Associations between genetic markers and mastitis resistance in Canadian Holsteins

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April, 2005

A thesis submitted to McGill University in partial fulfillment of the degree of Doctor of Philosophy

Your file Votre référence
ISBN: 978-0-494-21680-4
Our file Notre référence
ISBN: 978-0-494-21680-4

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ABSTRACT

The objective of this thesis was to test for associations between genetic polymorphisms of genes related to immune response (growth hormone (GH), growth hormone receptor (GHR), ornithine decarboxylase (ODC), insuline-like growth factor-1 (IGF-1), adrenocorticotropin hormone (ACTH), corticotrophin releasing hormone (CRH), and prolactin (PRL)) and mastitis resistance traits (incidence of clinical mastitis (ICM), occurrence of clinical mastitis (OCM), culling due to mastitis (CDM), and somatic cell scores (SCS)) in Canadian Holsteins.

Using lactation records of cows enrolled in milking recording in Québec (Programme d'Analyse des Troupeaux Laitiers du Québec, PATLQ) from 1980 to 1994 (411,291 first, 238,432 second, and 130,983 third lactations, respectively) Estimated Transmitting Abilities of traits were generated with a model that included the random effect of sire, and fixed effects of herd-year-season-of calving, age at calving, and genetic group. 721 bulls which had daughters in the phenotypic data sets were genotyped for twenty polymorphisms of the above genes located on autosomes (BTA) 5, 11, 14, 19, 20, and 23.

Two types of analysis of associations were performed: analysis across-population with a model that included the fixed effect of marker and random effect of the son of grandsire, and within-family analysis with a model that included the fixed effects of the grandsire, marker nested within grandsire, and the random effect of son nested within marker and grandsire. Permutation tests were performed to reduce Type I error probability.

Significant associations were found within families for markers of IGF-1 (BTA5), ODC (BTA11), GH (BTA 19), GHR (BTA 20), and PRL (BTA 23) for ICM, OCM, CDM, and SCS in different lactations. Some of these putative quantitative trait loci (QTL) are located on BTA where other authors have reported QTL affecting SCS and udder conformation. The results from this study may contribute to efforts to dissect the genetic basis of mastitis resistance in dairy cattle.

RÉSUMÉ

L'objectif de cette recherche était d'analyser les associations entre polymorphismes de gènes qui affectent la réponse immunitaire (l'hormone de croissance (HC), le récepteur de l'hormone de croissance (RHC), l'ornithine décarboxylase (ODC), l'IGF-1 (insuline-like growth factor-1), l'hormone adrénocorticotrope (ACTH), l'hormone corticotrope (CRH), et la prolactine (PRL)) et caractéristiques de résistance à la mammite (l'incidence de mammite clinique (IMC), l'occurrence de mammite clinique (OMC), la réforme pour cause de mammite (RPM), et le comptage de cellules somatiques (SCS)) à la première, deuxième et troisième lactation.

Le registre des lactations des vaches inscrites au Programme d'Analyse des Troupeaux Laitiers du Québec (PATLQ) de 1980 au 1994 (411, 291 inscriptions de première lactation, 238, 432 inscriptions de deuxième lactation, et 130, 983 inscriptions de troisième lactation) a été utilisé pour estimer les habilités de transmission des taureaux (HT) pour les caractéristiques à l'étude. Le modèle défini afin obtenir les HT incluait l'effet aléatoire du taureau, l'effet fixe pour le troupeau-année-saison de vêlage, l'âge au vêlage ainsi que le groupe génétique. Un total de 721 taureaux avec filles de la banque de données phénotypique a été génotypé pour vingt polymorphismes génétiques situés sur les autosomes (AUT) 5, 11, 14, 19, 20, et 23.

Deux types d'analyses ont été faites: une analyse à l'intérieur de la population avec un modèle qui inclus l'effet du marqueur génétique et l'effet aléatoire du taureau; et une analyse à l'intérieur de la famille, avec un modèle qui inclus l'effet du grand-père, l'effet du marqueur imbriqué dans l'effet grand-père, et l'effet aléatoire du taureau imbriqué dans les effets du marqueur et du grand-père. Des permutations ont été faites pour réduire la probabilité de l'erreur de type I. Des associations statistiquement significatives ont été trouvées pour les marqueurs IGF-1 (AUT 5), ODC (AUT 11), GH (AUT 19), GHR (AUT 20) et PRL (AUT 23) pour les traits IMC, OMC, RPM et SCS pour différentes lactations. Quelques uns de ces loci de caractères quantitatifs (QTL) ont été localisés sur des AUT où d'autres QTL affectant le SCS et conformation de la glande mammaire ont été trouvés par le

passé. Les résultats de cette recherche pourraient contribuer aux efforts entrepris pour disséquer les bases génétiques de la résistance à la mammite en production laitière.

ACKNOWLEDGMENTS

I want to thank the Consejo Nacional de Ciencia y Tecnología de México for the financial support, and the Programme d'analyse des troupeaux laitiers du Québec, Centre d'insémination artificielle du Québec, and Canadian Dairy Network for facilitating essential information used in this research.

I greatly thank the guidance and help (intellectual, technical, and financial) of my supervisor Dr. J. Flann Hayes through all the stages of my studies in Canada; I will always appreciate his genuine intentions towards my training and development as researcher. I thank Drs. Humberto Monardes and Roger I. Cue for their friendly disposition towards my graduate experience, either by being always available to respond my questions or providing me with valuable criticism and information. I appreciate the attentions given by Dr. David Zadworny, as well as the labor of his technical staff, especially Marilyn Richard who diligently processed a significant amount of samples required to culminate this research. Thanks to Drs. Kevin Wade, René Lacroix, and Diederick Pietersma for giving me the opportunity to use the facilities of the Infosys Group. I also thank Drs. K. F. Ng-Kwai-Hang (McGill University), Costas Karatzas (Pharmathene Canada Inc.) and David E. Kerr (University of Vermont) for serving in my committee and for their enriching comments and criticism to this thesis. I thank Drs. Dale Van Vleck (University of Nebraska) and Curtis Van Tassell (USDA) for their kind responses to my questions by email. Also thanks to Drs. Ciro Ruiz, Arif Mustafa, and Vilceu Bordignon, they were always available to discuss a wide variety of topics.

Thanks to other members of the staff of the Department of Animal Science: Barb Stewart, Carol Newman, Cinthya Horvath, and specially Sandra Nagy and Lise Ménard for their always friendly support. My gratefulness to all graduate students of the Department of

Animal Science, but especially to Fréderic Fortín and Annie St-Onge, both for their finest attentions towards me and my family; I also appreciate the friendship of Boda Gayatri, Gao Yongchun, and Mathieu Leduc; their kindness made student life easier. Thanks to Dana Praslickova, Sylvia Borucki, and Kenton Olivierre for sharing their time. Thanks to Robert L. Houde for his friendship and assistance in many passages of my writing.

I give my deeply appreciation to Claudia Báez, Guy Déry, and Amabilis Deveu (and all the people from the Carrefour Marguerite-Bourgeois) for supporting my wife in adapting to a different culture; their warm smiles and encouraging words really made us feel at home, even in winter. Thanks to Teresa García-Peniche (Virginia Tech, INIFAP), Consuelo Almazán-García (Oklahoma State University, UAT), Marino Rebolledo-Alvario (Intervet), and Juan Almaraz and Dagobiet Morales (McGill University, Colegio de Postgraduados) for the laughs or thoughts we shared by email or during lunch hour.

I thank the support, comprehension and love of my parents and my brother who due to this doctoral issue suffered the temporal absence of their first grandchild and nephew, respectively.

Last but not least, I thank Silvia and our son José; they were path and guide in this process; they are constant source of love, happiness, courage and determination. This endeavour simply could not have been done without them.

CONTRIBUTION TO THE KNOWLEDGE

This thesis has resulted in the following original contributions to knowledge:

1. The estimation of variance components for mastitis resistance traits (incidence of clinical mastitis, occurrence of clinical mastitis, culling due to mastitis) in Canada
2. Estimation of the generation interval for all the paths of selection in Canadian Holstein cattle (sires to breed cows, sires to breed bulls, dams to breed bulls, and dams to breed cows)
3. Relationships among mastitis resistance traits and SCS in Canadian Holsteins
4. Allelic frequencies for several genetic polymorphisms on genes affecting immune response (Growth hormone, Growth Hormone Receptor, Prolactin, ACTH, CRH, and ODC) in Canadian Holstein bulls
5. Detection of QTL affecting mastitis resistance traits and SCS in the Canadian Holstein population

ABBREVIATIONS

AccI	restriction enzyme obtained from *Acinetobacter calcoaceticus*
ACTH	adrenocorticotropic hormone
AluI	restriction enzyme obtained from *Arthrobacter luteus*
BoLa	bovine lymphocyte antigen
bp	base pair
BsT	bovine somatotropin
BTA	*Bos taurus* autosome
cM	centimorgan
CM	clinical mastitis
CRH	corticotropin releasing hormone
dATP	2' –deoxyadenosine-5'-triphosphate
DGTA1	acylCoA:diacylglycerol acyltransferase
DRB	D region associated B cell antigens
EBV	estimated breeding value
EDTA	ethylenediamine tetraacetic acid
ETA	estimated transmitting ability
et al.	*et alia* (and others)
e.g.	*exempli gratia* (for example)
GAS	gene assisted selection
GH	growth hormone
GHR	growth hormone receptor
h^2	heritability
ICAR	International Committee for Animal Recording
i.e.	*id est* (that is)
IFDP	International Federation of Dairy Producers
IGF-1	insulin-like growth factor-1
INTERBULL	International Bull Evaluation Service
IL	interleukins

IMI	intramammary infections
Kg	kilograms
LD	linkage disequilibrium
MAS	marker assisted selection
MHC	major histocompatibility complex
mL	milliliter
mM	millimolar
ng	nanogram
ODC	ornithine decarboxylase
PATLQ	Programme d'Analyse des Troupeaux Laitiers du Québec
PRL	prolactin
QTL	quantitative trait loci
r_g	genetic correlation
SCC	somatic cell count
SCS	somatic cell score
StuI	restriction enzyme obtained from *Streptomyces tubercidicus*
TaqI	restriction enzyme obtained from *Thermos aquaticusYtl*
TNF-α	tumor necrosis factor-α
χ^2	chi-square
μM	micro molar

CHAPTER 1

INTRODUCTION

Mastitis is a major cause of economic loss in the dairy industry. These losses can be classified as direct (reduction of milk production, reduction of milk quality, costs of veterinary treatments and drugs) or indirect (increase of involuntary culling and low fertility). As a consequence, many efforts have been made to reduce the economic impact of mastitis on the dairy industry. Although the results of a recent Canadian study regarding the economic impact of mastitis on the national dairy industry is not available, it may be expected to be (proportionally) similar to the US or Europe.

Needless to say, mastitis is a pathology which seriously threatens the profitability of dairy operations. Consequently, mastitis is the pathological condition that receives the greatest attention from producers, breeders, and geneticists.

One of the most promising approaches to control mastitis is the genetic improvement of dairy cattle. A genetic option widely used is the selection of animals with high milk yield and certain body conformation, such as deep udders with strong fore attachment. Evidence shows a favorable genetic correlation between udder conformation and somatic cell count in Holsteins (Monardes *et al*, 1990), arguably the beneficial effect of udder conformation may be due to better anatomical defenses against infections. This genetic selection has allowed the modern dairy cow to express high milk yields but also levels of mastitis that would be higher if selection for conformation or lower levels of SCS had not been taken into account. That is why some traits with indirect relationships with mastitis resistance, such as udder conformation, somatic cell scores and clinical mastitis incidence, have been included in selection programs (Swalve, 2000). Thus it has been demonstrated that the genetic trend for mastitis resistance can stay flat without a detrimental effect on the genetic trend for yield traits (Heringstad *et al*, 2003); however, clinical mastitis incidence has not declined over the years (Bradley, 2002).

Identifying genes with effects on traits of interest is a feasible approach for genetic improvement of disease resistance. Clinical mastitis has low heritability and consequently

marker assisted selection may have an important role in selection against mastitis resistance. Some of the research aimed at detecting Quantitative Trait Loci (QTL) which affect mastitis resistance has been carried out to study the role of the Major Histocompatibility Complex (MHC) genes, although the results have not been conclusive. However evidence suggests that genes other than MHC genes may be involved in the genetic control of immune response expression. Finding genes which control mastitis resistance may be a way to reduce the negative impact that selection for high yield might have on mastitis resistance.

Quantitative Trait Loci (QTL) have been detected affecting yield traits in dairy cattle, fewer have been detected affecting mastitis; despite that complex traits are more likely to be controlled by several genes, the first step to dissect the molecular basis of quantitative traits is to detect associations between genetic markers and quantitative traits.

In order to perform association studies between markers and quantitative traits, the knowledge of polymorphisms at the DNA level is required. The objective of this study was to test the relationship between genetic markers of genes with recognized effects on immune response and mastitis resistance in Canadian Holsteins.

This study provides evidence of QTL affecting mastitis resistance traits in Canadian Holsteins; further research will be needed in order to detect and reveal the complex interrelationships that might occur in the chromosomal regions that harbor these QTL. Many tools are being used to understand gene interactions (*i.e.* microarrays and DDRT-PCR); however association studies are still helpful in initial steps towards detecting genes with beneficial effects on economically important traits in dairy cattle.

CHAPTER 2

REVIEW OF LITERATURE

2.1 ECONOMIC LOSSES DUE TO MASTITIS

It has been estimated that around 60% of the losses due to mastitis are due to reduced milk production; Blosser (1979) estimated through a review of scientific literature that loss of milk yield from mastitis in the United States (US) was 386 kg/ cow per year; more recently, the reduction was estimated between 110 to 552 kg per lactation in Finnish Ayrshire (Rajala-Schultz *et al*, 1999).

Several studies have been carried out to estimate the economic importance of mastitis. In the United Kingdom (UK), the average cost of a case of clinical mastitis (CM) was £175/affected cow per year (Kossaibati and Esslemont, 2000) and considering an average incidence of 40 cases/100 cows/year, the total loss can be as much as £168 million per year (Bradley, 2002 In the US, the cost per episode of clinical mastitis was US$107 (Hoblet *et al*, 1991); in general, the cost of an individual mastitis clinical case varies between US$77 and US$136 (Kirk *et al*, 1994). The apparent difference between the estimates from UK and US reported here may be partially explained by the methodology used. Kossaibati and Esslemont (2000) considered the cost of affected cows per year, while the studies from US considered costs per clinical episode. The global cost for the US dairy industry is roughly US$ 2 billion per annum or 10% of the total value of milk sales (Harmon, 1996, Wells and Ott, 1998).

2.2 EFFECTS OF MASTITIS ON MILK YIELD AND QUALITY

The reduced yield associated with mastitis may be explained by the tissue damage and the udder's consequent reduced ability to synthesize milk (Harmon, 1994). Evidence shows that the loss in milk depends on parity and the stage of lactation when mastitis occurs, and the damage of the secretory tissue might be so important that the production

level observed before the onset of mastitis is not reached during the rest of the lactation period (Rajala-Schultz *et al*, 1999).

Tissue damage is a result of the inflammatory reaction caused by the proliferation of leukocytes[1] against the infecting pathogen (Bouchard *et al*, 1999). The inflammatory process within infected udders is characterized by increased migration of polymorphonuclear neutrophils (PMN). Neutrophilia (the augmented number of neutrophils) is the main factor in the increase of somatic cell counts (SCC) (Burton *et al*, 2001; Paape *et al*, 2002).

The quality of milk is a determining factor in the value of dairy products. Subclinical mastitis has been established as a key element causing reduction of milk quality. The resulting high SCC during subclinical mastitis has negative effects on milk, such as undesirable changes in the protein fraction, reduced shelf life, and changes in flavor quality (Urech *et al*, 1999; Ma *et al*, 2003; Santos *et al*, 2003).

Several proteolytic and lipolytic enzymes of various origins may be present at any time in bovine milk (Fox and McSweeney, 1998). The low milk quality results from the passage of extracellular fluid into the milk. The extracellular fluid contains many components, among them enzymes (lipases and proteases) that affect the composition of the milk. Once these enzymes are in contact with the milk, fat and casein break down, contents of fat, protein and lactose are reduced, and the content of whey proteins is increased.

High levels of SCC (3×10^5 to 5×10^5 somatic cells/ml milk) decreased the casein fraction as a percentage of the total protein in milk, and were associated with reduced

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[1] Leukocyte is a term used to encompass the totality of white cells (monocytes, eosinophils, basophils, and neutrophils) present in blood. In bovines, neutrophils represent between 20-30% of the leukocytes, when there is no infection (Tizard, 1996). Experimentally, it has been observed that 12 hours after an injection of 0.5 mg *E. coli* endotoxin, immature PMN represented 30% of the total circulating leukocytes (Paape *et al*, 2003).

capacity for producing cheddar cheese by decreasing the conversion of casein into cheese (Auldist *et al*, 1996). *Plasmin*, a proteolytic enzyme, is present in normal milk, and it shows increased activity in cows with mastitis. *Plasmin* promotes the hydrolysis of casein, the main consequence being altered rennet coagulation (Srinivasan and Lucey, 2002). Additionally, proteolytic activity may be increased due to the action of proteolytic enzymes from PMN and macrophages (Verdi and Barbano, 1991; Moussaoui, *et al*, 2002). The characteristic bitterness found in milk from udders with mastitis is a result of the combined action of these proteases and the proteases that pass from the extracellular compartments to the milk. The action of lipases causes the release of fatty acids from triglycerides, and the result is an undesirable rancid flavour (Santos *et al*, 2003). Normal milk contains lipoprotein lipase (LPL) (Fox and McSweeney, 1998); additionally, reduced shelf-life is caused by the action of bacterial enzymes that cause extensive lipolysis and proteolysis (Ma *et al*, 2003); Urech *et al* (1999) has pointed out that proteolysis can occur even during subclinical mastitis; in that study subclinical mastitis was defined as absence of clinical signs of mastitis and more than 100,000 cells/ml.

