 0.38 (P<0.06), respectively.

3.3.2. Herd 2

Cow, antibody group by parity, parity and week significantly contributed to the variation in antibody to *E. coli* J5 (P<0.0001) for Herd 2. The LS Mean of antibody for Group 3 animals at week -8 tended to be higher (OD value=0.386) than for animals of Group 1 (OD value=0.257; P<0.005) and Group 2 (OD value=0.292; P<0.05). Optical density values of antibody to *E. coli* were not significantly different between groups at weeks -3 to 6; however, low antibody animals had OD values that were consistent but not significantly lower than the population mean. (Fig. 3B) Least square means of antibody to *E. coli* J5 at weeks -3, 0, 3, and 6 were significantly higher (P<0.0001) than pre-immunization antibody at week -8 regardless of group (OD value=0.307). Correlation analysis between serum antibody to *E. coli* J5 and serum antibody to OVA indicated a positive and significant relationship (r=.49; P< 0.0001). The correlation between

antibody to *E. coli* J5 and antibody to OVA for Groups 1, 2, and 3 was 0.65(P<0.0001), 0.54 (P<0.0001), and 0.31 (P<0.08) respectively.

3.3.3. Herd 3

Cow, antibody group, week, and the interaction between week and antibody group significantly contributed to the variation in antibody to *E. coli* J5 (P<0.0001) in Herd 3. In this herd, antibody for Group 1 animals was significantly lower (P≤.05) at weeks -8 and -3 compared to Group 2 and 3 animals. At parturition, Group 1 and 2 animals had higher antibody to *E. coli* than Group 3 animals. At weeks 3 and 6, however, the rank of antibody group for antibody to *E. coli* was similar to the other herds, in that Group 1 >Group 2 > Group 3 (Fig. 3C). LS Means of antibody to *E. coli* J5 at weeks -3,0,3, and 6 were significantly different across time and were significantly higher (P<0.0001) than pre-immunization antibody regardless of group (OD value=0.224)(Fig. 3C). Correlation analysis between serum antibody to *E. coli* J5 and serum antibody to OVA indicated a positive and significant relationship (0.47; P< 0.0001). Correlation between serum antibody to *E. coli* J5 and antibody to OVA for Groups 1, 2, and 3 were 0.93(P<0.007), 0.48 (P<0.0001), and 0.36(P<0.006) respectively.

3.4. IgG_1 in serum and whey

Analysis of variance indicated that the effect of week contributed significantly (P<0.05)

and the effect of antibody group tended ($P<0.07$) to contribute to variation in serum IgG_1. Except at week 3, serum IgG_1 did not differ significantly between groups; however, Group 1 animals tended to have lower serum IgG_1 compared to animals of Group 2 and 3. Least square means of total IgG_1 in sera increased significantly ($P<0.0001$) from parturition (430.09mg/100mL) to week 3 (687.46 mg/100mL) and week 6 (799.51 mg/100mL) (Fig. 4A, population mean). Correlations between serum IgG_1 concentration and serum antibody to OVA and *E. coli* were not significant.

The effects of week and parity contributed significantly ($P<0.05$) to the variation in IgG_1 concentration in whey. Although antibody group did not significantly contribute to variation in whey IgG_1 (Fig. 4B), LS means of IgG_1 concentration (mg/100mL) at week 0 were significantly lower for Group 1 (768.16 mg/100 mL) and Group 3 (1081.39 mg/100mL) compared to Group 2 (1381.60 mg/100mL). Concentration of IgG_1 did not differ significantly between groups at weeks 3 and 6. Population LS Means of IgG_1 concentration in whey declined significantly from parturition (1046.28 mg/mL) to week 3 (44.93 mg/100mL, $P<0.0001$). There was no significant change at week 6 (43.25 mg/100mL). Correlation analysis between whey IgG_1 concentration and whey antibody to OVA indicated a positive and significant relationship ($r= 0.711$;$P<0.0001$). The correlation coefficients between whey IgG_1 and whey OVA antibody for Groups 1, 2, and 3 were 0.52($P<0.0001$), 0.76($P<0.0001$), and 0.69($P<0.0001$), respectively.

3.5. IgG_2 in sera

Herd contributed significantly ($P<0.0001$) to variation in serum IgG_2 concentration and therefore, herds were analyzed separately.

3.5.1. Herd 1

Effects of cow and the interaction between antibody group and week contributed significantly ($P \leq .05$) to variation in IgG_2 concentration for Herd 1. Antibody group did not significantly contribute to the variation in IgG_2; however, LS means of IgG_2 in sera at weeks 0 and 3 was lowest for Group 1 animals and highest for Group 3 animals. This trend reversed at week 6, such that Group 1 animals had the highest concentration of IgG_2 and Group 3 animals had the lowest. LS Means of IgG_2 significantly increased from 1019.43 mg/100mL at parturition to 1534.56 mg/100 mL at week 3 but declined significantly at week 6 to 1103.23 mg/100 mL. Correlation analysis, between antibody to OVA in sera and concentration of IgG_2, indicated a negative and significant relationship ($r=-0.23$, $P<0.03$). Correlations between antibody to OVA with serum IgG_2 concentration indicated for Group 1, 2, and 3 were 0.07(ns), -0.35($P<0.004$) and -0.33 (ns). Significant correlations were not observed between *E. coli* antibody and serum IgG_2 concentration, even when examined by group.

3.5.2. Herd 2

Cow significantly contributed ($P \leq .05$) to the variation of serum IgG_2 concentration while antibody group and the interaction between antibody group and parity tended to contribute to the variation in serum IgG_2 concentration. At parturition, groups did not significantly differ in serum IgG_2. At week 6, LS means of IgG_2 concentration for animals in Group 1 were significantly higher than for Group 3 animals. Least square means of IgG_2 concentration did not differ significantly between weeks 0, 3, and 6. Correlation analysis between serum IgG_2 concentration and serum antibody to OVA indicated a positive and significant relationship ($r=.15$; $P<0.03$). Significant correlations were not observed between serum IgG_2 concentration and serum antibody to OVA or serum antibody to *E. coli* J5.

3.5.3. Herd 3

Cow ($P<0.005$) and parity ($P<0.04$) contributed significantly to the variation of serum IgG_2 concentration. Week ($P<0.09$) tended to contribute to variation in serum IgG_2 concentration. Antibody group did not significantly contribute to variation in serum IgG_2 concentration. Correlations between serum IgG_2 concentration and antibody to OVA and *E. coli* were not significant.

3.6. IgG_2 in whey

Herd contributed significantly ($P<0.03$) to the variation in serum IgG_2 concentration and therefore, herds were analyzed separately.

3.6.1. Herd 1

Week contributed significantly to variation in IgG_2 concentration in whey. Whey IgG_2 concentration did not differ significantly between groups. LS Means of total IgG_2 concentration in whey declined significantly from week 0 (327.34 mg/100 mL) to week 3 (26.31 mg/100 mL). Correlation analysis between whey IgG_2 concentration and antibody to OVA indicated a positive and significant relationship ($r=0.7$; $P<0.0001$). Correlations between whey IgG_2 concentration and whey antibody to OVA were $r=0.9$ ($P<0.002$), 0.6 ($P<0.0003$), and 0.8 ($P<0.02$) for Groups 1, 2, and 3, respectively.

3.6.2. *Herd 2*

The combined effects in the linear model did not explain variation in whey IgG_2 concentration, therefore, LS means were not estimable. Correlations between whey IgG_2 concentration and whey antibody to OVA indicated a positive and significant relationship ($r=0.3$, $P<0.009$). Correlations between whey IgG_2 and whey antibody to OVA were 0.2(ns), 0.5($P<0.0001$), and 0.6 (ns) for Groups 1, 2, and 3, respectively.

3.6.3. *Herd 3*

Antibody group and week significantly contributed to the variation in whey IgG_2 concentration. Whey IgG_2 concentration did not significantly differ between groups at parturition, and concentration at week 3 could only be estimated for Group 3 animals because Groups 1 and 2 were either low or too low to be detected. Correlation analysis indicated no significant relationships between whey IgG_2 concentration and whey antibody to OVA.

3.7 *Somatic Cell Score*

For Herds 1 and 2, cow within antibody group and week significantly contributed to the variation in SCS (Table 1). In Herd 3, only the effect of cow within antibody group accounted for the variation in SCS. LS Means of SCS in Herd 1 tended to be lower for the high antibody group compared to low antibody group at weeks 3,4,5 and 6 following parturition (Fig. 6A). Conversely, LS Means of SCS in Herd 2 were significantly lower for low antibody animals compared to high antibody animals.

3.8. *Mastitis Occurrence*

Percent mastitis occurrence varied between groups and between herds. Rates of occurrence of clinical mastitis are presented in Table 2. Though groups were small,

mastitis did not occur in Group 1 of either Herds 1 or 3. Mastitis occurrence in Herd 1 was 21.7% and 33.3 % for Groups 2 and 3, respectively. Mastitis occurrence in Herd 3 was 11.5 and 10% for Groups 2 and 3 respectively. However, in Herd 2, Group 1 animals had the highest occurrence of mastitis (15.4%) and exceeded the percent occurrence of mastitis in Groups 2 (2.1%) and 3 (0 %) (Fig. 5). Animals with mastitis in Herds 1 (n=6 heifers; n=26 cows) and 3 (n=8 heifers; n=29 cows) were in their second or greater parity. Animals with mastitis in Herd 2 (n=34 heifers; n=33 cows) were all heifers. Across all herds, animals in Group 3 had the highest rate of mastitis occurrence (13.6%) compared to Group 1 (11.1%) and Group 2 (9.3%) (Table 2). These differences across herds however, were not significant.

3.9. *Odds-Ratio for Mastitis*

Within herd, odds-ratio calculations comparing animals of one antibody group with the other two groups indicated that only animals in Group 1 of Herd 2 had a statistically significant higher relative risk of having a mastitis event (by 7.57 times) compared to the animals in the rest of the herd. Although the risk of mastitis occurrence within Group 3 of Herds 1 and 3 was 2.16 and 1.8 times greater (respectively) than for other groups, these values were not significant.

4.0. Discussion

A previous study of one herd indicated that animals could be classified according to the amplitude and direction of their individual OVA antibody profiles, and that this ranking had some association with mastitis occurrence (Mallard et al., 1997; Ch. V). This herd (Herd 1) was evaluated with two more herds, Herds 2 and 3. The objective of the current study was to verify the relevance of high and low antibody profiles across the three herds and to determine if it would be possible to develop a quantitative measure of classification for antibody that reflected the initial biological assessment of animals. The results indicated substantial variation in antibody to OVA from the peripartum period to peak lactation and that animals could be ranked using a quantitative index. Animals that ranked high, average or low for serum antibody to OVA, also ranked similarly for whey antibody to OVA. Serum antibody to OVA was expected to be significantly different between groups because animals were classified into high or low groups based on their total antibody curve slopes. They were classified as either less or greater than one standard deviation from the population mean of the total index of antibody.

In all herds, measured antibody to the more biologically relevant antigen, *E. coli*, ranked similarly to the ranking for antibody to OVA, particularly at weeks 0, 3, and 6 after parturition. This may help identify animals that respond best following immunization. Nonetheless, ranking based on antibody to OVA is more practical since OVA is not normally encountered in the dairy cow's environment. Thus, the possibility of pre-

existing antibody to OVA is eliminated. In theory, any antibody to OVA produced was expected to be evoked only by the immunization protocol. Further, antibody to *E. coli* was significantly affected by herd making comparisons of populations difficult.

A mathematical approach to assess variation in innate and immune host resistance mechanisms during the peripartum period developed by Detilleux et al. (1994) used a fitted polynomial model to assess hyporesponsiveness during the peripartum period. These results were utilized in an animal model to detect variation between daughters of various sire groups. This method of assessment of hyporesponsiveness was not suitable for this study because it requires many data points across time. In this study, variation in antibody to OVA was partitioned using a simple model, wherein animals that had any hyporesponsiveness in the immediate peripartum period were ranked lower compared to animals that responded consistently and positively to OVA immunization.

Correlation analysis between antibody to OVA and $IgG_{1\&2}$ by antibody group in serum and whey indicated some significant relationships. Serum and whey IgG_2 distributions by antibody group differed for each herd. Consequently, relationships between herds were difficult to determine. Unpublished data from this laboratory, as well as other studies (Gilbert et al., 1994) have indicated that the serum antibody to OVA is largely of the IgG_2 subclass. Therefore these findings may have indicated some association between the two parameters investigated. However, given that herd differences existed, it was not feasible to relate previously published results with IgG_2 concentration

investigated in the current study.

The incidence of mastitis by antibody group was not consistent between herds. In Herd 1 and Herd 3, the incidence of mastitis was greatest for animals with low antibody (Group 3). All animals within these herds that had mastitis were in their second or later parity. Though not significant, odds-ratio assessment for these herds indicated that there was a 2.16 and a 1.80 times greater chance of having a mastitis event if animals were classified in Group 3 versus Groups 1 & 2. In contrast, animals from Herd 2 had a very different distribution of mastitis occurrence among groups. Animals in Group 1 had the greatest rate of mastitis occurrence and according to the odds-ratio parameter, were 7.57 ($P<0.05$) times more likely to have a mastitis event than Group 2 or 3 animals. Further, all animals that had mastitis within Herd 2 were first parity heifers. Differences in herd management and the distribution of heifers and cows within each herd and antibody group, may help explain the differences in the distribution of mastitis occurrence. Herd 1 (n=6 heifers; n=26 cows) and Herd 3 (n=8 heifers; n=29 cows) had a greater ratio of cows to heifers within each antibody group, while Herd 2 (n=34 heifers; n=33 cows) heifers and cows were more evenly distributed among all antibody groups. Previous studies have acknowledged an increase in the rate of occurrence of mastitis with advancing parity and (Todhunter et al., 1995, and McClure et al., 1994) may explain the disparity among herds. The unexpected distribution of mastitis in the Herd 2 might further be explained by a more recent investigation from Finland (Myllys et al., 1995). This study indicated that in well managed herds with high milk production and low somatic cell counts, the

rate of the treatment of heifers that had a mastitis episode increased from 1.8% to 4.4% over an 8 year period. In contrast to clinical mastitis observed in second parity and multiparous cows, that study further indicated that mastitis in heifers only resulted in small production losses, did not pre-dispose heifers to more mastitis or other diseases later in lactation, and the recovery rate from mastitis was high as indicated by a rapid decline in somatic cell counts (SCC) following infection. This may indicate that mastitis in heifers and in cows cannot be compared directly. That disease occurrence in this study was not consistent among herds, may be explained by a number of factors including the relatively small sample size evaluated, environmental (management) differences, distribution of heifers and multiparous cows, and type of mastitis (subclinical vs. clinical, and the infecting pathogen). Further research of a larger population of animals may be warranted because, in this study, Group 1 animals within two of the three herds evaluated were observed to have no occurrence of mastitis.

Alterations in antibody and the incidence of mastitis indicates that immune response phenotype may be a potential phenotypic marker for disease resistance and/or susceptibility. Several studies of Norwegian bulls have demonstrated that these associations do exist. Antibody responses to human serum albumin (HSA) of half sib sisters of Norwegian bulls were demonstrated by Lie et al.(1986) to associate with BoLA class I alleles. High responders to HSA were strongly associated with allele BoLA-w16, and were also determined to be susceptible to mastitis. Conversely, low responding animals, associated with the BoLA-w2 allele, were found to be relatively resistant to

mastitis. Alternatively, there may be greater benefit in selecting animals that have an average antibody response to a panel of antigens, such as was observed for the Group 2 animals, that have an average rate of mastitis occurrence within a particular herd. Further research is required to accurately assess whether animals within particular antibody group are more susceptible or resistant to mastitis.

It was determined that sufficient individual variation in antibody to OVA existed such that animals could be readily classified quantitatively into high, average and low antibody groups using a mathematical index based on OD values of antibody profiles from week -3 to week 6 relative to parturition. Further investigations of larger populations with more disease data would be required to accurately assess associations between antibody profiles and disease occurrence during the peripartum period. Detection of immune response traits such as antibody, that associate well with disease resistance may provide a useful phenotype to begin selective breeding of dairy cattle for improved inherent immune responsiveness and disease resistance.

Table 1. Analysis of variance of antibody response to ovalbumin (OVA) and *E. coli* J5, the concentration of immunoglobulin $G_{1\&2}$ in serum and whey, and somatic cell score (SCS)

			Source of Variation								
Dependent Variable	R^2[a] (%)	C.V.[b] (%)	Herd	Season-yr[c]	Cow[d]	Group[e]	Parity	Group*parity	Week	Group*Week	Parity*Week
Serum anti-OVA	79.41	27.63	–	–	0.0001	0.0001	0.096	ns[f]	0.0001	0.0001	–
Whey anti-OVA	73.73	32.16	0.02	–	0.0001	0.0001	ns	ns	0.0001	ns	–
Herd 1	75.34	-130.01[h]	–	–	0.0001	0.0001	0.0001	–	0.0001	0.05	–
Herd 2	71.29	-524.16	–	–	0.0001	0.007	–	–	0.0001	0.07	–
Herd 3	82.72	6682.1	–	–	0.0001	0.0002	–	–	0.0001	ns	–
Serum anti-*E. coli*	74.23	-43.72	0.003	–	0.0001	–	0.0004	–	0.0001	–	0.0001
Herd 1	78.63	-54.79	_	_	0.0001	ns	–	–	0.0001	0.06	–
Herd 2	76.89	-45.16	–	–	0.0001	ns	0.0001	0.0001	0.0001	ns	–
Herd 3	70.63	-31.53	–	–	0.0001	ns	–	–	0.0001	0.002	–
Immunoglobulin Concentration											
Serum IgG_1	49.74	7.53	–	–	ns	0.07	ns	ns	0.0001	ns	–
Serum IgG_2	63.14	4.34	0.0001	–	0.0001	ns	0.04	ns	0.025	ns	–
Herd 1	59.97	4.67	–	–	0.02	ns	–	–	0.0015	ns	–
Herd 2	48.49	4.36	–	–	0.021	0.08	ns	0.08	ns	ns	–
Herd 3	56.14	3.70	–	–	0.005	–	0.04	–	0.09	–	ns
Whey IgG_1	90.10	15.11	–	–	ns	ns	0.01	ns	0.0001	ns	–
Whey IgG_2	96.85	13.50	0.03	–	ns	ns	0.0009	ns	0.0001	ns	–
Herd 1	94.85	14.96	–	–	ns	ns	–	–	0.0009	ns	–
Herd 2	ns	ns	–	–	ns	ns	0.08	ns	0.02	ns	–
Herd 3	97.41	12.94	–	–	ns	0.097	–	–	0.0001	ns	–
Somatic Cell Score											
SCS(Herd 1)	83.51	44.89	–	–	0.0001	ns	–	–	0.0013	ns	–
SCS(Herd 2)	81.43	46.70	–	–	0.0001	ns	–	–	0.0001	ns	–
SCS(Herd 3)	78.84	26.54	–	–	0.0001	ns	–	–	ns	ns	–

a R^2 = coefficient of determination
b C.V. = coefficient of variation
c Season-Year = season and year of calving
d Cow nested or 'grouped' within the interaction between antibody group and parity. i.e. Cow(group*parity). If parity is not significant, parity is removed and the model becomes cow nested within antibody group only.
e Group = variation due to antibody group of animals classified with high, average or low antibody to OVA
f ns= not significant
g ——— = not significant therefore removed and no longer relevant to that dependent variable
h Coefficient of variation is negative due to analysis of variance of natural loganthm transformed data

Table 2. Percent Occurrence (%) of clinical mastitis by antibody group within herd

		% Occurrence of Mastitis within an Antibody Response Group			
Herd		Group 1	Group 2	Group 3	Overall Mastitis Frequency by Herd
Herd 1	# of animals	n= 4	n=22	n=6	n=32
	% with mastitis	0	21.7	33.3	21.2
Herd 2	# of animals	n=13	n=47	n=7	n=67
	% with mastitis	15.4	2.1	0	4.5
Herd 3	# of animals	n=1	n=26	n=10	n=37
	% with mastitis	0	11.5	10	10.8
All herds	# of animals	n=18	n=95	n=23	n=136
	% Overall Mastitis Frequency by Group	11.1	9.3	13.6	--

Figure 1. LS Means of serum antibody to ovalbumin (OVA) by antibody group. Group 1 = high antibody, Group 2 = average antibody, and Group 3 = low antibody based on described index, and Population mean (PM). Significant differences between groups are indicated with lower case letters between groups and differences over time are indicated by different uppercase letters ($P<0.05$).

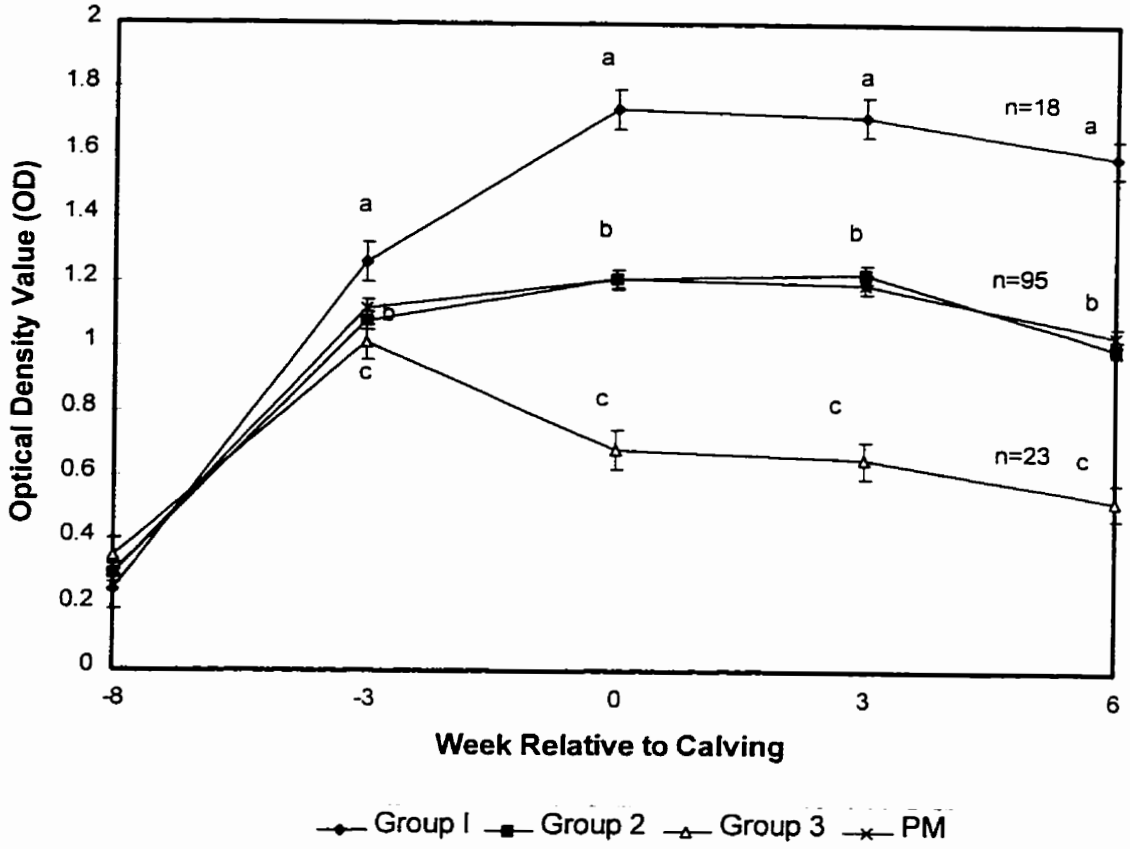

Optical Density Value (OD)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-8
-3
0
3
6
Week Relative to Calving
a
a
a
n=18
a
b
b
b
n=95
b
c
c
c
n=23
c
Group I
Group 2
Group 3
PM

Figure 2. LS Means of whey antibody to ovalbumin (OVA) by antibody group for A) Herd 1, B) Herd 2, and C) Herd 3. Group 1 = high antibody, Group 2 = average antibody, and Group 3 = low antibody based on described index, and Population mean (PM). Significant differences between groups are indicated with lower case letters and differences over time are indicated by different uppercase letters ($P<0.05$).

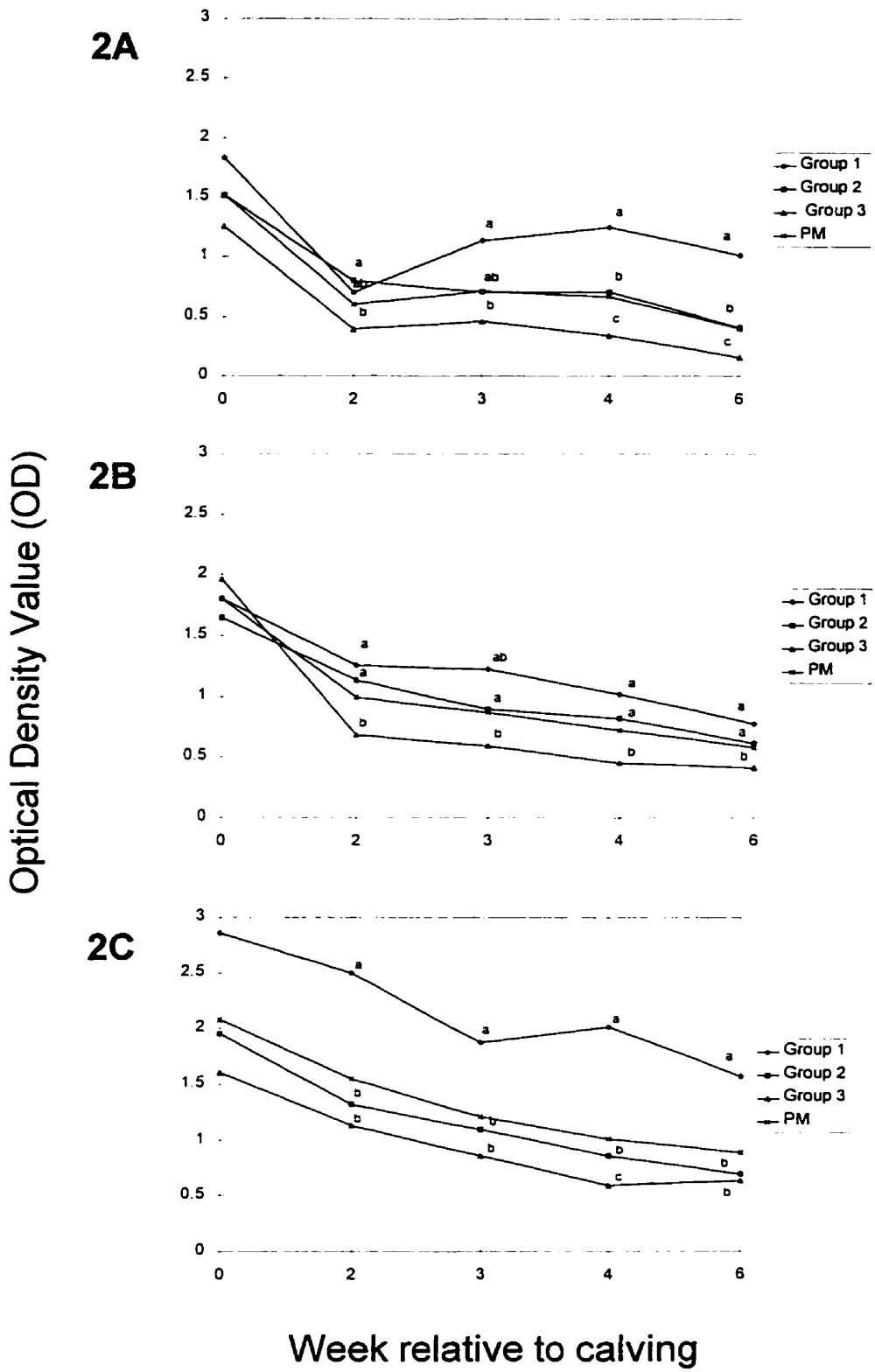

2A
2B
2C
Optical Density Value (OD)
Week relative to calving
Group 1
Group 2
Group 3
PM
0
0.5
1
1.5
2
2.5
3
0
2
3
4
6

Figure 3. LS Means of serum antibody to *E. coli* J5 by antibody group for A) Herd 1, B) Herd 2, and C) Herd 3. Group 1 = high antibody, Group 2 = average antibody, and Group 3 = low antibody based on described index, and population mean (PM). Significant differences between groups are indicated with different lower case letters ($P<0.05$).