Understandably, these problems derived from high SCC directly concern the industrial milk processors. In an attempt to obtain raw milk with reduced cell counts, milk pasteurizing plants penalize milk with high SCC. This is why producers have been giving more attention to programs aimed at reducing infections in their herds. Standards for bulk SCC on delivery varies depending on countries; for instance, 500,000 cells per ml in Canada, 750,000 cells per ml in US; 400,000 cells per ml in the European Union. One of the most common approaches to reduce SCC in bulk tank milk is to administer antibiotics to cows with mastitis. However, this approach may bring unwanted complications. In some countries there are monetary penalties to producers that deliver milk with antibiotic residues; thus discarded milk is another cause of losses. Therefore several management programs have been proposed in order to reduce the incidence of mastitis. In Québec, for instance, the impact of programs oriented to reduce mastitis incidence is evident when comparing the trend in the distribution of herds according to various levels of SCC (Table 2.1):

Table 2. 1 Percentage of herds by SCC in milk recording in Québec from 1997 to 2003.

SCC / ml	1997	1998	1999	2000	2001	2002	2003
< 100,001	11.75	8.51	7.05	7.19	7.45	8.19	7.93
100,001-200,000	34.19	29.51	27.73	29.33	29.72	30.08	29.36
200,001-300,000	27.31	27.35	28.16	28.73	28.87	28.26	28.40
300,001-400,000	14.70	16.86	18.18	17.77	17.52	17.23	17.49
400,001-500,000	6.69	9.0	9.81	8.91	8.68	8.58	8.98
> 500,000	5.17	8.72	9.09	7.74	7.76	7.66	7.85

References: PATLQ, Rapports annuels de Production, 1997-2003

The proportion of herds with more than 300,000 cells per ml has actually increased from 26% in 1997 to 34% in 2003, however, the average SCC per herd has decreased through the years: 366,000, 312,000, and 272,000 cells per ml in 1983, 1993, and 2003, respectively (FPLQ, 1983 and 1993, PATLQ, 2003). Several factors might be responsible for the reduction in average SCC per herd; some of them are: better hygienic management, culling of cows with chronic mastitis, and selection for better udder conformation. The average of SCC per herd will be further reduced in the future, as a recently approved resolution voted by dairy farmers will reduce the standard for total bacterial count from 100,000 per ml to 50,000 per ml (FPLQ, 2002), and therefore more prophylactic and therapeutic actions are likely to be taken by farmers. So far, as previously mentioned, the prophylactic and therapeutic approach more frequently used is the use of antibiotics. However, with regard to antibiotics, the standard requirements for milk in Canada establish a zero-limit for residues, otherwise milk is destroyed. In this respect, in Québec, there was an increase of 11% in milk loads destroyed in 2002 compared with 2001 (FPLQ, 2002). Therefore, many precautions should be taken by farmers when using antibiotics as prophylactic or therapeutic choice.

For reasons to be discussed in the next two sections, mastitis, as a global problem for the dairy industry, is not likely to be efficiently controlled solely by therapeutic means. Various approaches have to be combined to reduce the actual level of losses caused by this pathological condition.

2.3 PATHOGENESIS OF MASTITIS

Two main factors affect the incidence of mastitis: i) the virulence of the pathogens, and ii) the response of the animal's immune system. Animals have several defense mechanisms against infections: anatomical structures (the skin, the teat canal), inflammation response (the initial inflammation is characterized by the presence of several cells, mainly PMN and macrophages whose main goal is to destroy foreign particles, especially bacteria, by phagocytosis) which is a general response localized near the site of entrance of the pathogen(s), and a more specific response, through the production of antibodies (i.e immunoglobulins) by lymphocytes. Other mechanisms such as the complement system, a system of serum proteins that are activated by specific combinations of antigen-immunoglobulin, collaborate with the destruction of the invading pathogens. In the case of the udder, additional defense mechanisms involve proteins (lactoferrin) and enzymes (lactoperoxidase). All these mechanisms coordinate to provide the animal with an adequate defense against infections.

The main cause of mastitis is the colonization of the udder by pathogens, and its immediate result is the udder's inflammation and reduced capacity for milk secretion.

Once an infecting microorganism (mainly bacteria, less frequently viruses) colonizes the cistern of the udder, after passing the first barrier of defense that is the skin and the teat canal with its keratin layer, the innate immune system enters into action. The immune response is defined according to the type of mechanism involved, either cellular and/or humoral. The initial immune response is based on the intervention of several types of cells with the purpose of eliminating the infecting microorganisms through bactericidal effects (*i.e.* cellular immune response). In brief, the innate immune system integrates three main components that interact in response to an infection: the complement system, macrophages and neutrophils.

Proteins from the complement system exhibit bactericidal activities (for example by making channels on the surface of the pathogens, which leads to their destruction), and in addition, some of these proteins opsonize[2] the invading pathogens and turn them into targets for macrophages.

In turn, activated macrophages produce interleukins (IL) and Tumor Necrosis Factor α (TNF-α) which activate Natural Killer Cells (NK cells). Cytokines produced by macrophages also play a role in the expression of *selectin*, a protein (expressed on the surface of endothelial cells) essential for attracting neutrophils to the site of infection. Macrophages release a neutrophil chemotactic factor which promotes the migration of large amounts of PMN to the site of the infection. These events occur within hours after infection, hence their relevance in clearing out the causal agent. Many infections are in fact cleared via the interaction of the complement system, macrophages, neutrophils and lymphocytes.

The role of neutrophils is probably the most important within the udder's defense. The proportion of neutrophils in SCC depends on the health status of the udder and on the type of microorganism involved in the infection. Both healthy and affected udders have been reported to show 107 x 10^3, and 2 x 10^6 cells per ml, respectively; in infections caused by *E. coli* and *S. aureus*, 90% of somatic cells were neutrophils (Leitner *et al*, 2000). Somatic cells must be seen as a normal part of the udder's environment; however, sometimes the equilibrium is broken and oxidant substances released by PMN during the process of phagocytosis may damage the secretory tissue of the udder. There is however a controversy on how to define a normal level of SCC; a proposed level has been less than 10^5 cells per ml normally present in healthy udders (Kehrli and Shuster, 1994). Traditionally, it has been considered that the key bactericidal action of the neutrophils occurred exclusively at the intracellular level, through phagocytosis, respiratory burst,

[2] Foreign particles are coated (opsonized) with antibodies or other proteins from the Complement System, which make them susceptible to be phagocytosed.

and antibacterial peptides; however, evidence suggests that neutrophils also have the capacity to release extracellular traps (NETs) composed of DNA, histones and elastase. These NETs, abundant especially in acute inflammation, have bactericidal effects (against gram positive and gram negative bacteria) which remain to be understood. A hypothesis is that even before neutrophils engulf the pathogens, NETs kill bacteria through the effect of histones and elastases; in addition NETs may prevent the diffusion of harmful proteins of bacterial origin far from the site of inflammation (Brinkmann *et al*, 2004).

When the innate immune system is not enough to deal with pathogen colonization of the udder, the humoral mechanism (also called adaptative immune response) enters into action. The humoral immune response is determined by the action of antibodies (proteins that opsonize the pathogen cells and prepare them to be phagocytosed); the antigens from bacteria (or viruses) are processed within macrophages and presented on the surface of the macrophage in association with the MHC class II antigens. Lymphocytes are the only cells that have the ability to recognize antibodies through membrane receptors specific for foreign antigens (Sordillo *et al*, 1997). It has been proposed that the adaptative immune system is more efficient against viruses than against bacteria.

With regard to mastitis, the diversity of pathogenic agents is a determining factor in the immune response. Most of infections caused by coliforms (*i.e. E. coli*), are acute infections commonly occurring in early lactation that are cleared out within hours or days, with clinical signs that generally last less than 7 days (Erskine, 2001). As will be pointed out later, this causes a practical problem for milk recording systems; several cases of CM of this type, when present between test days, may be missed before the information is collected. In the case of mastitis caused by *Staphylococcus aureus, Streptococcus agalactiae* or *Corynebacterium bovis*, it is known that they tend to cause chronic infections that may be expressed as subclinical mastitis as the lactation progresses (Erskine, 2001). Hence, the proliferation of lymphocytes against infection is crucial within the immune response, especially in cases of infections of longer duration. Additionally, the ability of the organism to detect the infections is crucial in dealing with any pathogen. This ability to detect pathogens has been attributed to Toll-like receptors located in the cellular membrane. These receptors trigger the innate immune response, as

soon as invading bacteria are detected. Recent studies have shown the role of some Toll-like receptors in mounting immune response against mastitis in cattle (Goldammer *et al*, 2004).

Thus, the genes that regulate the proliferation of neutrophils and lymphocytes (i.e. Growth hormone, ornithine decarboxylase, IGF-1) are candidates to promote resistance against infections such as mastitis.

2.3.1 Profile of infections

Various methods to improve the health status of udders have been studied, and since the early 1970s, programs to improve the hygiene of milking procedures have been in place. These programs include the washing of the udders prior the milking, the use of teat dip after milking, and the application of antibiotic formulae for the dry period. Nowadays, most programs applied are variations of these procedures (Erskine, 2001) and they have proved successful to control infectious mastitis. In Ontario dairy farms, for instance, analysis of the economics of mastitis control by management indicates that some practices such as teat dipping after milking, the use of hand held sprayers to wash the udders or dry cow treatment to selected cows were associated with economic benefits that ranged from CAD$60 to CAD$75 per cow per lactation (Gill *et al*, 1990).

The hygiene of herds has improved, and with it, the prevalence of mastitis has been reduced. However, despite successful efforts to improve health management of herds, the incidence of mastitis has not declined over the years (Hillerton *et al*, 1995; Bradley, 2002). In this context, the term prevalence of mastitis in a herd refers to the number of cows (or quarters) that are diagnosed as infected, divided by the total number of cows (or quarters) currently at risk of infection. The term incidence of mastitis refers to the number of new cases of clinical mastitis in the population at risk during a given period of time (Erskine, 2001).

It has been hypothesized that the reduction in infectious microorganisms brought about by application of control plans has led to a new profile of infections with environmental microorganisms, such as *E. coli* (Kossaibati *et al*, 1998). There is some evidence to support this idea. Table 2.2 summarizes information collected by Bradley

(2002) from several sources regarding incidence of mastitis according the infecting pathogen:

Table 2. 2 Incidence and etiology of clinical mastitis in UK dairy herds (quarter cases/ 100 cows/year).

Pathogen	1967	1982	1998
Staphylococcus aureus	67	7	2.2
Streptococcus agalactiae	6	7	-
Streptococcus dysgalactiae	16	4	2
Streptococcus uberis	7	9	5.3
Escherichia coli	7	10	14.4
Other	50	9	17.7
Total	153	40	41.6

Adapted from Bradley (2002)

It may be observed that, for instance, in 1998, *E. coli* was the major cause of clinical mastitis with 14.4 quarter cases/100 cows/year, whereas in 1967 it was responsible for 7 quarter cases/100 cows/year.

In the Netherlands, prevalence and incidence of clinical mastitis were monitored over a 5-year period in a research herd of between 160 and 220 Friesian cows run on a commercial basis. Data indicated that the prevalence of clinical mastitis caused by coagulase-positive staphylococci declined (22 affected cows over a total of 128 cows in 1985 versus 2 affected cows over a total of 175 cows in 1991), but the total incidence of mastitis did not, showing an average of 24 cases per month from July-1985 to June-1990 (Hillerton *et al*, 1995).

It is generally accepted that most of contagious mastitis cases are caused by pathogens like *Streptococcus agalactiae* (> 40% of the infections), and *Staphylococcus aureus* (30-40 % of all infections); given that the main source of infections are infected udders, the reduction of this type of mastitis is indeed feasible by following measures of hygiene. On the other hand, environmental mastitis caused by Streptococci (*uberis, bovis,*

dysgalactiae), Enterococci (*faecalis, faecium*), coliform (*E. coli, K. pneumoniae*) and *Arcanobacterium pyogenes* accounts for 1-10% of all infections, and are difficult to control since the main source of infection is the environment of the animal. Environmental mastitis is more common in summer and is spread mainly by flies (*Hydrotaea irritans*) (Nickerson *et al*, 1995). Importantly, there is evidence suggesting that mastitis from different etiology (*i.e.* different causal agent) may have different resistance mechanism, assuming that different genes determine it (Nash *et al*, 2000).

It has been reported that low SCC herds exhibit a low prevalence of intramammary infections (IMI) caused by contagious pathogens and a high incidence of CM when compared to high SCC herds that show high prevalence of contagious pathogens and low incidence of CM. Erskine *et al* (1988) studied the profile of infections in herds classified according the level of bulk somatic cell counts; Table 2.3 summarizes those results:

Table 2. 3 Percentage of clinical mastitis caused by various pathogens in isolates from low and high bulk SCC herds.

	High > 700,000	Low < 150,000
Mastitis incidence	35	51
Streptococcus agalactiae	41.5	0.0
Staphylococcus aureus	18.3	2.2
Other streptococci	12.6	12.3
Coagulase-negative staphylococci	7.6	7.8
Coliforms	8	43.5
Other major pathogens	3.4	5.4
No isolate	8.8	28.6

Adapted from Erskine *et al* (1988)

In general it has been proposed by Erskine (2001) that in low SCC herds, up to 50% of the infections is caused by coliforms, and in high SCC herds the main cause of infections is generally *Streptococcus agalactiae* and *Staphylococcus aureus* (60% and 22%, respectively).

2.3.2 Mastitis: risks factors

The facts discussed above have encouraged the assessment of several risk factors for mastitis in order to find more efficient control programs. One of these factors is the system in which cows are maintained. The lowest content of somatic cells in tanks occurs in rotationally grazed herds (Goldberg *et al*, 1992), which can be expected, as healthy cows in a more open space are not closely exposed to cows with mastitis. Thus, when using confinement, differences in clinical mastitis incidence can be the result of differences in the refinement of the farmer to perform activities tending to increase hygiene of housing of cows. In this sense, it has been shown that quick and dirty management is associated with higher CM incidence, when compared with herds under clean and accurate management (Barkema *et al*, 1999b). The use of confinement housing is an anomaly with regard to the natural grazing behavior of ruminants, and it has some well documented consequences.

Regarding the potential of cows to resist mastitis infections, it has been observed that the incidence of clinical mastitis increases with age at calving (Waage *et al*, 1998; Wanner *et al*, 1998). It can be expected that older heifers at calving show an increased susceptibility to mastitis; a possible explanation for this is that longer exposure to bacteria causes higher incidence of intramammary infections (Wanner *et al*, 1998). As well, it can be expected that cows in second and later lactations exhibit more mastitis than heifers. The major risk factors for clinical mastitis are milking and the hygiene of housing; hence the more time exposed to these risks the more chances to get mastitis. Confinement allows full exploitation of cows; however slight differences in management can have a large effect on CM incidence. Additionally, some conformation traits that are associated with low mastitis incidence such as the udder support ligaments suffer deterioration as animals grow older.

Combining cows and heifers in the same location is another factor favoring CM (Barkema *et al*, 1999); for instance, 25 percent of heifers before first parturition have been found to test positive for mastitis (Nickerson *et al*, 1995). Some differences in CM have been found to be associated with the size of the herd. The explanation may be that with more cows on a farm, management tends to be quicker and laxer, hence more chances to create a dirty environment which in turn causes elevated SCC. It has to be

noted that the trend in the dairy industry is a decreasing number of herds of increasing size (number of heads). In Canada the average herd size increased from 20 cows per farm in 1970 to 61 cows per farm during 2002[3]. In large herds, conditions for transmission of mastitis are commonly present. Hence, the intricate relationships between farm management and udder health may turn mastitis outbreaks into a permanent threat for modern dairy operations.

2.3.3 Options for control of mastitis

Normally, contagious mastitis is considered to be easy to control with antibiotics and prophylactic measures; however, that is not the case with environmental mastitis. Nevertheless, environmental infections are so important that, for instance, bacteriemia in cows with naturally occurring acute coliform mastitis has been considered high enough to prescribe parental antimicrobial treatment (Wenz *et al*, 2001). Although this observation is based on a study with a small sample (144 dairy cows in six herds), it is a clear indication of the new infection profile that producers are increasingly facing.

Several factors may limit the use of antibiotics to combat mastitis incidence. Nowadays, there are increasing concerns regarding consumer's worries towards antibiotics residues in milk as well as bacterial antibiotic resistance (Fang and Pysörälä, 1996; White and McDermott, 2001; Guérin-Faublée *et al*, 2002). These concerns have encouraged the development of new therapies (cytokines), delivery systems (micro and nano particles) or products (recombinant mucolytic protein) (Gruet, 2001), or even the rediscovery of approaches such as the use of bacteriophages (Barrow and Soothill, 1997), that might be alternatives to antibiotic therapy. A gene therapy to combat mastitis in goats has been evaluated, and despite not qualifying as currently feasible (Fan *et al*, 2004), it

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[3] Agriculture and Agri-food Canada. 2002. Canadian dairy industry profile, p.16. Available in http://dairyinfo.gc.ca/dairyprofile.pdf, accessed January 10, 2004.

stresses the importance of reaching a deeper understanding of candidate genes to increase mastitis resistance.

The eradication of mastitis appears difficult, if not impossible, due to the dynamics of its epidemiology. The epidemiology of mastitis is different according to the causal agent, and risk factors might have varying importance between pathogens (Peeler *et al*, 2000). Logically, plans recommended to reduce mastitis incidence should be designed for the specific conditions of the herd and their infection profile. Researchers are looking into more general options in developing strategies to increase cow's mastitis resistance; among these, genetic selection is an option with a major role. The genetic improvement of any economically important trait has been based on reliable recording systems. Several countries have developed recording systems for dairy cattle that provide valuable information to enhance the management of the dairy cows, and as a result the efficiency of dairy enterprises has continued to improve.