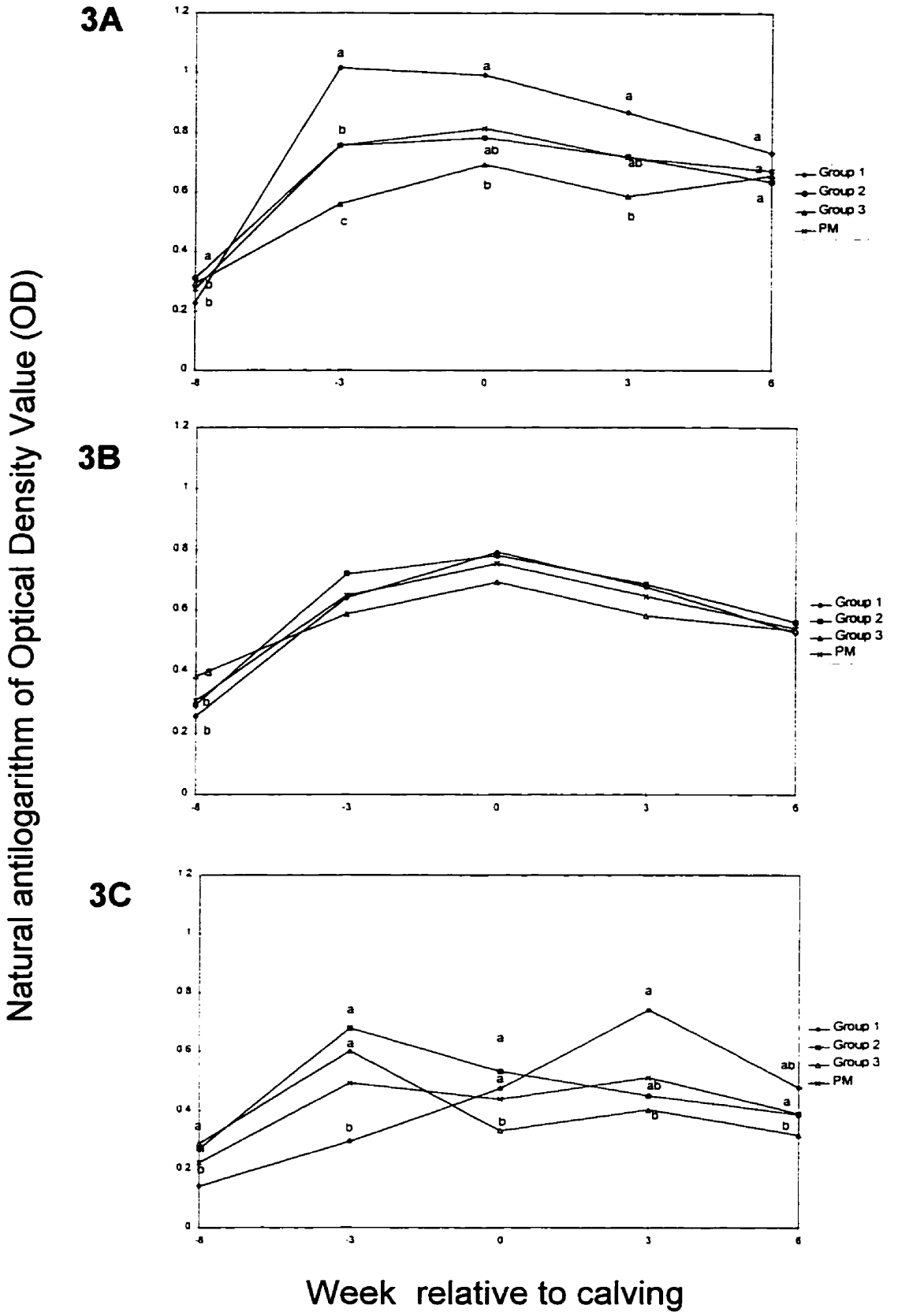

3A
3B
3C
Natural antilogarithm of Optical Density Value (OD)
Week relative to calving
Group 1
Group 2
Group 3
PM

Figure 4. LS Means of IgG_1 by antibody group in A) sera and B) whey. Group 1 = high antibody, group 2 = average antibody, and Group 3 = low antibody based on described index, and Population mean (PM). Significant differences between groups are indicated with lower case letters and differences over time are indicated by different upper case letters($P<0.05$).

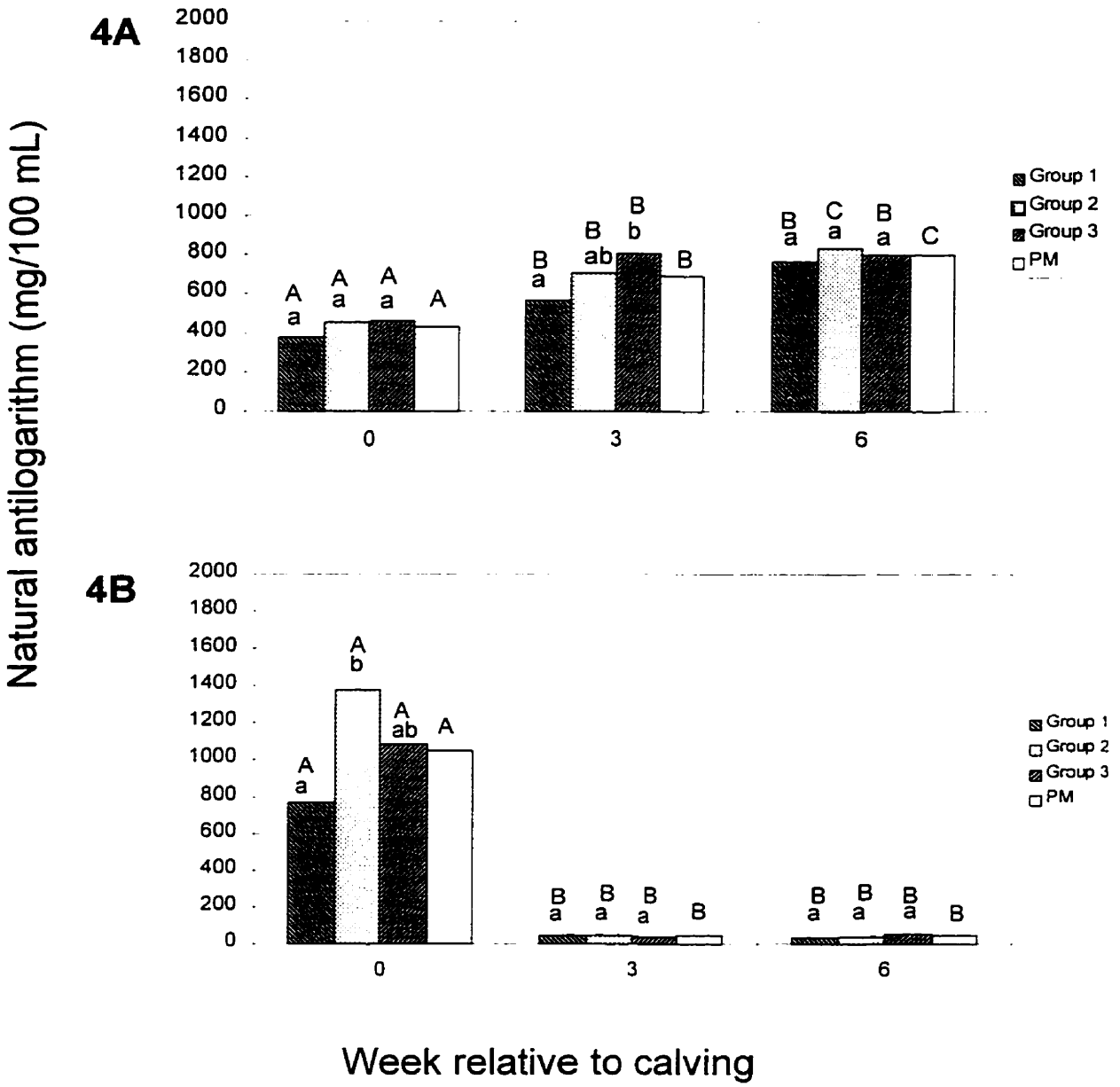
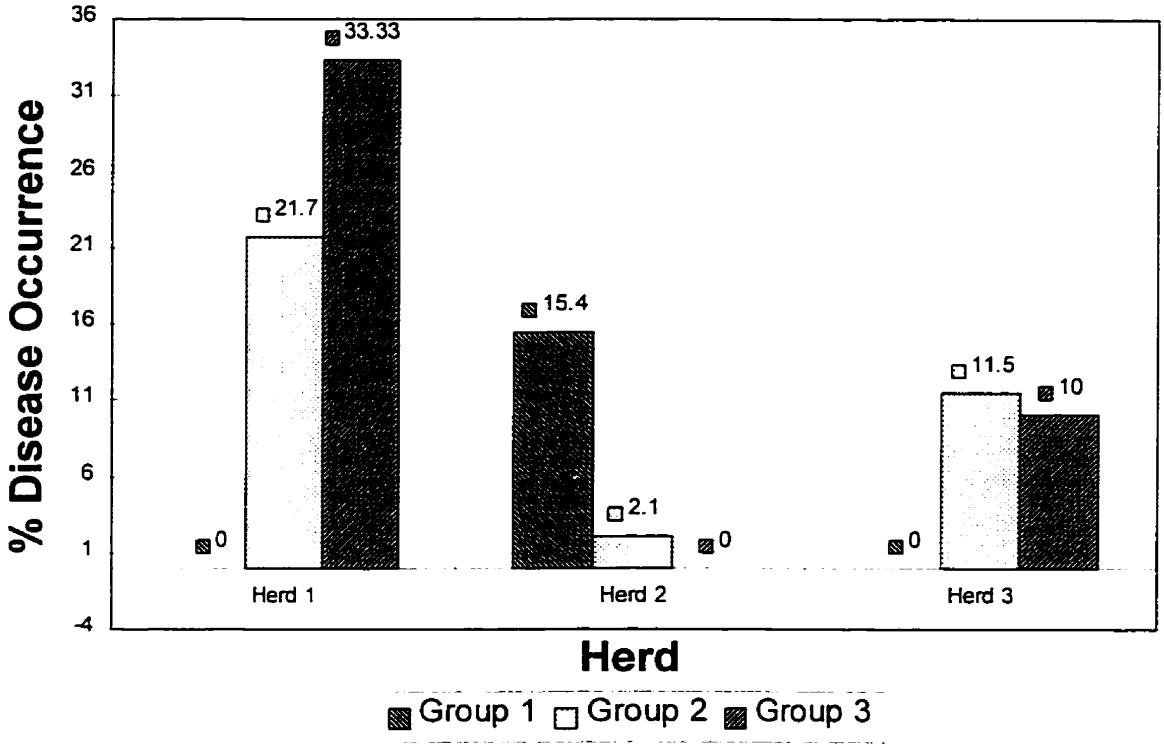

4A
4B
Natural antilogarithm (mg/100 mL)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Group 1
Group 2
Group 3
PM
0
3
6
Week relative to calving

Figure 5. Percent (%) mastitis occurrence by antibody group within herd.


% Disease Occurrence
36
31
26
21
16
11
6
1
-4
33.33
21.7
15.4
11.5
10
2.1
0
0
0
Herd 1
Herd 2
Herd 3
Herd
Group 1
Group 2
Group 3

CHAPTER III

Relationships Between Cell Mediated Immune Response (CMIR) and Antibody in Periparturient Holstein Dairy Cows

Abstract

To examine variation in cell mediated immune response (CMIR) as a function of peripartum serum antibody to ovalbumin (OVA), 136 Holstein cows and heifers from three herds were evaluated from three weeks before parturition to week 6 following parturition for lymphocyte proliferative responses to OVA and concanavalin A (Con A), delayed type hypersensitivity (DTH) to purified protein derivative (PPD) of tuberculin, differential complete blood cell counts, and somatic cell score (SCS). Using a mathematical index, animals were quantitatively classified based on their antibody to OVA into high (Group 1), average (Group 2) or low (Group 3) antibody phenotypes. Lymphocyte proliferative responses to OVA ($r=-0.28$; $P<0.0001$) and Con A ($r=-0.14$; $P<0.0001$) were negatively correlated with antibody to OVA. Animals classified with low antibody (Group 3) had the highest unstimulated and OVA-stimulated lymphocyte proliferative responses. Unstimulated lymphocyte proliferative responses were depressed between week -3 and parturition. Con A stimulated lymphocyte proliferative responses were also depressed at parturition but this was significant ($P\leq0.05$) only for Group 1 cows with high antibody to OVA. Although animals exhibiting high and low DTH response phenotypes could be identified, DTH was not significantly associated to

antibody to OVA. Delayed type hypersensitivity at 48 and 72 hours were negatively and significantly correlated with unstimulated (r=-0.21; P<0.002; r=-.17; P<0.01) and Con A stimulated (r=-0.29, P<0.0001; r=-0.28, P<0.0001) lymphocyte proliferation, respectively. Lymphocyte number in peripheral blood declined significantly from week -3 to week 0. Milk somatic cell score (SCS) was negatively, and significantly, correlated with *in vitro* lymphocyte proliferative response to OVA in Herd 2 (r=.-13; P<0.0001) only. SCS was not significantly correlated with Con A stimulation. SCS was also negatively and significantly correlated with DTH at 48 hours post-challenge (r=-0.21; P<0.01). Cumulative results indicate a variety of negative phenotypic associations between measures of antibody and CMIR, and among indicators of CMIR. Because both antibody and CMIR are important in host resistance to infectious disease, use of a selection index would be required to simultaneously enhance both parameters, assuming there are beneficial associations with cow health.

Keywords: periparturient, CMIR, antibody, and SCS

1.0. Introduction

Innate and immune response mechanisms of dairy cows are impaired during the peripartum period. Neutrophil function (Detilleux et al., 1995, Kehrli et al., 1989b, Gilbert et al., 1994 and Cai et al., 1988), complement activity (Detilleux et al., 1995),

conglutinin concentration (Detilleux et al., 1995), IgG_1 (Detilleux et al., 1995), milk somatic cell count (Shuster et al., 1996) and lymphocyte proliferation (Saad et al., 1989; Kehrli et al., 1989a; Ishikawa, 1987; Kashiwazaki, 1985; Wells et al.1977) are impaired either pre- or postpartum. Some investigations however, indicated that not all animals exhibit a period of hyporesponsiveness, at least with respect to antibody. Mallard et al. (1997; Ch. V) demonstrated that peripartum antibody to ovalbumin (OVA) are continuous in nature, and that this variability allowed animals to be readily classified as low, average or high antibody producers. Further, animals of the high group had lower mastitis occurrence than animals with average and low antibody (Ch. II) in two of the three herds investigated.

Given that both antibody and cell mediated immune mechanisms are involved in response to infectious disease, it is relevant to evaluate the relationships between antibody and indicators of CMIR. Because negative associations have been reported between antibody and aspects of CMIR, animals categorized on the basis of antibody to OVA may have the inverse rank for CMIR responses (Biozzi et al., 1972; Arthur and Mason, 1986). This would have practical implications if antibody was proposed as a candidate marker of disease resistance of dairy cattle. The objectives of this paper were to evaluate CMI responses with respect to antibody group and to determine if any associations exist with SCS as an indicator of udder health.

2.0. Materials and Methods

2.1. Experimental Design

To evaluate phenotypic variation in CMIR of dairy cattle, 136 Holstein animals from two research herds (Herds 1 and 2, respectively) and one commercial herd (Herd 3) were examined every three weeks from week -3 to six weeks postpartum (week 6). Eighty-eight animals were multiparous cows and 48 were primiparous heifers. To stimulate immune response during the peripartum period, animals received an intramuscular (im) injection of ovalbumin (OVA, Type VII, Sigma Chemical Co., St. Louis, MO) and with a mastitis endotoxemia preventive vaccine, an Rc mutant of *Escherichia coli* O111:B4 (Rhône Mérieux *Escherichia coli* J5, Rhône Mérieux, Lenexa, KS) approximately eight weeks (4 mg OVA) and three weeks (2 mg OVA) prior to predicted parturition dates. At parturition (week 0), animals received a single im immunization injection of OVA (2 mg) dissolved in phosphate buffered saline (PBS - 0.1 M, pH 7.4). Using a mathematical index, animals were classified based on serum antibody to OVA into high (Group 1), average (Group 2) or low (Group 3) response groups (Ch.II). At weeks -3, 0, 3, and 6, peripheral blood mononuclear celss (PBMC) were stimulated *in vitro* with OVA (5 mg/mL) and concanavalin A (Con A) (5 mg/mL), and proliferative response was measured as described below (section 2.4). For lymphocyte proliferative response, week-3 responses of animals that calved early or later than predicted parturition dates were

adjusted to reflect the true time point evaluated (i.e. week -2 or week -4). To evaluate delayed type hypersensitivity (DTH) as a measure of CMIR, a subset (n=36; 15 cows and 21 heifers) of animals from Herd 2 were given a 1.5mg/mL intradermal injection of the Bacillus Calmette Guerin (BCG; Connaught, Mississauga, Ont.) vaccine in the left caudal tail fold at week 1 postpartum.

2.2. Delayed Type Hypersensitivity

Animals vaccinated with BCG (1.5mg/mL) received a 0.1 mL intradermal injection of the PPD of tuberculin (250 US Tuberculin Units: Connaught, Mississauga, Ont.) and for control, received 0.1 mL injection of PBS at week 3 int the right caudal tail fold. The PPD was injected in a designated site approximately 4 cm from the PBS designated site (Appendix III, Fig. 1) and both were located 10 cm from the base of the tail. Prior to injection, sites were encircled with a coloured marker and and double skin thickness measurement was taken in triplicate (time=0), using Harpenden skin thickness calipers (John Bull, England, UK). Forty eight and 72 hours after intradermal injection of PPD and PBS, double skin thickness was measured again. Skin thickness increase at 48 and 72 hours was calculated as follows:

% increase in skin thickness = (((A-B)/B)-(C-D)/D)))X100

where A=mean test thickness (at time=48, 72 hours)

B=mean of pre-test thickness (at time=0 hours)

C=mean of control thickness (at time=48, 72 hours)

D=mean of pre-control thickness (at time=0 hours)

Prior to conducting these experiments it was confirmed that the herd was tuberculin test negative on the basis of negative results in 10 randomly selected animals.

2.3. Lymphocyte Proliferative Response

Lymphocyte proliferation assays were performed according to the procedure of Chang et al. (1993). Briefly, blood was centrifuged (850 *x g*, 15 min) and whole blood buffy coats were diluted in phosphate buffered saline (PBS 0.1M, pH 7.4). Peripheral blood mononuclear cells (PBMCs) were separated from diluted whole blood buffy coats by density gradient centrifugation (1000 *x g*, 30 min) using aqueous Histopaque 1.077 (Sigma Chemical Co. St. Louis, MO.) Cell pellets were washed by centrifugation in PBS (400 *x g*, 7 min) and suspended in culture medium (Rosewell Park Memorial Institute: RPMI- 1640, and 100 I.U. penicillin-streptomycin, prepared by Central Media Laboratory; Ontario Veterinary College, University of Guelph, Guelph, Ont.) and 10% FCS and brought to a final concentration of 2.0 x 10^6 cells/mL. To determine specific clonal proliferative responses to antigen, a stock solution (50 µg/mL) of OVA (Sigma Chemical Co., St. Louis, MO) dissolved in RPMI - 1640 was prepared and stored in small aliquots at -70°C. Five µg/mL of OVA was added to each of 6 replicates of test

PBMC in 96 well flat-bottom plates (Nunc, Fisher Scientific, Don Mills, Ont.). Medium only was added to 6 well replicates of PBMC as non-stimulated controls, to obtain background values for unstimulated cell proliferation. The mitogen, concanavalin A (Con A; Sigma Chemical Co., St. Louis, MO) prepared from stock solution (50 μg/mL) and diluted to (5 μg/mL) for addition was added to 6 replicates of cells on a plate with 6 non-stimulated control replicates. Following 24 h of incubation with OVA or Con A(37°C, 6% CO_2) cells were incubated for 18 h with 0.5 μCi methyl tritiated thymidine per well (ICN Biochemical, Canada Ltd. Montreal, Que.). Plates were frozen until cells were harvested using a plate harvesting system (LKB Wallac, Turku, Finland) onto fiberglass filter mats (LKB Wallac, Turku, Finland). Radioactivity was recorded as counts per minute (cpm) by a beta plate liquid scintillation counter (LKB Wallac,Turku, Finland).

2.4. Flow Cytometric Assay for the Detection of CD Surface Molecules of Peripheral Blood Lymphocytes

Cell phenotypes were characterized after stimulation with either Con A or OVA, by staining with monoclonal antibodies recognizing five cell surface markers as described by Van Kampen and Mallard (1997). The monoclonal antibodies were kindly provided by Dr. Jan Naessens of ILRI (ILRI, Nairobi, Kenya) and included antibodies to the following bovine cell surface markers: CD2+ (IL-A43), CD4+ (IL-A11), CD8+ (IL-A105), WCI

(IL-A29), and IgM (IL-A30). Peripheral blood lymphocytes from a subset of animals (n=10) from Herd 2 (n=7) and Herd 3 (n=3) were evaluated for these lymphocyte cell surface markers at weeks -3, 0, 3, and 6 relative to parturition. Lymphocytes were prepared and cultured as previously described for lymphocyte proliferation assays, however, each 96 well plate was divided into quadrants each with 24 wells. Twenty four replicates each of Con A stimulated (5 μg/mL), OVA stimulated (at 5μg/mL and 20μg/mL) and non-stimulated controls were cultured for 42 hours (the same total duration used in the lymphocyte proliferation assays). After 42 hours, cells were harvested by pipette, washed with PBS and transferred to 10 mL glass test tubes. Cells were centrifuged (400 x *g*, 10 min), and supernatants decanted and cells were resuspended in 250 μL PBS + 0.1M sodium azide (Fisher Scientific, Fairlawn, NJ). Immunostaining was performed in 96-well round-bottom plates (Corning, New York, NY). Fifty μL of cells and 50 μL of diluted primary antibody were added to each well and plates were incubated (20 min, rt). After incubation, 100 μL of PBS 0.1M Azide was added to each well to wash the cells. Cells were suspended by mixing on a shaker and centrifuged (400 *x g*, 6 min). Supernatants were then removed using an aspirator. This washing procedure was performed twice. Fifty μL of FITC-conjugated goat anti-mouse IgG(H+L) (Cedarlane Laboratories, Hornby, Ont.) was then added to the cells and cells were incubated (20 min, rt). After incubation, plates were washed twice as described above. Cells were fixed in 1% paraformaldehyde and transferred into 3 mL polystyrene tubes (Becton Dickinson, Lincoln Park, NJ) containing 300 μL of 1% paraformaldehyde.

Tubes were covered with Parafilm and refrigerated (4°C) μg/mL until time of assay.

A FACS Scan flow cytometer (Becton Dickinson, Lincoln Park, NJ) was used to acquire lymphocyte subset data. LYSIS II software (Becton Dickinson, Lincoln Park, NJ) was used for analyzing data describing the frequency of positively stained cells. Lymphocytes were gated out from other populations based on their forward and side scatter characteristics. Histograms representing fluorescence of cells experessing CD2 (pan T cell), CD4 (helper T cells), CD8 (cytotoxic/suppressor T cells), WC1 ($\gamma\delta$ T cells), and IgM (B cells) cell surface markers were plotted for each cow, timepoint, and culture condition observed. The region of background fluorescence was established with the negative control marker, M1. Events accumulated to the right of this marker were considered positive. (Appendix III, Fig. 2).

2.5. Complete Blood Cell Counts

Complete Blood Cell Counts were determined by the Clinical Pathology Laboratory at the Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada. Counts included the percent and total number of leukocytes, erythrocytes, banded neutrophils, segmented neutrophils, lymphocytes, monocytes, basophils, and eosinophils.

2.6. Milk Somatic Cell Counts

Weekly milk somatic cell counts (SCC), an indicator of subclinical mammary gland infection, were obtained from animals of Herd 1 using the weekly sampling service offered by the Ontario Dairy Herd Improvement Corporation (Ontario DHI). Weekly samples of animals in Herd 2 and Herd 3 sampled 1-4 hours after morning milking were tested for SCC by the Mastitis Laboratory at the Ontario Veterinary College, University of Guelph, Guelph, Ontario Canada. Monthly SCC were obtained from Ontario DHI for all three herds. Somatic cell counts were transformed to somatic cell score (SCS) for analysis. Somatic cell score is the log-linear transformation of SCC in cells/mL and is calculated as follows:

$$SCS=[\log_e(SCC/100)\div\log_e(2)] + 3 \quad \text{(Shook, 1993)}$$

2.7. Statistical Methods

Type III least squares analysis of variance (ANOVA) and corrected means (least square means, LS Means) were generated using the General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS; Helwig and Council, 1982) to evaluate the effects of herd, season-year, cow, antibody group, parity, week, and their interactions on lymphocyte proliferation to OVA and Con A, DTH, complete blood cell counts and SCS

(Table 1). Sources of variation were tested against the mean square (MS) for cow grouped within antibody group and parity to determine significance in the GLM. Sources of variation that were not significant were removed from the model in order to generate LS Means. Unstimulated lymphocyte proliferation was used as a covariate in the GLM for OVA and Con A stimulated lymphocyte proliferation because some variability in unstimulated responses between dairy animals has been described (Burton et al., 1991). Data that did not show a normal distribution (unstimulated lymphocyte proliferation, OVA and Con A stimulated lymphocyte proliferation, and total neutrophils) as indicated by the univariate procedure of SAS (Helwig and Council, 1982), were transformed to natural logarithms. Lymphocyte count data was transformed using a square root transformation. Least square means were converted back to original units from $\log_e$ or square root transformed data. Consequently, standard errors of means are not shown. The Proc CORR procedure of SAS was used to generate Pearson product moment correlation coefficients. Results were considered to be statistically significant if the P-value was <0.05 and trends were reported at the p-value <0.10.