2.4 MASTITIS: METHODS OF MEASUREMENT

The objective of milk recording systems is to store information on each individual cow's performance in each of its lactations. With this information, several reports may be generated and used by farmers within daily management of their farms. International organizations, such as the International Committee for Animal Recording (ICAR), develop standards for the operation of milk recording systems to ensure quality of the information.

In general, each lactation record should include the animal identification and information regarding dates (birth, services, calving), measurements of yield traits such as milk, fat and protein (from several tests along the lactation), Conditions Affecting Records (CAR) codes and reason for culling. The CAR can be defined as a means to help identify various factors that affect the lactation record.

2.4.1 Milk recording in North America

In Canada the report on CAR codes and reason for culling is done by the farmer on the testing day. A CAR for mastitis is included within the options of these reports.

However such databases have a major disadvantage: the accuracy of the incidence of mastitis depends heavily on the accuracy of reporting individual cases. Thus, if a cow had mastitis (clinical or subclinical, or a high SCC value) between the test-day visits, the episode of mastitis may be missed, and the cow may appear as being without mastitis. This situation has been described by other researchers (Almeida, 1996; Dürr *et al*, 1997, 1997b) and it is common in milk recording systems where direct recording of clinical mastitis incidence is not performed.

The need to capture a mastitis case on the test day presents additional complications. It has been stated that as a result of prophylactic programs, more cases of mastitis are being caused by environmental microorganisms; generally these infections last less than a month (Harmon, 1994; Erskine, 2001), and thus it is possible to miss cases of mastitis caused by environmental microorganisms. This causes an underestimation of mastitis incidence.

2.4.2 Milk recording in the Nordic countries

In contrast, in Nordic countries (Sweden, Denmark, Norway and Finland) the recording system is more accurate regarding clinical mastitis. Reports of mastitis cases are done by veterinarians treating each cow (Heringstad *et al*, 2000; Heringstad *et al*, 2001b). Because each mastitis case is diagnosed and treated by an animal health professional this information is considered accurate and more reliable. However, this system is not exempt from problems. One weak point of this system may be the homogeneity in diagnostic criteria for each case of mastitis. Recently, the quality of the recording for udder diseases in the National Herd Cards System for Cattle in Norway was evaluated (Sviland and Waade, 2000). Veterinarians were provided with seven theoretical cases of mastitis in a questionnaire, and asked to classify them. The authors scored the accuracy to classify cases to be between 67 to 100%. Two cases of subclinical mastitis were classified as clinical mastitis, but almost all clinical cases were correctly classified. Because of this, the authors recommended further improvements of the recording quality through the formulation of more precise diagnostic criteria.

2.4.3 Application of milk recording

The need for an objective measure of mastitis has prompted the dairy industry to adapt and continuously improve milk recording systems. Given that recording each case of clinical mastitis is a difficult task, other related traits have been recorded. The use of automated systems to measure the number of somatic cells in milk samples has facilitated the recording of that trait. One of the most popular systems makes use of laser-based flow cytometry to carry out the quantitative determination of somatic cells in milk. There are an increasing number of producers participating in such programs. In Ontario, 40% of DHI herds were using SCC service in 1994. In UK since 1997 SCC is registered in milk recording. In Quebec, for instance, 6,210 dairy herds are enrolled in milk recording, and of these 6,068 (97.7%) are sampling milk to measure percentage of fat, protein, milk urea nitrogen, lactose and SCC every month (PATLQ, 2003). PATLQ processes the milk samples for milk components (fat and protein) using infrared system, and SCC using cytometry (Lefebvre D. Research and Development PATLQ, personal communication, 2003).

Some approaches to reducing the incidence and prevalence of mastitis have been based on genetic evaluations for several measures of mastitis. Technically, there are several measures that can be extracted from milk recordings, and arbitrarily they can be classified into two classes: direct, such as the clinical information regarding health status of cows (*i.e.*: recorded cases of mastitis that occur over the lactation of each cow); and, indirect, such as traits related with presence of mastitis such as somatic cell counts (SCC) and udder conformation.

In Canada, several measurements have been recommended for reporting and calculating incidences of diseases in dairy cattle. With respect to retrospective studies of clinical mastitis, a Lactational Incidence Risk expressed as affected lactations per 100 lactations at risk (number of lactations with one or more cases of clinical mastitis/number of lactations) has been proposed (Kelton *et al*, 1998).

Recording SCC or mastitis incidence is not only a valuable managerial tool but a source of information for genetic selection purposes. Somatic Cell Score (SCS) has been widely used to estimate genetic parameters. Because milk yield and SCS show a strong genetic correlation, the latter has been included in selection indices used in breeding

programs in countries like Germany, Canada and the United States (Swalve, 2000). Other countries, such as Norway, Sweden, Finland and Denmark directly include mastitis incidence in their selection programs (Heringstad *et al*, 2000, 2001).

There is not a standard measure of mastitis to be stored in milk recording. Each milk recording system includes those traits that are useful considering present and future characteristics of the industry. In addition to CM and/or SCS, other traits, summarized in Table 2.4, have been used as indicators of mastitis.

Table 2. 4 Traits (other than CM and/or SCS) used to monitor mastitis and their respective mechanism of action

Trait (ICAR, 2002)	Description of the mechanism of action
Milking speed	Unfavorable genetic correlation between milking speed and SCC
Electrical conductivity	In milk from cows with mastitis the electrical conductivity increases because ions concentration of NA+ and Cl- increase, and K+ decreases (Kitchen, 1981)
N-acetyl-β-D-glucosaminidase (NAGase)	Used as a measure of inflammation (Pyörälä, 2003); Log NAGase (NAGase units/mililiter) goes from 0.80 in foremilk from healthy quarters to 1.02 in foremilk from unhealthy quarters (Urech *et al*, 1999)
Bovine serum albumin and antitrypsin	Increases from 0.25 mg/ml to 0.6 mg/ml (Sandholm *et al*, 1984)
Sodium (Na), potassium (K) and lactose	Na increases from 0.057 mg/100 ml to 0.105 mg/100ml; K goes from 0.170 mg/100 ml to 0.150 mg/100 ml; normal milk contains 48 mg/ml of lactose; with mastitis, lactose levels are reduced.

ICAR = International Committee on Animal Recording

However, the use of these traits within national breeding programs is still limited (INTERBULL, 2002). According to the International Committee in Animal Recording (ICAR, 2002) the most reliable traits to evaluate udder health are: SCC, udder conformation, milking speed and CM incidence. Thus, it is recommended to estimate

variance components for these variables, and breeding values of animals in order to support selection decisions.

2.5 MASTITIS: QUANTITATIVE GENETICS

The information obtained from milk recording systems and breeding associations is basic for obtaining a general understanding of the genetic properties of a population from a quantitative genetic point of view. Data obtained from milk recording programs has been fundamental in increasing yield average observed in dairy cows. Annual rates of genetic improvement are function of intensity of selection, accuracy of selection, genetic standard deviation, and generation interval. Some parameters illustrate how intense selection may be applied to the dairy population within AI progeny-testing schemes in the various pathways of selection (Dekkers, 1992):

- Sires of bulls: 2.42
- Sires of cows: 1.76
- Dams of bulls: 2.42
- Dams of cows: 0.35

In addition, generation intervals have decreased in the Canadian Holstein population in each of the selection pathways outlined above. The trends in generation interval for each of the several pathways of selection in Canadian Holstein are shown in Figures 4.5 and 4.6.

These two factors (selection intensity and generation interval) may partially explain the annual rate of genetic improvement for milk, milk components, and SCS in the Canadian Holstein population. In 1992 the annual average milk production per cow was 8,028 kg; by 2002 this average had risen to 9,717 kg (Agriculture and Agri-Food Canada, 2003). The 1689 kg difference has a significant contribution from genetic selection based on parameters obtained from information provided by milk recording. Table 2.5 shows the genetic trends for several economically important traits in Canadian Holsteins between the periods from 1989-1999 and 1994-1999.

Table 2. 5 Average annual genetic trends for milk, fat, protein and SCS in Canadian Holsteins. Values represent the annual trend for the period for kg EBV (milk, fat, protein) or scores EBV (somatic cell score).

Trait	1989-1999	1994-1999
Milk	131 kg	159 kg
Fat	4.0 kg	4.6 kg
Protein	4.4 kg	5.0 kg
SCS	0.0005 scores	0.004 scores

Canadian Dairy Network, 2003. Genetic trends in Canadian Dairy Breeds. http://cdn.ca/Articles/0305/trends/genetictrends.html. Accessed August 2, 2003.

It can be appreciated that for all traits the genetic gain has been faster from 1994 to 1999.

2.5.1 Genetic parameters: heritability and genetic correlations

Two of the most useful parameters to genetically characterize a population are heritability and genetic correlation.

Heritability indicates the extent to which the phenotypic variation is explained by the additive genetic values of the individuals in a population.

Clinical mastitis and SCS are traits of low to medium heritability. Values of heritability for clinical mastitis are found to be within the range from 0.011 to 0.42 (Kadarmideen and Pryce 2000; Rupp and Boichard, 1999; Heringstad *et al*, 1999, 2001, and 2003; Nash *et al*, 2000). In Canada, there are only three reports on genetic parameters for clinical mastitis incidence (Monardes, 1980; Uribe *et al*, 1995; Van Doorp *et al*, 1996), but none for occurrence of clinical mastitis per lactation, or culling due to mastitis. Estimates of heritability for clinical mastitis were reported by Monardes (1980), Uribe *et al* (1995) and Van Dorp *et al* (1996), at 0.04, 0.15 and 0.04, respectively; these three

studies used small data sets (8914, 4368 and 7416 cows, respectively) which can be a partial explanation for the difference between the estimates of heritability.

With regard to heritability for SCS, estimates have been shown to be within the range from 0.018 to 0.60 (DeGroot, 2002; Amin and Tibor, 2001; Mulder *et al*, 2002; Mrode *et al*, 2001; Haile-Mariam *et al*, 2001; Rupp and Boichard, 1999). Culling due to mastitis is a trait that has been little studied, with a heritability of 0.011 to 0.017 (Heringstad *et al*, 2003). In Canada, Monardes (1984) studied monthly somatic cell counts of cows in milk recording in Québec between February 1977 and February 1982, and found heritabilities for log SCC between 0.082 and 0.126 for several lactations (from first lactation to fifth or more). In this same study, an examination of test-day SCC throughout individual lactations of 18 heifers was performed in order to detect possible correspondence between SCC profiles and various measures of cell counts; results suggested that the profiles were highly variable between individuals which could be due to differences in resistance to external challenges (i.e. bacteria, climate stress, and management).

Some estimates of heritability for mastitis caused by various pathogens were reported by Nash (2000); values ranged from 0.11 (for environmental microorganisms in first lactation cows) to 0.25 (Streptococci other than *Strep. agalactiae* in second lactation cows). The heritability of clinical mastitis incidence from all microorganisms was 0.14 and 0.01 for first and second lactation, respectively. To explain the differences in estimates, the authors hypothesize that fewer genes may control resistance against mastitis produced by a group of related pathogens.

A low heritability indicates that standard genetic improvement schemes would have a small effect on the improvement of the trait. Marker assisted selection (MAS) has been proposed to make genetic improvement for low-heritable traits. The main motivation of QTL mapping studies is to identify genes for incorporation into MAS schemes.

The other genetic parameter of importance in the genetic improvement of domestic animals is the genetic correlation. This parameter indicates how strong a genetic relationship is between two traits. The main genetic cause of genetic correlation is pleiotropy: the property of a gene to affect more then one trait. Clinical mastitis and SCS show moderate to high genetic correlation (0.60 to 0.72) (Lund *et al*, 1999; Rupp and

Boichard, 1999). More cases of clinical mastitis are likely to occur with high SCS. There is evidence of a linear relationship between breeding values for SCC and breeding values for CM (Philipsson *et al*, 1995). This relationship is expected because, as discussed before, high SCC may indicate the presence of a clinical infection.

2.5.2 Selection for milk yield, conformation, and mastitis

The current high levels of milk yield per cow have been reached through intense selection; at the same time an increase in health problems, including mastitis, has been observed (Shook and Schutz, 1994). This can be partially explained by the genetic correlations of clinical mastitis and SCS with milk yield (0.15 to 0.70, and 0.60 to 0.80, respectively) (Uribe *et al*, 1995, Heringstad *et al*, 1999; Lund *et al*, 1999; Rupp and Boichard, 1999). These estimates suggest that some genes that have a favorable influence on milk yield also have a negative effect on resistance to mastitis.

Selecting cows for high milk yield may lead to changes in conformation, such as increased udder size, but also it increases the need for metabolic adaptations; as a result, cows may be more prone to infection due to changes in circulating hormone profiles (Nikolic *et al*, 2003).

Udder capacity has to be increased in order to house a large amount of secretory tissue and ductular system, both necessary for the synthesis and storage of milk. However, at the same time the increased capacity of the udder can weaken fore udder attachment and cleft (De Groot *et al*, 2002). In general well balanced deep udders, with strong fore-udder attachment are associated with lower levels of SCS and clinical mastitis; the genetic correlations range from -0.29 to -0.46 (Rupp and Boichard, 1999). Cows with better udders, which offer more efficient protection mechanisms against risk of infection, are less susceptible to mastitis.

Although evidence is scarce with regard to genetic associations between energy balance and diseases in dairy cattle, the negative energy balance observed at the end of pregnancy and at the beginning of lactation has been considered partially responsible for the immunosupression observed in dairy cows in that stage. The explanation is simple: at the beginning of lactation when milk yield is rising, and also after the lactation peak, cows (especially high-yielding cows) will have difficulties meeting their nutritional

requirements. The first sign of this imbalance is weight loss. This situation may lead to a low immune status. In support of this, a genetic correlation between dairy character and clinical mastitis of 0.24 has been observed which indicates a modest increase of clinical mastitis as the dairy character is improved (Hansen *et al*, 2002); the same authors argue that the deleterious effects of extreme dairy character on health may partially be explained by the high positive genetic correlation between dairy character and milk yield. As well, they have stressed that a potential genetic deterioration of disease resistance may occur if either selection for high yield only is applied, or if positive weights for dairy character are used in selection indexes for type.

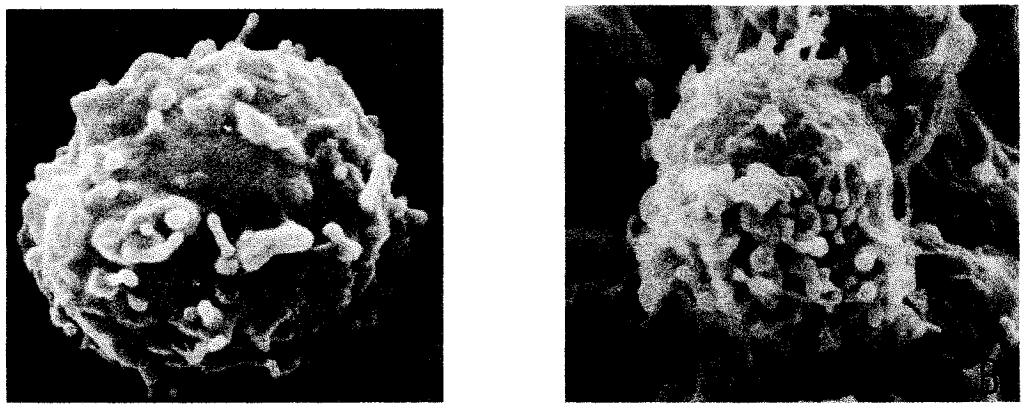
Clinical mastitis in first and second lactation is negatively correlated with longer productive life, shallower udders, deeper udder cleft and strongly attached fore udder (Nash, 2000). Similar relationships among SCS and conformation traits have been reported in Canadian Holsteins (Monardes *et al*, 1990). Selection for predicted transmitting ability for type has shown favorable effects on the SCS response; evidence indicates that selecting for higher udders with tighter attachments and closer teats would reduce SCS (De Groot *et al*, 2002).

There are additional explanations for the mechanisms producing reduced mastitis resistance in dairy cattle selected for high yield. It has been suggested that selection for low SCC can lead to selection of cows with less capacity to mobilize leukocytes, and hence with higher susceptibility to mastitis (Kehrli and Shuster, 1994; Schukken *et al*, 1999). In support of this, it has been shown that following a challenge with *S. aureus* to two groups of cows with high and low SCC, respectively, the first group showed less CM than the second one, suggesting that cows with more SCC had better defense mechanisms to counteract the infection than cows with less SCC (Schukken *et al*, 1999).

However, other studies have failed to find any relationship between low SCC and higher susceptibility to mastitis (Rupp *et al*, 2000). On the one hand, evidence shows that selection for high milk production did not produce unfavorable correlated responses in the functional capacity of immune function traits (Detilleux *et al*, 1995), suggesting that bulls highly selected for milk yield might have enough variation regarding mastitis resistance to be used in selection programs, and in that way in fact balance the intense selection for

milk yield with mastitis resistance. On the other hand, Philipsson *et al* (1995) found that daughters of sires selected for low SCC show low incidence of CM.

The presence of milk at the site of infection makes the onset of an efficient immune response more difficult. It is known that milk reduces the bactericidal activity of the complement system (Hogan and Smith, 2003); meanwhile a hypothesis suggests high yield may act as a mere physical barrier to neutrophils reaching the infection site (Kerhli and Schuster, 1994). In addition, it is known that macrophages engulf milk fat and casein which reduces their capability to phagocytose pathogens, mainly because of the loss of pseudopods, essential in the process of phagocytosis (Figure 2.1 adapted from Paape *et al*, 2002).



A B

Figure 2. 1 Scanning electron micrograph of a leukocyte isolated from milk (A), and a leukocyte isolated from blood (B). During phagocytosis of milk fat globules and casein, leukocytes lost pseudopods (A) required for phagocytosis; as comparison, note the higher convoluted membrane in the leukocyte in blood (B). Photos taken from Paape *et al*, (2002).

Many factors other than conformation and enhanced milk production may interact to generate susceptibility to mastitis. In this regard, the effect of administering bovine somatotropin (BsT) on clinical mastitis in high producing cows has been evaluated. BsT was not found to affect clinical mastitis, and therefore it was suggested that the effect of high milk yield on clinical mastitis might be mediated through mechanisms others than

increased physiological function of the udder (Judge *et al.*, 1997). However, this study used a relatively small sample, and hence the conclusions have to be taken cautiously. Despite that, other studies seem to support that hypothesis. Gene expression analysis in dairy cows was used to detect 18 genes with $\geq$ 1.2-fold higher expression 14 days prepartum than 6 hours postpartum (Burton *et al*, 2001). Some genes had no known name or function at the time of the experiment, but most of them showed sequence homology to genes whose function are surveillance, migration, phagocytosis, respiratory burst, and phagosome-lysosome[5] fusion functions. It was implied that genes involving functions other than milk yield, such as neutrophil function, may be associated with the increased disease susceptibility. The authors propose selection for leukocyte function as a way to counteract any long-term effect that selection for milk yield might have on mammary immunity.

From genetic correlations between milk yield and mastitis resistance, it can be inferred that some genes show pleiotropy. A clearer interpretation of the cause of the correlation will be possible when associations between genes that influence both milk yield and mastitis resistance can be obtained. As it will be presented later, some genes, such as the Growth Hormone gene or the Prolactin gene, have products related to increased milk yield and are involved in the immune response as well.

The inclusion of clinical mastitis as a breeding goal could have a major impact on a dairy population. In countries with records of all veterinary treatments, clinical mastitis is directly used in breeding programs (Heringstad *et al*, 1999; Heringstad 2000; Ødergard *et al*, 2002). In Norway, the genetic trend for clinical mastitis incidence for bulls born between 1974 and 1990 was approximately flat, while the genetic trend for protein production yield increased from -2 to 9 kg (Heringstad *et al*, 2001). The main explanation

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[5] When a neutrophil engulfs a foreign particle, it encloses it into a vacuole called phagosome; then, the primary granules of the neutrophil fuse with the phagosome releasing enzymes into it. These enzymes destroy bacterial wall.

for this is that selection for increased production might after all not to be in conflict with mastitis resistance. Given the low heritability of clinical mastitis and its genetic correlation with milk yield, other genetic approaches may be explored in order to improve mastitis resistance.

In light of these results, some questions may be formulated: if the increased susceptibility to mastitis is produced by mechanisms other than increased physiological functions of the udder, and if there is genetic variation for mastitis resistance in bulls highly selected for milk production, then which genes actually increase resistance to mastitis? If they exist, what is their mode of action? These questions can be answered using a combination of quantitative and molecular genetic tools. An alternative to breeding programs that include reduced SCS or clinical mastitis within their breeding goals can be the direct use of genes affecting these traits to identify cows with increased mastitis resistance.

Recently, association studies between genetic markers and estimated transmitting abilities (ETA) for several economically important traits have allowed researchers to map Quantitative Trait Loci (QTL) of importance for animal breeding. There have been some attempts to study associations between genetic markers and QTL for SCC or SCS, but there is no attempt to map QTL for incidence of CM, occurrence of CM or/and culling due to mastitis.

In the milk recording system of Québec (PATLQ), there are three sources of information that can be used to generate ETA for mastitis resistance to be used in association studies to map QTL with effect on mastitis resistance: SCS, incidence of CM, the number of CM cases over the lactation, and culling due to mastitis.

2.6 QTL AND GENETIC MARKERS

The most important quantitative traits in agriculture are thought to have several loci underlying them. These loci have been termed Quantitative Trait Loci (Geldermann, 1975). A complete understanding of how a phenotype is genetically determined requires the knowledge of all the genes affecting a trait, as well as their mechanism of action. Mackay (2001) has stated that the complete dissection of a quantitative trait requires, among other things, complete knowledge of the identities and number of all genes

defining the phenotypic trait, mutation rates of these loci, the number and identities of genes affecting the trait within the population, between populations and within species. It has to be added that evidence encourages the study of genomic imprinting that may affect gene expression and alter the resulting phenotype through interactions with QTL. Nowadays no trait has been analyzed at that level of resolution. Despite that, there are ways to partially dissect the underlying genetics of quantitative traits. A first step is to map QTL affecting economically important traits.

Different markers have been used to map QTL affecting quantitative traits. For instance, the first study performed to detect a QTL was that of Sax in 1923. In that study QTL affecting the weight of beans (*Phaseolus vulgaris*) were mapped using the pigment of the seeds as a phenotypic marker. Another type of marker is protein polymorphism, such as milk protein polymorphisms and blood groups. The latter were used during the 1960's to map QTL; Neimann-Sorensen and Robertson (1961) reported an attempt to map QTL affecting milk production in cattle in Norway. The significance of this study is that it was the first attempt to use analysis of variance to find differences in performance attributable to polymorphisms. Over the years, molecular tools to find polymorphisms at the DNA level have helped generate genetic markers.

A genetic marker is a sequence variation at the DNA level that can be used to identify certain segments of the genome that influence quantitative traits. During the 1980's and 1990's several types of genetic markers were developed, for example: restriction fragment length polymorphisms (RFLP), randomly amplified polymorphic DNA (RAPD) and single nucleotide polymorphism (SNP) (Lynch and Walsh, 1998; Griffiths and Miller, 1999; Gibson and Muse, 2002). A genetic marker should be highly polymorphic, and co-dominant (Lynch and Walsh, 1998; Falconer and MacKay, 1996).

Although traditionally, in most cases genetic markers have been considered not biologically significant[6], they have been successfully used to identify the position of genes along chromosomes.

2.7 QTL MAPPING

There are several methods used to detect major genes or QTL. They represent different approaches for obtaining evidence of segregating genes affecting quantitative traits. While some methods make no use of genetic markers, others rely on them and indeed have become an initial step towards the genetic dissection of quantitative traits.

The most powerful application in the category of marker-free methods is segregation analysis[7]. This method, as proposed by Elston and Stewart (1971), was based on the comparison of the likelihood obtained from two models: a model with transmission probabilities (0, ½, and 1; which can be obtained from pedigrees), and a model with equal transmission probabilities (referred as to non-genetic model). A significant increase in the likelihood was an indication that a major gene was segregating in the population. Modifications proposed by Morton and Maclean (1974) comprised fitting an initial model with fixed non-genetic effects and then a model with a major locus effect.

The advantage of segregation analysis is that the phenotypic observations may be used to detect major genes or QTL when DNA markers are not available. Although segregation analysis is a powerful marker-free approach, it gives no information regarding the position of the gene responsible for the variation in the quantitative trait.

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[6] The traditional assertion that genetic markers located in the intronic regions (i.e. non-coding region) are not biologically significant has changed. New evidence points out that RNA that has been spliced during the transcription process (intronic RNA) may have a role in gene regulation through a mechanism of gene silencing called RNA interference (RNAi) (Mattick, 2002).

[7] Other marker-free approaches are based on analysis of multimodal distribution, departure from normal distribution, heterogeneity of variances, offspring-parent resemblance (Hill and Knott, 1990; Falconer and Mackay, 1996).

Methods that make use of genetic markers are linkage analysis and association analysis. These methods detect chromosomal segments that harbor genes affecting quantitative traits. Linkage analysis allows one to infer the probability that a genetic marker is linked to a QTL affecting a quantitative trait. The principle of this method relies on the fact that two alleles on different tightly linked loci will be inherited together (non-random segregation) with higher frequency than two alleles located in loci widely separated on the same chromosome. Linkage analysis makes use of markers scattered along the genome; measuring the rate of recombination between markers allows measurement of linkage, the LOD score. LOD score is the logarithm of likelihood ratio of the probability of each pedigree given a value of recombination to that of independent recombination (this is recombination = 0.5). Then, LOD score is plotted against the recombination and, generally a score greater than three is considered evidence that two loci are closely linked. In linkage analysis the identity of the linked gene is unknown, but its chromosomal position is assumed to be near the marker used to track it.

Within the category of methods that use genetic markers to detect QTL the most powerful approach is association analysis. In this approach, analysis of variance is used to test whether genetic markers have a statistical association with quantitative traits[8]. These association studies take advantage of linkage disequilibrium (LD) present in the population under study. If LD is present between a genetic marker and a QTL underlying a quantitative trait, significant associations may be found through statistical analyses.

The use of genetic markers in association studies to map QTL relies on the kind of association that a genetic marker can have with a candidate QTL. A genetic marker can be either: 1) Directly affecting the trait, *i.e.*: the marker is the QTL, or 2) in LD with QTL affecting the trait because, though no necessarily, it is (physically) linked to the QTL. It has to be emphasized that LD does not require (physical) linkage.

In association studies it is difficult to distinguish whether the marker is tightly linked to a QTL (or a cluster of QTL) or whether it is actually the gene affecting the trait. When recombination between the marker and the QTL is zero, there is no way, in association studies, to differentiate the marker from the QTL and for all practical purposes the marker is the QTL. Furthermore, it should be noted that not all alleles segregating together are (physically) linked. If two genes segregate together they are said to be in LD. In other words, LD occurs when two genes (*e.g.* the marker and the QTL) segregate in a non-random association. Physical linkage can produce LD, but even two alleles in different chromosomes (*i.e.*: not linked) can be in LD (Farnir *et al*, 2000),

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[8] With regard to the genetic markers, there are two main ways to perform association analysis: 1) using anonymous markers, or 2) using markers of genes with known physiological function. The latter option may have two variants according to the genetic nature of the marker: the comparative and the candidate gene. The comparative consists in using markers of genes that have a known function in a species, and screening them to search for candidate genes in a different species. The candidate gene approach consists in using genetic markers of genes with a known physiological effect on one trait to test them as candidate genes for traits.

although this LD has a shorter duration than the LD of physically linked genes. The duration of the LD from linked genes depends on the rate of recombination. The more apart two genes are on the same chromosome, the higher the rate of recombination between them.

All the rationale for the use of linkage disequilibrium described above applies when association studies are performed either at population level or at within-family level. Association studies performed to detect segregating QTL affecting quantitative traits at a population level are referred as to across-population analyses. For dairy cattle, two experimental designs facilitate the across-population analysis: the daughter design and the granddaughter design.

The daughter design consists in the use of the phenotypic information of the progeny (i.e. genotyped daughters) from heterozygous sires (for the relevant markers) to test the hypothesis of association between the markers and the quantitative trait. In this design the statistical model includes the effect of the marker (inherited by the daughters) and the other environmental effects. A significant effect for the marker indicates that there is a QTL segregating in the population. Because the analysis is performed across all the sires from which phenotypic information has been collected (through their daughters), the term across-population is used to refer it.

The granddaughter design allows testing for associations to map QTL at within-family level. It consists in genotyping sons of heterozygous (for the markers of interest) grandsires, and retrieving phenotypic measurements (*i. e.* ETA for the traits of interest) from the progeny (*i. e.* granddaughters in milk recording). In this design, the model includes the effect of grandsires, the marker inherited by the sons, and the sons. The main difference with respect to the across population analysis is that a significant effect for the marker indicates that within some families the marker is physically linked to a QTL affecting the trait. The analysis may be performed pooling all the grandsire families, or within each grandsire-family.

Despite the number of QTL which influence economically important traits, the use of these QTL in breeding programs appears quite limited. Spelman and Bovenhuis (1998) discussed factors affecting the use of detected QTL in breeding programs. They attributed the delay in the use of QTL within marker assisted selection (MAS) schemes to the

uncertainty about whether the QTL are real and whether the QTL are segregating in the breeding population. This uncertainty is in part related with the fact that some significant effects reported in the literature may occur by chance. For instance, Ashwell *et al* (1998) reported that from 2392 significance tests (performed to map QTL affecting milk yield and composition, conformation, and health), 32 significant effects at $P < 0.01$ were observed; at that significance level, one would expect 24 significant effects by chance. This is a common problem of multiple comparisons when several hypotheses are tested with the same data set.

The problem of multiple comparisons in QTL mapping is increased by the fact that genetic markers on the same chromosome are not independent; hence multiple tests done with the same data set will increase the chance of detecting a QTL even when the QTL does not exist. In other words, there will be more possibilities to reject the null hypothesis of no association between the markers and the QTL when in fact the null hypothesis is true (*i.e.* Type I error).

The problem of multiple comparisons in QTL mapping has been addressed by several authors, the most frequent citation being Churchill and Doerge (1994). These authors developed an empirical method to obtain threshold values based on permutation tests, an approach first proposed by Fisher in his *Design of experiments*, published in 1935. Basically, the method consists in the repeated random shuffling of the quantitative trait with regard to the individuals under study. The permuted data are used to analyze QTL effects. The test statistics obtained from each permuted data set are stored and used to obtain critical values to test hypothesis with the original data. They determined that 1000 permutations are sufficient to estimate experiment-wise *p-values* and hence reduce the probability of type I errors.

Other possible cause of delay in the use of QTL in breeding programs may be that some of the positive effects reported in the literature concerning QTL may be due to LD produced by linkage between a specific marker allele and QTL within some families, rather than to LD between a marker and a QTL with effect on the trait across the population. For example, Ashwell *et al* (2001) found that some of the detected QTL affecting SCS had an effect in some of the eight grandsire families analyzed, but not in all

of them. Only one marker allele was found to be associated with lower daughter yield deviation (DYD) values for SCS, regardless of grandsire family.

Among the factors which create LD (even among genes that are not linked) are selection, migration, mutation, and random drift. All of these factors are present in dairy cattle populations. Therefore, methods chosen to study QTL-markers associations, and interpretation of results, have to take these into account in order to formulate conclusions. The resolution of association studies has been delimited to about 20 cM (Weller, 2001). Within this range hundreds of genes may be located; hence the importance of refining and constantly analyzing the region where a QTL has been mapped, in order to obtain a more precise location of the underlying genes. Ultimately, a combination of techniques (linkage analysis, fine mapping, comparative mapping, evolutionary tree mapping, positional cloning, association analysis, epigenetic analysis) appears more appropriate to identify the gene underlying the QTL (Meuwissen and Goddard, 2000; Andersson and Georges, 2004).

2.8 QTL STUDIES IN DAIRY CATTLE: YIELD TRAITS

There are many designs to map QTL in different animal species, but specifically in dairy cattle the process of mapping QTL tends to be based on the breeding structure of the population. The Granddaughter Design (GDD) (Weller, 1990) is the design of choice. It consists in genotyping groups of bulls and their sires for the genetic markers of interest. The offspring from the sires are recorded for the phenotype. This GDD is preferred due to the low cost of obtaining genotypes, when compared with the daughter design (DD) that requires the genotyping of all daughters of the bulls under study. With the GDD one has the opportunity to run analyses across the population (although GDD is not a requirement for that) and within grandsire families.

Information about the genetic control of economically important traits has accumulated since the first major study to map QTL in dairy cattle was published (Georges *et al*, 1995). By now, almost all the bovine autosomes (BTA) have been reported to harbor QTL with effects on both yield and health traits. Khatkar *et al*, (2004) made an extensive review of QTL for dairy cattle; as a result a combined QTL map is available online (www.vetsci.usyd.edu.au/reprogen/QTL_Map).

Although results vary, some conclusions and lessons can be drawn from these QTL mapping studies.

Georges *et al* (1995) mapped QTL for milk production by genotyping 159 DNA markers on 1518 US Holstein sires (with more than 150, 000 daughters). Using multilocus linkage analysis they found QTL affecting milk yield on five chromosomes (1, 6, 9, 10 and 20). The analyses were performed independently for each of the half-sib pedigrees (i.e. 14 families).

In another study Lien *et al* (1995) used 13 families of Norwegian cattle to study associations between milk yield traits and casein haplotypes. The model for analyses included the effect of the grandsire, the haplotypes nested within grandsire, and the random effect of sire nested within haplotype. They found a significant effect of one of the haplotypes in five grandsire families; however, they did not reject the null hypothesis of no associations when they performed the analysis pooling all grandsire families. The authors suggest that at least in some families a particular haplotype was associated with a favorable QTL allele affecting protein yield.

Relationships between genetic variants within the 3rd intron of the bovine Growth Hormone gene and the estimated breeding values (EBV) of milk, fat and protein yields in 172 Canadian bulls (100 Holstein, 51 Ayrshire, and 21 Jersey) were studied with an across-population analysis by Sabour *et al* (1997). The model included the fixed effect of the growth hormone genotype and the random residual effect. From this analysis they did not find significant effects of any of the genotypes. In a different analysis, they also studied an allelic variation at amino acid position 127 of bovine GH (locus L/V; L=leucine, V=valine); using χ^2 tests they found differences in the genotypic proportions of LL and LV genotypes among bulls classified as top, middle and bottom in ETA for milk, fat and protein; however, the small sample and the extreme genotypic frequencies were reasons for which the authors suggested further studies in order to elucidate the relationships among GH and milk yield.

Associations between the growth hormone factor-1 (Pit-1), a transcription factor that activates the expression of Prolactin and Growth Hormone, and milk yield and conformation traits were studied in Italian Holstein bulls (Renaville *et al*, 1997). This study comprised 89 commercially available sires; the authors calculated daughter yield

deviations for yield traits (milk, fat and protein yields, and fat and protein percent), and 16 conformation traits. Relationships among sires were included in the model for analysis across-population. Although the results suggest that one allele of Pit-1 was associated with high milk and protein yield and better conformation (deep, angular body, and straight rear leg set), these results were not conclusive since the study was based on a small sample of bulls, and the lack of within-family analyses failed to corroborate whether the associations were present in some of the families of grandsires.