3.0. Results

3.1. Unstimulated in vitro lymphocyte proliferation

Individual cow, week relative to parturition, the interaction between antibody group and

week contributed significantly to variation in unstimulated lymphocyte proliferation (Table 1). Herd did not significantly affect the variation in unstimulated lymphocyte proliferative response. Unstimulated lymphocyte proliferative response significantly ($P<0.05$) declined at parturition, but increased again at week 3 of lactation. When these responses were evaluated by antibody group and week, lymphocyte proliferative responses were significantly higher ($P<0.05$) for animals of the low antibody group compared to animals in the high or average antibody groups (Fig. 1A). The correlation between antibody to OVA and unstimulated lymphocyte proliferation across all groups was negative and significant ($r=-0.26$, $P<0.0001$; Table 2).

3.2. OVA stimulated lymphocyte proliferation

Individual cow and the interaction between antibody group and parity, replicate and unstimulated lymphocyte proliferation, significantly contributed to variation in OVA lymphocyte proliferative response (Table 1). Herd did not significantly contribute to the variation in lymphocyte proliferative responses to OVA. Least square means of lymphocyte proliferation did not differ signficantly across weeks. At weeks 0 and 3, lymphocyte proliferation to OVA was significantly lower for Group 1 ($P<0.01$) compared to Group 3 (Fig. 1B). At week 6, the response of these groups was reversed. The correlation between antibody to OVA across all groups and OVA stimulated lymphocyte proliferation across all groups was negative and significant ($r=-0.27$, $P<0.0001$).

3.3. Con A Stimulated Lymphocyte Proliferation

Individual cow, parity, the interaction between parity and antibody group, antibody group, week and the interaction between week and antibody group significantly contributed to variation in Con A stimulated lymphocyte proliferation. Herd did not signficantly contribute to variation in lymphocyte proliferation to Con A. Least square means of Con A stimulated lymphocyte proliferation declined, though not signficantly, from week -4 and -3 to parturition (Fig. 1C). Proliferative responses increased significantly ($P<0.05$) at week 3 compared to parturition. Group 1 animals had the highest Con A-induced lymphocyte proliferation at weeks -4, -3, 0, 3, and 6. Response decreased in Group 1 (high response) animals from week -4 to parturition and significantly increased from parturition to week 3. Antibody to OVA and Con A-stimulated lymphocyte proliferation was negatively correlated ($r=-0.14$, $P<0.0001$).

3.4. Delayed Type Hypersensitivity (DTH)

Antibody group did not significantly affect variation in DTH response. Cutaneous DTH responses at 48 and 72 hours were highly correlated ($r=0.90$; $P<0.0001$). At 48 hours DTH ranged from 0 to 75% skin thickness increase with a mean of 30.7% (Fig. 2) while 72 hour values ranged from 0 to 79% with a mean of 29.5%. Antibody to OVA at week 3 did not correlate significantly with DTH responses. The DTH response at 48 and 72

hours was negatively and significantly correlated with unstimulated (r=-.21; P<0.002; r=-.17; P<0.01) and Con A (r=-0.29; P<0.0001; r=-.28; P<0.0002) stimulated lymphocyte proliferative responses, respectively. DTH response at 48 hours was significantly and negatively (r=-0.21; P<0.01) correlated with SCS.

3.5. Differential Complete Blood Cell Counts

Counts of segmented neutrophils varied between animals within all herds, but were not significantly affected by week or antibody group. Because banded neutrophils were not observed in every animal, a general linear model could not be used to explain the variation in this response. Counts of lymphocytes tended to decline (P<0.08) from week -3 (4.8 X 10^9 cells /mL) to week 0 (3.9 X 10^9 cells/mL). Differences in lymphocyte numbers between antibody groups were observed only at weeks 3 and 6 of lactation when Group 3 animals tended to have more lymphocytes compared to Groups 1 and 2. Across time, only Group 3 animals had the greatest decline in percent and total numbers of lymphocytes from week -3 to parturition.

3.6. Milk Somatic Cell Score

For Herds 1 and 2, individual cow, week relative to parturition contributed significantly to variation in response. In Herd 3, only the effect of cow accounted for the variation in

response. Least square means of SCS in Herd 1 were lowest for animals of the high antibody group, and greatest in animals of the low antibody group at weeks 3,4,5 and 6 following parturition. Conversely, LS Means of SCS in Herd 2 tended to be lower for animals of the low antibody group compared to animals of the high antibody group. Somatic cell score was negatively and significantly correlated with OVA stimulated lymphocyte proliferative responses in Herd 2 ($r=-.13$; $P<0.0001$). Delayed type hypersensitivity at 48 hours was negatively and significantly correlated with SCS ($r=-0.21$; $P<0.01$).

3.7. Lymphocyte Subsets After Culture

Although lymphocyte subset proportions varied depending on week relative to parturition (week -3 to week +6), the percentage of cells positively expressing CD2+, CD4+, CD8+, WC1+, and IgM were not significantly different between unstimulated control and treatment groups (Appendix III, Fig. 4). Cells expressing IgM were most frequent (38-60%) regardless of treatment and WC1+ cells were least numerous (5-15%) at all time points. Only at week 3 relative to parturition were there more Con A-stimulated lymphocytes expressing IgM (60%) compared to unstimulated controls (40%) or OVA stimulated PBMC (38-40%)(Appendix III, Fig. 3).

4.0. Discussion

Mallard et al. (1997; Ch. V) indicated that in the peripartum period, Holstein animals varied in antibody to OVA and that animals could be grouped into high, average and low antibody groups. The objectives of this study were to evaluate CMI responses with respect to antibody group and to evaluate possible associations with SCS as an indicator of udder health.

The current Th1/Th2 paradigm indicates that host response to infection in mice and humans may result in a profile of cytokines supporting either a cell-mediated or antibody-mediated immune response mechanism (Romagnani, 1997). This paradigm may hold true for cattle and could indicate that animals with a high antibody are likely to have low cell mediated immune responses and visa versa. A study of swine leukocyte antigen (SLA)-defined pigs demonstrated this inverse relationship between antibody and indicators of CMI. Pigs with low antibody response to sheep red blood cells (SRBCs) and (T,G)-A--L had significantly higher DTH responses to dinitrochlorbenzene (DNCB), measured as increased skin thickness, than high antibody response pigs (Mallard et al., 1989). In contrast, a positive relationship apparently exists between antibody response and CMI response in mice. Mice selectively bred for high (H line) and low (L line) antibody response to sheep and pigeon erythrocytes demonstrated similar high and low responses to measures of T cell response such as skin graft rejection (Liacopoulos-Briot

et al., 1972a), graft versus host reaction (Byfield and Howard, 1972), DTH response (Mouton et al., 1974) and *in vitro* proliferation to phytohemaglutinin antigen (Liacopoulos-Briot et al., 1972b). Further, T cell responses among H and L lines of mice was highest for the H line. These H and L lines of mice, and mice selectively bred for high and low antibody responsiveness to Salmonellae flagellar and somatic antigen, were evaluated for DTH response to sheep erythrocytes, *Salmonella typhimurium* and purified protein derivative of *Mycobacterium tuberculosis* (de Oliveira et al., 1985). Although DTH responses to selection antigens and unrelated antigens varied within lines, antibody responsiveness and DTH responses were not correlated. In pigs and mice, therefore, expression of CMI and antibody response are likely polygenic and subject to independent quantitative regulation. Further, the relationship between CMIR and antibody response may differ from species to species. In the current study, animals with the highest antibody (Group 1) had significantly ($P \leq 0.05$) lower unstimulated and OVA-stimulated lymphocyte proliferative responses during the peripartum period while low antibody animals (Group 3) had the highest lymphocyte proliferative responses. Con A-induced lymphocyte proliferation and antibody to OVA however, were not inversely related, because animals with high antibody also had high Con-A stimulated lymphocyte proliferative responses, indicating that relationships between antibody and CMIR may vary. Although no differences in DTH were observed between antibody groups, DTH responses were demonstrated to vary between individuals. This variation in DTH, a measure of CMI, indicates that it may be possible to select animals for enhanced CMI.

Mallard et al. (1992,1998) demonstrated in Yorkshire pigs that both antibody response and CMIR can be used together in a selection index to simultaneously direct both aspects of immune response, with the goal of developing broad-based disease resistance. The results presented here suggest that a similar approach may be valid in dairy cattle once a strong link between immune response traits and disease resistance is established.

Depression of lymphocyte proliferation during the postpartum period has been demonstrated previously in humans (Weinberg, 1984), sheep (Burrels et al., 1978), and dairy cattle (Wells et al., 1977; Manak et al., 1982; Kashiwazaki et al., 1985; Ishikawa, 1987; and Kehrli et al., 1989a). Ishikawa (1987) demonstrated in cows a decreased blastogenic response of PBMC stimulated with Con A and pokeweed mitogen from the third trimester of pregnancy, and reached a minimum at parturition. Saad et al. (1989) described a depressed Con A-, phytohemagglutinin (PHA)-, and PWM-stimulated lymphocyte proliferation that started only 1 week prior to parturition and was minimal one day before parturition. Saad et al. (1989) also evaluated milk mononuclear cell (MC) proliferative responses and, in contrast to PBMC, milk MC did not increase in proliferative response two weeks after lactation. Peripartum depression of lymphocyte proliferation was observed in unstimulated lymphocyte proliferative responses between weeks -3 and parturition (week 0), and a depression of response to Con A was also observed. The largest ($P<0.05$) depression of Con A stimulated lymphocyte proliferative responses at parturition were observed in animals with a high antibody phenotype (Group

1). Again, this may indicate a negative association between high antibody response and certain indicators of CMIR in dairy animals, that would need to be considered in the development of a selection index for high and low immune response.

Table 1. Analysis of Variance of unstimulated and stimulated lymphocyte proliferation to ovalbumin (OVA) and concanavalin A (Con A), lymphocyte and neutrophil number, and somatic cell score

Dependent Variable	$R2^a$ (%)	Cv^b (%)	Herd	Season-Year[c]	Cow[d]	Group[e]	Parity	Group *Parity	Week	Group *Week	Parity* Week
Lymphocyte Proliferation											
Unstimulated	58.69	11.50	--[f]	--	0.0001	ns[g]	ns	0.0001	0.0001	0.05	--
OVA	85.75	6.34	--	--	0.0001	0.01	0.0006	0.0001	ns	0.009	--
Con A	67.20	5.15	--	--	0.0001	ns	0.004	0.0001	0.0001	0.0002	--
Complete Blood Cell Counts											
Lymphocytes	83.16	17.31	--	--	0.0001	ns	0.0001	0.0001	0.08	ns	--
Segmented Neutrophils	37.42	2.81	--	--	0.003	ns	ns	ns	ns	ns	--
Banded Neutrophils	ns	ns	--	--	ns	ns	ns	ns	ns	ns	--
Somatic Cell Score											
Herd 1	83.50	44.89	--	--	0.0001	ns	--	--	0.001	ns	--
Herd 2	81.43	46.70	--	--	0.0001	ns	--	--	0.0001	ns	--
Herd 3	78.84	26.54	--	--	0.0001	ns	--	--	ns	ns	--

a R^2 = coefficient of determination

b CV= coefficient of variation

c Season-Year = season and year of calving

d Cow nested or 'grouped' within the interaction term between antibody group and parity i.e. cow(Group*parity). If parity is not significant, it is removed and the cow term then becomes 'grouped' within antibody group

e Group = variation due to antibody group of animals classified with high, average or low antibody to OVA

f -- = not relevant to that dependent variable and therefore removed from the model

g ns= not significant

Table 2. Correlation analysis of antibody to ovalbumin (OVA) with unstimulated and stimulated lymphocyte proliferation to OVA and concanavalin A (Con A), and delayed type hypersensitivity (DTH) response to purified protein derivative (PPD) of *M. tuberculosis*.

Dependent Variable	Independent Variable	r	P-value
Unstimulated Lymphocyte Proliferation	Antibody to OVA	-0.26	0.0001
OVA-Stimulated Lymphocyte Proliferation	Antibody to OVA	-0.27	0.0001
Con A-Stimulated Proliferation	Antibody to OVA	-0.14	0.0001
DTH - 48 hours	Antibody to OVA	ns	ns
DTH - 72 hours	Antibody to OVA	ns	ns

Figure 1. Type III LS Means of counts per minute (cpm) measuring unstimulated (1A) and stimulated lymphocyte proliferation to ovalbumin (OVA; 1B) and concanavalin A (Con A; 1C). Group 1 = high antibody to OVA, Group 2 = average antibody to OVA, and Group 3 = low antibody to OVA and Population mean =PM. Significant differences between groups are indicated with lower case letters and differences over time are indicated by different upper case letters($P<0.05$).

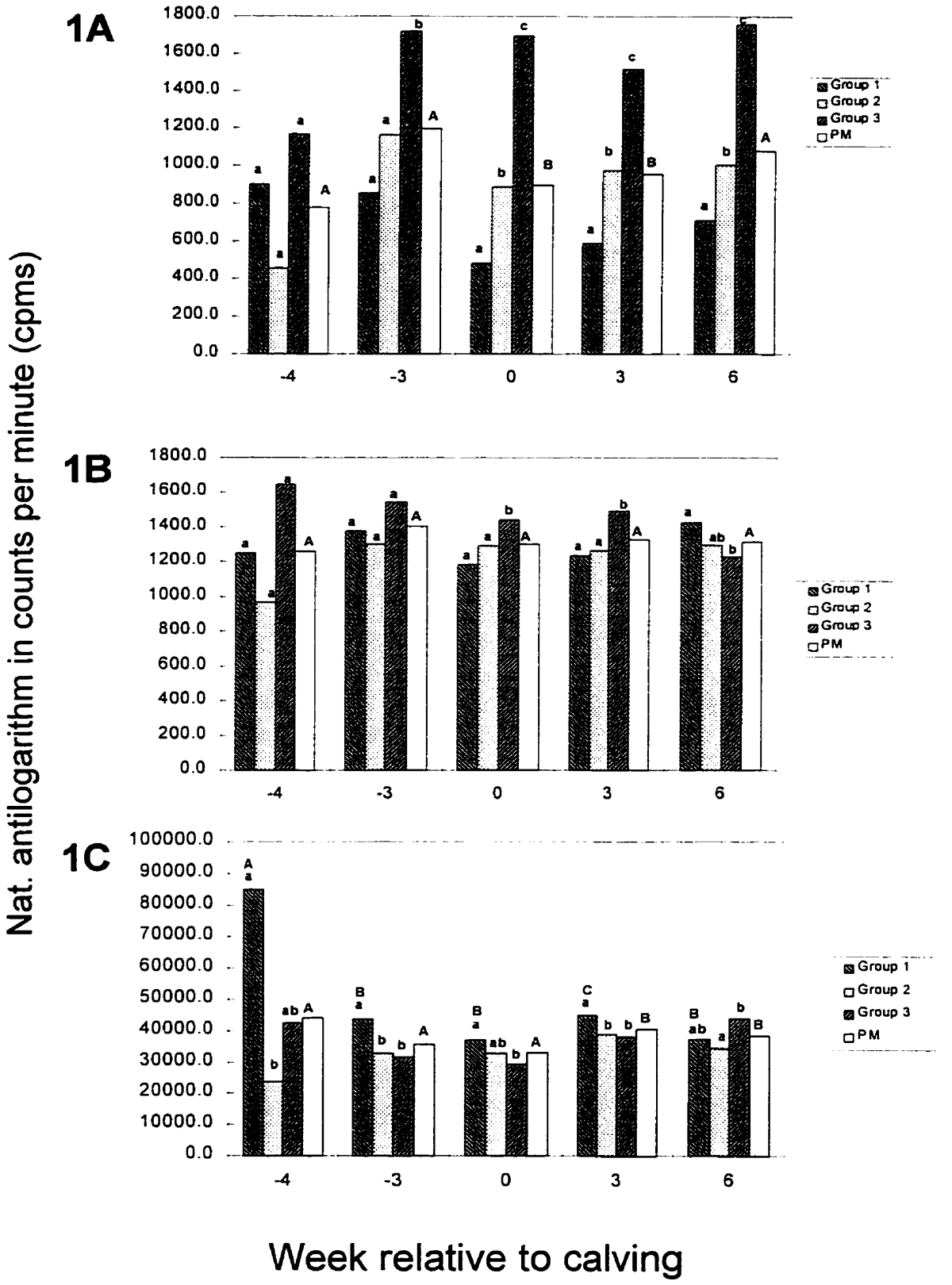
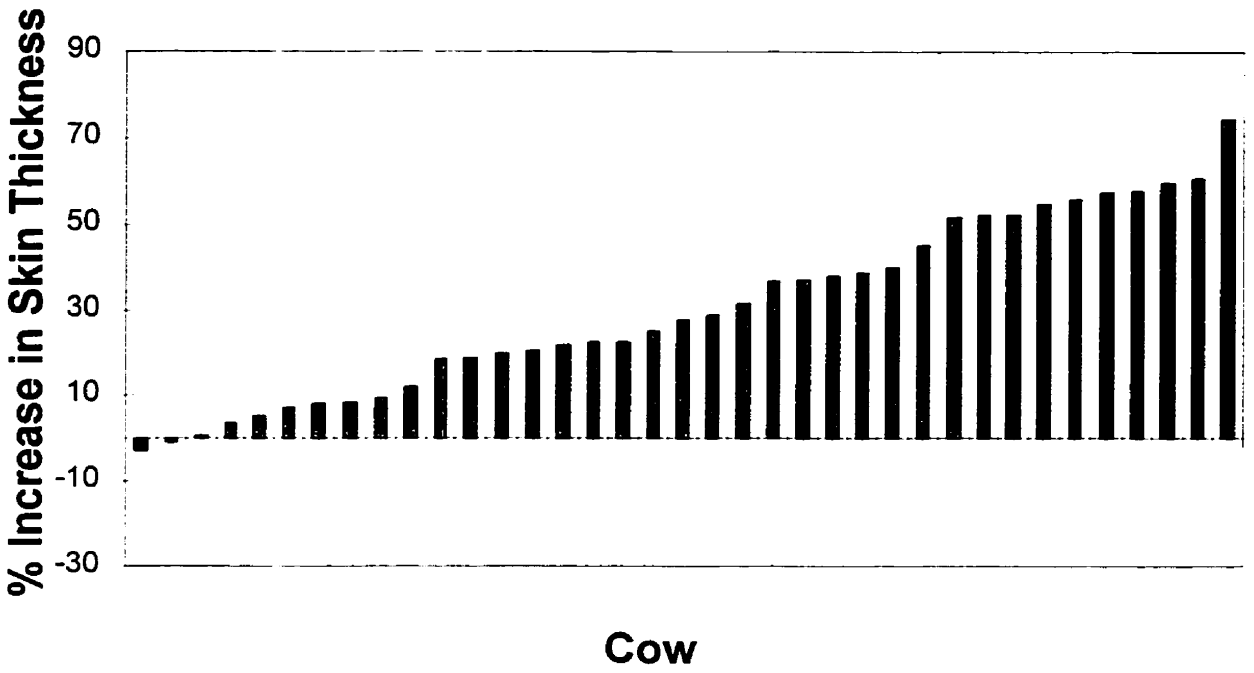

1A
1B
1C
Nat. antilogarithm in counts per minute (cpms)
Week relative to calving
Group 1
Group 2
Group 3
PM
-4
-3
0
3
6

Figure 2. Percent (%) increase in skin thickness 48 hours after challenge with the purified protein derivative of tuberculin (PPD) in cows and heifers previously sensitized to BCG. Response of individual animals is presented in order of rank from lowest to highest (N=36). Each bar respresents the response of one animal.


% Increase in Skin Thickness
90
70
50
30
10
-10
-30
Cow

CHAPTER IV

The Relationship Between Milk Production and Antibody to Ovalbumin (OVA) During the Peripartum Period

Abstract

Suboptimal innate and immune mechanisms of host resistance during the peripartum period may contribute to increased incidence of mastitis. To evaluate associations between antibody to OVA and milk production variables during the peripartum period, 136 Holstein cows and heifers from 3 herds with known antibody profiles, were evaluated for projected 305-day milk, protein, and fat yield. Using a mathematical index, animals were quantitatively classified based on their profile of antibody to OVA into high (Group 1), average (Group 2) or low (Group 3) antibody groups. Group 3 had the highest projected ($P<0.0001$) milk yield (8448.6 kg) compared to Groups 1 (8191.2 kg) and 2 (8174.8 kg). Group 3 also had the highest 305-day projected protein (279.8 kg) and fat yield (343.1 kg) compared to Groups 1 (263.5 kg, 314.0 kg) and 2 (261.4 kg, 314.9 kg) respectively. However, in two out of the three herds investigated, Group 1 animals had no incidence of clinical mastitis compared to other antibody groups. Although this suggests that animals with low antibody produce more milk, fat and protein, and therefore more income, mastitis occurrence was observed to be highest for these animals in two out of three herds investigated.

Keywords: periparturient cattle, antibody, and production

1.0 Introduction

Selection of dairy cows with superior milk production traits has resulted in a steady increase in the incidence of clinical mastitis (Harmon, 1984; Emmanuelson, 1988). Clinical mastitis accounts for a large proportion of costs due to antibiotic therapy, milk withdrawal, veterinary costs, and losses due to culling and death. Losses attributed to subclinical disease include an overall reduction in the quantity and the quality of milk. To help prevent continued increases in mastitis, transmitting abilities of somatic cell score (SCS; the log-linear transformation of SCC) are calculated and are currently incorporated into the total merit index for sire to help prevent continued increases in mastitis (Reents et al., 1995). Various host resistance mechanisms are compromised during the peripartum period, when mastitis and other diseases are frequent. It may be beneficial to identify sires and cows on the basis of potential health-related markers with which to select for indirect reduction in prevalence of mastitis and other infectious diseases. There are several reports of depressed innate and immune response traits in periparturient dairy cows (Kehrli et al., 1989a&b; Gröhn et al., 1992; Detilleux et al., 1995; Cai et al., 1996; Dietz et al., 1997). However, Mallard et al., 1997 (Ch. I) have indicated that animalsvary in their antibody to ovalbumin (OVA) during the peripartum period and that not all animals show depressed antibody around parturition or peak lactation. Further, in Herds 1 and 3, animals with high antibody had no mastitis in contrast to animals in average and low antibody groups (Ch. II). Since sufficient

variation existed to classify animals based on their peripartum antibody to OVA, it may be useful as a candidate marker for selection for disease resistance. However, relationships with production traits are unknown. In pigs selected for high antibody and cell mediated immune responses, favourable associations were observed with production traits such as days to reach market weight, compared to low responding pigs (Wilkie et al., 1997; Mallard et al., 1998). The objective of this study was to evaluate the effect of antibody group on 305-day projected production traits (milk, fat, and protein) and relate production and immune response associations with disease occurrence.

2.0 Materials and Methods

2.1. Animals and Treatments

Phenotypic variation in immune responses of 136 Holstein cows and heifers from 2 research herds (n=32; n=67) and 1 commercial herd (n=37) were examined from week -3 relative to calving (week 0) to six weeks postpartum (week 6). Eighty-eight animals were multiparous cows and 48 were primiparous heifers. As described previously (Mallard et al., 1997; Ch. V), to stimulate antibody during the peripartum period, animals received an intramuscular (im) injection of ovalbumin antigen (OVA , Type VII, Sigma Chemical Co., St. Louis, MO) and a mastitis endotoxemia preventive vaccine, an Rc mutant of *Escherichia coli* O111:B4 (Rhône Mérieux *Escherichia coli* J5, Rhône Mérieux, Lenexa,

KS) approximately 8 weeks (4 mg OVA) and 3 weeks (2 mg OVA) prior to predicted calving dates. At parturition (week 0), animals received a single immunization of the OVA dissolved in phosphate buffered saline (PBS - 0.1 M, pH 7.4) (2 mg, im). Using a mathematical model described previously (Ch. II), animals were categorized based on their antibody to OVA and grouped into high (Group 1), average (Group 2) and low (Group 3) antibody phenotypes.

2.2. Production Variables

Projected 305 day milk, fat, and protein yields were obtained from the Ontario Dairy Herd Improvement Corporation (Ontario DHI). The last test day before the end of lactation was used to calculate projected 305-day milk, fat and protein and was based on at least 100 days in milk (DIM).

2.3. Statistical Methods

Type III least squares analysis of variance (ANOVA) and corrected means (least square means, LS Means) were generated using the General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS; Helwig and Council, 1982) to evaluate the effects of herd, season-year, antibody group, parity, and their interactions on milk, fat, and protein yield (Table 1). Results were considered to be statistically significant if the p-

value was <0.05 and trends were reported at the p-value <0.10.

3.0. Results

3.1. Effects of antibody group on milk production variables

3.1.1. Milk yield

Parity and the interaction between antibody group and parity contributed significantly ($P \leq .0001$) and antibody group tended ($P \leq 0.06$) tended to contribute to variation in projected 305-day milk yield (Table 1). Group 3 animals had a significantly higher ($P<0.0001$) 305-day cumulative milk yield (8448.6 kg) compared to average (8174.8 kg) and high (8191.2 kg) antibody responding dairy animals (Fig. 1A).

3.1.2. Protein

Antibody group, parity and the interaction between antibody group and parity contributed significantly ($P<0.0001$) to variation in protein yield (Table 1). Group 3 animals had a significantly higher ($P<0.0001$) 305-day protein yield (279.8 kg) compared to average (261.3 kg) and high (263.5 kg) antibody responder animals (Fig. 1B).

3.1.3. Fat

Antibody group, parity, and the interaction between antibody group and parity significantly ($P \leq .0001$) contributed to variation in 305-day fat yield (Table 1). Group 3 animals had a significantly higher ($P<0.0001$) 305-day cumulative fat yield (343.1 kg) compared to average (314.9 kg) and high (314.0 kg) antibody responding animals (Fig. 1C).