In Germany, 20 markers were tested for their associations with EBV for yield traits in five grandsire families of Holstein bulls (Kühn *et al*, 1999). This study focused on BTA6. The authors reported finding a significant effect on protein yield in one of the families. They presumed that the QTL was located between two of the polymorphisms (TGLA37 and FBN13), within a 3 cM interval in the middle section of the chromosome. However, caution should be exercised because no mention was made regarding the adjustment in the threshold for the LOD; the non-independence of the markers used was a factor that should have been addressed to reduce the chance of false positives, a significant effect of a QTL that does not exist in the interval. This problem arises when fitting a single-locus model using interval mapping (Ronin *et al*, 1999). The interval between the marker TGLA37 (one of the flanking markers of the putative segment with the QTL) and the marker IL90 was covered with no markers, and its length was around 30 cM. This leaves open the possibility markers TGLA37 and IL90 may be in LD with other QTL located in this interval. It has been shown that a substantial amount of LD between syntenic alleles may extend up to 50 cM in bovine populations (Farnir *et al*, 2000).

Another Canadian study further investigated the relationships between genetic markers of the GH gene and milk yield In Holsteins (Yao *et al*, 1996). They found significant effects for four markers, and reported the average effect of the gene substitution for alleles of Gh4.1 (43 and -253.6 milk, kg for the favorable allele and unfavorable allele, respectively) and Gh6.2 (44.9 and -283 milk, kg for the favorable allele and unfavorable allele, respectively). These analyses were done across population, not accounting for the relationships among sires.

Nadesalingam *et al* (2001) used interval mapping and 6 grandsire families (432 sons) to search for evidence of favorable QTL affecting yield traits (production of milk, fat, and protein, and percentages of fat and protein); they found that there were five putative QTL located on chromosome 1, and two QTL locations on chromosome 6, all related to increased milk yield.

Recently, also in Canada, Richard (2002) studied the effects of five markers on milk yield, and percentages of protein and fat, and confirmed that a marker on chromosome 20 for Growth Hormone Receptor had a significant effect on protein percentage.

A QTL that is being included in selection schemes to improve milk yield and composition is acylCoA:diacylglycerol acyltransferase (DGTA1), mapped on BTA (*Bos taurus* autosome) 14. The detection of this QTL is also an example of how to combine molecular and quantitative genetic tools to dissect the genetic variation underlying a QTL. Using a whole genome scan, a QTL located in the centromeric end of BTA 14 was first reported by Coppieters *et al* (1998) in Holsteins from Netherlands and New Zealand. Subsequently the position of the QTL was refined (Riquet *et al*, 1999) to a chromosomal segment of 9.5 cM. Later, Grisart *et al* (2002) cloned the QTL and identified a missense mutation in the DGTA1 gene with effects on milk fat content. Recently the causative mutation was characterized as a nonconservative lysine to alanine substitution (DGTA1 K23A) (Grisart *et al*, 2004).

Despite risks of false positives in many studies, some Artificial Insemination Companies are using MAS within their selection procedures, at least for some disease and yield traits. However, it is difficult to say how intensive the use of MAS is, especially in the latter traits, or what the procedures are to incorporate the QTL information. MAS is likely being used in selection within families. An example of such application is the use of MAS for yield traits in dairy cattle in France described by Boichard *et al* (2002).

2.9 QTL STUDIES IN DAIRY CATTLE: MASTITIS RESISTANCE

Despite the importance of mastitis for the dairy industry, few studies have been carried out to detect QTL or major genes affecting mastitis resistance. The main studies performed in this sense have investigated the role of the Major Histocompatibility Complex (MHC) genes, known as BoLA genes.

Aarestrup *et al* (1995) analyzed the association between BoLA class I haplotypes and subclinical mastitis. They used 657 cows from various breeds and determined their SCC and bacteriological status, defined as infected when bacteria (*Staphylococcus aureus*, coagulase-negative staphylococci, or both) were present in one or more samples from a cow. They used a model including fixed effects of herd, lactation number, breed and haplotype. They found that two alleles were associated with a decreased SCC and another two alleles were associated with increased SCC. Two alleles were associated with increased likelihood of isolating bacteria, and two alleles were associated with decreased likelihood.

In another study, Kelm *et al* (1997) studied genetic associations between measures of mastitis prevalence and genotypes of the MHC class II DRB3.2 and IgG2 loci, and the CD18 mutation responsible for BLAD (bovine leukocyte adhesion deficiency). Periparturient Holstein cows (n=137) were under study and the measures of mastitis used were EBV for SCS, clinical mastitis (CM), and intramammary infections (IMI) caused by major and minor pathogens. They found that the marker DRB3.2*16 was associated with increased EBV for SCS; DRB3.2*23 was associated with decreased EBV for CM; the other allele, DRB3.2*24 was in association with decreased EBV for SCS and also in association with increased EBV for IMI.

Dietz *et al* (1997) used 1100 cows to analyze associations between alleles of the DRB3.2 locus with levels of SCC. The authors compared animals with elevated SCC with control animals. Cows with elevated SCC were classified either as acutely elevated SCC (one test of more than 500,000 cells) or chronically elevated SCC (three consecutive tests of more than 500,000 cells). Cows that were not classified in neither of the above-mentioned groups were considered as control cows. The authors found that alleles DRB3.2*8, DRB3.2*16 and CRB3.2*23 were associated with an increased risk of disease in cows with an acute SCC in first, second and third lactation, respectively; allele DRB3.2*22 was associated with reduced risk of high cell count in second lactation cows. However, Sharif *et al* (1998) found that allele DRB3.2*16 was significantly associated with lower SCS, conflicting with the findings by Kelm *et al* (1997) and Dietz *et al* (1997). Sharif *et al* (1998) used information from 901 cows, included parity, season of parity and markers as fixed effects in the model.

Results from association studies between MHC class II genes and mastitis may seem disappointing as most of the literature shows contradictory results. The studied MHC genes (Kelm *et al*, 1997; Dietz *et al*, 1997; Sharif *et al*, 1998) can explain only partially the variation in mastitis resistance. There are a number of possible explanations for the conflicting results. One can be the use of logistic regression (Dietz *et al*, 1997; Sharif *et al*, 1998). This is a statistical technique more appropriate for epidemiological studies, where the odds regarding the likelihood of specific risks are the indicators of the possible relationships between variables; however, these studies did not take into account the population structure and factors such as linkage disequilibrium. Furthermore, they have in common that they did not include the additive relationships among animals. This lack of information has been considered as a possible cause of spurious effects in association studies (Kennedy *et al*, 1992).

However evidence suggests that indeed these genes still should be considered strong candidate genes for mastitis resistance. Pan *et al* (2001) carried out a segregation analysis of SCS data of Ontario Holsteins. They used simulation data and field data to analyze evidence of major genes affecting SCS. Their results suggest that a major gene affects SCS in the population under study.

In summary, so far markers have been postulated to be linked to QTL affecting milk yield and mastitis resistance. However, almost all studies, regardless of the trait studied, recommend a cautionary interpretation of the results. This arises from the uncertain causes of the detected associations. A number of factors may affect the results observed: structure of the population, relationships among animals, control of the type I error. Consequently, the information on putative QTL affecting mastitis resistance has not been included into commercial selection programs in dairy cattle. In addition, some diseases are more likely to be explained with the action of genes of the MHC, but more complex diseases are not. Possibly these complex diseases, such as mastitis, can be explained by the complementary action of other genes affecting the immune response. Additionally, reservations concerning the use of MHC allele within MAS have been expressed; selection for resistance to a specific disease may result in susceptibility to another disease (Mallard *et al*, 1998). The MHC is essential in the antigen presentation; hence the genetic diversity of the MHC is crucial in recognition of a variety of pathogens. This hypothesis

is supported by simulation studies. Using stochastic simulation, Springbett *et al* (2003) tested several scenarios in order to evaluate the effect of genetic diversity on the transmission of infectious diseases. Genetic heterogeneity was implied in the protection of populations from epidemics. These authors proposed that a homogeneous population may show fewer epidemics on average, but may in fact be susceptible to catastrophic ones. This can be interpreted on the basis that selection for a specific allele-haplotype of the MHC conferring resistance to mastitis may result in susceptibility to other diseases. There are, at least, two ruminant species that challenge some of these generalizations; the first one being several subspecies of bighorn sheep or *Ovis canadiensis* (*nelsoni*, *mexicana*, *cremnobates*, and *canadensis*). This native North American sheep has a declining population (in number and distribution), and it shows high disease susceptibility (blue-tongue, pneumonia, and scabies), although other factors are also in play: over-hunting, habitat loss due to competition for food and water from livestock. However, it has been shown that the high disease susceptibility of these animals may not be caused by low MHC variation, given the average 63% of observed heterozygosity found in the Ovca-DRB gene (MHC type II) of 231 specimens from several locations in California, Arizona, and New Mexico (Gutierrez-Espeleta *et al*, 2001). The other example is a population of 49 heads of Chillingham cattle that live in isolation in a park in the North region of England. This population has been isolated for about three hundred years; hence, it is expected that inbreeding increases homozygosity. After analyzing 25 microsatellites (scattered along 15 autosomes) in thirteen animals, Visscher *et al* (2001) found identical homozygous genotype for 24 of the 25 markers. The authors stressed the fact that even after a long period of time living as close herd, the animals do not show loss of fertility or viability. No comment was made regarding diversity in MHC alleles. They suggest that selection has removed deleterious genes from this population and that genetic uniformity does not necessarily reduce viability. One can generally expect lower viability in animals with lower disease resistance; hence the existence of this herd also suggests that sometimes disease resistance is not affected by homozygosity resulting from inbreeding.

2.10 CANDIDATE GENES FOR MASTITIS RESISTANCE

In the light of the evidence from quantitative genetics regarding the importance of the genetic associations between milk yield and mastitis resistance, it is pertinent to ask the following questions: Is it possible to explain the genetic variation in mastitis resistance? Furthermore, do we have adequate available information on genetic markers to perform association studies to map QTL affecting mastitis resistance?

In the Animal Science Department of McGill University, there have been efforts to develop molecular markers for genes whose products affect the immune response, such as Growth Hormone (GH), Growth Hormone Receptor (GHR), Ornithine Decarboxylase (ODC). The motivation behind the development of these markers in the bovine was to test for their associations with milk yield, fat and protein yields and percentages. Some investigations were carried out in this department to detect associations between these markers and milk yield (Aggrey *et al*, 1999, Richard, 2002), but any attempt to study their association with mastitis resistance is still lacking.

It has been proposed that selection for improved immune response may be achieved without any negative effect on milk production (Detilleux *et al*, 1995). Therefore, association studies to map QTL for mastitis resistance can be explored given that evidence indicates both immune and endocrine systems are genetically determined and regulated. It has been shown that there are peptides that regulate the brain and endocrine system, and act as endogenous immuno-modulatory substances, as well as bidirectional communicators between the immune and neuroendocrine systems (Blalock, 1994). With current techniques, genes can be studied in order to dissect, at least partially at the moment, their genetic properties as well as their role on mastitis resistance. The following is a brief discussion regarding the role of the above-mentioned genes in the immune response.

2.10.1 Molecular Features of genes linked to immune response: GH, GHR, IGF-1, PRL, ODC, ACTH, and CRH

The bovine GH gene, a 3000 bp gene comprised of 5 exons, is located on chromosome 19 (65.7 cM). The gene product is a protein hormone, composed of 198

amino acids, synthesized in the anterior pituitary gland with effects on several physiological processes, such as body growth, metabolism of insulin, and immune response. In order to carry out its metabolic role, GH has to bind with two receptors. Hence, the GHR gene (2014 bp), located at 59 cM on chromosome 20, has an important role in the control of the transduction of GH. Most of the body tissues have receptors for GH; however, these receptors are more common in liver tissue. The Growth Hormone Receptor Factor (GHRF) induces GH release from pituitary, while somatotropin inhibits it through a negative feedback mechanism.

GH has two types of effects: direct and indirect. The direct effects result when the GH binds to two Growth Hormone Receptors (GHRs) that are located on the membranes of cells. This causes a dimerization of the GHRs, activation of cytoplasmic tyrosine kinases (JAK1, JAK2, JAK3 and tyk2) and phosphorylation of the JAK system and the GHRs. These events lead to the recruitment and/or activation of several intercellular signaling molecules such as MAP kinases and insulin receptor substrates (IRS1 and IRS2); MAP kinases are implicated in the regulation of cellular growth and/or differentiation, and IRS are thought to be responsible for insulin-like metabolic effects of GH (Carter-Su *et al*, 1996).

Eppard *et al* (1996) measured a decreased incidence of clinical mastitis in cows treated with BsT. They showed although with a small experimental group, that cows with a low level of GH, had a higher incidence of mastitis, when compared with cows with a higher level of GH. The results suggest that GH has an effect on immune response.

Other authors (Weigent *et al*, 1991) offered an explanation for this; they suggested GH is an endogenous autocrine growth factor for lymphocytes and this naturally is related to an increased capacity to deal with infections. Postel-Vinay *et al*, (1997) proposed that the interaction of GH with the lymphohematopoietic system is on the basis of the following arguments: a positive effect of GH on T cell development has been observed in hypophysectomized or GH-deficient animals, and conversely, proliferation of lymphocytes is blocked by specific antibodies to GH. Naturally, the effect of GH on the immune response may be modified by genetic variation in GH and GHR genes.

In addition, immune response is affected by other hormones such as Insulin-like growth factor-1 (IGF-1) (the gene spans more than 3660 bp and contains 5 exons),

located on chromosome 5 at position 74 cM) which is a mediator of the effects of GH. IGF-1 is secreted mainly in liver and its mechanism of action may be explained as follows: GH circulates in the blood, and stimulates the production of IGF-1 in the liver. One of the actions of IGF-1 on the immune system is to modulate lymphoid tissue size. Another effect of IGF-1 is on the maturation of lymphocytes in bone marrow and on their function in the periphery. Two possible mechanisms have been proposed for these effects: IGF-1 could act positively in the differentiation of cells in marrow bone or it could act passively by reducing apoptosis. It has been proposed that IGF-1 increases lymphoid cell numbers by inhibiting apoptosis (Clark, 1997).

The PRL gene (9388 bp) is mapped on chromosome 23 (at 43.2 cM), and its product is a neuroendocrine hormone with a lactotrophic effect; it is related to the onset and persistency of lactation (Tucker, 2000). It has however an additional role in the regulation of the immune response. Specifically in regard to its immunoregulatory effect, PRL binds to receptors expressed in lymphocytes; it then triggers the phosphorylation of the receptor and several cellular proteins. It has been proposed that PRL serves as a messenger for the synthesis of interleukin 2 (IL-2), a small signal protein which stimulates proliferation of cytotoxic T cells (T cells) and Helper T cells (HT cells), through the stimulation of growth-promoting genes, such as c-myc, c-fos, and IRF-1 (Gouilleux *et al*, 1995). The importance of PRL on the immune response is clear, as T and HT cells are major players in the cellular immune response. Additionally, PRL increases levels of ODC (9452 bp, located at 8.97 cM on chromosome 11), an enzyme that contributes to the proliferation of antibody-producing cells.

During a stress response, for instance, when animals have an infection of the mammary glands, or otherwise under intensive milking, the Central Nervous System (CNS) causes the release of CRH. This hormone is synthesized in the hypothalamus and released into portal circulation. CRH causes the release of ACTH from the pituitary; ACTH in turn stimulates the secretion of glucocorticoids by the adrenal cortex. Cortisol, one of the glucocorticoids released, causes immunosuppression. Cortisol reduces counts of eosinophils in the blood, through increased diapedesis and increased release of neutrophils from bone marrow (Nelson and Cox, 2000). The hypothesis behind the immunosupression caused by cortisol is that the neutrophils are less capable of migrating

to the site of inflammation because of their early release. This causes an animal's reduced ability to deal with infections. Lower neutrophil migration is a factor that makes the animal more susceptible to diseases (Kulberg *et al*, 2002).

The effect of selection for high protein yield or for low clinical mastitis has been investigated in Norwegian cattle (Kulberg *et al*, 2002) at different stages of lactation: changes were found in the total number of white cells (mainly neutrophils), in the level of cortisol, and in the neutrophil/lymphocyte ratio in peripheral blood. The group of cows selected for low clinical mastitis showed a trend of low total white cells, high number of blood neutrophils, and low cortisol levels. A possible explanation for the difference in mastitis susceptibility may be that cows with low clinical mastitis showed an increased ability to recruit neutrophils, and an increased capability of neutrophils to face infections. Other research has shown that cows selected for high yield had a significantly higher number of circulating neutrophils, although, these neutrophils had a higher ability to migrate compared with neutrophils from cows with average production potential (Detilleux *et al*, 1995); this is in agreement with the findings for the Norwegian cattle. Other research showed as well that CRH and TSH stimulate cellular response against infections through increasing Natural Killer Cell activity (Carr *et al*, 1990). Hence, the increased ability of neutrophils to eliminate pathogenic microorganisms may be considered a feature of animals with higher resistance to diseases. This variation is considered to be partially due to genetic differences, and understandably the CRH and ACTH genes become candidate genes for resistance to mastitis. The CRH gene (584 bp) is located on chromosome 14 (68 cM) and the POMC (propriomelanocorticotropin) gene is approximately 7300 bp in length (1084 bp mRNA available in NCBI) is mapped on chromosome 11 (81 cM). The POMC gene synthesizes a large molecule of which ACTH is part.

2.11 HYPOTHESIS AND OBJECTIVES

Given the roles of the genes discussed above in preparing the organism against infections, markers available for these genes may be used in studies to analyze their associations with mastitis resistance. From the direct role of some of these genes on the immune response it is logical to look for associations between markers for these genes

and resistance to mastitis. This project will test the hypothesis that there are associations between DNA markers in genes related to immune response (Growth hormone, Growth hormone receptor, Ornithine Decarboxylase, Prolactin, Adrenocorticotropic hormone, Insuline-like growth factor-1, and Corticotropin releasing hormone) and incidence of clinical mastitis, occurrence of clinical mastitis, culling due to mastitis, and somatic cell scores in Canadian Holsteins. The objectives are to examine both across-population and within family associations between these markers and mastitis resistance in Holstein cattle.

CHAPTER 3

MATERIAL AND METHODS

3.1 GENETIC POLYMORPHISMS

The compiling of a bovine DNA bank was started in the early nineties in the Animal Science Department of McGill University. The original motivation for the construction of the DNA bank was to support studies of associations between genetic markers of genes involved in the regulation of growth and immune responsiveness and milk yield traits in Holstein cattle. Initially, five markers for GH, three for GHR, and two for ODC were included.

By 2000, the bank was comprised of genetic polymorphisms of the above ten markers for 26 Holstein grandsire families that included 1738 Holstein bulls.

Until 2000, several studies of associations were performed by merging the molecular information contained in the bovine DNA bank with the information on bull's genetic evaluations for milk production traits obtained from the Canadian Dairy Network (CDN) (Zadworny and Kuhnlein, 1990; Yao *et al*, 1996; Aggrey *et al*, 1999, Richard, 2002).

More recently, the present study was initiated to analyze associations between genetic polymorphisms and mastitis resistance. The base for this study was the DNA bank described above, and information obtained from the provincial milk recording (PATLQ) that has been used in the Animal Science Department of McGill University for several other research purposes. The phenotypic information consisted in several files containing information recorded by the PATLQ (Programme d' Analysis de Troupeaux Laitiers du Québec) that included reports of cases of clinical mastitis, culling due to mastitis and SCC. The data base included records of cows born between 1975 and 1993. This phenotypic data base was used to generate measurements of mastitis resistance (the description is provided later, in section 3.5).

Several activities were done between 2001 and 2003 intended to increase the size of the DNA bank. The goal was to increase: a) the number of genotyped bulls, as well as b) the number of markers and genes.

For the first aim, a search was performed to identify more bulls to increase the number of grandsire families (*i.e.* to detect new grandsire families that were not already genotyped),

and, consequently, to increase the number of bulls to perform across-population analysis. To do so, 482,895 first lactation records (before any editing), were used to detect bulls that matched the following criteria: to have at least 15 daughters in the phenotypic data set and have at least 19 paternal male half-sibs. As result, 1002 bulls were found matching these criteria. From these bulls, 282 bulls were already genotyped for the ten markers above mentioned (*i.e.* they were on the 1738-bull list). The genotypic frequencies (for the original ten markers) of these 282 bulls are presented in table 3.1. The table displays the identities for the original ten genetic markers.

Table 3. 1 Genotypic and allelic frequencies for the ten initial markers included in the DNA bank. The frequencies are calculated for the bulls originally included in the DNA bank and that had at least 15 daughters in the phenotypic data set, and at least 19 paternal male half-sibs (n=282 bulls)

Marker	Genotypic frequencies			Total number of	Allelic frequencies	
	+/+	+/-	-/-	sires	+	-
ODC1	12 (0.04)	93 (0.34)	166 (0.61)	271	0.21	0.78
ODC2	136 (0.48)	138 (0.49)	7 (0.02)	281	0.73	0.27
GHRAL	102 (0.39)	127 (0.48)	35 (0.13)	264	0.63	0.37
GHRAC	128 (0.48)	120 (0.44)	21 (0.08)	269	0.70	0.30
GHRST	239 (0.90)	27 (0.10)	-	266	0.95	0.05
GH61	248 (0.89)	30 (0.11)	1 (0.003)	279	0.95	0.05
GH62	146 (0.56)	104 (0.40)	9 (0.04)	259	0.76	0.24
GH1	27 (0.12)	144 (0.63)	59 (0.26)	230	0.43	0.57
GH41	167 (0.66)	82 (0.32)	4 (0.02)	253	0.82	0.18
GH42	161 (0.67)	75 (0.31)	5 (0.02)	241	0.82	0.18

For a description of the genetic markers as well as their code name, see section 3.3.

Thus, 720 bulls (out of the 1002 bulls matching the search criteria) had not been typed previously. No additional grandsire families were identified after the search process.

During the above search, 157 additional bulls were found to have at least 15 daughters in the phenotypic data set, but did not have at least 19 paternal male half-sibs. It was

considered that by genotyping these bulls, more information to perform across-population analysis could be obtained. It has to be noted that to perform across-population analysis no information regarding the grandsire is needed, thus all bulls genotyped without regard to their status as sons of grandsires may be included.

Hence, a total of 877 additional bulls (with daughters in the phenotypic data set) were identified. From them, samples of frozen semen of 336 bulls were available from CIAQ to genotype. From the remaining 541 bulls no frozen samples were available.

In order to increase the number of markers and genes in the DNA bank, from 2001 to 2003 ten additional new markers were added: four new markers for GH, and additionally, six markers of genes not previously included (two for ACTH, one for CRH, two for Prolactin, and one for IGF-I). As pointed out in sections 2.10 to 2.13, these genes are involved in the regulation of the immune response; hence they were included in the DNA bank.

The 336 new bulls from which semen samples were available were submitted for genotyping for the complete set of twenty genetic markers. The 282 original bulls (with at least 19 paternal male half-sibs, each with at least 15 daughters in the phenotypic data set) were submitted for genotyping for the new ten markers.

Most of the genotyping was done by personal from the Molecular Genetics Laboratory of the Animal Science Department (McGill University), with the genotyping for the new set of ten markers performed by DNA Landmarks, a biotechnology company based in Quebec, to expedite the process.

3.1.1 DNA extraction and amplification

Complete details of the extraction, amplification, and generation of the genetic markers may be found in the references that are provided below in the sections corresponding to every gene. However, a brief description of the DNA extraction and amplification processes is provided next.

The DNA extraction process was described by Zadworny and Kuhnlein (1990). DNA was extracted from 1-2 semen straws; the content of the straws was deposited in Eppendorf tubes, and centrifugation was applied to obtain a pellet; once the pellet was obtained, it was washed 4-5 times with phosphate buffered saline (PBS). Cells were suspended in 100 µl PBS to which 400 µl of a solution (2% 2-mercaptoethanol, 10 mM triethenolamine, pH 8.0,

100mM NaCl, 10 mM EDTA, pH 8.0, and 0.5% sodium dodecyl sulfate) was added. The mixture was incubated (50° c, 30 min). Proteinase K was added to the final concentration (200 µl/ml). The incubation was continued by 12-16 h. Following phenol and chloroform-isoamyl alcohol extractions, DNA was recovered by ethanol precipitation.

Markers for GH were obtained using the PCR-SSCP method. PCR-SSCP method allows detect polymorphisms through mobility shifts due to a mutational change in the conformation of the single strand.[9] After SSCP analysis, those fragments displaying electrophoretic pattern are sequenced. The DNA sequence of these segments was obtained by the method of Sanger. The markers for GHR and ODC were obtained using a PCR-RFLP method.

GH gene (Yao *et al*, 1996).

Primers. Pairs of oligonucleotide primers were synthesized to amplify five fragments of the GH gene: GH1, GH4.1, GH4.2, GH6.1, and GH6.2 (table 3.1). The PCR was performed in a reaction volume of 25 µl using 100 ng of DNA, 0.5 µM of each primer, 1X PCR buffer (10 nM tris-HCl, 1.5 mM MgCl2 and 50 mM KCl, pH 9.0), 5% deionized formamide, 200 µM deoxinucleoside triphosphate (dNTP) and 0.625 units of Thermus thermophilus (Tth) DNA polymerase. The amplification was carried out for 35 cycles at 92°C X 30 sec, 59°C X 80 sec and 72°C X 90 sec using a DNA thermal cycler.

SSCP analysis: The SSCP analysis was performed with a Bio-Rad "Mini-Protein II" vertical gel. One µl of the PCR product was diluted with 15 µl of a solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol). The mixture was denatured at 100°C for 5 min, cooled in ice for 5 min and loaded on a nondenaturing 12-20%

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[9] In SSCP analysis the PCR product is denatured, and the separate strands adopt a particular conformation that depends on the nucleotide sequence. The change in the conformation also changes the electrophoretic mobility. The length of the PCR product must be between 150 to 300 base pairs to obtain maximum results.

acrylamide:bis-acrylamide (49:1) gel. Electrophoresis was performed in 1X Tris borate (pH 8.3)-EDTA buffer at 10-12.5 volts/cm for 6-24 h at room temperature. DNA was detected by silver staining. The polymorphisms for GH1 are shown in figures 7.1 to 7.3; for GH4.1 and GH4.2 in figures 7.4 to 7.5; for GH6.1 and GH6.2 in figures 7.6 to 7.8 (appendix 2).

Sequence analysis: In order to reveal the base change responsible of the difference in mobility sequence analysis, sequences of DNA fragments with different electrophoretic pattern were determined by the dideoxy-chain termination method of Sanger with [35S] dATP using a T7 sequencing kit.

GHR (Aggrey *et al*, 1999).

Primers. A pair of primers was synthesized to amplify two fragments of the GHR gene. The features of the primers and the fragments are shown in table 3.1. The PCR for the 836 bp fragment was performed in a reaction volume of 25 µl using 100 ng of DNA, 0.25 µM of each primer, 1X PCR buffer (10 mM tris-HCl, 1.5 mM MgCl2 and 50 mM KCl, pH 9.0), 5% deionized formamide, 160 µM dNTP and 0.625 units of Tth DNA polymerase. The amplification was carried out for 35 cycles at 92°C X 60 sec, 66°C X 80 sec and 72°C X 120 sec using a DNA thermal cycler. Similar conditions were used for the amplification of the 1119 bp fragment with the exemption that no deionized formamide was included.

RFLP analysis. For the 836 bp fragment 10 µl of the PCR product were digested with 5 units of AluI, and for the 1119 bp fragment two 10 µl aliquots of the PCR product were digested with 5 units of AccI or StuI. The conditions for the digestion were 37°C for at least 2 h. The digested fragments were separated by electrophoresis (2% agarose gel in 1X TPE [90mM Tris-phosphate, 2 mM EDTA]). The gel was stained with ethidium bromide and visualized under UV light. The polymorphisms for GHR-Stu, GHR-AccI and GHR-Alu are shown in figures 7.9 to 7.11 (appendix 2).

Sequence analysis. For sequencing the amplified DNA fragments were cloned into pUC18 plasmid; sequences were determined by the dideoxy-chain termination method of Sanger using a T7 sequencing kit with [35S] dATP as the labeled nucleotide.

ODC (Yao *et al*, 1998). Primers. A pair of primers was synthesized to amplify two fragments of the ODC gene. The features of the primers and the fragments used are in table 3.1. One fragment of 1393 bp contained a polymorphic site (TaqI); the second fragment of

796 bp fragment was contained a polymorphic site (MspI). The PCR for both fragments was performed in a reaction volume of 25 µl using 100 ng of DNA, 0.5 µM of each primer, 1X PCR buffer (10 mM tris-HCl, 1.5 mM MgCl2 and 50 mM KCl, pH 9.0), 5% deionized formamide, 2000 µM dNTP and 0.625 units of Tth DNA polymerase. The amplification was carried out for 35 cycles at 92°C X 30 sec, 50°C X 80 sec and 72°C X 120 sec for the 1393 bp fragment, and at 92°C X 30 sec, 61°C X 80 sec and 72°C X 90 sec for the 796 bp fragment using a DNA thermal cycler.

RFLP analysis. For both PCR products (1393 and 796 bp fragments) 7 µl were digested with 5 units of TaqI, and MspI, respectively, at 37°C for 2 h. The digested DNA fragments were then separated by electrophoresis (2% agarose gel in 1X TPE [90mM Tris-phosphate, 2 mM EDTA]). Then, the gel was stained with ethidium bromide and visualized under UV light. The polymorphisms for ODC are shown in figures 7.12 to 7.14.

In all the cases the process of genotyping included proper controls to increase the reliability of the determination of the genotypes. In the cases in which a genotype was not possible to be determined the information was discarded.

Table 3.2 shows the main features of all the primers used to detect and amplify the genomic regions under analysis. The markers for ACTH, CRH, PRL, IGF-1, and the following markers for GH: GH1-258, GH1-300, GH5-183, GH5-255, were obtained by using automated sequence analysis using fluorescent dyes. Figure 7.15 displays the sequences used to generate the polymorphisms for ACTH, CRH, PRL, and IGF-1. The amplified sequences shows in each case the polymorphisms detected.

Table 3. 2 Primers for each marker and length of the amplified gene fragments

Marker	Type	Fragment	Primer's sequence (5' → 3')	Length of the amplified gene fragment
CRH		CRH291	CCGCCTGGGTAACCTCGATGAG	294 bp (position 224 to
			CTGTGCTAACTGATCGGCCTTG	518)
ACTH		ACTH341	GGCACCTCGGACCGTGTCTA	264 bp (position 439 to
(POMC)		ACTH388	AACAACAGATGGCTGGCAACTA	703)
IGF-1		IGF1-390	TTCCATTGCGCAGGCTCTATCT	723 bp (position 1433 to
			GGGCCAAGCAGCAGAGTAGAAG	2156)
ODC[1]	RFLP	ODC1	GTCAGGAAGATTCTCTAGAGA	1393bp (position 2342 to
		(TaqI)	TGGATTTGCATAGATAATCC	3734)
		ODC2	ACCACAGGATATGCCAGACTGG	796 bp (position 5413 to
		(MspI)	GCACCCATGTTCTCAAAAGAGC	6208)
GHR[2]	RFLP	GHRAL	TGCGTGCACAGCAGCTCAACC	836 bp (position
			ATGCCCAGCAGTGGGGTTGCT	-1871 to -1036)
		GHRAC		
			ATGCCCAGCAGTGGGGTTGCT	1119 bp (position
		GHRSTU	GGCAAACAGTGCGGGGTTGGA	-1056 to 63)
GH[3]	SSCP	GH6.2	TAGGGGAGGGTGGAAAATGGA	404 bp (position 2054 to
		GH6.1	GACACCTACTCAGACAATGCG	2457)
		GH4.2	GGACAGAGATACTCCATCCAG	345 bp (position 1380 to
		GH4.1	AGATGCGAAGCAGCTCCAAGT	1724)
		GH1	GGTGGGTTGCCTTTCTCTTCT	464 bp (position 8 to 471)
			TGTCATCATCCCGTCTCCACT	
		GH1-258	Same primer as for GH1	
		GH1-300		
		GH5-183	TTGGAGCTGCTTCGCATCTCA	366 bp (position 1706 to
		GH5-255	ATTTTCCACCCTCCCGTACAG	2071); obtained by direct sequencing
PRL		PRL1-152	ATCCCAAGATATCCTCTACTGA	611 bp (position 8841 to
		PRL2-361	TCTTAGATTTTGACATCGCTAC	9452)

1= Yao *et al* (1998); 2= Aggrey *et al* (1999); 3= Yao *et al* (1996)

3.2 CHROMOSOMAL POSITION OF THE POLYMORPHISMS

Table 3.3 shows the chromosomal position of each gene, the names assigned to each polymorphism, as well as the base substitution that determined the genetic variation. The information regarding the genetic maps was retrieved from databases online; the information of the URL of such databases is provided at the bottom of table 3.3.

Table 3. 3 Position of polymorphisms on chromosomes and base substitution

Gene	BTA	Gene position (Map name)	Genetic markers	
			Polymorphism	Position base substitution
CRH	14	68.0 cM (IBRP97)	CRH1_291	500 (A →G)
ACTH (POMC)	11	81 cM (INRA)	ACTH1_388	643 (A → C)
			ACTH2_341	573 (A → G)
IGF-1	5	74 cM (MARC)	IGF1_390	1407 (C → T)
ODC	11	8.97 cM	ODC1	2512 (G → T)
			ODC2	5664 (G → A)
GHR	20	59.0 cM (IBRP97)	GHR22StuI	-232 (C → T)
			GHR21AccI	-892 (C → T)
			GHR1Alu	-1182 (A→T)
GH	19	65.7 cM (MARC97)	GH6.2	2291 (A → C)
			GH6.1	2141 (C → G)
			GH4.2	1692 (T → C)
			GH4.1	1547 (C → T)
			GH1	125-142(DEL→INS TGC)
			GH1-258	258 (C → T)
			GH1-300	300 (C → T)
			GH5-183	183 (G → T)
			GH5-255	255 (C → T)
PRL	23	43.2 cM (MARC97)	PRL1_152	9017 (A → G)
			PRL2_361	9218 (C → T)

Adapted from: Yao *et al* (1996); Zadworny and Künhlein (1990); Yao *et al* (1998); Aggrey *et al* (1999); www.thearkdb.org; http://dga.jouy.inra.fr; www.marc.usda.gov/genome/genome.html

As mentioned above graphical representations of the polymorphisms are shown in Appendix 2 in Figures 7.1 to 7.13 (pages 136-141). Figures 7.1 to 7.7 depict the genetic map of BTA19 where the bovine GH gene is located, as well as the position of the genetic markers for this gene; figures 7.8 to 7.10 show the polymorphisms of GHR, located on BTA20, and figures 7.11 to 7.13 show the localization of the polymorphisms for ODC. The genotypic and allelic frequencies were calculated by direct count.

3.3 GENETIC EVALUATIONS FOR MASTITIS RESISTANCE TRAITS AND SCS

3.3.1 Edits

Lactation records from the PATLQ (*Programme d'analyse des troupeaux laitiers du Québec*) were used to obtain estimated transmitting abilities (ETA) for several mastitis resistance traits and SCS for Holstein sires. The period of time covered by these records was from November, 1979 to March 1995, and before edits the number of lactation records was 1,874,091.