4.0. Discussion

Although based on relatively few animals, the current study suggests that animals with the highest antibody have lower milk, fat and protein yield. Given the positive genetic correlation between the selection for increased milk production and the increased rate of clinical mastitis occurrence (Emmanuelson et al., 1988), one might hypothesize that superior production could be associated with unfavourable changes in host defense that could result in a higher occurrence of mastitis. The fact that animals of average and high antibody (Groups 1 and 2) tended to produce less milk and milk solids per lactation than animals of the low antibody group might indicate that selection based on antibody to OVA is not economically feasible in the short term. At a price of $5.15/kg of fat and $8.39/kg protein (Ontario Milk Producer, Oct. 1997), animals with low antibody would earn an estimated revenue of Cdn$ 4114.49/lactation (based on fat and protein

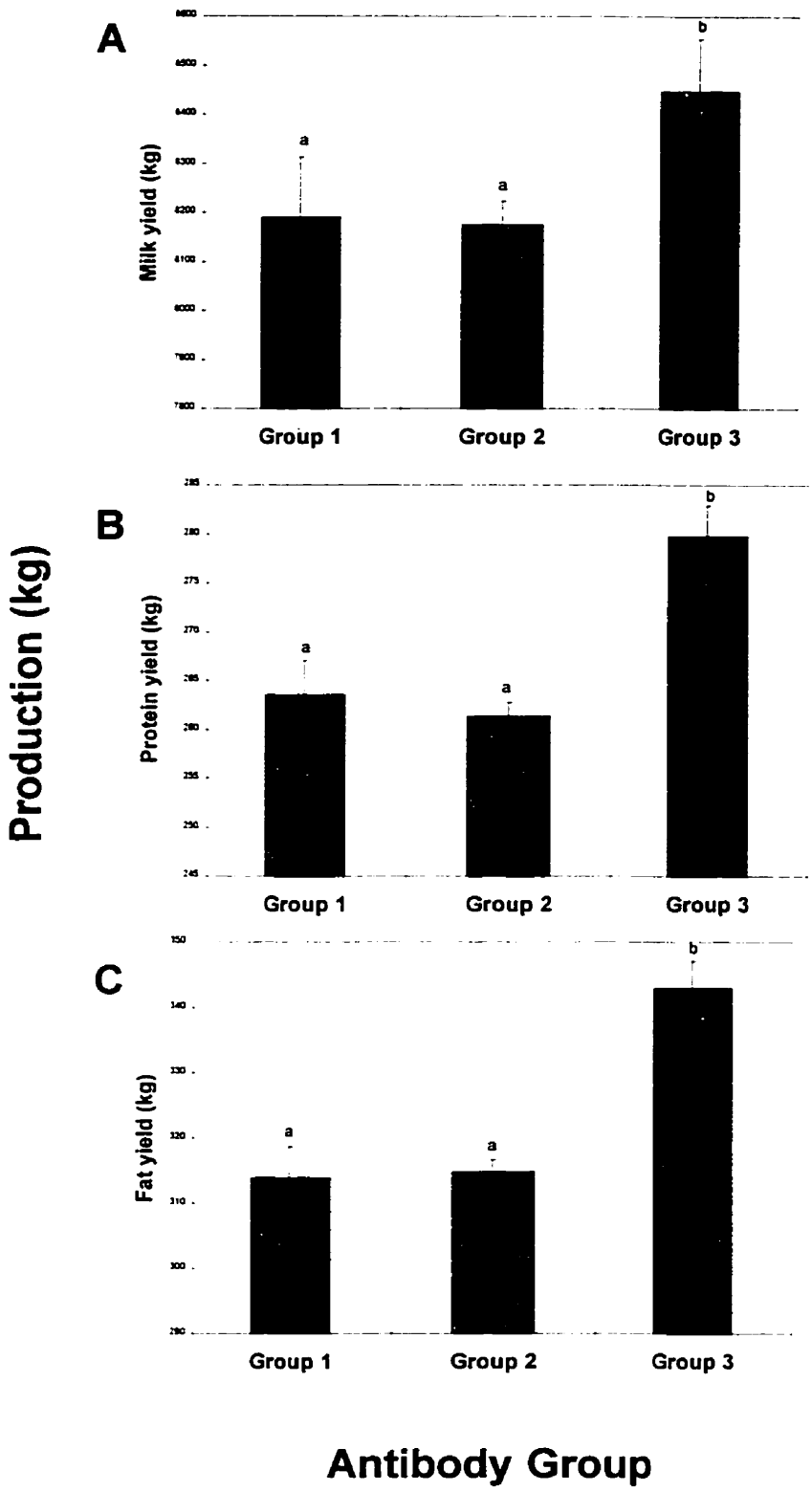
component pricing only) followed by animals with high antibody at $3827.87 per lactation ($286.62 less than Group 3 animals), and animals with average antibody at $3814.04 per lactation ($300.45 less than low antibody animals and $13.82 less than high antibody animals). In the long term however, it may be more beneficial to own animals with superior health traits that minimize disease-related costs (approximately $140-300/cow/lactation in Ontario; Zhang et al., 1993) and still produce milk at an optimal level of production quantity and quality. A previous U.S. study (Dunklee et al., 1994) determined that health costs were positively associated with higher production, however, health costs did not outweigh profit potential. Regardless of whether health costs do or do not have an impact on the production profit potential of dairy animals, reduced occurrence of mastitis will nonetheless be mutually beneficial to dairy producers, processors and consumers. Milk producers will benefit through a reduction in economic loss incurred by mastitis, processors manufacturing milk products will benefit from an enhancement in milk quality, and consumers concerned about animal welfare and food safety standards will appreciate knowing that antibiotic usage to treat mastitis has been reduced as a direct result of reduced mastitis occurrence.

Table 1. Analysis of Variance (ANOVA) of projected 305-day milk, protein and fat yield

Dependent Variable	R^2[a](%)	CV[b](%)	Herd	Season-Year[c]	Group[d]	Parity	Group *Parity
Milk yield	19.50	14.98	--[e]	--	.06	.0001	.0001
Protein yield	15.26	14.76	--	--	.0001	.0001	.0001
Fat Yield	17.51	13.53	--	--	.0001	.0001	.0001

a R^2 = coefficient of determination
b CV= coefficient of variation
c Season-Year = season and year of calving
d Group = variation due to antibody group of animals classified with high, average, or low antibody to OVA
e -- = not relevant to that dependent variable and therefore removed from the model

Figure 1. LS Means of projected 305 day yield for A) milk, B) protein, and C) fat. Group 1 = high antibody, Group 2 = average antibody, and Group 3 = low antibody based on described index, and Population mean (PM). Significant differences between groups are indicated with lower case letters ($P<0.05$).


A
Milk yield (kg)
a
a
b
Group 1
Group 2
Group 3
B
Protein yield (kg)
a
a
b
Group 1
Group 2
Group 3
C
Fat yield (kg)
a
a
b
Group 1
Group 2
Group 3
Production (kg)
Antibody Group

CHAPTER V

Effects of Growth Hormone, Insulin-like Growth Factor-I, and Cortisol on Periparturient Antibody Profiles of Dairy Cattle

Bonnie A. Mallard[1], Lauraine C. Wagter[1], Mary Jane Ireland[1] and Jack C.M. Dekkers[2]

[1]Department of Pathobiology, Ontario Veterinary College,[2] Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

Vet. Immunol. Immunopath. (In press)

Please address all correspondence to:

Dr. Bonnie A. Mallard

Phone: (519) 824-4120 Ext. 4736 Fax: (519) 767-0809
E-mail: bmallard@ovcnet.uoguelph.ca

Department of Pathobiology
Ontario Veterinary College, University of Guelph
Guelph, Ontario, Canada, N1G 2W1

Abstract

The objectives of this study were to determine hormone and antibody profiles from the prepartum period to peak lactation, and evaluate potential immunomodulatory effects of the classic endocrine hormones, growth hormone (GH), insulin-like growth factor-I (IGF-I) and cortisol. Specifically, 33 Holstein cows and heifers were immunized with ovalbumin (OVA) and *Escherichia coli* J5 at weeks -8 and -3 prior to parturition. At parturition (week 0), animals received an additional immunization of OVA. Blood was collected at weeks -8, -3, 0, 3 and 6 relative to parturition and various samples were used to determine plasma hormone concentration, serum immunoglobulin (Ig), and antibody to OVA and *E. coli*. Colostrum and milk samples were also collected post-parturition to monitor local immunoglobulin and antibody. Results indicated that not all periparturient animals exhibited depressed immune response, and that antibody to OVA could be used to partition animals into 3 groups recognizing animals with sustained measurable antibody before and after parturition (Group 1), animals which responded poorly to immunization at parturition (Group 2), and animals which did not respond to immunizations at week -3 or parturition (Group 3). Animals with the highest antibody to OVA (Group 1) also tended ($P \leq 0.10$) to have the highest antibody to *E. coli* J5 at parturition and had the lowest incidence of disease, particularly mastitis. Antibody to OVA measured in milk tended to be higher in Group 1 cows, particularly at week 0 ($P \leq 0.06$) compared to animals of Group 3. IGF-I was higher ($P \leq 0.05$) in animals of Group 1 than Group 3 at peak lactation (week 6).

Keywords: stress, hormones, disease resistance, antibody

1. Introduction

The immune and endocrine systems are integrated biological circuits interacting to maintain homeostasis in changing environments constantly challenged by internal and external stimuli (Griffin, 1989; Blalock, 1994). The bidirectional communication theory, substantiated by the identification of hormone receptors on lymphocytes and the ability of these cells to secrete classic hormones, such as growth hormone (GH) and insulin-like growth factor-I (IGF-I), demonstrates a functional link, and has heralded a new field of research, neuroendocrine-immune modulation (Badolato et al., 1994; Blalock et al., 1994). In light of this functional link, and due to its suggested ability to regulate immune response, considerable research has now focussed on the impact of stress and hormones on immunocompetence. The objectives of this study were to determine peripartum hormone and immune response profiles, and to investigate possible immunomodulatory effects of classic endocrine hormones, GH, IGF-I and cortisol, on antibody and immunoglobulin concentration around parturition using the periparturient dairy cow as a large animal stress model. Throughout her life the dairy cow experiences repeated cycles of physical and metabolic stresses associated with gestation, parturition, and lactational demands. Coincident with these events are well documented changes in hormone release and immunomodulation characterized by depressed innate and immune mediated responses, which often associate with culmination of disease (Peter and Bosu, 1986; Heyneman et al.,

1990; Detilleux et al., 1994). Many studies have reported immuno-enhancement immediately following administration of acute stress (Bachen et al., 1992; Morrow-Tesch et al., 1993) or exercise (Hoffman-Goetz, 1994; Nash, 1994), but long-term application of stress, as with the periparturient dairy cow, seems to lead to depressed innate and immune mechanisms of host defense (Weinberg, 1984; Kehrli et al., 1989; Saad et al., 1989; Gilbert et al., 1993) However, the impact of the peripartum period on antibody has not been previously investigated. Previous studies indicate that the duration and type of stress may have profound influence on both health and performance. The release of glucocorticoids (GC) in response to stress is well characterized in many species and may in part serve to control the magnitude of an immune response (Blalock 1994; Derijk and Sternberg, 1994). Cytokines released during inflammation or infection can stimulate the eventual release of GC, which may act in a feedback loop to control the magnitude of the immune response (Vassilopoulou-Sellin, 1994; Spangelo and Gorospe, 1995). Chronic activation of the hypothalamic pituitary adrenal axis can also result in the suppressed release of GH, which possesses immuno-enhancing properties (Franco et al., 1990; Burton et al., 1991). A reduction of GH may result in the inhibition of IGF-I action on target tissues, such as lymphocytes. In addition, GC may desensitize tissue to IGF-I and thereby diminish its immuno-regulatory effects (Burton et al., 1992).

To further understand the complex endocrine-immune interactions that occur around parturition and their impact on host resistance, we utilized the dairy cow as a large animal stress model of pregnancy, parturition, and lactation. To evaluate peripartum and peak

lactation immune response and hormone profiles, 33 cows and heifers were immunized with ovalbumin (OVA) and *Escherichia coli (E. coli)* J5. Blood samples were collected to measure antibody, GH, IGF-I, and cortisol concentrations at dry-off (approximately 8 weeks prepartum) and weekly from week -3 to week 6 postpartum.

2. Materials and methods

2.1. *Animals and Treatments*

Antibody and hormone profiles of 33 Holstein cows and heifers were examined from approximately eight weeks prepartum (week -8) based on predicted calving dates to six weeks postpartum (week 6). Twenty-six animals were multiparous cows and seven were primiparous heifers. To determine associations between periparturient immune response and hormone profiles, animals received an intramuscular (im) injection of a mastitis endotoxemia preventive vaccine with the manufacturer's adjuvant (Rhône Mérieux *E. coli* J5, Rhône Mérieux, Lenexa, KS) along with the antigen, OVA (Type VII, Sigma Chemical Co., St. Louis MO), at weeks -8 (4 mg) and -3 (2 mg). At parturition (week 0), animals received an additional immunization of OVA without adjuvant dissolved in phosphate buffered saline (PBS - 0.1 M, pH 7.4) (2 mg, im). OVA was chosen as an inert antigen to which these animals had not been previously exposed. *E. coli* J5 was used as an antigen previously recognized by most dairy cows and of more complex response, but of biological relevance. Animals were initially classified according to their serum antibody curve kinetics to OVA

as either high (Group 1) relative to cows that exhibited a reduction in antibody following tertiary immunization postpartum (Group 2) or following both secondary and tertiary immunization postpartum (Group 3) (Fig. 1A).

2.2. *Blood and Milk Sampling Schedule*

Peripheral blood was collected via tail venipuncture at week -8, and weekly from weeks -3 to 6 relative to parturition. Various samples were used to monitor plasma hormone concentrations (GH, IGF-I, cortisol), serum immunoglobulin $G_{1\&2}$, and antibody to OVA and *E. coli* J5. Colostrum and milk samples were collected to monitor antibody to OVA and to monitor total IgG_1 (weeks 0, 3, 6) and IgG_2 (weeks 0 and 3). Colostrum was collected at the first milking following parturition. Milk samples were stripped from all quarters approximately 2-4 hr after morning milking. Colostrum and milk samples were stored frozen without preservative at -20°C until time of whey separation and immunoglobulin quantification.

2.3. *ELISA for OVA Antibody Detection In Serum and Whey*

Serum was separated from coagulated peripheral blood by centrifugation and stored frozen (-20° C) until time of assay. Milk samples were stored frozen (-20° C) until time of assay when they were centrifuged twice (11,000 g, 15 min) to separate fat from whey. Antibody to OVA was detected by ELISA and quantified based on optical density measurements

according to the procedure described by Burton, et al. 1993. Briefly, 96-well polystyrene plates (Fisher Scientific, Don Mills, Ont.) were coated with a 3.11×10^{-5} M solution of OVA (OVA, Type VII, Sigma Chemical Co., St. Louis MO) dissolved in carbonate-bicarbonate coating buffer (pH 9.6). Plates were incubated (4°C, 48h), then washed with PBS and .05% Tween 20 solution, (pH 7.4). Plates were blocked with a PBS-3% Tween 20 solution and incubated (room temperature; rt, 1h). Plates were washed and diluted test sera (1/50 and 1/200) or milk whey (Neat, 1/10, 1/100 and 1/400) and controls were added using a quadrant system (Wright, 1987). Sera samples were added in duplicate, and whey samples were added in quadruplicate. Negative and positive controls included a pooled sample of pre-immunization sera and a pooled sample of sera from animals 14 days post secondary immunization respectively. Plates were incubated at rt for 2h. Subsequently, alkaline phosphatase conjugate rabbit anti-bovine IgG (whole molecule) (Sigma Chemical Co., St. Louis, MO) was dissolved in wash buffer, added to the plates and incubated (rt, 2h). P-Nitrophenyl Phosphate Disodium tablets (pNPP) (Sigma, St. Louis, MO) were dissolved in a 10% diethanolamine substrate buffer, (pH 9.8). Plates were washed with wash buffer. pNPP was added to the plates and was then incubated at rt for 30 minutes (min). Plates were read on a EL311 automatic ELISA plate reader (BIO-TEK Instruments, Highland Park, VT) and the optical density (OD) was recorded at 405 and 630 nanometres (nm) when the positive control reached OD≥.999. The mean of the number of replicates added to each plate was corrected to an OD = 1.0 by multiplying by the inverse of the mean of the positive controls. Corrected means of each dilution were then added together to give an additive OD value.

2.4. *ELISA for E. coli J5 Antibody Detection In Serum*

According to the method described by Rhône-Mérieux Animal Health (Lenexa, KS; 1994 personal communication), heat-killed *E. coli* strain J5 (ATCC, Rockville, MD) was coated at a concentration of 6.25 x 10^7 colony forming units per mL onto Dynatech Immulon II polystyrene 96-well flat bottom plates overnight at 4°C. After washing with wash buffer (PBS plus .05% Tween 20), 1% gelatin was added to block non-specific binding and plates were incubated (rt, 1h). Plates were washed and four replicates of test serum (dilutions of 1/1000, 1/1500, 1/2000 and 1/2500) were added using a modified quadrant system. PBS-.05%Tween 20 was used as a blank and fetal calf serum (FCS, Bockneck Laboratories, Can Sera, Rexdale, Ont.) was used as a negative control. Negative and positive controls prepared from pooled pre- and post immunization sera were plated respectively. Test sera were incubated (rt, 2h), and plates were washed with PBS-.05% Tween 20. Horseradish peroxidase conjugate goat anti-bovine IgG whole molecule in PBS (1/4000; The Binding Site, Birmingham, UK) was added and the plates were incubated (rt, 1h). After washing, the substrate, 2,2'-azino-di-(3-ethyl-benzthiazoline sulphonate-6) (ABTS; Boehringer Mannheim, Laval, Que.) was added and plates were incubated (rt, 30 min). Plates were then read on an EL311 automatic ELISA plate reader (BIO-TEK Instruments, Highland Park, VT) and OD recorded at 405 nm and 490 nm. The mean OD of the four sample replicates were corrected to an OD=1.0. Based on the immunization protocol and phenotypic observation of antibody curve kinetics of all dilutions tested, the 1/1000 dilution consistently allowed for differentiation between positive and negative controls, exhibiting minimal prozone effect and

therefore was the dilution of choice for comparison between animals.

2.5. *Radial Immunodiffusion Assay*

Radial immunodiffusion was used according to a method previously described (Mallard et al. 1992) to determine the concentrations of $IgG_{1\&2}$ in serum and whey from colostrum and milk. Whey from weeks 0 and 3 were tested for the $IgG_{1\&2}$ subclasses. At week 6 however, IgG_1 only was tested in whey since very low concentrations of IgG_2 exist in normal milk (Butler, 1980).

2.6. *Disease Occurrence*

Occurrence of infectious and metabolic diseases were recorded throughout the study period since connections within the endocrine-immune axis may conceivably affect both. All disease events were recorded by the herd manager. If an animal had two or more episodes of the same disease event, it was recorded as one event for the study period.

2.7. *Somatic Cell Count*

Milk (AM/PM composite sample) was collected weekly during milking to determine somatic cell count (SCC). Only the SCC counts which coincided with the day of blood sample collection for each week are reported. SCC, an indicator of subclinical mammary gland

infection, was transformed to somatic cell score (SCS) for analysis. SCS is the natural logarithm of SCC in cells/μL and is calculated as follows (Shook, 1993):

$$SCS=[\log_e(SCC/100) \div \log_e(2)] + 3$$

2.8. *Hormone Assays*

Peripheral blood samples collected for hormone assay were immediately put on ice. Samples were centrifuged at 4°C and the plasma was removed. Plasma from each cow was individually aliquoted into multiple 1 mL containers and stored frozen at -20°C until time of each hormone assay.

2.8.1. *Cortisol*

Plasma cortisol concentration (μg/dL) was determined using a commercially available Gamma Coat Cortisol ^{125}I, RIA kit (INCSTAR Corporation, Stillwater, MN). The assay sensitivity was 0.21 μg/dL and the inter- and intra-assay CV were less than 10%.

2.8.2. *IGF-I and GH*

Radioimmunoassay (RIA), as described previously by Elsasser et al. (1989), was used to determine the IGF-I concentration (ng/mL) of samples. The IGF-I used for tracer and

standards was recombinant threonine-59-substituted human IGF-I (Amgen; Thousand Oaks, CA). Based on duplicate samples, all performed on the same day, the intra-assay CV was less than 10%. GH concentration (ng/mL) was quantified using RIA (Elsasser et al., 1988).

2.9. *Statistical Methods*

Least squares analysis of variance (ANOVA) and corrected means (least square means, LS Means) were generated using the General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS; Helwig and Council, 1979). The statistical models used in this study included fixed effects of antibody groups (1,2,3), cow nested within antibody group, and week relative to parturition (weeks -3, 0, 3, and 6). In preliminary analysis, the effect of parity was not significant and was therefore removed from all subsequent models. A model was constructed for the following dependent variables: antibody to OVA in sera and whey, antibody to *E. coli* J5 in sera, and the concentration of $IgG_{1\&2}$ in serum and whey. Sources of variation included in the model for each dependent variable are summarized in Table 1. Hormone concentrations (GH, IGF-I, cortisol) were included as covariates in all models. Data that did not show a normal distribution as indicated by the univariate procedure of SAS, were transformed to natural logarithms. Pearson product moment correlation coefficients between immune response variables and hormone concentrations were generated using the correlations procedure of SAS (Proc CORR). Results were considered to be statistically significant if the P-value was $\leq .05$ and trends were reported at the P-value $\leq .10$.

3. Results

3.1. *Antibody to OVA*

3.1.1. *Serum*

Serum antibody to OVA varied significantly over the peripartum period and individuals could be readily classified into three antibody groups: the high antibody group (Group 1, n=12; 6 heifers, 6 cows) relative to animals which exhibited a reduction in antibody following immunization either postpartum (Group 2, n=12 cows) or pre- and postpartum (Group 3, n=9; 8 cows, 1 heifer). Approximately 1/3 (Group 1) of the animals showed consistent, above average serum antibody to OVA following immunization at weeks -8, -3, and 0 relative to parturition. The remaining animals had either an average amount of antibody, or had antibody lower than the population mean and had a reduction following immunization at week -3 and/or 0 relative to parturition (Fig. 1A). At week -3 , all animals including those of Group 3 exhibited had greater antibody than background (week -8). At week -3 therefore, Group 3 animals were considered to have low antibody rather than have no exhibited no response to immunization. ANOVA indicated that the statistical model accounted for 94.19% of the total variation in serum antibody to OVA over the peripartum period, and that the effects of cow ($P \leq .0001$), antibody group ($P \leq .005$), and the interaction between antibody group and week ($P \leq .0001$), contributed significantly to the variation in antibody to OVA (Table 1). Growth hormone (GH) exhibited some tendency to be positively associated with antibody to OVA ($P \leq .15$). Animals in Group 1, with the highest

antibody to OVA, tended to have the highest GH concentrations in plasma at each sample week in comparison to animals in Groups 2 and 3, but these differences were not significant (Fig. 2A). Correlation analysis also indicated a significant and positive relationship (r^2 = .29, P≤.001) between antibody to OVA and GH, regardless of week or antibody group (Table 2). LS Means of IGF-I and cortisol concentrations in plasma (Fig. 2B,C) were not significantly different between antibody groups, except at week 6 when Group 1 animals had a higher concentrations of IGF-I (P≤.05) compared to animals in Group 3 (Fig. 2B). Correlation analysis of antibody to OVA indicated relationships with IGF-I (r^2=-.19, P≤.04) and cortisol (r^2=.17, P≤.06) (Table 2).

3.1.2. *Whey*

ANOVA indicated that cow (P≤.006), antibody group (P≤.003) and the interaction between IGF-I concentration and week (P≤.005) contributed significantly to the variation in whey antibody (Table 1). There was a tendency for week relative to parturition (P≤.06) to associate with antibody to OVA in whey. Corrected population least square means (LS Means) of antibody to OVA in whey declined significantly following parturition, such that at week 0 the OD value was 1.68 ± .17 compared to .85 ± .17 (P≤.004) at week 3 and .50 ● .20 (P≤.0001) at week 6 (Fig. 1B). At parturition, there was a tendency (P≤.06) for antibody to OVA in whey to differ between Groups 1 (1.96±.26) and 3 (1.33±.23). Comparable to the antibody to OVA in serum, correlation analysis indicated a significant relationship between OVA antibody in whey with GH (r^2 = .31,

P≤.0005) and IGF-I (r^2 = -.22, P≤.01) (Table 2).

3.2. *Antibody to E. coli J5*

Only the effect of cow (P≤.0002) contributed significantly to the variation in antibody to *E. coli* J5 . Pre-immunization sera (week -8) indicated that these animals had minimal background OD values of measurable *E. coli* J5 antibody prior to vaccination (population mean ± SEM = .314 ± .11; n=33) compared to post-vaccination natural antilogarithm OD values at week -3 (.663) and week 0 (.830). Antibody to *E. coli* when grouped by antibody group (1, 2, or 3), indicated that only at parturition (week 0) did Group 1 animals tend (P≤.10) to have a higher concentration of *E. coli* antibody (OD value = 1.053) than Group 3 animals (OD value =.702). Correlation analysis indicated that GH was significantly correlated with antibody to J5 *E. coli* (r^2=.18, P≤.04) (Table 2). Antibody to *E. coli* J5 was positively correlated with antibody to OVA (r^2=.59, P≤.0001).

3.3. *IgG_1 & IgG_2 in serum, colostrum, and milk*

Antibody group significantly contributed to the variation of serum IgG_2 (P≤.002) only. There was a tendency for the interaction between IGF-I and week relative to parturition to account for variation in total whey IgG_1 concentration (P≤.07). The model constructed for whey IgG_2 was unable to explain the variation in this response. Correlation analysis indicated a significant, negative relationship between GH and IgG_1 in serum (r^2=-.26;

P≤.01). Conversely, IGF-I tended to correlate positively with total IgG_1 in serum (r^2=.19, P≤.07). Growth hormone (r^2=.26, P≤.03) and IGF-I (r^2=-.20, P≤.10) correlations with IgG_1 in whey were reversed from that in serum.

3.4. *Disease Occurrence*

Records of disease events indicated that 54.5% of the 33 animals evaluated were considered healthy during this study. Of the diseased animals, 7 animals had mastitis events (21.21%), 7 had ketosis events (21.21%) and 3 animals had other disease events (9.09%) while on this study. Group 1 animals which showed a consistent above average antibody to OVA, had the lowest percent occurrence of disease overall (Fig. 3) and actually had no occurrence of clinical mastitis. According to a t test of significance, disease occurrence overall was not significantly different between antibody groups, but mastitis occurrence for Group 1 animals was significantly (P<0.05) lower than for Group 3 animals.

3.5. *Somatic Cell Score (SCS)*

At parturition, LS Means of SCS were lowest for Group 2 animals (SCS=3.2) compared to Group 1 (SCS=4.36) and Group 3 (SCS=4.98) cows. At weeks 2,3,4, and 6 after parturition, Group 1 animals consistently had the lowest SCS while Group 3 animals consistently had the highest SCS.

3.6. *Hormones*

At parturition, least square mean (LS Mean) concentrations of GH (Fig. 2A) and cortisol (Figure 2C) were at a maximum while IGF-I (Fig. 2B) was at a minimum. After parturition, GH concentrations decreased ($P \leq .05$) until week 6. Cortisol concentrations also decreased ($P \leq .05$) post-parturition and then increased slightly after week 3. In contrast, IGF-I concentrations decreased ($P \leq .05$) at parturition and then continued to increase ($P \leq .05$) toward peak lactation.