An editing process was carried out to eliminate records belonging to cows of breed other than Holstein, records with irrecoverable errors (e.g.: lactation number less than 1, erroneous identities of cows, records with mistakes in the identity of the sires, mistakes in dates of birth and calving). Other criteria were set to eliminate lactation records with extreme previous calving interval lengths (less than 350 days or more than 650 days) to avoid including records with mistakes in dates of calving.

Only cows born between January 1, 1975 and December 31, 1993, and calving from January, 1980 until December, 1994 were accepted to ensure a homogeneous data set in terms of the possibility to record complete lactations. Edits to exclude records of cows with extreme ages at calving at different lactations (from first to third lactation) were set by considering ranges of age at calving. After these edits a further criterion was imposed to keep only cows with consecutive lactation records and to eliminate 4th and later lactations. Then, for each lactation record a Herd-Year-Season of calving (HYS) variable was created. The procedure considered two seasons, one from September 1st to the last day of February (the procedure was done considering leap years), and another season from March 1st to August 31st. These seasons were used by other authors in independent studies of the same data set.

The sequential order of the editing process is summarized in the table 3.4, where the number of records deleted for each of the editing criteria used, and the remaining records after each edit are shown.

Table 3. 4 Editing process to create lactation files (first, second, and third lactation). Initial number of lactation records, number of records removed sequentially for editing criteria, and final number of lactation records

Criteria for deletion	Number of lactation records deleted	Remaining records
Total records	-	1,874,091
Records of breed other than Holstein	119,694	1,754,397
Parity number < 1	7,102	1,747,295
Parity number > 14	82	1,747,213
Cows with mistake in registration number	137,021	1,610,192
Cows not purebred Holstein	52,275	1,557,917
Repeated records by herd-cow-lactation number	46,325	1,511,592
Records with irreparable mistake in sire's registration number	1,170	1,510,422
Calving interval < 300 days	2,098	1,508,324
Calving interval > 650 days	8,371	1,499,953
Cows born before 1/January/1975	33,883	1,466,070
Cows born after 31/December/1993	4	1,466,066
Cows with calving before 1/January/1980	10,085	1,455,981
Cows with calving after 31/December/1994	89,684	1,366,297
Date of birth greater than date of calving	7	1,366,290
Cows with 1st calving < 19 months old	140	1,366,150
Cows with 1st calving > 44 months old	1,325	1,364,825
Cows with 2nd calving < 32 months old	7,037	1,357,788
Cows with 2nd calving > 57 months old	1,773	1,356,015
Cows with 3rd calving < 44 months old	5,163	1,350,852
Cows with 3rd calving > 69 months old	2,757	1,348,095
Non-consecutive lactations	310,139	1,037,956
4th lactations or later	171,249	866,707

After all edits were imposed the remaining data set included 866,707 lactation records; of these 445,891 were first lactation records, 235,333 second lactation and 155,483 third lactation.

The next step after the initial editing described above was to integrate the data sets required for genetic evaluations. Two criteria were imposed to keep records for the generation of the genetic evaluations: i) records of sires with at least 10 daughters in 10 herds, and ii) HYS with at least two sires. In order to do so, an iterative process was performed on every data set (*i.e.*: first, second, and third lactation). Table 3.5 shows a summary of the editing process to create the files for the genetic evaluations for each data set.

Table 3. 5 Editing process to create lactation files (first, second, and third lactations) to be used in the genetic evaluations

Lactation Number	Original number of lactation records	Number of lactation records deleted for: 1) sires with less than 10 daughters in 10 herds, and 2) herd-year-season of calving with at less than 2 sires	Remaining lactation records (used in genetic evaluations)
First	445,891	34,600	411,291
Second	265,333	26,901	238,432
Third	155,483	24,500	130,983

Table 3.6 shows the total number of sires, HYS, and lactation records by parity after applying the edits to keep records for genetic evaluations.

Table 3. 6 Number of sires, HYS and records by lactation number and number of cows used in the genetic evaluations

Lactation	Number of sires	Herd-Year-Season of calving	Number of cows
1st	1,913	61,681	411,291
2nd	1,638	50,013	238,432
3rd	1,320	35,383	130,983

3.3.2 Creation of mastitis variables (binary traits)

One of the features of Québec's milk recording between 1979 and 1995 is that records of mastitis incidence were kept in addition to the information regarding cause of culling and SCC. The lactation records used in this study had six fields where different Code Affecting Records (CAR) reported on the test-day visit could be recorded. The information stored on these fields classified important events that might have an impact on the lactation, such as clinical mastitis. These events were recorded voluntarily by producers each month. Additionally, in the other field available for that purpose, a disposal code that indicates the main reason for culling the cow was recorded. The system did not record secondary reason for culling. The causes of culling were classified in previous studies as low production, reproductive problems, involuntary reasons, mastitis, udder breakdown, feet and leg problems, death or sickness, and injury (Dürr *et al*, 1997; 1997b).

It is recognized that susceptibility to mastitis increases with age and stage of lactation, and hence in this study records from the first to the third lactations were kept; this would allow monitoring the performance of the cows with regard to their resistance against mastitis by lactation and across several lactations.

Binary traits were created for incidence of clinical mastitis in two ways: a) by taking lactations independently and b) over the three first lactations collectively. Thus, if a cow had at least a case of clinical mastitis reported in any of its first three lactations (*i.e.*: at least one CAR = 16, that was the code for clinical mastitis used in the PATLQ) the binary trait (for the appropriate lactation) was coded as 1, otherwise the lactation record was coded as 0 (*i.e.*: no clinical mastitis along the lactation). The definition of the binary trait across three lactations collectively was as follows: if any of the lactation records (from the 1st to the 3rd) of a cow had a mastitis case reported (*i.e.*: at least one CAR coded as 16) then the binary trait for mastitis incidence across lactations was coded as 1, otherwise as 0 (*i.e.*: no clinical mastitis reported in any of the lactations, from 1st to 3rd).

The occurrence of clinical mastitis was calculated as the total number of cases of clinical mastitis that were reported along each lactation; in this case every report of clinical mastitis (CAR = 16) was considered a case, and the total number of reports of clinical mastitis represented the occurrence of clinical mastitis in each lactation. Another variable created, with regard to occurrence of clinical mastitis was the total number of cases of

clinical mastitis over the three lactations (taking into account cows with all the three lactations). This is, all the reports of CM in each lactation from 1st to 3rd were added up to obtain the total number of clinical mastitis cases over the three lactations collectively, which represents the occurrence of clinical mastitis over the three lactations.

With regard to culling due to mastitis, each lactation record was coded as 1 if the cow was culled due to mastitis (CAR = 54) before the end of each lactation, and 0 otherwise.

Recording health traits (*i.e.* mastitis) in milk recording systems on a voluntary basis has been criticized as lacking precision; indeed, this is an important drawback in genetic studies of mastitis using field data collected in this way. The main outcome is an underestimation of mastitis incidence that normally ranges between 3 and 43 infected quarters/100 cows/year (Erskine, 1988; Kossaibati *et al*, 1998; Bradley, 2002). However, the importance of the disease for the Canadian dairy industry encourages the analysis of any relevant information available in order to find possible ways to alleviate the economic losses due to mastitis.

As stated above, culling reasons in Québec herds were reported by means of a disposal code that is assumed to be the main reason for the culling of the cow. When a milk recording system only records a primary reason for disposal, differences in criteria to decide the culling of animals may not be properly represented. For instance, a cow culled due to low milk yield may have been affected by chronic mastitis (this condition may be responsible for the low production), hence if the disposal code is recorded as "Low Milk Production" (i.e.: code 50) it may create an inaccuracy that is not possible to correct when analyzing historical files such as those used in this study. Another example of uncertainty resulting from the sole use of primary disposal codes may be culling due to "Reproductive Problems" (*i.e.*: code 57). It is accepted that mastitis may have an effect on fertility of cows. Endocrine changes occur under stress and they may affect follicular function, as a consequence fertility may be reduced (Nikolic *et al*, 2003; Barker *et al*, 1998; Hockett *et al*, 2002).

Situations such as those described above may indeed occur in milk recording data. This lack of precision has been discussed by other researchers using the same database (Dürr *et al*, 1997; 1997b) who concluded that it should not prevent analysis of this database in order to obtain research results.

With regard to somatic cells, every lactation record had a lactation measure of somatic cell count (LSCC), and somatic cell score (SCS) were obtained by using the log

transformation (SCS=log2[Lactation SCC/100000]+3) proposed by Shook and Schutz (1994).

3.3.3 Creation of pedigree files and phantom parents

For each data set (first, second and third lactation) a list of sires with progeny in the data set was created and was used to retrieve their pedigree (sire and dam) and ancestors (from both paternal and maternal sides) from a pedigree file with 6,345,935 records. The pedigree file was provided by CDN. The number of pedigrees and animals in the files for each lactation is provided in table 3.7.

Table 3. 7 Number of animals in pedigree

Lactation number	Number of animals in pedigree (sires, ancestors)	Sires with genetic evaluation (with daughters in mastitis file)
1st	5326	1913
2nd	4673	1638
3rd	3889	1320

In the case of the 1st lactations, there were 5326 animals in the pedigree file. This number may be partitioned into 2280 males (from which 1863 were Canadian, and 417 from the US), and 3046 females (1646 Canadian, and 1400 US). This file contained 1913 sires with progeny in the data file, 3413 of their ancestors (367 males, and 3046 females).

In the case of the 2nd lactations, there were 4673 animals in the pedigree file. This number may be partitioned into 1993 males (from which 1697 were Canadian, and 296 from the US), and 2680 were females (1478 Canadian, and 1202 US). This file contained 1638 sires with progeny in data file, 3035 of their ancestors (355 males, and 2680 females).

In the case of the 3rd lactations, there were 3889 animals in the pedigree file. This number may be partitioned into 1654 males (from which 1428 were Canadian, and 226 from the US), and 2235 females (1208 Canadian, and 1027 US). This file contained 1320 sires with progeny in data file, 2569 of their ancestors (334 males, and 2235 females).

In almost every pedigree file integrated for genetic evaluation purposes it is not possible (at least in commercial dairy cattle populations) to identify the parents (both or either of them) of all animals; thus some animals end up with no identified parent(s). Furthermore there is a point where the pedigrees stop (*i.e.*: where there is no more information regarding parents of some animals). The animals from which it is not possible to retrieve parental identification are called base animals (*i.e.* the ancestors are unknown animals). If no consideration is given to this situation, genetic evaluations may be biased because all unidentified parents will be considered as part of the same generation (*i.e.*: born in the same genetic group) when in reality they may belong to different years of birth and hence belonging to different generations each with different genetic merit. A solution for this problem has been proposed by Thompson (1971) and it has been elaborated by Westell *et al* (1988). The approach consists in the creation of phantom parents (PP) on the basis of the year of birth of the animals with unknown parents. The procedure accounts for the genetic trends present in the population under study.

In the file for first lactations 1687 ancestors were base animals (226 males, 1461 females); in the second lactation file 1311 ancestors were base animals (198 males, and 1113 females), and in the third lactation file 1520 ancestors were base animals (213 males, and 1307 females). Thus, the first step in order to create genetic groups (GG) was the estimation of the generation interval for each path of selection (e.g.: sires to breed cows, sires to breed bulls, dams to breed cows, and dams to breed bulls). To do so, the pedigree file with 6,345,935 pedigrees was used, and only pairs of animals with relevant information (*i.e.* the identity of the animal, the identity of its sire, and dam, and its year of birth) were considered. Each record had the following columns: animal identity, sire identity, dam identity, date of birth of the animal, and sex of the animal. Each record was used to obtain all the pair of animals corresponding to each path of selection, this is: female animal-sire (sire to breed cows), female animal-dam (dam to breed cows), male animal-sire (sire to breed bulls), and male animal-dam (dam to breed bulls). Each record corresponding to each pair of animals had the animal identity, the parent identity, and the dates of birth of the animal and the parent. The date of birth of the animal was obtained directly at the moment of retrieving the pair of animals from the pedigree file; the date of birth of the parent was extracted from the pedigree file by searching the parent in the column of animal (because a parent might appear as animal on the same pedigree file, occupying the appropriate column), then the date of birth

of the parent was included in the file (containing the pair of animals). With that layout, the period between the dates of birth of the animal and the parent was calculated in months (accounting for leap years); with that information the average generation interval was calculated for each path of selection.

Once obtained the generation intervals, for each animal with unknown pedigree, phantom parents were assigned a year of birth according the year of birth of the animal. The procedure was to subtract from the year of birth of the animal the appropriate GI estimated previously (e.g.: if the animal was female, to create the phantom sire the generation interval corresponding to the selection path Sire to breed cows was used; to create the phantom dam, the generation interval corresponding to Dam to breed cows was used, and so on). Once the PP was assigned, GG for the PP was defined by grouping PP according their assigned year of birth; in this way eighteen GG were defined for the PP. The definition of the genetic groups will be explained later.

3.3.4 Models for genetic evaluations

Before using phenotypic data to map QTL, it is necessary to eliminate the nuisance background variation from the data set under study. Several measures have been used as phenotypic indicators in the detection of QTL in dairy cattle: DYD (daughter yield deviation) (Georges et al, 1995; Ashwell et al, 1998; Nadeselingam et al, 2001), EBV (estimated breeding value) (Weller et al, 2003; Ron et al, 2004), or ETA (estimated transmitting abilities, also termed PTA, predicted transmitting abilities) (Falaki et al, 1996; Zhang et al, 1998; Ashwell et al, 2004).

Evidence suggests that there is no major difference in terms of QTL detection when using any of them. Nadesalingam (1999) compared the use of EBV, DYD, or DRP (deregressed proofs) in the detection of QTL using LS interval mapping. Similar results were obtained with EBV and DYD. Some minor differences were found with DRP. The conclusions of that study were that the choice of the trait measure had minimal effect on the search for QTL, and that any of the traits under study could be used for both the detection of QTL and the estimation of their effects. Rodriguez-Zas et al (2002) compared the use of DYD and PTA in the detection of QTL for somatic cell scores and components of milk in

dairy cattle using interval mapping and composite interval mapping. They found identical results in the location of QTL, regardless the type of phenotypic indicator used.

The following model was fitted for the analysis of each response variable described above to obtain variance components for the traits and estimated transmitting abilities for the sires which afterwards were used in the detection of QTL:

$$Y_{ijklm} = \mu + HYS_i + AGE_j + GG_k + S_{kl} + e_{ijklm}$$

where Y_{ijklm} is the observation (*i.e.*: incidence of clinical mastitis, occurrence of clinical mastitis, culling due to mastitis, or SCS, for any of the parity numbers); μ is the population mean; HYS_i is the fixed effect of the $_i$th Herd-Year-Season of calving subclass (61,681 HYS levels for first lactation; 50,013 for second lactation; 35,383 for third lactation); AGE_j is the fixed effect of the $_j$th age at calving of the cow subclass (26 levels for first, second and third lactations); GG_k is the fixed effect of the $_k$th genetic group (18 genetic groups for first, second and third lactations); S_{kl} is the random effect of the $_l$th sire within the $_k$th genetic group; and e_{ijklm} is the random residual error associated with the $_{ijklm}$th record.

The subset of programs MTDFREML (Boldman *et al*, 1995) was used to obtain REML estimates of variance components was. Additionally it was used to obtain sires's ETA. This subset of programs uses different files: one with the pedigree and another one with the phenotypic information. In order to process the information of pedigree (to form the non-zero elements of A^{-1}), all the animals must have a unique identification, and the file must be logically sorted (from animal 1 to animal n). Recodification of the original animal identities, and their logical sorting was performed using two separate Fortran programs (Cue, I, personal communication). The file with the phenotypic information (i.e. the information of incidence of clinical mastitis, occurrence of clinical mastitis, culling due to mastitis, and SCS), contained also information of HYS, age at calving and the identity of the animal (in this case the sire of the cow). MTDFREML allows the definition of genetic groups. In the pedigree file each missing parent was identified with the number of the genetic groups to which was assigned (see description in section 3.3.3). A column vector with the number of genetic groups was defined. The model with groups, such as the mentioned above is intended

to account for differences in the structure of the subpopulations (see section 3.3.3) and reduce the bias in the estimation of the prediction of the transmitting abilities. For models that include genetic groups MTDFREML uses rules elaborated by Westell *et al* (1988) for calculating the coefficients associated with group effects.

HYS of calving and age at calving corresponding to the lactation were considered when analyzing lactations separately; in the case of the analysis of the variables defined over all lactations the HYS and the age at calving at first lactation were considered. A derivative-free algorithm to obtain REML estimates of variance components was used. The heritability estimates were estimated as $4\sigma^2_s / (\sigma^2_s + \sigma^2_e)$, where σ^2_s and σ^2_e are sire and residual variances, respectively.

3.4 ASSOCIATION ANALYSIS

Once the ETA were obtained for all the traits under study, data sets were created by merging the information of each genetic polymorphism and the ETA by sire. These data sets were used to perform the two type of analysis to look for associations between the genetic markers and the ETA.

3.4.1 Across-population analyses

Collecting information in a GDD is not a requirement to test for candidate genes (*i.e.* genes that due to their physiological function are directly involved in the control of a quantitative trait; here the marker is the QTL). However, a data set collected in a GDD can be used to fit a model to test the hypothesis of association between a genetic marker (for a candidate gene) and a quantitative trait. This analysis allows one to test whether the marker is associated with the value a quantitative trait in the population. A statistically significant association would means that there is a QTL affecting the trait and it is in population-wide linkage disequilibrium with the marker.