4.0. Discussion

It is well accepted that aspects of host defense are impaired at parturition in many species, including the dairy cow (Weinberg, 1984; Clarke and Kendall, 1994; Detilleux et al. 1994; Detilleux et al., 1995). Further, it has been suggested that normal physiological conditions, specifically hormone fluctuations occurring concurrently with the progression of pregnancy and in response to the stress of parturition, may contribute to immunodepression. Although previous investigations have established suboptimal polymorphonuclear (PMN) cell counts and mitogen-driven lymphocyte proliferation during parturition in dairy cows, this is the first study to challenge the humoral component of the periparturient immune system, as measured by antibody to antigen before and after parturition.

Antibody to OVA, an inert antigen to which these animals had not been previously exposed, could be utilized to partition animals into 3 antibody groups recognizing animals with sustained antibody before and after parturition (Group 1), animals which had a reduction in antibody following immunization at parturition (Group 2), and animals which had a reduction in antibody after immunization pre- and postpartum (Group 3). Variation in antibody to *E. coli* J5, a biologically relevant antigen , was more difficult to partition. This may be due to previous or concurrent exposure to gram negative bacteria. Although antibody to OVA and *E. coli* J5 were significantly correlated, only at parturition did Group 1 animals tend ($P \leq .10$) to have the highest antibody to *E. coli* J5.

Nagahata et al. (1992), examined B lymphocyte populations to evaluate host defense in dairy cows during the periparturient period and found no significant changes in the number of B lymphocytes of cows from 2 weeks before until 2 weeks after parturition. however they did report a significant decrease in plaque forming cells immediately after parturition. They felt this indicated a decrease in function of B lymphocytes during the immediate postpartum period. This may correspond to the low peripartum antibody seen in some animals in this study.

Although it has been reported that low serum antibodies in cattle at parturition may be due to sequestration of immunoglobulin (Ig) into the mammary gland (Detilleux et al., 1995), our study would suggest that lower antibody in serum does not necessarily relate to Ig transport. For instance, Group 1 cows with the highest serum antibody also tended

to have higher whey antibodies to OVA postpartum when compared to cows of Groups 2 and 3. Initially, it was questioned whether low serum antibody may be associated with higher local antibody in the colostrum or milk. Instead, this data suggests that animals with high serum antibody also supply higher concentrations of antibody to the mammary gland. This may be important when trying to devise vaccination strategies which provide optimum immunity to both the peripartum cow and her calf.

Although more study is required to evaluate the consistency and breadth of antibody response before and after parturition in subsequent lactations, this study has demonstrated significant individual variation during the peripartum period, and shows that not all cows experience peripartum depression of antibody. It is worth noting that in swine, inherently high and low immune response individuals can also be identified in a population (Mallard et al., 1992). In light of previously reported heritability estimates of bovine antibody response (Burton et al., 1989) this data may suggest that Group 1 animals could be inherently better able to produce antibody in spite of the metabolic and physical stresses experienced during parturition. Peripartum heritability estimates of antibody response remain to be established, as does how these animals respond to other test antigens. However, animals in Group 1 did have the lowest incidence of peripartum disease, particularly mastitis (0% occurrence), and lower SCS scores than Group 2 and 3 animals following parturition, thus indicating that antibody response should be evaluated further as a potential marker of peripartum disease resistance.

The present study is the first to simultaneously evaluate antibody and hormone profiles during the peripartum period and has revealed some associations between IGF-1 and antibody, however, no actual cause and effect relationship can be established from this study. In addition, an elevation of plasma IGF-I during the latter stages of pregnancy followed by a dramatic decline around parturition with a steady increase in concentrations following parturition was demonstrated. Some of these observations have been confirmed in the literature; for instance, Vega et al. (1991) attributed changes in IGF-1 and GH to the decrease in metabolic demands associated with the cessation of milk production, during late gestation, followed by an increase in metabolic demand associated with the onset of lactation at parturition. As well, the demand of the mammary gland may alter the transport of IGF-I by sequestering it from the blood. Lactogenic hormones, such as prolactin and cortisol, may also prevent the synthesis of IGF-I and IGF-I binding proteins (Vega et al., 1991).

As previously reported (Hoshino et al., 1991), circulating concentrations of GH increased around parturition, concurrent with early milk production, and decreased as lactation progressed. The inverse relationship between peripartum IGF-I and GH is noteworthy in that IGF-I production is normally dependent on GH as blood concentrations influence liver production of IGF-I (Burton et al., 1992). However, due to the peripartum uncoupling between these two hormones, it may be possible to evaluate the influence of each hormone separately on both the innate and humoral aspects of the immune system.

Although the interaction of GH, and to a lesser extent IGF-I, with the immune system has been widely reported in a variety of species including dogs, humans and mice, direct effects of GH on lymphoid cells have not been unequivocally demonstrated. For example, GH deficient patients often are not found to be immuno-compromised (Fornari et al., 1994). Furthermore, various studies have demonstrated that the immune systems of GH deficient children treated with GH can be normal, suppressed or even enhanced (Gupta et al., 1983; Kelley, 1990; Petersen et al., 1990). It is also suggested that some effects of GH on the immune system are a result of IGF-I (Burton et al., 1992; Badolato et al., 1994). In general these studies indicate that the precise relationships between these hormones and immune responsiveness will be challenging to untangle. In the present study, GH concentration was positively, and IGF-I negatively correlated with antibody. Although not statistically significant, animals in Group 1 with the highest antibody to OVA, tended to have the highest GH concentrations. For the most part, IGF-I concentrations were not different among immune response groups, except at week 6 when animals of Group 1 had significantly higher concentrations than Group 3 cows. These results are consistent with the work of Yoshida et al., (1992) which demonstrated that GH stimulates B cell growth and Ig synthesis by B cells and B cell lines. Growth hormone has been reported to alter antibody synthesis in response to T-dependent antigens, as well as increase activity of T lymphocytes and natural killer (NK) cells (Geffner et al., 1990; Schurmann et al., 1995). Badolato et al., (1994) found that B cells displayed relatively high numbers of GH receptors, whereas T and NK cells showed much lower numbers of receptors. In addition, increased GH concentrations can enhance otherwise suppressed

antibody response due to stress released glucocorticoids (Franco et al., 1990). Again, it has been suggested that the effects of GH may be mediated through IGF-I, a lymphocyte growth factor (Franco et al., 1990), but whether this is true during the peripartum period when these hormones become uncoupled seems unlikely.

5.0. Acknowledgements

Special thanks are extended to Dr. Essi Evans and the Shurgain Agresearch staff, Marion Wilke and Sophia Lim for technical assistance, Dr. B.N. Wilkie and Dr. Catherine Bomben for their helpful comments in the preparation of the manuscript, and the Natural Sciences and Engineering Research Council for financial support.

Table 1. Analysis of variance of antibody to ovalbumin (OVA) and *E. coli* J5, and the concentration of immunoglobulin $G_{1\&2}$ in serum and whey

			Source of Variation									
Dependent Variable	R^2[a] (%)	C.V.[b] (%)	Cow (Group)[c]	Week	Group[d]	Group* Week	GH[e]	GH* Week	IGF-I[f]	IGF-I* Week	Cort[g]	Cort* week
Antibody												
Serum OVA	94.19	14.01	.0001	ns[h]	.005	.0001	.15	ns	ns	ns	ns	ns
Whey OVA	83.81	37.36	.006	.06	.003	ns	ns	ns	.12	.005	ns	ns
E. coli	79.10	97.18	.0002	ns	ns	ns	ns	ns	ns	ns	ns	ns
Immunoglobulin												
Serum IgG_1	73.17	7.88	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Serum IgG_2	75.47	4.63	ns	ns	.001	ns	ns	ns	ns	ns	ns	ns
Whey IgG_1	94.66	16.46	ns	ns	ns	ns	ns	ns	ns	.07	ns	ns
Whey IgG_2	87.25	30.92	ns	-------[i]	ns	--------	ns	-------	ns	-------	ns	--------

a R^2 = coefficient of determination
b C.V. = coefficient of variation
c Cow(Group) = Cow nested within group
d Group= variation due to antibody group of animals classified with high, average, or antibody to OVA
e,f,g = growth hormone, insulin-like growth factor-I, cortisol,
h ns= not significant
i ------- = not relevant to that dependent variable

Table 2. Correlation analysis of hormone concentration with antibody to ovalbumin (OVA), and *E. coli* J5, and the concentration of $IgG_{1\&2}$ in serum and whey

Dependent Variable	Independent Variable	r[a]	P value
Antibody			
Serum OVA	GH[b]	.29	.001
	IGF-I[c]	-.19	.04
	Cortisol[d]	.17	.06
Whey OVA	GH	.31	.0005
	IGF-I	-.22	.01
	Cortisol	--	ns
E. coli J5	GH	.18	.04
	IGF-I	--	ns
	Cortisol	--	ns
Radial Immunodiffusion			
Serum IgG_1	GH	-.26	.01
	IGF-I	.19	.07
	Cortisol	--	ns
Serum IgG_2	GH	--	ns
	IGF-I	--	ns
	Cortisol	--	ns
Whey IgG_1	GH	.26	.03
	IGF-I	-.20	.10
	Cortisol	--	ns
Whey IgG_2	GH	--	ns
	IGF-I	--	ns
	Cortisol	--	ns

a r^2 = SAS Pearson Product Moment Correlation Coefficient
b, c, d, = growth hormone, insulin-like growth factor-I, cortisol

Fig. 1. LS Means of antibody to OVA in A) serum and B) whey by antibody group following immunization at weeks -8, -3, and 0 as measured by enzyme linked immunosorbent assay (ELISA). Group 1 = high antibody; Group 2 = reduction in antibody following tertiary immunization postpartum (week 0); Group 3 = reduction in antibody following secondary and tertiary immunizations pre- and postpartum, respectively; Pop = population mean. Animal classification is based on serum antibody to OVA. Significant differences between animals in the three groups are indicated by different letters above error bars ($P \leq .05$).

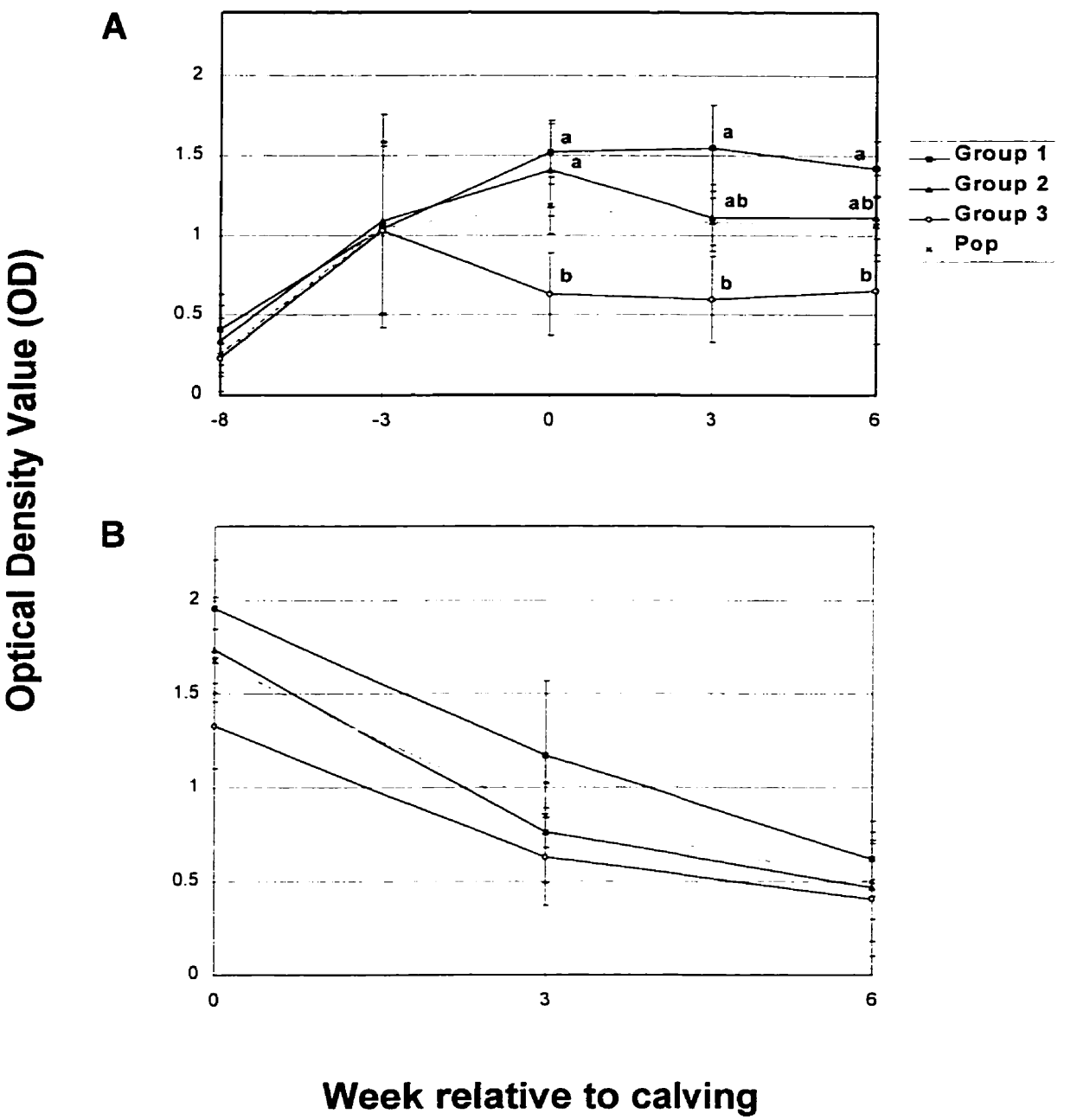

A
B
Optical Density Value (OD)
Week relative to calving
Group 1
Group 2
Group 3
Pop
a
a
a
a
ab
ab
b
b
b
2
1.5
1
0.5
0
-8
-3
0
3
6

Fig. 2. LS Means of hormone concentrations by antibody group as determined by radioimmunoassay (RIA). A) growth hormone (GH), B) insulin-like growth factor-I (IGF-I), and C) cortisol. Group 1 = high antibody; Group 2 = reduction in antibody following tertiary immunization postpartum (week 0); Group 3 = reduction in antibody following secondary and tertiary immunizations; Pop = population mean. Animal classification is based on serum antibody to ovalbumin (OVA). Nat. log. = natural logarithm. Significant differences between animals in the three groups are indicated by different letters above standard error bars ($P \leq .05$).

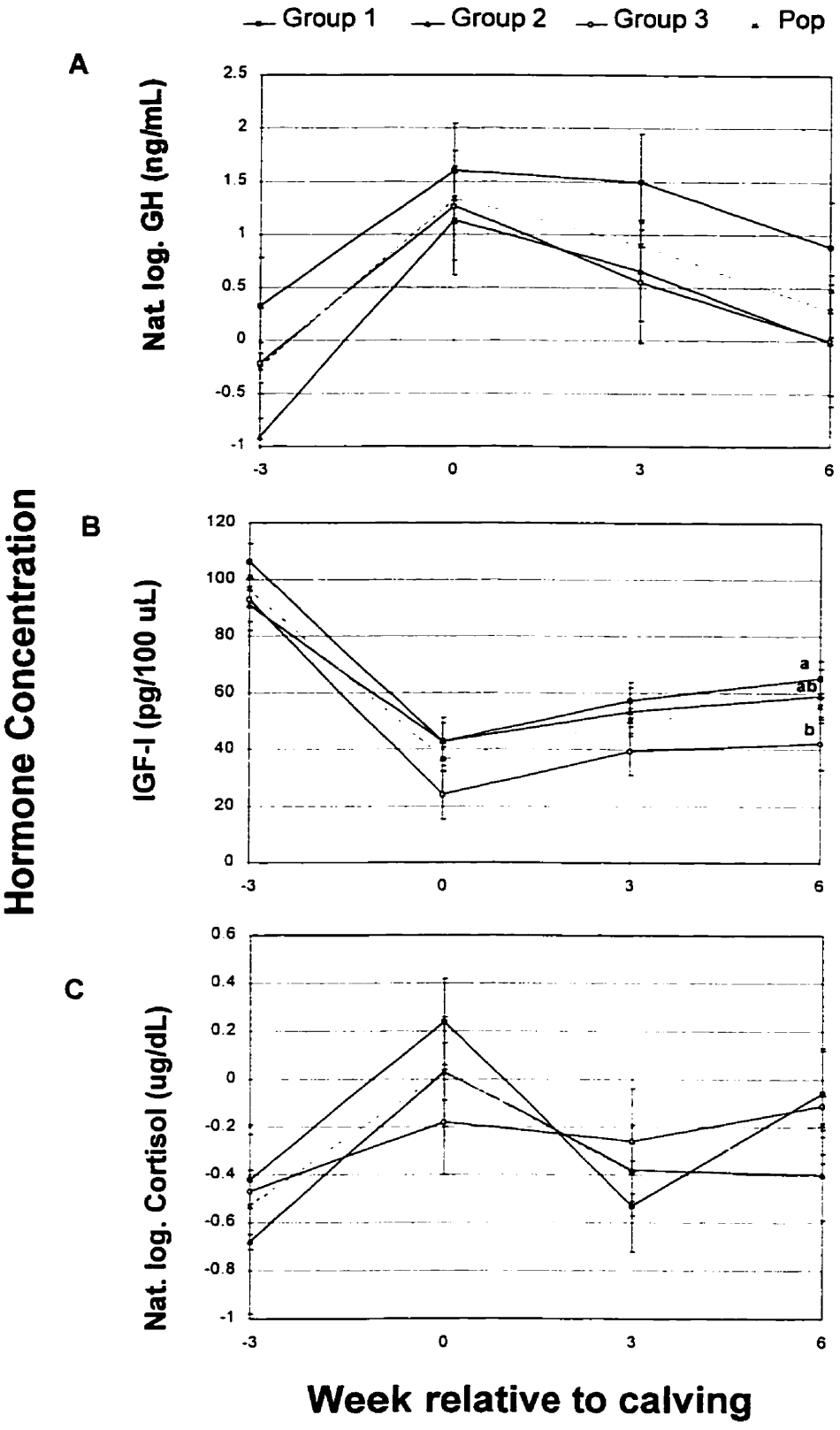
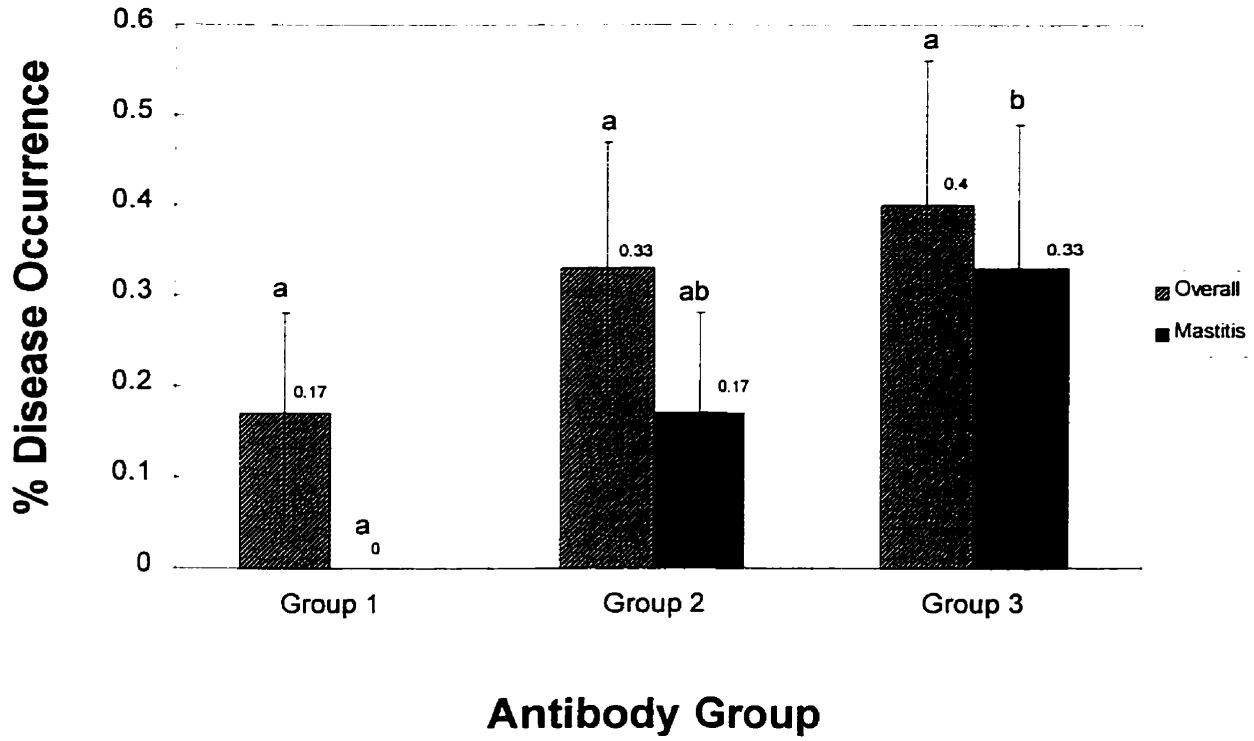

Group 1
Group 2
Group 3
Pop
A
Nat. log. GH (ng/mL)
2.5
2
1.5
1
0.5
0
-0.5
-1
-3
0
3
6
B
IGF-I (pg/100 uL)
120
100
80
60
40
20
0
a
ab
b
C
Nat. log. Cortisol (ug/dL)
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
Hormone Concentration
Week relative to calving

Fig. 3. Percent (%) overall disease and mastitis occurrence by antibody group. Group 1 = high antibody; Group 2 = reduction in antibody following immunization postpartum (week 0); Group 3 = reduction in antibody following immunization pre- and postpartum. Animal classification is based on serum antibody to ovalbumin (OVA). Numbers above bars represent precise percent disease occurrence in each group. Standard error was calculated based on the proportion of animals having (p) or not having (q) disease, and the sample size (N) within each antibody group. $SEM=\sqrt{(pq/N)}$. Significant differences between animals in the three groups are indicated by different letters above error bars ($P \leq .05$).


% Disease Occurrence
0.6
0.5
0.4
0.3
0.2
0.1
0
a
0.17
a
0
a
0.33
ab
0.17
a
0.4
b
0.33
Group 1
Group 2
Group 3
Antibody Group
Overall
Mastitis

CHAPTER VI

Summary and General Discussion

Impairment of host defense(s) has been documented to occur at parturition in many species (Weinberg, 1984; Clarke and Kendall, 1994; Detilleux et al. 1994; Detilleux et al., 1995). Coincident with this impairment, is an increased prevalence of clinical mastitis. The most common pathogen(s) causing mastitis at this time are the coliforms *(E. coli*, *Klebsiella*, and *Pasteurella* species) which are encountered in the natural environment. Hormone fluctuations that occur concurrently with the progression of pregnancy and in response to the stress of parturition may contribute to immunodepression and consequently increased susceptibility to mastitis. Previous investigations have established suboptimal polymorphonuclear (PMN) cell counts and mitogen-driven lymphocyte proliferation during the peripartum period in dairy cows, however, the research presented here is the first to investigate variation in antibody and DTH phenotype during the peripartum period.

A Biological Assessment of Antibody Variation in One Herd

In a preliminary study, one herd was evaluated for antibody to ovalbumin (OVA), an antigen not normally encountered by the dairy cow. It was determined that antibody to OVA could be utilized to group animals biologically into three antibody groups, recognizing animals with sustained antibody before and after parturition (Group 1),

animals with a reduction in antibody following tertiary immunization at parturition (Group 2), and following secondary and tertiary immunizations (Group 3). Variation in antibody to *E. coli* J5, a biologically relevant pathogen known to cause mastitis, was more difficult to partition. This might have been due to previous or concurrent exposure to Gram negative bacteria in the environment. Significant differences were not determined between groups for $IgG_{1\&2}$ concentrations in serum or whey nor were there any significant associations with antibody group. Clinical mastitis occurrence was lowest for Group 1 animals (0%) in contrast to Groups 2 & 3. These disease estimates were supported by somatic cell scores (SCS), which serve as an indicator of subclinical udder infection. Group 1 animals with no mastitis, had the lowest SCS while Group 3 animals had the highest at weeks 3,4,5, and 6 following parturition. This study indicated that antibody variation existed within a herd but it would be necessary to observe antibody in more herds within the population.

Evaluation of Antibody Variation in Three Herds

Following the preliminary study of one herd, a second study of antibody further evaluated two more herds for serum and whey antibody to OVA. While the preliminary study indicated that animals could be grouped biologically according to the amplitude and direction of their individual OVA antibody profiles, the second study indicated that antibody in three herds were on a continuum and therefore a more objective means of grouping animals would be required. The objective of this study was to verify the

relevance of high and low antibody profiles across three herds and to determine if it would be possible to develop a quantitative measure of classification for antibody that reflected the initial biological assessment of animals identified in the first herd studied. The results indicated substantial variation in antibody to OVA during the peripartum period and that animals could be classified according to the magnitude of antibody. Animals that had any reduction in antibody in the immediate peripartum period were ranked lower, compared to animals that responded consistently and positively to OVA immunization. Animals that ranked high, average or low for serum antibody, also ranked similarly for their whey antibody to OVA. In all herds investigated, antibody to the more biologically relevant antigen, *E. coli*, showed that animals ranked similarly to the ranking for serum antibody to OVA, particularly at parturition and weeks 3 and 6 after parturition. Impairment of antibody profiles observed in Group 3 might be explained by a previous study by Nagahata et al. (1992), who examined B lymphocyte populations in order to evaluate host defense in dairy animals during the periparturient period and found a significant decrease in the number of antibody producing cells immediately after parturition. They felt this indicated a decrease in function of B lymphocytes during the immediate postpartum period. Kollman et al. (1993) evaluated antibody response to *Eimeria* sp. and reported that during the course of *Eimeria* infection in dairy cattle followed under field conditions, dairy animals had a decline in serum IgG antibody at parturition. Although it has been reported that low serum Ig in cattle at parturition may be due to sequestration of immunoglobulin (Ig) into the mammary gland (Detilleux et al., 1995), the results presented here would suggest that lower antibody in serum is not a

consequence of Ig transport. For instance, Group 1 animals, with the highest serum antibody, also had high whey antibodies to OVA postpartum when compared to animals of Groups 2 and 3. This data suggests that animals with high serum antibody also supply higher concentrations of antibody to the mammary gland. More study is required to evaluate the consistency and breadth of antibody response before and after parturition and in subsequent lactations, however, this study has demonstrated significant individual variation during the peripartum period, and that not all animals experience peripartum depression of antibody. In light of previously reported heritability estimates of bovine antibody response (Burton et al., 1989), this data may suggest that Group 1 animals could be inherently better able to produce antibody, in spite of the metabolic and physical stresses experienced during parturition. Although peripartum heritability estimates of antibody response remain to be established, the results presented here on two out of three herds indicates that there may be beneficial to use antibody as a potential marker of selection for peripartum disease resistance.

Evaluation of Cell Mediated Immune Response in Three Herds

Results presented in chapters I & II indicated that Holstein cows and heifers varied in their peripartum antibody to OVA and that animals could be grouped into high, average and low antibody groups based on the magnitude and direction of response to immunization. The objectives of this study were to evaluate CMI responses with respect to antibody group and evaluate any relationships with SCS, an sub-clinical indicator of

udder health. Literature on the current Th1/Th2 paradigm indicates that host response to infection in mice and humans may result in a profile of cytokines, supporting either cell mediated or antibody mediated immune response mechanisms (Romagnani, 1997). This paradigm may hold true for cattle and could indicate that animals with a high antibody are likely to have low cell mediated immune responses and visa versa. A study of swine leukocyte antigen (SLA)-defined pigs demonstrated an inverse relationship between antibody response and indicators of CMI. Pigs exhibiting low antibody response to sheep red blood cells (SRBCs) and (T,G)-A--L antigen had significantly higher DTH responses to dinitrochlorbenzene (DNCB), measured as increased skin thickness, than high antibody response pigs (Mallard et al., 1989). In contrast, studies in mice have demonstrated a positive relationship between antibody response and CMIR. Mice selectively bred for high (H line) and low (L line) antibody response to sheep and pigeon erythrocytes demonstrated similar high and low responses to measures of T cell response such as skin graft rejection (Liacopoulos-Briot et al., 1972a), graft versus host reaction (Byfield and Howard, 1972), DTH response (Mouton et al., 1974) and *in vitro* proliferation to phytohemaglutinin antigen (Liacopoulos-Briot et al., 1972b). Further, antibody production against antigens evaluated to induce these T cell responses was found to be highest in the H line mice. Another study de Oliveira et al. (1985) using these lines of mice and mice selectively bred for high and low antibody responsiveness to Salmonellae flagellar and somatic antigen were evaluated for DTH response to sheep erythrocytes, *Salmonella typhimurium* and purified protein derivative of *Mycobacterium tuberculosis*. Although DTH responses to selection antigens and unrelated antigens varied within lines,

no correlations between antibody responsiveness and DTH responses were found. These findings in pigs and mice indicate that expression of CMIR and antibody response are likely polygenic and subject to independent quantitative regulation. Further, the relationship between CMIR and antibody response may differ from species to species. In the current study, animals with the highest antibody (Group 1) had the lowest unstimulated and OVA-stimulated lymphocyte proliferative responses during the peripartum period. This is in contrast to low antibody animals (Group 3), which had the highest lymphocyte proliferative responses. Concanavalin A (Con A) lymphocyte proliferation and antibody to OVA, however, did not demonstrate an observable inverse relationship since animals with high antibody also had high Con A-stimulated lymphocyte proliferative responses, indicating that relationships between antibody and CMIR may vary depending on the measurements examined. Although no differences in DTH were observed between antibody group, DTH responses were demonstrated to vary between individuals (0-79% increase in skin thickness), an indication that selection based on DTH may be possible for enhanced CMIR.

Depression of lymphocyte proliferation was observed to occur for unstimulated lymphocyte proliferation responses between weeks -3 and parturition (week 0). Significant ($P<0.05$) depression of Con A stimulated lymphocyte proliferative responses at parturition were only observed in Group 1 animals with the high antibody phenotype. Again, this may indicate a negative association between high antibody and certain indicators of CMI.

Evaluation of complete blood cell counts indicated a non-significant decline in lymphocyte number at parturition. Group 3 animals experienced the sharpest decline in lymphocyte numbers from week -3 to parturition but had higher unstimulated and OVA stimulated *in vitro* lymphocyte proliferation than other antibody groups. In contrast to the literature (Kehrli et al., 1989b), PMN number did not significantly change during the peripartum period.

Mallard et al. (1992,1998) demonstrated in Yorkshire pigs that antibody response and CMIR can be used together in a selection index to simultaneously direct both aspects of immune response, with the goal of developing broad-based disease resistance. The results presented here suggest that a selection index which employs indicators of antibody and CMIR could be adapted for dairy cattle with a goal to enhance broad-based disease resistance during the peripartum period.

Peripartum Mastitis Occurrence in Three Herds And the Relationship Between Mastitis, SCS, and Antibody

The distribution of mastitis by antibody group was not consistent between herds. In Herd 1 and Herd 3, the rate of mastitis occurrence was greatest for animals with low antibody (Group 3). All animals within these herds that had a mastitis event were in their second or later parity. Odds-ratio assessment for these herds indicated that there was a 2.16 and

1.80 times greater chance of having a mastitis event if animals were classified in Group 3 versus Groups 1 & 2. In contrast, animals from Herd 2 had a very different distribution of mastitis occurrence among groups. Animals in Group 1 had the greatest rate of mastitis occurrence and, according to the odds-ratio parameter, were 7.57 ($P<0.05$) times more likely to have a mastitis event. Further, all animals within Herd 2 that had a mastitis event were first parity heifers. SCS for Herds 1 and 2 underscored the distribution of clinical mastitis; however, SCS was not significantly different between antibody groups. Nonetheless, animals of the low antibody group in Herd 1 had the highest rate of clinical mastitis and had the highest SCS. In contrast, animals of the high antibody group in Herd 2 had the highest SCS and the highest rate of clinical mastitis. Differences in herd management and the distribution of heifers and cows within each herd and antibody group, may help explain the differences in the distribution of mastitis occurrence. Herd 1 (n=6 heifers; n=26 cows) and Herd 3 (n=8 heifers; n=29 cows) had a greater ratio of cows to heifers within each antibody group, while for Herd 2 (n=34 heifers; n=33 cows) heifers and cows were more evenly distributed within herd and among all antibody groups. Previous studies have acknowledged an increase in the rate of occurrence of mastitis with advancing parity (Todhunter et al., 1995, and McClure et al., 1994), which may explain the disparity among herds. The unexpected distribution of mastitis in Herd 2 might further be explained by a more recent investigation from Finland (Myllys et al., 1995), which indicated that in well managed herds with high milk production and low somatic cell counts, the rate of the treatment of heifers that had a mastitis episode increased from 1.8% to 4.4% over an 8 year period. In contrast to

clinical mastitis observed in second parity and multiparous cows, that study further indicated that heifer mastitis only resulted in small production losses, did not pre-dispose a heifer to more mastitis or other diseases later in lactation, and the recovery rate of mastitis was high, as indicated by a rapid decline in somatic cell counts (SCC) following infection. This may indicate that heifer mastitis and cow mastitis cannot be compared. That disease occurrence in this study was not consistent among herds, may be explained by a number of factors, including the relatively small sample size evaluated, environmental (management) differences, distribution of heifers and multiparous cows, and type of mastitis (subclinical vs. clinical and the infecting pathogen). Further research of a larger population of animals may be warranted since, in this study, Group 1 animals were observed to have no occurrence of mastitis in two of the herds studied.

A previous study by Solbu et. al. (1982), relating disease with immune response reported an association between mastitis and high antibody response. Among half sisters of Norwegian bulls, cows with low antibody response to human serum albumin (HSA) were associated with allele w16. Cows with this allele was also determined to be susceptible to mastitis. Conversely, low HSA-responding cows, associated with the BoLA-w2 allele, were found to be relatively resistant to mastitis. Alternatively, there may be a greater good in selecting cows which have an average antibody response to a panel of antigens, such as was observed for the Group 2 animals, and have an average rate of mastitis occurrence within a particular herd. Further research is required to accurately assess whether animals within particular antibody group are more susceptible or resistant to

mastitis.

Further investigations of larger populations with more disease data would be required to accurately assess associations between antibody profiles and disease occurrence during the peripartum period. Detection of immune response traits, such as antibody to antigen, which associate well with disease resistance and/or susceptibility, may provide a useful phenotype to begin selective breeding of dairy cattle for improved inherent immune responsiveness and disease resistance.

Peripartum Antibody and Production

The fourth investigation indicates a potential economic advantage of animals with low antibody in that they produce more milk, fat and protein than average and high antibody animals. However, animals that have high antibody in two out of the three herds evaluated had the lowest occurrence of mastitis in contrast to low antibody responder animals. Given the knowledge of a positive correlation between the selection for increased milk production and the increased rate of clinical mastitis occurrence, one might hypothesize that superior production could be associated with changes in host defense which result in the higher occurrence of clinical mastitis. This study supports this hypothesis in two of the three herds investigated. The observation that animals with high and average antibody (Groups 1 and 2) produced less milk and milk solids per lactation than the animals of the low antibody group would indicate that selection based

on antibody to OVA is not economically feasible in the short term. In the long term however, it may be more beneficial to own a cow with superior health traits that has minimal disease costs and still has optimal production traits. A previous US study (Dunklee et. al, 1994) determined that although health costs are positively and unfavourably associated with higher production, health costs do not outweigh profit potential. Regardless of whether health costs do or do not have an impact on the production profit potential of dairy animals, reduced occurrence of mastitis will nonetheless be mutually beneficial to dairy producers, processors and consumers. Milk producers will benefit through a reduction in economic loss incurred by mastitis, processors manufacturing milk products will benefit from an enhancement in milk quality, and consumers concerned about animal welfare and food safety standards will appreciate knowing that antibiotic usage to treat mastitis has been reduced as a direct result of reduced mastitis occurrence.

Effect of Peripartum Hormones on Immune Responsiveness

The fifth and final study was the first to simultaneously evaluate antibody and hormone profiles during the peripartum period and has revealed some associations between IGF-I and antibody. However, no actual cause and effect relationship could be established from this study. Plasma IGF-I elevated during the latter stages of pregnancy and was followed by a dramatic decline around parturition, with a steady increase in concentrations following parturition. Some of these observations have been confirmed in the literature;

for instance, Vega et al. (1991) attributed changes in IGF-1 and GH to the decrease in metabolic demands associated with the cessation of milk production, during late gestation, followed by an increase in metabolic demand associated with the onset of lactation at parturition. As well, the demand of the mammary gland may alter the transport of IGF-I by sequestering it from the blood. Lactogenic hormones, such as prolactin and cortisol, may also prevent the synthesis of IGF-I and IGF-I binding proteins (Vega et al., 1991).

As previously reported (Hoshino et al., 1991), circulating concentrations of GH in this study increased around parturition, concurrent with early milk production, and decreased as lactation progressed. The inverse relationship between peripartum IGF-I and GH is noteworthy, in that IGF-I production is normally dependant on GH, as blood concentrations influence liver production of IGF-I (Burton et al., 1992). However, due to the peripartum uncoupling between these two hormones, it may be possible to evaluate the influence of each hormone separately on both the innate and antibody-mediated aspects of the immune system.

It has also been suggested that some effects of GH on the immune system are a result of IGF-I (Burton et al., 1992; Badolato et al., 1994). In general these studies indicated that the precise relationships between these hormones and immune responsiveness will be challenging to determine. In the present study, GH concentration was positively, and IGF-I was negatively correlated with antibody. Although not statistically significant,

animals in Group 1 with the highest antibody to OVA, tended to have the highest GH concentrations. For the most part, IGF-I concentrations were not different among antibody groups, except at week 6, when animals of Group 1 had significantly higher concentrations than Group 3 animals. These results are consistent with the work of Yoshida et al. (1992), which demonstrated that GH stimulates B cell growth and immunoglobulin synthesis by B cells and B cell lines. GH has been reported to alter antibody synthesis in response to T-dependent antigens, as well as to increase activity of T lymphocytes and natural killer (NK) cells (Geffner et al., 1990; Schurmann et al., 1995). Badolato et al., (1994) found that B cells displayed relatively high numbers of GH receptors, whereas T and NK cells showed much lower numbers of receptors. In addition, increased GH concentrations can enhance otherwise suppressed antibody response due to stress released glucocorticoids (Franco et al., 1990). Again, it has been suggested that the effects of GH may be mediated through IGF-I, a lymphocyte growth factor (Franco et al., 1990), but whether this is true during the peripartum period when these hormones become uncoupled seems unlikely.

Conclusions

* Dairy cows and heifers could be classified using a mathematical index into three phenotypic groups according to the quality and quantity of their peripartum OVA antibody patterns.

* Whey OVA and serum *Escherichia coli* antibody reflected the ranking based on serum antibody groups.

* Enough variation existed in *E. coli* antibody such that the antibody could also be partitioned into low and high antibody phenotypes, but grouping would be more complex due to possible environmental exposure and significant herd differences.

* Unstimulated and OVA-stimulated lymphocyte proliferative responses were inversely related to OVA antibody.

* Serum and whey $IgG_{1\&2}$, and DTH response did not significantly associate with antibody group.

* DTH response varied between animals

* In two out of the three herds investigated, animals with low antibody had the highest occurrence of mastitis and animals with high antibody had no mastitis. This may be related to management factors and/or the distribution of heifers and mature cows within each herd and antibody group. Whether OVA is the most useful antigen for determining associations between antibody and mastitis incidence remains to be determined.

* In two out of the three herds investigated, SCS, a subclinical measure of infection, reflected the distribution of clinical mastitis.

* Animals with low antibody had a higher projected profit potential based on component pricing for protein and fat.

* Elevated growth hormone and cortisol, and decreased insulin-like growth factor-I concentrations at parturition appear to contribute to variation in peripartum immune response.

Practical Implications to the Industry

Increased milk production has resulted in a gradual increase in the incidence of clinical mastitis. Immune response phenotype may be useful as a selection tool to increase immune responsiveness and enhance resistance to mastitis. Selection to reduce the occurrence of mastitis will be mutually beneficial to producers, processors, and consumers. Producers will benefit through a reduction in economic loss incurred by mastitis, processors will benefit through the enhancement of milk quality, and consumers concerned about animal welfare and food safety standards will appreciate knowing that antibiotic usage to treat mastitis has been reduced as a direct result of reduced mastitis occurrence

Future Direction

* To evaluate more animals for immune response phenotype and disease occurrence, particularly mastitis, to increase the accuracy of associations between immune response and resistance and/or susceptibility to disease.

* To relate genotype with innate and immune response phenotype: 1) by association with candidate markers such as genes which encode MHC, Ig and TcR; or 2) by association with anonymous markers (i.e. QTL, micro- and mini-satellites).

References

AArestrup, F.M., Jensen, N.E., and Østergård, H., 1995. Analysis of associations between major histocompatibility complex (BoLA) class I haplotypes and subclinical mastitis of dairy cows. J. Dairy Sci., 78:1684-1692.

Arthur, R.P., and Mason, D., 1986. T cells that help B cell responses to soluble antigen are distinguishable from those producing interleukin 2 on mitogenic or allogenic stimulation. J. Exp. Med., 163:774-786.

Bachen, E.A., Manuck, S.B., Marsland, A.L., Cohen, S., Malkoff, S.B., Muldoon, M.F., and Rabin, B.S., 1992. Lymphocyte subset and cellular immune responses to a brief experimental stressor. Psychosom. Med., 54:673-679.

Badolato, R., Bond, H.M., Valerio, G., Petrella, A., Morrone, G., Waters, M.J., Venuta, S., and Tenore, A., 1994. Differential expression of surface membrane growth hormone receptor on human peripheral blood lymphocytes detected by dual fluorochrome flow cytometry. J. Clin. Endocrinol. Metab., 79:984-990.

Banos, G., and Shook, G.E., 1990. Genotype by environment interaction and genetic correlations among parities for somatic cell count and milk yield. J. Dairy Sci., 73:2563-2573.

Bauman, D.E., Eppard, P.J., DeGeeter, M.J. and Lanza, G.M., 1985. Responses of high-producing dairy cows to long-term treatment with pituitary somatotropin and recombinant somatotropin. J. Dairy Sci., 68:1352-1362.

Biozzi, G., Stiffel, C., Mouton, D., Bouthillier, Y., and Deereusefound, C., 1968. A kinetic study of antibody producing cells in the spleen of mice immunized intravenously with sheep erythrocytes. Immunol., 14:7-20.

Biozzi, G., Stiffel, C., Mouton, D., Bouthillier, Y., and Decreusefond, C., 1972. Cytodynamics of the immune response in two lines of mice genetically selected for high and low antibody synthesis. J. Exp. Med., 135:1071-1094.

Biozzi, G., Mouton, D., Sant'Anna, O.A., Passos, H.C., Gennari, M., Bouthillier, Y. Ibanez, O.M., Stiffer, C., and Siquerira, M., 1979. Genetics of immunoresponsiveness to natural antigens in the mouse. Curr. Top. Microbiol. Immunol., 85:31-98.

Blalock, J. E., 1994. The syntax of immune-neuroendocrine communication. Immunol.Today, 15:504-511.

Boettcher, P.J., Hansen, L.B., Van Raden, P.M. and Ernst, C.A., 1992. Genetic evaluation of Holstein bulls for somatic cells in milk of daughters. J. Dairy Sci., 75:1127-1137.

Bruecker, K.A. and Schwartz, L.W., 1982. Bovine peripheral blood polymorphnuclear neutrophil chemotactic response to Pastuerella hemolytica or zymosan-activated serum. Am. J. Vet. Res., 43:1879-1881.

Burrells, C., Wells, P.W., and Sutherland, A.D., 1978. Reactivity of ovine lymphocytes to phytohemagglutinin and pokeweed mitogen during pregnancy and in the immediate post-parturient period. Clin. Exp. Immunol., 55:410-415.

Burton, J.L., Mallard, B.A., and Mowat, D.N., 1993. Effects of supplemental chromium on immune resposes of periparturient and early lactation dairy cows. J. Anim. Sci., 71(6):1532-1539.

Burton, J.L., Burnside, E.B., Kennedy, B.W., Wilkie, B.N., and Burton, J.H., 1989a. Antibody responses to human erythrocytes and ovalbumin as marker traits of disease resistance in dairy calves. J. Dairy Sci., 72:1252-1265.

Burton, J.L., Kennedy, B.W., Burnside, E.B., Wilkie, B.N., and Burton, J.H., 1989b. Dinitrochlorobenzene contact hypersensitivity as a marker trait for selection to improve disease resistance in calves. J. Dairy Sci., 72:2351-2361.

Burton, J.L., McBride, B.W., Kennedy, B.W., Burton, J.H., Elsasser, T.H. and Woodward, B., 1991. Influence of exogenous bovine somatotropin on the responsiveness of peripheral blood lymphocyte to mitogen. J. Dairy Sci., 74:916-928.

Burton, J.L., McBride, B.W., Kennedy, B.W., Burton, J.H., Elsasser, T.H., and Woodward, B., 1992. Contact sensitivity and systemic antibody responses in dairy cows treated with recombinant somatotropin. J. Dairy Sci., 75:747-755.

Burton, J.L., Nonnecke, B.J., Elsasser, T.H., Mallard, B.A., Yang, W.Z., and Mowat, D.N., 1995. Immunomodulatory activity of blood serum from chromium-supplemented periparturient dairy cows. Vet. Immunol. Immunopath., 49:29-38.

Burvenich, C., Paape, M.J., Hill, A.W., Guidry, A.J., Miller, R.H., Heyneman, R., Kremer, W.D.J. and Brand A., 1994. Role of the neutrophil leukocyte in the local and systemic reactions during experimentally induced *E.coli* mastitis in cows immediately after calving. Vet. Quart., 16:45-50.

Butler, J.E., 1980. A concept of humoral immunity among ruminants and an approach to its investigations. The Ruminant Immune System: Proc. Int. Symp. Rum.Imm. Sys., Plymouth, New Hampshire, pp. 3-55.

Butler, W.R., Everett, R.W., and Coppock, C.E., 1981. The relationships between energy balance, milk production, and ovulations in postpartum Holstein cows. J. Anim. Sci., 53:742-748.

Byfield, P.E., and Howard, J.G., 1972. Equivalent graft-versus-host reactivity of spleen cells from two lines of mice genetically selected for high and low humoral antibody formation. Transplant. 14:133-134.

Cai, T., Weston, P.G., Lund, L.A., Brodie, B., McKenna, D.J., Wagner, W.C., 1994. Association between neutrophil functions and periparturient disorders in cows. Am. J. Vet. Res., 55:934-943.

Caroll, E.J., Mueller, R. and Panico, L., 1982. Chemotactic factors for bovine leukocytes. Am. J. Vet. Res., 43:1661-1664.

Caroll, E.J., 1983. Immunological aspects of coliform mastitis. Vet. Res. Comm. 7:247.

Chang, X., Mallard, B.A., and Mowat, D.N., 1994. Proliferation of peripheral blood lymphocytes of feeder calves in response to chromium. Nut. Res., 14:851-864.

Clarke, A.G. and Kendall, M.D., 1994. The thymus in pregnancy: the interplay of neural, endocrine and immune influences. Immunol. Today, 15(11): 545-551.

Colditz, I.G., and Maas, P.J.C., 1987. The inflammatory activity of activated complement in ovine and bovine mammary glands. Immunol. Cell Biol., 65:433-436.

Corbeil, L.B., Watt, B., Corbeil, R.R., Betzen, T.G., Brownson, R.K., and Morrill, J.L. Immunoglobulin concentrations in serum and nasal secretions of calves at the onset of pneumonia. Am. J. Vet. Res., 45:773-778.

Craven, N., 1986. Chemotactic factors for bovine neutrophil in relation to mastitis. Comp. Immunol. Microbiol. Inf. Dis., 9:29-36.

Craven, N., and Williams, M.R., 1985. Defences of the bovine mammary gland against infection and prospects for their enhancement. Vet. Immunol. Immunopath. 10:71-127.

Cullor, J., 1991. The *Escherichia coli* J5 vaccine: Investigating a new tool to combat coliform mastitis. Vet. Med., 836-844.

Daley, M.J., Oldham, E.R., Williams, T.J., and Coyle, P.A., 1991. Quantitative and qualitative properties of host polymorphnuclear cells during experimentally induced *Staphylococcus aureus* mastitis in cows. Am. J. Vet. Res., 52(3):474-479.

Davis, R.S., Gluckman, P.D., Hart, I.C., and Henderson, H.V., 1987. Effects of injecting growth hormone or throixine on milk production and blood plasma concentrations of insulin-like growth factors I and II in dairy cows. J. Endocrinol., 114:17-24.

Dehoff, M.H., Elgin, R.G., Collier, R.J., and Clemmons, D.R., 1988. Both type I an II insulin-like growth factor receptory binding increase during lactogenesis in bovine mammary tissue. Endocrinol., 122:2412-2417.

Dekkers, J.C.M. and Burnside, E.B., 1994. Sire selection for udder health. Ont. Milk Prod. Sept. 1994, pp. 31-34.

Dekkers, J.C.M., TenHag, J.H., and Weersink, A., 1996. Economic aspects of persistency of lactation. 1996 Dairy Research Report, Ontario Ministry of Agriculture, Food and Rural Affairs, Publ. No. 0396, pp. 81-85.

Dekkers, J.C.M., Kolstad, B., Jairath, L.K., and Liu, Z., 1996. A new economic index for selection of sires. 1996 Dairy Research Report, Ontario Ministry of Agriculture, Food and Rural Affairs, Publ. No. 0396, pp.15-25.

Dekkers, J.C.M., Boettcher, P.J., and Mallard, B.A., 1998. Genetic improvement of udder health. 6th World Congr. Genet. Appl. Livest. Prod., Australia.

Derijk, R. and Sternberg, E. M., 1994. Corticosteroid action and neuroendocrine-immune interactions. Ann. N. Y. Acad. Sci., 746:33-44.

Detilleux, J.C., Koehler, K.J., Freeman, A.E., Kehrili, Jr., M.E., and Kelley, D.H., 1994. Immunological parameters of periparturient Holstein cattle: genetic variation. J. Dairy Sci., 77:2640-2650.

Detilleux, J.C., Kehrli, Jr., M.E., Freeman, A.E., Whetston, C.A., and Kelley, D.H., 1995a. Two retroviral infections of periparturient Holstein cattle: A phenotypic and genetic study. J. Dairy Sci., 78:2294-2298.

Detilleux, J.C., Kehrli, Jr., M.E., Stabel, J.R., Freeman, A.E., and Kelley, D.H., 1995b. Study of immunological dysfunction in periparturient Holstein cattle selected for high and average milk production. Vet. Immunol. Immunopath., 44:251-267.

Detilleux, J.C., Kehrli, Jr., M.E., Stabel, J.R., Freeman, A.E., Fox, L., and Kelley, D.H. 1995c. Mastitis of periparturient Holstein cattle: A phenotypic and genetic study. J. Dairy Sci., 78:2285-2293.

Dietz, A.B., Cohen, N.D., and Timms, L., 1997a. Bovine lymphocyte antigen cl. II alleles as risk factors for high somatic cell counts in milk of lactating dairy cows. J. Dairy Sci., 80:406-412.

Dietz, A.B., Detilleux, J.C., Freeman, A.E., Kelley, D.H., Stabel, J.R. and Kehrli Jr., M.E., 1997b. Genetic association of bovine lymphocyte antigen DRB3 alleles with immunological traits of Holstein cattle. J. Dairy Sci., 80(2):400-405.

Dubey, D.P., 1992. Histocompabitiblity and transplantation immunology. Manual of laboratory immunology. ASM Press. Editors: N.R. Rose, E.C. DeMacario, J.L. Fahey, H. Friedman, G.M. Penn., pp. 870-871.

Dunklee, T.S., Freeman, A.E., and Kell, D.H., 1994. Comparison of Holsteins selected for high and average milk production. 2. Health and reproductive response to selection for milk. J. Dairy Sci., 77:3683-3690.

Dürr, J.W., Monardes, H.B. and Turner, J.D., 1996. Correlations of neutrophil phagocytosis for proven bulls with traits of economic importance of their daughters. J. Dairy Sci., 79:133-139.

Durum, S.K., Schmidt, J., and Oppenheim, J.J., 1985. Interleukin-1: an immunological perspective. Ann. Rev. Immunol., 3:263-287.

Eichmann, K., Braun, D.G., and Krause, R.M., 1971. Influence of genetic factors on the magnitude and the heterogeneity of the immune response in the rabbit. J. Exp. Med., 134:48-65

Elsasser, T.H., Rumsey T.S., Hammond, A.C., and Fayer, R., 1988. Influence of parisitism on plasma concentrations of growth hormone, somatomedin-C and somatomedin-binding proteins in calves. J. Endocrinol., 116:191-200.

Elsasser, T.H., Rumsey, T.S., and Hammond, A.C., 1989. Influence of diet on basal and growth hormone-stimulated plasma concentrations of IGF-I in beef cattle. J. Anim. Sci., 67:128-141.

Emmanuelson, U., Danell, B., and Phillipsson, J., 1988. Genetic parameters for clinical mastitis, somatic cell counts, and milk production estimated by multiple-trait restricted maximum likelihood. J. Dairy Sci., 71:467-475.

Envoldsen, C., Hindhede, J., and Kristensen, T., 1996. Dairy herd management types assessed from indicators of health, reproduction, replacement, and milk production. J. Dairy Sci., 79:1221-1236.

Fornari, M.C., Scolnik, M.P., Palacios, M.F., Intebi, A.D., and Diez, R.A., 1994. Growth hormone inhibits normal B cell differentiation and neutrophils' chemotaxis *in vitro*. Int. J. Immunopharmac., 16(8):667-673.

Franco, P., Marelli, O., Lattuda, D., Locatelli, V., Cocchi, D., and Muller, E.E., 1990. Influence of growth hormone on the immunosuppressive effect of prednisolone in mice. Acta Endocrinologica, 123:339-344.

Franklin, S.T., Young, J.W., and Nonnecke, B.J., 1991. Effects of ketones, acetate, butyrate, and glucose on lymphocyte proliferation., J. Dairy Sci., 74:2507-2514.

Fries, R., Hediger, R., and Stranzinger, G., 1986. Tentative chromosomal localization of the bovine majorhistocompatibility complex by *in situ* hybridization. Anim. Genet., 17:287-294.

Gambel, P.I., and Ferguson, F.G., 1982. An *in vitro* and *in vivo* analysis of murine immunocompetence during pregnancy and lactation. J. Reprod. Immunol., 4:107-119.

Gavora, J.S., Spencer, J.L., Gowe, S., and Harris, D.L., 1980. Lymphoid leukosis virus infection: Effects on production, mortality, and consequences for selection for high egg production. Poultry Sci., 59:2165-2178.

Gavora, J.S., and Spencer, J.L., 1983. Breeding for immune responsiveness and disease resistance. Anim. Blood Groups Biochem. Genet., 14:159-180.

Gawazdauskas, F.C., Keys, J.E., and McGilliard, M.L., 1986. Adrenal response during periparturient period to adrenocorticotropin in dairy cattle fed corn silage and grass legume silage. J. Dairy Sci., 69:2134-2139.

Geffner, M.E., Bersch, N., Lippe, B.M., Rosenfeld, R.G., Hintz, R.L., and Golde, D.W., 1990. Growth hormone mediates the growth of T-lymphoblast cell lines via locally generated insulin-like growth factor-I. J. Clin. Endocrinol. Metab., 71:464-469.

Gilbert, R.O., Gröhn, Y.T., Miller, P.M., and Hoffman, D.J., 1993a. Effect of parity on periparturient neutrophil function in dairy cows. Vet. Immunol. Immunopath., 36:75-82.

Gilbert, R.O., Gröhn, Y.T., Guard, C.L., Surman, V., Neilsen, N., and Slauson, D.O., 1993b. Impaired post partum neutrophil function in cows which retain fetal membranes. Res. Vet. Sci., 55:15-19.

Gilbert, F.B., Poutrel, B. and Sutra, L., 1994. Immunogenecity in cows of *Staphylococcus aureus* type 5 capsular polysaccharide-ovalbumin conjugate. Vaccine, 12(4):369-374.

Gillis, S., Ferris, M., Ou., W., and Smith, K., 1978. T cell growth factor: parameters for production and a quantitiative microassay for activity. J. Immunol., 120:2027-2032.

Giraudo, J.A., Calzolari, A., Rampone, H., Rampone, A., Giraudo, A.T., Bogni, C., Larriestra, A., and Nagel, R., 1997. Field trials of a vaccine against bovine mastitis. 1. Evaluation in heifers. J. Dairy Sci., 80:845-853.

Glass, E.J., and Spooner, R.L., 1990. Generation and characterization of bovine antigen-specific T cell lines. J. Immunol. Methods, 128:267-275.

Glass, E.J., Oliver, R.A., and Spooner, R.L., 1990. Variation in T cell responses to ovalbumin in cattle: evidence for Ir gene control. Anim. Genet., 21:15-28.

Goff, J.P., and Horst, R.L., 1997. Phsiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci., 80:1260-1268.

Goff, J.P., and Stabel, J.R., 1990. Decreased plasma retinol, α-tocopherol, and zinc concentration during the periparturient period: effect of milk fever. J. Dairy Sci., 73(11):3195-3199.

Gonzalez, R.L., Cullor, J.S., Jasper, J.E., Farver, T.B., Bushnell, R.B. and Oliver, M.N., 1989. Prevention of clinical coliform mastitis in dairy cows by a mutant *Escherichia coli* vaccine. Can. J. Vet. Res., 53(3):301-305.

Gray, G.D., Knight, K.A., Nelson, R.D., and Herron, M.J., 1982. Chemotactic requirements of bovine leukocytes. Am. J. Vet. Res., 43: 757-759.

Griffin, J.F.T., 1989. Stress and Immunity: a Unifying Concept. Vet. Immunol. Immunopathol., 20: 263-312.

Gröhn, Y.T., Eicker, S.W., and Hertl, J.A. 1995. The association between previous 305-day milk yield and disease in New York state dairy cows. J. Dairy Sci., 78:1693-1702.

Guidry, A.J., Paape, M.J., and Pearson, R.E., 1976. Effects of parturition and lactation on blood and milk cell concentrations, corticosteroids, and neutrophil phagocytosis of the cow. Am. J. Vet. Res., 37:1195-1200.

Gupta, S., Fikrig, S.M., and Noval, M.S., 1983. Immunological studies in patients isolated with growth hormone deficiency. Clin. Exp. Immunol., 54:87-90.

Harmon, R.J., Schanbacker, F.L., Ferguson, L.C., and Smith, K.L., 1975. Concentration of lactoferrin in milk of normal lactating cows and changes occurring during mastitis. Am. J. Vet. Res., 36:1001-1007.

Harmon, R.J., 1994. Physiology of mastitis and factors affecting somatic cell counts. 1994. J. Dairy Sci., 77: 2103-2112.

Harp, J.A., Kehrli, M.E., Hurley, D.J., Wilson, R.A., and Boone, T.C., 1991. Number and percent of T lymphocytes in bovine peripheral blood during the peripartum period. Vet. Immunol. Immunopath., 28:29-35.

Helwig, T.T. and Council, K.A., 1982. SAS User's Guide. SAS Institute, Raleigh, NC.

Hein, W.R. and Mackay, C.R., 1991. Prominence of $\gamma\delta$ T cells in the ruminant immune system. Immunol. Today, 12:30-34.

Hessing, M.J.C., Coenen, G.J., Vaiman, M., and Renard, C., 1995. Individual differences in cell-mediated and humoral immunity in pigs. Vet Immunol. Immunopath., 45:97-113.

Heyneman, R., Burvenich, C., and Vercauteren, R., 1990. Interaction between the respiratory burst activity of neutrophils, leukocytes and experimentally induced *Escherichia coli* mastitis in cows. J. Dairy Sci., 73:985-994.

Hill, A.W., Heneghan, D.J.S., and Williams, M.R., 1983. The opsonic activity of bovine milk whey for the phagocytosis and killing by neutrophils of encapsulated and non-encapsulated *Escherichia coli*. Vet. Microbiol., 8:293-300.

Hoffman-Goetz, L. and Pedersen, B.K., 1994. Exercise and the immune system: a model of the stress response? Immunol. Today, 15(8):382-387.

Hoshino, S., Wakita, M., Kobayashi, Y., Sakauchi, R., Nishiguchi, Y., Ozawa, A., Hodate, K., Hamaguchi, I. and Yotani, Y., 1991. Variations in serum level of insulin-like growth factor-1, growth hormone, and thyroid hormones during lactation in dairy cows. Comp. Biochem. Physiol., 99(12):61-64.

Hutt, F.B., 1958. Genetic resistance to disease in domestic animals. Ithaca, NY, Cornell University Press; London., 198pp.

Ibanez, O.M., Reis, M.S., Gennari, M., Ferreira, V.C.A, Sant'Anna, O.A., Siqueira, M., and Biozzi, G., 1980. Selective breeding of high and low antibody-responder lines of guinea pigs. Immunogenet., 10:283-293.

Ishikawa, H., 1983. Depression of B lymphocytes by mastitis and treatment with levamisole. J. Dairy Sci., 66:556-561.

Ishikawa, H., 1987. Observation of lymphocyte function in perinatal cows and neonatal calves. Jpn. J. Vet. Sci., 49(3):469-475.

Kashiwazaki, Y., 1984. Lymphocyte activities in dairy cows with special preference to outbreak of mastitis pre-and postpartus. Jpn. J. Vet. Res., 32:101.

Kashiwazaki, Y., 1985. Transformation of bovine peripheral blood lymphocytes in the perinatal period. Jpn. J. Vet. Sci., 47(2):337-339.

Kehrli, M.E.,Jr., Nonnecke, B.J., and Roth, J.A., 1989a. Alterations in bovine lymphocyte function during the periparturient period. Am. J. Vet. Res., 50(2):215-220.

Kehrli, M.E. Jr, Nonnecke, B.J., and Roth, J.A., 1989b. Alterations in bovine neutrophil function during the periparturient period. Am. J. Vet. Res., 50(2):207-214.

Kehrli, M.E. Jr., Goff, J.P., Harp, J.A., Thurston, J.R., Norcross, N.L., 1990a. Effects of preventing periparturient hypocalcemia in cow by parathyroid hormone administration on hematology, conglutinin, immunoglobulin and shedding of *Staphylococcus aureus* in milk. J. Dairy Sci., 73(8):2103-2111.

Kehrli, M.E., Schmalstieg, F.C., Anderson, D.C., Van Der Maaten M.J., Hughes, B.J., Ackermann, M.R., Willhelmsen, C.L., Brown, G.B., Stevens, M.G., and Whetstone, C.A., 1990b. Molecular definition of the bovine granulocytopathy syndrome: Identification of deficiency of the Mac-I (CD 11b/CD18) glycoprotein. Am. J. Vet. Res., 51:1826-1836.

Kehrli, M.E.,Jr., Weigel, K.E., Freeman, A.E., Thurston, J.R., and Kelley, D.H., 1991. Bovine sire effects on daughters' in vitro blood neutrophil functions, lymphocyte blastogenesis, serum complement and conglutinin levels. Vet. Immunol. Immunopath., 27:303-319.

Kelley, K.W., 1990. The role of growth hormone in modulation of the immune response. Ann. N.Y. Acad. Sci., 594:95-103.

Kelley, K.W., Greenfield, R.E., Evermann, J.F., Parish, S.M., and Perryman, L.E., 1982. Delayed-type hypersensitivity, contact hypersensitivity, and phytohemagglutinin skin-test responses of heat and cold-stressed calves. Am. J. Vet. Res., 43(5):775-779.

Kelm, S.C., Detilleux, J.C., Freeman, A.E., Kehrli, Jr., M.E., Dietz, A.B., Fox, L.K., Butler, J.E., Kasckovics, I., and Kelley, D.H. Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. J. Dairy Sci. (submitted).

Kensinger, M.H., Hurley, D.J., and Wilson, R.A., 1990. Culture conditions for blastogenic responses of bovine mammary mononuclear cells. Vet. Immunol. Immunopath., 24:323-330.

Khansari, D.N., Murgo, A.J., and Faith, R.E., 1990. Effects of stress on the immune system. Immunol. Today, 11(5):170-175.

Kollmann, D., 1993. Thesis: *Eimeria* infections in cows and their calves during the periparturient phase. Veterinarmedizin, pp. 1-150. (in German, with English abstract).

Kremer, W.D.J., Noordhuizen-Stassen, E.N., and Lohuis, J.A.C.M. 1990. Host defence mechanisms and bovine coliform mastitis - a review. Vet. Quart. 12(2):103-113.

Kuby, J., 1997. Immunology. Third edition., W.H. Freeman and Company, USA.

Larsen, B., Jensen, N.E., Madsen, P., Nielsen, S.M., Klastrup, O. and Madsen, P.S., 1985. Association of the M blood group system with bovine mastitis. Anim. Blood Groups Biochem. Genet., 16:165-173.

Lewin, H., 1989. Disease resistance and immune response genes in cattle: Strategies for their detection and evidence of their existence. J. Dairy Sci., 72(50):1334-1348.

Lewin, H., 1994., Host genetic mechanism of resistance and susceptibility to a bovine retroviral infection. Anim. Biotech., 5(2):183-191.

Liacopoulos-Briot, M., Bouthillier, Y., Mouton, D., Lambert, F., Dcreusefond, C., Stiffel, C., and Biozzi, G., 1972. Comparison of skin allograft rejection and cytotoxic antibody production in tow line os mice genetically selected for 'high' and 'low' antibody synthesis. Transplant., 14:590-596

Liacopoulos-Briot, M., Lambert, F., Mouton, D., Bouthillier, Y., Decreusefond, C., Stiffel, C., and Biozzi, G., 1972. Stimulation des lymphocytes par la phytohémagglutinine chez les souris des lignées génétiquement sélectionnées d'après le caractère 'production d'anticorps'. Ann. Immunol., Paris, 123:135.

Lie, Ø. 1979. Genetic analysis of some immunological traits in young bulls. Acta Vet. Scand., 20:372-386.

Lie, Ø., 1985. Genetic approach to mastitis control. Kiel. Milchwirtsch. Forshungsber., 37:487.

Lie, Ø., Solbu, H., Larsen, H.J., and Spooner, R.L., 1986. Possible association of antibody responses to human serum albumin and (T,G)-A--L with the bovine major histocompatibility complex (BoLA). Vet. Immunol. Immunopath., 11:333-350.

Logg, M.H., 1942. Effect of pregnancy and parturition on pulmonary tuberculosis. Br. Med. J., 1:468-469.

Lundén, A., Sigurdardottir, S., Edfors-Lilja, I., Danell, B., Rendel, J. and Andersson, L., 1990. The relationship between bovine major histocompatibility complex class II polymorphism and disease studied by use of bull breeding values. Anim. Genet., 21:221-232.

Lutje, V. and Black, S.J., 1991. Cellular interactions regulating the *in vitro* response of bovine lymphocytes to ovalbumin. Vet. Immunol. Immunopath., 28(3-4):275-288.

MacPhee, I.A.M., Antoni, F.A., and Mason, D.W., 1989. Spontaneous recovery of rats from experimental allergic encephalomyelitis is dependent on regulation of the immune system by endogenous adrenal corticosteroids. J. Exp. Med., 169:431-445.

Madsen, P., 1989. Genetic resistance to bovine mastitis. Curr. Top. Vet. Med. Anim. Sci., 52:169-177.

Mallard, B.A., Wilkie, B.N., and Kennedy, B.W., 1989. Genetic and other effects on antibody and cell mediated immune response in SLA-defined miniature pigs. Anim. Genet., 20:167-178.

Mallard, B.A., Wilkie, B.N., Kennedy, B.W., and Quinton, M., 1992. Use of Estimated Breeding Values in a selection index to breed Yorkshire pigs for high and low immune and innate resistance factors. Anim. Biotech., 3(2):257-280.

Mallard, B.A., Wagter, L.C., Ireland, M.J., and Dekkers, J.C.M., 1997. Effects of growth hormone, insulin-like growth factor I, and cortisol on periparturient antibody response profiles of dairy cattle. Vet. Immunol. Immunopath. (In press).

Mallard, B.A., Wilkie, B.N., Kennedy, B.W., Gibson, J., and Quinton, M., 1998. Immune responsiveness in swine: Eight generations of selection for high and low immune response in Yorkshire pigs. 6th World Congr. Genet. Appl. Livest. Prod., Australia.

Mallard, B.A., Leslie, K.E., Dekkers, J.C.M., Hedge, R., Bauman, M., and Stear, M.J., 1995. Differences in bovine lymphocyte antigen associations between immune response and risk of disease following intramammary infection with *Staphylococcus aureus*. J. Dairy Sci., 78:1937-1944.

Mallard, B.A., Dekkers, J.C., Ireland, M.J., Leslie, K.E., Sharif, S, Lacey-Van Kampen, C., Wagter, L. and Wilkie, B.N., 1997. Alteration in immune responsiveness during the peripartum period. J. Dairy Sci. (accepted for publication).

Malo, D., Hu, J., Skamene, E., and Schurr, E., 1994. Population and molecular genetics of susceptibility to intracellular pathogens. Anim. Biotech., 5(2):173-182.

Manak, R.C., 1982. Mitogenic responses of peripheral blood lymphocytes from pregnant and ovariectomized heifers and their modulation by serum. J. Reprod. Immunol., 4:263-276.

Martin, W., Meek, A., and Willeberg, P., 1987. Veterinary Epidemiology: principles and methdods. 1st Edition. Iowa State University Press. pp. 129-131.

Mason, D., 1991. Genetic variation in the stress response: susceptibility to experimental allergic encephalomyelitis and implication for human inflammatory disesase. Immunol. Today, 12(2):57-60.

Matthews, K.R., Harmon, R.J. and Langlois, B.E., 1992. Prevalence of *Staphylococcus* species during the periparturient period in primiparous and multiparous cows. J. Dairy Sci., 75:1835-1839.

McClure, A.M., Christopher, E.E., Wolff, W.A., Fales, W.H., Krause, G.F., and Miramonti, J., 1994. Effect of Re-17 mutant *Salmonella typhimurium* bacterin toxoid on clinical coliform mastitis. J. Dairy Sci., 77:2272-2280.

Mejdell, C.M., Lie, Ø., Solbu, H., Arnet, E.F., and Spooner, R.L., 1994. Association of major histocompatibility complex antigens (BoLA-A) with AI bull progeny test results for mastitis, ketosis, and fertility in Norwegian cattle. Anim. Genet., 25:99-104.

Miglior, F., Burnside, E.B., and Dekkers, J.C.M., 1995. Nonadditive genetic effects and inbreeding depression for somatic cell counts of Holstein cattle. J. Dairy Sci., 78:1168-1173.

Mouton, D., Bouthillier, Y., Oriol, R., Decreusefond, C., Stiffel, C., and Biozzi, G., 1981. Intensité de la réaction d'hypersensibilité retardée chez les souris des lignées sélectionnées <<bonnes>> et <<mauvaises>> productrices d'anticorps. Ann. Immunol., Paris, 125C:581-588.

Morrow-Tesch, J.L., McGlone, J.J., and Norman, R.L., 1993. Consequences of restraint on natural killer cell activity, behaviour, and hormone levels in rhesus macaques. Psychoneuroendocrinol., 18(5-6):383-385.

Morrow-Tesch, J.L., Wollen, N., and Hahn, L., 1996. Response of γδ T-lymphocytes to heat stress in *Bos taurus* and *Bos indicus* crossbred cattle. J. Therm. Biol., 21(2):101-108.

Myllys, V. and Rautala, H., 1995. Characterization of clinical mastitis in primiparous heifers. J. Dairy Sci., 78:538-545.

Nardone, A., Lacetera, N., Bernabucci, U., and Ronchi, B., 1997. Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and the early postpartum period. J. Dairy Sci., 80:838-844.

Nagahata, H., Ogawa, A., Sanada, Y., Noda, H., and Yamamota, S., 1992. Peripartum changes in antibody producing capability of lymphocytes from dairy cows. Vet. Quart., 14(1):39-40.

Nagahata, H., Makino, S., Takeda, S., Takahashi, H., and Noda, H., 1988. Assessment of neutrophil function in the dairy cow during the perinatal period. J. Vet. Med., 35:747-751.

Nakao, T., and Grunnert, E., 1990. Adrenocortical function in cows with the downer cow syndrome. A preliminary report. J. Vet. Med., 37(8):610-613.

Nansen, P., 1972. Selective immunoglobulin deficiency in cattle and susceptibility to infection. Acta Pathologica Microbiologica Scandinavica 80B:49-54.

Nardone, A., Lacetera, N., Bernabucci, U., and Ronchi, B., 1997. Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and the early postpartum period. J. Dairy Sci., 80:838-844.

Nash, M.S., 1994. Excercise and immunology. Med. Sci. Sports Exerc., 26(2):125-127.

National Mastitis Council. 1994. Udder Topics, 17(4):1-4.

Newbould, F.H.S., 1976. Phagocytic activity of bovine leukocytes during pregnancy. Can. J. Comp. Med., 40:111-116.

Nickerson, S.C., Owens, W.E., Boddie, R.L., and Boddie, N.T., 1992. The effect of chronic immunostimulation of the nonlactating bovine mammary gland with interleukin-2, pokeweed mitogen and lipopolysaccharide. J. Dairy Sci., 75(12):3339-3351.

Nickerson, S.C., 1993. Vaccination programs for preventing and controlling mastitis. Natl. Mast. Coun. Reg. Mtg. Proc., pp. 64-72.

Nickerson, S.C., Owens, W.E., Rejman, J.J., and Oliver, S.P., 1993. Effects of interleukin-1 and interleukin-2 on mammary gland leukocyte populations and histology during the early nonlactation period. J. Vet. Med., 40:621-633.

Nickerson, S.C., Baker, P.A., and Trinidad, P., 1989. Local immunostimulations of the bovine mammary gland with interleukin-2. J.Dairy Sci., 72:1764-1773.

Nielson, U.S., Pedersen, G.A., Pedersen, J., and Jensen, J. 1997., In Proc. Genet. Improvement Func. Traits in Cattle., Uppsula, Sweden.

Nonnecke, B.J., and Harp, J.A., 1985. Effect of chronic staphylococcal mastitis on mitogenic responses of bovine lymphocytes. J. Dairy Sci., 68:3323-3328.

de Oliveira, S.L., Ibanez, O.M., Mouton, D., Sant'Anna, O.A., Siqueira, M., and Biozzi, G., 1985. Independent polygenic regulation of quantitative antibody responsiveness and expression of delayed-type hypersensitivity (DTH). Expl. Clin. Immunogenet., 2:223-233.

Ontario Milk Producer, October 1997. Prices., p.30.

Oppenheim, J.J., Kovacs, E.J., Matsuchima, K., and Durams, S.K., 1986. There is more than one interleukin-1. Immunol. Today, 7:45-56.

Paape, M.J., Hafs, H.D. and Snyder, W.W., 1963. Variation of estimated numbers of milk somatic cells stained with wright's stain or pyronin y-methyl green stain. J. Dairy Sci., 46: 1211-1216.

Park, Y.H., Fox, L.K., Hamilton, M.J. and Davis, W.C., 1992. Bovine mononuclear leukocyte subpopulations in peripheral blood and mammary gland secretions during lactation. J. Dairy Sci., 75:998-1006.

Parker, K.A., Leyh, R., Field, M.F., and Anderson, G.A., 1994. Serologic response of cattle to core antigen vaccination. Natl. Mast. Coun. Ann. Mtg. Proc., pp. 326-327.

Peter, A.T. and Bosu, W.T.K., 1987. Peripartal endocrine changes associated with retained placenta in dairy cows. Theriogenol., 28:383-393.

Petersen, B.H., Rapaport, R., Henry, D.P., Huseman, C., and Moore, W.V., 1990. Effect of treatment with biosynthethic human growth hormone (GH) on peripheral blood lymphocyte populations and function in growth hormone-deficient children. J. Clin. Endocrinol. Metab., 70:1756-1760.

Pinard, M. H. and Van der Zijpp, A.J., 1992. Divergent selection for immune resposiveness in chickens: Estimation of realized heritability with an animal model. J. Anim. Sci., 70(10):2986-2993.

Politis, I., Hidiroglou, M., Batra, T.R., Gilmore, J.A., Gorewit, R.C., and Scherf, H., 1995. Effects of vitamin E on immune function of dairy cows. Am. J. Vet. Res., 56(2):179-184.

Puel, A, and Mouton, D., 1996. Genes responsible for quantitative regulation of antibody production. Crit. Rev. Immunol., 16:223-250.

Reents, R., Dekkers, J.C.M., and Schaeffer, L.R., 1995. Genetic evaluation for somatic cell score with a test day model for multiple lactations. J. Dairy Sci., 78:2858-2870.

Rich, A.R., 1951. The influence of sex and age. In: The pathogenesis of tuberculosis. 2nd ed. Springfield: Charles C Thomas. pp. 189-195.

Rogers, M.P., Dubey, D., and Rech P., 1979. The influence of psyche and the brain on immunity and disease susceptibility: a critical review. Psychosom. Med., 41:147-164.

Romagnani, S., 1997. The Th1/Th2 paradigm. Immunol. Today, 18(6):263-266.

Roth, J.A., and Kaeberle, M.L., 1982. Effect of glucocorticoids on the bovine immune system. J. Am. Vet. Med. Assoc., 180:894-901.

Saad, A.M., Concha, C., and Astrom, G., 1989. Alterations in neutrophil phagocytosis lymphocyte blastogenesis in dairy cows around parturition. J. Vet. Med., 36:337-345.

Sandholm, M. and Mattila, T., 1986. Mechanisms of infection and inflammation of the mammary gland- an overview. Proceedings of symposion on mastitis control and hygienic production of milk. Espoo, Finland.

Schaeffer, L.R., Minder, C.E., McMillan, I., and Burnside, E.B., 1977. Nonlinear techniques for predicting 305-day lactation production of Holsteins and Jerseys. J. Dairy Sci., 60:1636-1644.

Schiebel, I.F., 1943. Hereditary differences in the capacity of guinea-pigs for the production of diptheria antitoxin. Acta Path. Microbiol. Scand., 20:464-484.

Schukken, Y.H., Mallard, B.A., Dekkers, J.C.M., Leslie, K.E., and Stear, M.J., 1994. Genetic impact on the risk of intramammary infection following *Staphylococcus aureus* challenge. J. Dairy Sci., 77:639-647.

Schmutz, S.M., Berryere, T.G., Robbins, J.W., and Carruthers, T.D., 1992. Resistance to *Staphylococcus aureus* mastitis detected by a DNA marker. Proc. Nation. Mast. Council. pp. 124-133.

Schurmann, A., Spencer, G.S.G., and Berry, C.J., 1995. Growth hormone alters lymphocyte sub-populations and antibody production in dwarf rats in vivo. Experientia, 51:780-785.

Schutz, M.M., 1994. Genetic evaluation of somatic cell scores for united states dairy cattle. J. Dairy Sci., 77:2113-2129.

Sharif, S., Mallard, B.A., Wilkie, B.N., Sargeant, J.M., Scott, H.M., Dekkers, J.C.M., and Leslie, K.E., 1997. Associations of the bovine major histocompatibility complex DRB3 (BoLA-DRB3) alleles with occurrence of disease and milk somatic cell score in Canadian dairy cattle., Anim. Genet. (submitted).

Shook, G.E., 1989. Selection for disease resistance. J. Dairy Sci., 72:1349-1362.

Shook, G.E., 1993. Genetic Improvement of mastitis through selection on somatic cell count. Veterinary clinics of north america: Food animal practice, 9(3):563-580.

Shook, G.E. and Schutz, M.M., 1994. Selection on somatic cell score to improve resistance to mastitis in the United States. J. Dairy Sci., 77:648-658.

Shuster, D.E., Lee, E.K., and Kehrli, M.E., 1996. Bacterial growth, inflammatory cytokine production and neutrophil recruitment during coliform mastitis in cows within ten days after calving, compared with cows at midlactation. Am. J. Vet. Res., 57(11):1569-1575.

Siegel, B.P., and Gross, W.B., 1980. Production and persistence of antibodies in chickens to sheep erythrocytes. 1. Directional selection. Poultry Sci., 59:1-5.

Sigguradóttir, S., Lunden, A., and Andersson, L., 1988. Restriction fragment length polymorphis of DQ and DR class II genes of the bovine major histocompatibility complex. Anim. Genet., 19:133-150.

Smith, K.L., and Hogan, J.S., 1994. Understanding environmental mastitis. Natl. Mast. Coun. Reg. Mtg. Proc., pp. 33-38.

Smith, K.L., Todhunter, D.A., and Schoenberger, P.S., 1985. Environmental pathogens and intramammary infection during the dry period. J. Dairy Sci., 68:402-417.

Smith, V.G., Edgerton, L.A., Hafs, H.D., and Convey, E.M., 1973. Bovine serum estrogens progestins, and glucocorticoids during late pregnancy, parturition, and early lactation. J. Anim. Sci., 36:391-396.

Solbu, H., Spooner, R.L. and Lie, Ø. 1982. A possible influence of the bovine major histocompatibility complex (BoLA) on mastitis. Proc. 2nd World Congr. Genet. Appl. Livest. Prod., 7:368.

Soller, M., 1994. Marker assisted-selection-an overview. Anim. Biotech., 5:193-207.

Sordillo, L.M., 1995. Vaccination as a protection against bovine mastitis. Natl. Mast. Coun. Reg. Mtg. Proc., pp. 64-68.

Sordillo, L.M., and Babiuk, L.A., 1991. Controlling acute *Escherichia coli* mastitis during the periparturient period with recombinant bovine interferon gamma. 1991. Vet. Microbiol., 28:189-198.

Sordillo, L.M., Redmon, M., Campos, M., Warren, L., and Babiuk, L.A., 1992. Cytokine activity in bovine mammary gland secretions during the periparturient period. Can. J. Vet. Res., 55:298-301.

Spangelo, B.L. and Gorospe, W.C., 1995. Role of cytokines in the neuroendocrine-immune system axis. Front. Neuroendocrinol., 16(1):1-22.

Sridama, V., Pacini, F., Yang, S-L., Moawad, A., Reilly, M., and DeGroot, L.J., 1982. Decreased levels of helper T cells. N. Engl. J. Med., 307:352-356.

Sternberg, E.M., Hill, J.M., Chrousos, G.P., Kamilarus, T., Listwak, S.J., Gold, P.W., and Wilder, R.L., 1989. Inflammatory mediator-induced hypothalamic-pituitary-adrenal axis activation is defective in streptococcal cell wall arthritis-susceptible Lewis rats. Proc. Natl. Acad. Sci. USA, 86(7):2374-2378.

Sulimova, G.E., Udina, I.G., Shaikhaev, G.O., and Zakharov, I.A., 1995. DNA polymorphism at the BoLA-DRB3 gene of cattle in relation to resistance to susceptibility to leukemia. Russ. J. Genet., 31:1105-1109.

Todhunter, D.A., Smith, K.L., and Hogan, J.S., 1990. Growth of Gram-negative bacteria in dry cow secretions. J. Dairy Sci., 73:363-372.

Van der Zijpp, A.J., Frankena, K., Boneschanscher, J., and Nieuwland, M.G.B., 1983. Genetic analysis of primary and secondary immune response in the chicken. Poultry Sci., 6: 565-572.

Van Kampen, C. and Mallard, B.A., 1997. Effects of peripartum stress and disease on bovine lymphocyte subsets. Vet. Immunol. Immunopath. (In press).

Van Werven, T., Noordhuizen-Stassen, E.N., Daemen, A.J.J.M., Schukken, Y.H., Brand, A. and Burvenich, C., 1997. Preinfection *in vitro* chemotaxis, phagocytosis, oxidative burst, and expression of CD11/CD18 receptors and their predictive capacity on the outcome of mastitis induced in dairy cows with *Escherichia coli.* J. Dairy Sci., 80:67-74.

Vassilopoulou-Sellin, R., 1994. Endocrine effects of cytokines. Oncology. Huntingt., 8(10):43-46.

Vega, J.R., Gibson, C.A., Skaar, T.C., Hadsell, D.L. and Baumrucker, C.R., 1991. Insulin-like growth factor (IGF) 1 and 2 and IGF binding proteins in serum and mammary secretions during the dry period and early lactation in dairy cows. J. Anim. Sci., 69:2538-2547.

Watson, D.L., and Schwartskoff, C.L., 1990. A field trial to test the efficacy of a staphylococcal mastitis vaccine in commercial dairies in Australia. In: Proc. Int. Symp. Bov. Mast., Indianapoli, IN, pp.73.

Weigel, K.A., Kehrli, Jr., M.E., Freeman, A.E., Thurston, J.R., Stear, M.J., and Kelley, D.H., 1991. Associations of class I bovine lymphocyte antigen complex alleles with in vitro blood neutrophil functions, lymphocyte blastogenesis, serum complement and conglutinin levels in dairy cattle. Vet. Immunol. Immunopath., 27:321-335.

Weiland, F., and Straub, O.C., 1976. Differences in the in vitro response of lymphocytes from leukotic and normal cattle to concanavalin A. Res. Vet. Sci., 20:340-341.

Weinberg, E.D., 1984. Pregnancy-associated depression of cell-mediated immunity. Rev. Inf. Dis., 6(6):814-831.

Wells, P.W., Burrells, C., and Martin, W.B., 1977. Reduced mitogenic responses in cultures of lymphocytes from newly calved cows. Clin. Exp. Immunol., 29:159-161.

Weller, J.I., Saran, A., and Zeliger, Y., 1992. Genetic and environmental relationships among somatic cell count, bacterial infection, and clinical mastitis. J. Dairy Sci., 75:2532-2540.

Wijngaard, P.L.J., Metzelaar, M.J., MacHugh, N.D., Morrison, W.I., and Clevers, H.C., 1992. Molecular characterization of the WC1 antigen expressed specifically on bovine $CD4^{-}CD8^{-}$ $\gamma\delta$ T lymphocytes. J. Immunol., 149:3273-3277.

Williams, M.R., Maxwell, D.A.G., and R.L. Spooner, 1975. Quantitative studies on bovine immunoglobulins, normal plasma levels of IgG2, IgG1, IgM, and IgA. 18:314-321.

Williams, M.R., and Hill, A.W., 1982. A role for IgM in the *in vitro* opsonisation of *Staphylococcus aureus* and *Escherichia coli* by bovine polymorphonuclear leucocytes. Res. Vet. Sci., 33:47-53.

Wright, P., 1987. Enzyme Immunoassay: observations on aspects of quality control. Vet. Immunol. Immunopath., 17:441-452.

Xu, A., van Eijk, M.J.T., Park, C., and Lewin, H.A., 1993. Polymorphism in BoLA-DRB3 exon 2 correlates with resistance to persistent lymphocytosis caused by bovine leukemia virus. J. Immunol., 151:6977-6985.

Yang, T.J., Mather, J.F., and Rabinovsky, E.D., 1988. Changes in subpopulations of lymphocytes in peripheral blood, and supramammary and prescapular lymph nodes of cows with mastitis and normal cows. Vet. Immunol. Immunopath., 18:279-285.

Yoshida, A., Ishioka, C., Kimata, H., and Mikawa, H., 1992. Recombinant human growth hormone stimulates B cell immunoglobulin synthesis and proliferation in serum-free medium. Acta Endocrinologica, 126:524-529.

Zanotti, M., Poli, G., Ponti, W., Polli, M., Rocchi, M., Bolzani, E., Longeri, M., Russo, S., Lewin, H.A., van Eijk, M.J.T., 1996. Association of BoLA class II haplotypes with subclinical progression of bovine leukemia virus infection in Holstein-Friesian cattle. Anim. Genet. 27:337-341.

Zhang, W.C., Dekkers, J.C.M., Banos, G., and Burnside, E.B., 1993. Sire genetic evaluation for somatic cell score and relationships with other traits. 1993 Dairy Research Report, Ontario Ministry of Agriculture, Food and Rural Affairs, Publ. No. 0193, pp., 106-109.

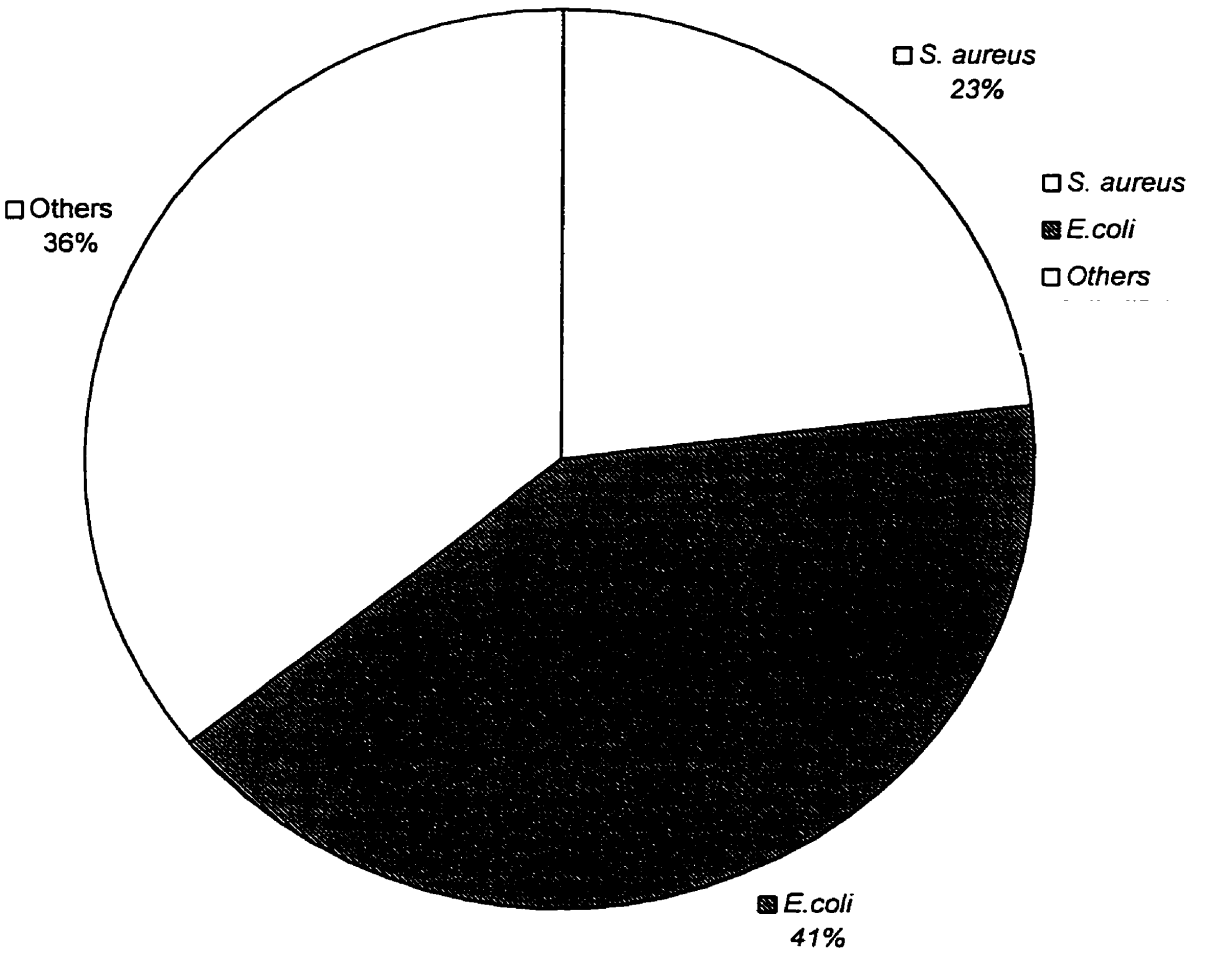
Appendix I

Table 1 Pathogens which cause mastitis

Major Pathogens			
Contagious			Source
Staphylococcus aureus			Harmon, 1994
Streptococcus agalactiae			Harmon, 1994
Sporadic			
Pseudomonas spp			Harmon, 1994
Actinomyces pyogenes			Harmon, 1994
Environmental			
Coliforms	-	*Escherichia coli*	Harmon, 1994, Kremer, 1994
		Klebsiella spp.	Harmon, 1994, Kremer, 1994
		Enterobacter spp.	Harmon, 1994, Kremer, 1994
		Citrobacter spp.	Harmon, 1994
		Proteus	Kremer, 1994
		Serratia	Lohuis, 1988
Environmental *Streptococci*			Harmon, 1994
Environmental *Enterococci*			Harmon, 1994
Streptococcus dysgalactiae			Harmon, 1994
Streptococcus uberis			Harmon, 1994
Streptococcus bovis			Harmon, 1994
Minor Pathogens			
Coagulase negative *staphylococci*			Harmon, 1994
Corynebacterium bovis			Harmon, 1994

Major pathogens, in contrast to minor pathogens result in the greatest compositional changes in milk and have the most economic impact.

Figure 1. Frequency of Major pathogens causing mastitis isolated at -30, 0, and 30 days post claving



Source: Adapted from Detilleux et. al. 1995. J.Dairy Sci. 78:2285-2293.

Appendix II

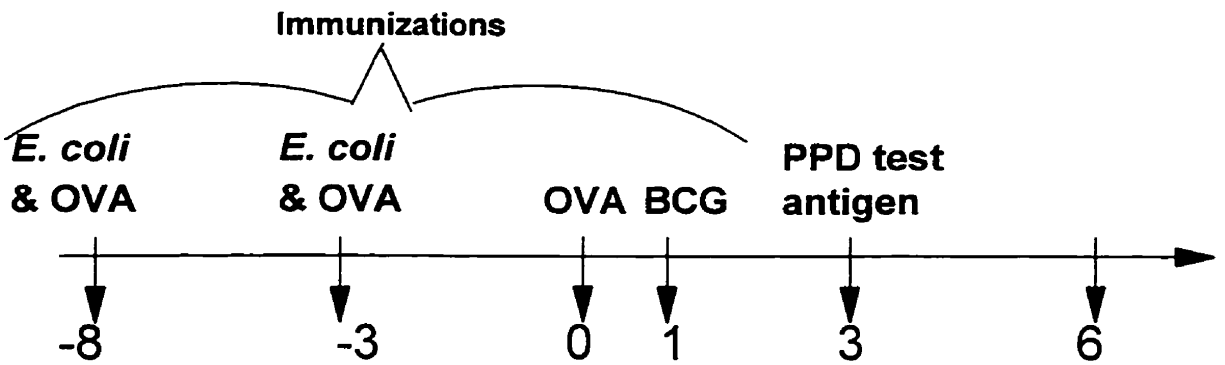
Table I. Subclass immunoglobulin concentration in serum, colostrum, and milk

Source	Concentration (mg/mL)			
	IgG_1	IgG_2	IgA	IgM
Serum	10.06	9.04	0.34	3.69
Colostrum	64.9	2.2	3.5	8.7
Milk	0.64	0.05	0.13	0.04

Source: Butler, J.E. A concept of humoral immunity among ruminants and an approach to its investigation. International Symposium on the Ruminant Immune System, Plymouth, NH. 1980.

Appendix III

Figure 1. Immunization and sampling schedule



Milk collection for:

1) whey antibody at Weeks 0,2,3,4 and 6 relative to calving
2) whey immunoglobulin at weeks 0, 3, and 6

Blood collection for:

1) lymphocyte proliferation: at week -3,0,3, and 6
2) serum antibody: at week -8 then weekly from week-3 to week +6
3) serum IgG: at weeks 0, 3, and 6

Figure. 2 Diagram of injection sites of BCG, PPD and PBS in the left and right caudal tail folds of the tailhead of the dairy cow

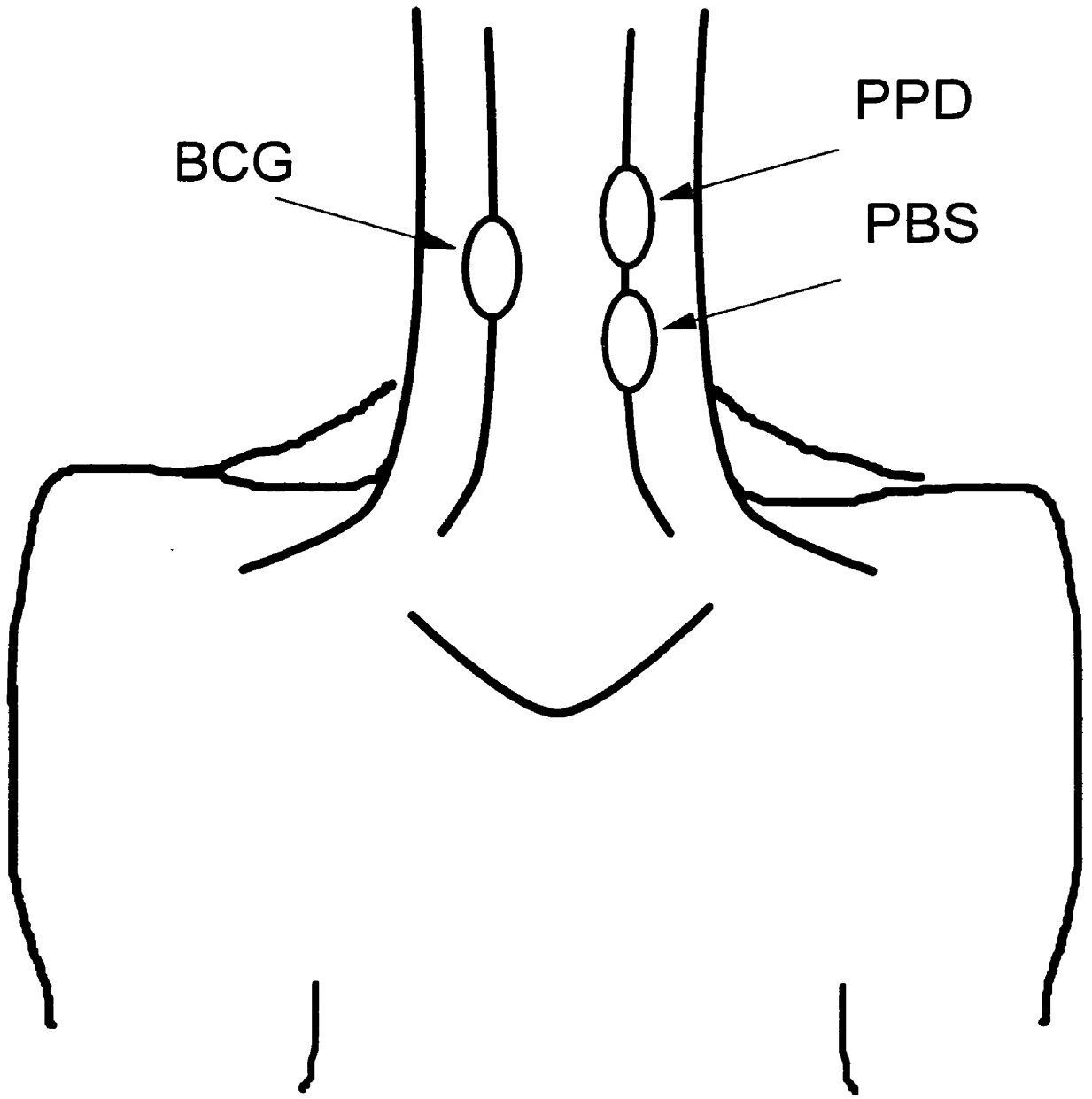
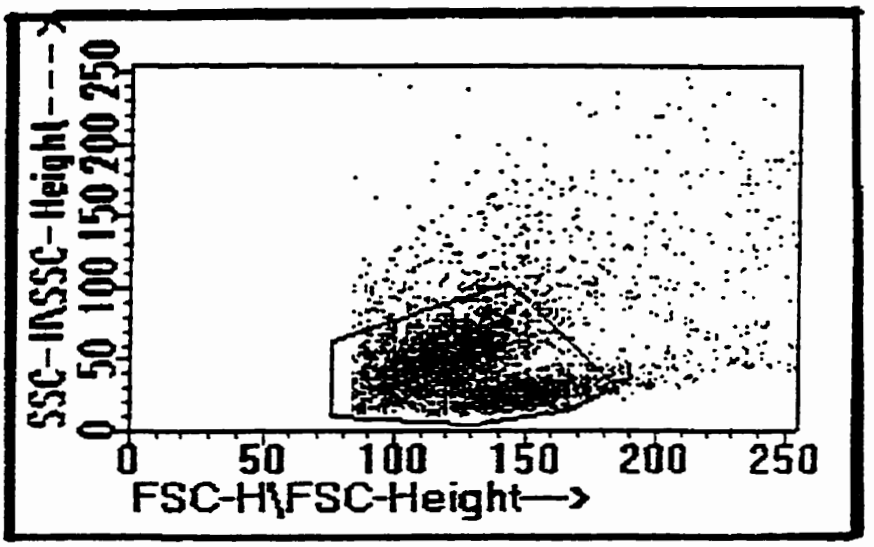


Figure 3. A) Lymphocytes stimulated in culture gated out from other cell populations based on forward (FSC) and side (SSC) scatter characteristics. B) Lymphocytes to the left of the marker M1 represent cells which naturally fluoresce but do not possess the CD surface marker of interest. Lymphocytes possessing the CD surface marker of interest will show fluorescence events to the right of M1. In this example, lymphocytes stimulated with ovalbumin (OVA) which possess the CD4 surface marker, are identified to the right of M1 and the left of M2.

A)



B)

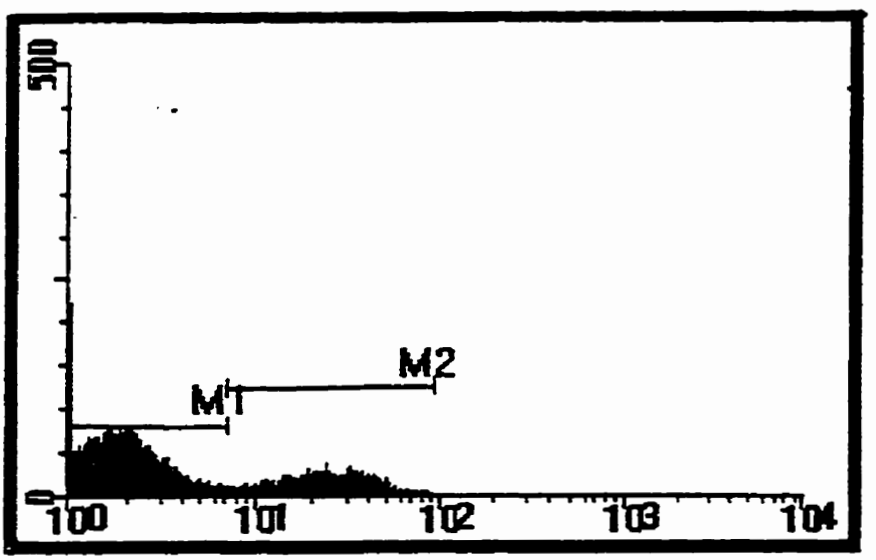


Figure 4. Percent of peripheral blood lymphocytes of dairy cows expressing A) CD2, B) CD4, C) CD8, D) WC1+, and E) IgM following 42 hours of culture with media, concanavalin A (Con A;5 ug/mL) or ovalbumin (OVA ;5ug/mL and 20 ug/mL).

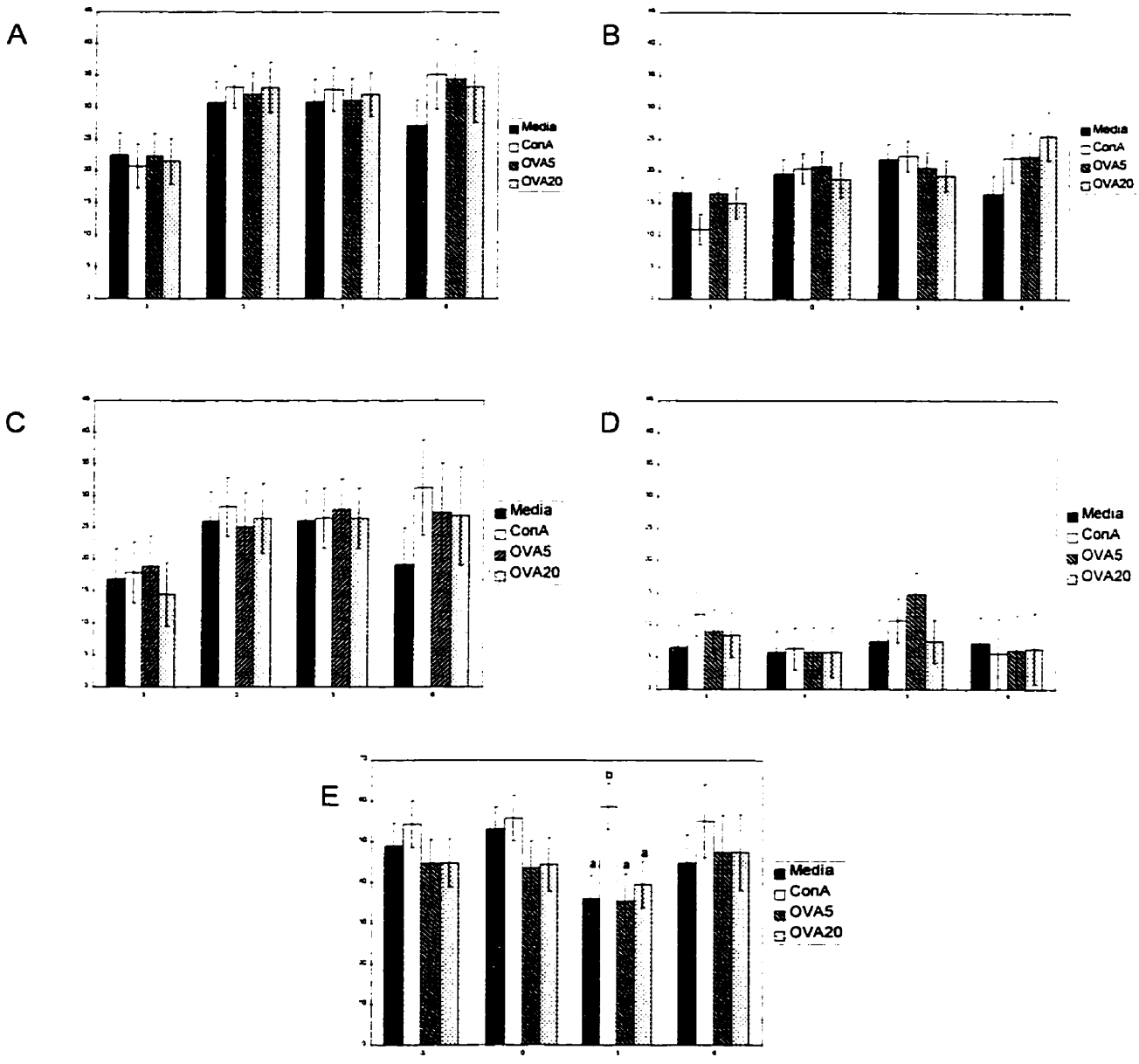


IMAGE EVALUATION TEST TARGET (QA-3)

150mm

6"

APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989