MODEL FOR ACROSS POPULATION ANALYSES

The following model was fitted to test for marker effects across-population:

$$Y_{ij} = \mu + m_i + a_{ij} + e_{ij}$$

where:

Y_{ij} = ETA for bull i that inherited marker allele j (*i.e.*: ETA for clinical mastitis, culling due mastitis, occurrence of clinical mastitis, or SCS); μ = the population mean; m_i = fixed effect of marker genotype (*i.e.*: +/+, +/-, or -/-); a_{ij} = random additive effect of $bull_{ij}$; e_{ij} = residual effect.

The bull effects a_{ij} were assumed to be ~ N (0, $A\sigma^2_s$) where A is the numerator relationship matrix among bulls, and σ^2_s the sire variance for the trait under analysis. The residual effects are assumed to be ~ N (0, $I\sigma^2_e$) where I is an identity matrix, and σ^2_e is the residual variance of the trait under analysis.

Kennedy et al, (1992) pointed out that tests of associations between putative QTL (*i.e.* genetic markers) and quantitative traits might be spurious if the genetic structure of the population and the gene flow is not taken into account. As a result significant associations could be detected when in reality they are not present (Type I error). The relationship matrix among all animals takes into account the gene flow and the genetic structure of the population.

Thus, in this study the matrix (A) with all the relationships among sires was created and incorporated into the across population analysis. The steps to do so are described next: the file with the pedigree to perform the genetic evaluations, previously described, was read into the procedure INBREED of SAS© (Statistical Analysis System) in order to obtain the relationship matrix (A); the elements of the matrix A corresponding to the bulls with information on genetic polymorphisms were extracted from matrix A, and arranged in a file that later was read into the procedure MIXED of SAS©, where the analyses to test associations were run. The values of σ^2_s and σ^2_e were used in the analyses with the procedure MIXED of SAS©.

CONTRASTS AND TEST OF HYPOTHESIS

The null hypothesis of association between the marker allele and a QTL was tested with the model described in the previous section. The contrast of group of animals that inherited the marker-allele '+' versus the group of animals that inherited the marker-allele '-'

was obtained with k'=(1-0-1). A trait-wise significance was obtained through permutation tests to assess the statistical significance of the difference between both groups.

3.4.2 Within-family analysis

In outbred populations many of the marker-alleles will be in population-wide linkage equilibrium with the genes controlling the traits of interest even if the marker is linked to the genes controlling the trait (QTL), and since population-wide disequilibrium is required so as to detect QTL in an across-population analysis a QTL may go undetected. However, LD always may be found within families if the marker is linked to the QTL. Hence, a database under a Granddaughter design should be used to test markers for linkage to genes controlling the traits, nesting the markers within the grandsires. The model describing this situation must include the effects of grandsire, marker nested within grandsire, and effect of sire (son). By doing within-family analyses, one can make a further differentiation from the results obtained with the across population analysis. A significant result indicates the presence of a segregating QTL, physically linked to the marker allele (hence in LD with it) with an effect on the trait.

MODEL FOR WITHIN-FAMILY ANALYSES

The model to test for associations within families was:

$$Y_{ijk} = \mu + gs_i + m_{ij} + s_{ijk} + e_{ijk}$$

where:

Y_{ijk} = ETA of the $_k^{th}$ son that inherited the $_j^{th}$ marker allele from the $_i^{th}$ grandsire (*i.e.*: ETA for clinical mastitis, culling due mastitis, occurrence of clinical mastitis, or SCS).

μ = the population mean.

gs_i = fixed effect of $_i^{th}$ grandsire.

m_{ij} = fixed effect of $_j^{th}$ marker allele nested within $_i^{th}$ grandsire.

s_{ijk} = effect of $_k^{th}$ son with $_j^{th}$ marker inherited from $_i^{th}$ grandsire, $N \sim (0, I\sigma^2_s)$.

e_{ijk} = residual effect, $N \sim (0, I\sigma^2_e)$.

This model includes the random effect of the son nested within marker, and in turn marker nested within grandsire. In this way, due to the nature of the GDD it is possible to analyze the effect of the marker within families (*i.e.*: within grandsires). A significant result means the marker is linked to a QTL.

CONTRASTS AND TEST OF HYPOTHESIS

To test the hypothesis of association within families between the genetic markers and the quantitative trait, contrasts to test the alternative allele-markers inherited for the bulls were run within each grandsire family, provided that the GS was heterozygote for the genetic marker.

3.4.3 Permutation tests

In order to obtain appropriate threshold values to detect QTL effects permutation tests were performed. An algorithm aimed to perform permutation tests was implemented for both across-population and within-family analyses.

The data sets used for analysis across population contained three columns: sire identity, marker genotype of the sire, and ETA of the sire (for the trait under analysis). In this case the algorithm consisted in shuffling the data sets of the ETA, keeping the original correspondence between the sire and their marker genotype.

In the case of the analysis within families, the data sets contained: grandsire identity, marker genotype of the grandsire, son identity, and ETA of the sons (for the trait under analysis). In this case the algorithm consisted in shuffling the data sets of the ETA, keeping the original correspondence between grandsire, the marker genotype, and the sons.

The shuffling was programmed within a SAS© routine and consisted in reading the variables from the original data set (both across population and within family); then, randomly reassigning each ETA to a different sire (son); this random shuffling intends to break any possible relation between marker genotypes and the trait (in this case, ETA). After the shuffling the statistical analyses (using the models described in sections 3.4.1 and 3.4.2) were run (*i.e.* analysis of the shuffled data set). The appropriate *p-values* obtained in each

analysis in each cycle of permutation were stored in permanent files (one for *p-values* obtained in the permutation tests for across population analysis, and another one for *p-values* from the permutation tests with within family analysis) in order to select the threshold for each individual analysis (either across-population or within-family). Each file consisted of 1000 *p-values*. Churchill and Doerge (1994) recommended 1000 shuffles as the minimum to obtain threshold values at significance level of 5%; that was the number of shuffles done in this study for each data set.

Therefore, at a significance level of $\alpha = 0.05$ the shuffled test statistics were ordered and the N $(1-\alpha)$ percentile was located. N is the number of statistics tests obtained; thus from 1000 permutations, the 950^{th} value of the ordered shuffled *p-values* was selected as the threshold value for the corresponding analysis.

The threshold values (*p-values*) obtained through permutation tests were used to compare with the *p-values* that were obtained in the analysis using the original data set in order to decide on the significance of the effects under study.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 GENETIC POLYMORPHISMS

The genotypic and allelic frequencies for each marker considering all the information available in the DNA data bank are shown in Table 4.1. The number of bulls by marker varies from 505 (for GH1-300) to 2061 (for ODC2). GS are included in these numbers. The main reason why not all sires were genotyped for all the markers is that two criteria were required in order to type new bulls: 1) to have daughters in the file from which mastitis information was extracted; and 2) to have semen sample available. During the years of the formation of the DNA bank, for some bulls the stocks of frozen samples were running out, and new semen samples were not possible to find. The lower numbers of animals genotyped correspond to the new sires genotyped in the period 2001-2003 for a new set of markers, *i.e.* those for ACTH, IGF-1, Prolactin, CRH, and some new markers for GH (GH1-258, GH1-300, GH5-183, and GH5-255).

There is no information available elsewhere regarding the use of a bovine DNA data bank with markers on genes such as those studied here to map QTL affecting clinical mastitis. As will be shown later, only three studies (Klungland *et al*, 2001; Holmberg and Andersson-Eklund, 2004, and Schulman *et al*, 2004) report mapping of QTL affecting clinical mastitis and SCC or SCS. Anonymous markers were used in those studies. Other studies of association performed to look for QTL affecting mastitis resistance traits have used MHC genes (Dietz *et al*, 1997; Kelm *et al*, 1997; Sharif *et al*, 19998) and were discussed in the Literature Review (section 2.9).

Table 4. 1 Genotypic and allelic frequencies per marker (all information available)

Marker	Number of sires (genotypic frequencies)			Total sires	Allelic frequencies	
	+/+	+/-	-/-		+	-
GH1	160(0.13)	736 (0.59)	350 (0.28)	1246	0.43	0.57
GH1_258	73 (0.14)	269 (0.53)	170 (0.33)	512	0.41	0.59
GH1_300	157 (0.31)	252 (0.50)	96 (0.19)	505	0.56	0.44
GH4.1	859 (0.67)	395 (0.31)	34 (0.02)	1288	0.83	0.17
GH4.2	796 (0.74)	256 (0.24)	24 (0.02)	1076	0.86	0.14
GH5_183	13 (0.02)	206 (0.25)	593 (0.73)	812	0.15	0.85
GH5_255	190 (0.24)	418 (0.52)	196 (0.24)	804	0.50	0.50
GH6.1	1736 (0.88)	235 (0.12)	12 (0.006)	1983	0.94	0.06
GH6.2	991 (0.55)	675 (0.38)	124 (0.07)	1790	0.74	0.16
GHR1-Alu	693 (0.35)	1001 (0.50)	313 (0.15)	2007	0.60	0.40
GHR2.1-AccI	1013 (0.51)	841 (0.42)	148 (0.07)	2002	0.72	0.28
GHR2.2-StuI	1858 (0.93)	141 (0.07)	2 (0.001)	2001	0.97	0.03
ODC1	113 (0.06)	612 (0.31)	1258 (0.63)	1983	0.22	0.78
ODC2	1372 (0.67)	660 (0.32)	29 (0.01)	2061	0.83	0.17
ACTH1-388	543 (0.75)	169 (0.23)	14 (0.02)	726	0.87	0.13
ACTH2-341	168 (0.27)	310 (0.50)	138 (0.23)	616	0.52	0.48
CRH-291	375 (0.55)	273 (0.40)	38 (0.05)	686	0.75	0.25
PRL1-152	4 (0.005)	125 (0.16)	664 (0.84)	793	0.09	0.92
PRL2-361	543 (0.69)	224 (0.29)	18 (0.02)	785	0.84	0.16
IGF1-390	155 (0.21)	371 (0.49)	225 (0.30)	751	0.45	0.55

Code for genotypes (base substitutions are shown in Table 3.3):

GH1: + denotes deletion of TGC repeat; - denotes insertion of TGC repeat. **GH1_258**: + denotes C at position 258; - denotes T. ***GH1_300***: + denotes C at position 300; - denotes T. **GH4.1**: + denotes C at position 1547; - denotes T. **GH4.2**: + denotes T at position 1692; - denotes C. **GH5_183:** + denotes G at position 183; - denotes T. **GH5_255:** + denotes C at position 255; - denotes T. **GH6.1:** + denotes C at position 2141; - denotes G. **GH6.2:** + denotes A at position 2291; - denotes C. **GHR1-ALU:** + denotes A at position -1182; - denotes T. **GHR2.1-ACCI:** + denotes C at position -892; - denotes T. **GHR2.2-STU**: + denotes C at position -232; - denotes T. **ODC1**: + denotes A at position 2512; - denotes T. **ODC2**: + denotes G at position 5654; - denotes A at position 5654. **ACTH1-388**: + denotes A at position 643; - denotes C at position 643. **ACTH2-341**: + denotes A at position 573; - denotes G at position 573. **CRH-291**: + denotes A at position 500; - denotes G at position 500. **PRL1-152**: + denotes A at position 9017; - denotes G at position 9017. **PRL2-361**: + denotes C at position 9218; - denotes T at position 9218. **IGF1-390**: + denotes C at position 1407; - denotes T at position 1407.

A= adenine; C = cytosine; T = thymine; G = guanine.

From the information in Table 4.1 it is noticeable that some genotypes show low heterozygosity (H), such as GH6.1 (0.11-0.12), GHR2.2 StuI (0.07), and PRL 1-152 (0.13). The lack of H may be an indicator of selection favoring one of the alleles. Noticeably, some genotypes show high frequency, for instance the +/+ genotype for GH61 and GHR2.2 StuI (0.88 and 0.93, respectively) and the genotype -/- for PRL1-152 (0.84-0.86). Other genotypes show a higher degree of H such as GH1 (0.59), GH1258 (0.53), GH1-300 (0.50), GH5-255 (0.52), GHR1-Alu (0.50), ACTH2-341 (0.50), IGF-390 (0.49).

Several marker alleles show high frequency: GH4.1(+), GH4.2(+), GH5-183(-), GH6.1(+), GH6.2(+), GHR2.1-AccI(+), GHR2.2-StuI(+), ODC1(-), ODC2(+), ACTH1-388(+), CRH-291(+), PRL1-152(-), and PRL2-361(+).

Given that GH4.1(+) has been reported to have a positive effect on milk yield (Yao *et al*, 1996), selection on milk yield may explain its high frequency; although GH4.2(+) was not found to have a positive effect on yield traits by the authors, its high frequency may be the result of its close position to GH4.1(+), only 145 base pairs away. A similar situation may occur in the case of GH6.1(+) and GH6.2(+); in the same study, GH6.1(+) was associated with high milk yield, and its distance from GH6.2(+) is 150 base pairs.

Unfortunately, despite those apparently high numbers, in association studies carried out on outbred populations, the initial large number of genotyped animals available in a DNA data bank to be used in association analyses is greatly reduced, basically due to two factors: i) sometimes, it is not possible to obtain information regarding the phenotype (*i.e.*: quantitative trait) of each animal with polymorphic information, and ii) the need to exclusively use the informative animals to perform statistical analyses. In the case of across-population analyses the presence of both homozygous genotypes is required in order to perform the appropriate statistical contrasts. In the case of within-family analyses, the informative animals are heterozygous grandsires and their homozygous sons. Only in this way is it possible to perform contrasts between groups of sons that alternatively have inherited one of the alleles from the heterozygous grandsire. In the first case GHR2.2-StuI and PRL1-152 show extremely low frequency of -/- and +/+ animals, respectively. In the second case, GH61 did not show genetic diversity to provide heterozygous grandsires, hence analysis of association within families for that marker were not possible to perform.

Table 4.2 shows the number of bulls per marker that were used in the association analyses that are described in section 4.8.

Table 4. 2 Genotypic and allelic frequencies per marker (for the bulls with daughters with data on mastitis traits).

Marker	Number of sires (genotypic frequencies)			Total sires	Allelic frequencies	
	+/+	+/-	-/-		+	-
GH1	38 (0.12)	183 (0.59)	88 (0.28)	309	0.42	0.58
GH1258	61 (0.14)	227 (0.53)	143 (0.33)	431	0.41	0.59
GH1300	131 (0.31)	213 (0.50)	852 (0.19)	426	0.56	0.44
GH41	468 (0.74)	150 (0.24)	11 (0.02)	629	0.86	0.14
GH42	339 (0.76)	94 (0.21)	13 (0.03)	446	0.87	0.13
GH5183	8 (0.01)	149 (0.24)	466 (0.75)	623	0.13	0.87
GH5255	150 (0.24)	320 (0.52)	146 (0.24)	616	0.50	0.50
GH61	610 (0.86)	97 (0.14)	3 (0.0042)	710	0.93	0.07
GH62	451 (0.69)	183 (0.28)	21 (0.03)	655	0.83	0.17
GHRAL	239 (0.34)	338 (0.48)	124 (0.18)	701	0.58	0.42
GHRAC	346 (0.50)	298 (0.42)	53 (0.08)	697	0.71	0.29
GHRST	636 (0.91)	63 (0.08)	2 (0.003)	701	0.95	0.05
ODC1	25 (0.037)	253 (0.37)	403 (0.59)	681	0.22	0.78
ODC2	481 (0.67)	233 (0.32)	7 (0.01)	721	0.83	0.17
ACTH388	425 (0.76)	121 (0.22)	10 (0.02)	556	0.87	0.13
ACTH341	120 (0.26)	235 (0.50)	112 (0.24)	467	0.51	0.49
CRH291	291 (0.55)	204 (0.39)	30 (0.06)	525	0.75	0.25
PRL152	2 (0.003)	101 (0.17)	506 (0.83)	609	0.08	0.92
PRL361	425 (0.70)	171 (0.28)	11 (0.02)	607	0.84	0.16
IGF390	115 (0.20)	297 (0.51)	173 (0.30)	585	0.45	0.55

In comparing the number of bulls showed in Table 4.2 with the size of the original DNA data bank presented in Table 4.1, one can see that the number of bulls in the association analyses were reduced; in the case of some markers, almost 65% (of ODC2 with 2061 sires in the original DNA bank *versus* 721 sires with daughters in the file with information on mastitis and thus available to perform association analyses). This is one of the main limitations studies of this nature face. Several options may be available to solve this situation, although they may be time consuming and costly. However, the allelic frequencies were not modified by the reduction in the number of bulls.

4.2 DESCRIPTIVE ANALYSIS OF THE RESPONSE VARIABLES

The complementary source of information in mapping QTL is the phenotypic data on the animals to be used in the association analysis. Before using the phenotypic information directly, procedures aimed at the elimination of the nuisance environmental variation must be applied. A measure that is widely used in the research to map QTL is Estimated Transmitting Abilities (ETA).

This chapter describes the generation of ETA for several traits related to mastitis resistance. First, a description of the response variables generated for each lactation number is displayed in Table 4.3 and then a more deep insight into how mastitis is expressed through years is given in sections 4.2.1 through 4.2.4.

From the descriptive analysis in Table 4.3, it can be generalized that that the clinical mastitis incidence, the occurrence of clinical mastitis, the frequency of culling due to mastitis, SCC, and SCS increased by parity number. These results are supported by evidence showing that parity is a risk factor, not only for increased clinical mastitis but also for subclinical mastitis (Erskine, 2001). Erskine (2001) has speculated that heifers may have a better function of PMN than older cows hence heifers may express a better immune response.

Table 4. 3 Descriptive statistics (derived from test-day data) for frequency of clinical mastitis, occurrence of clinical mastitis, culling due to mastitis, somatic cell counts (SCC), and somatic cell scores (SCS)

Incidence of clinical mastitis by lactation

Data set	No. of cows	No. of affected cows	Mastitis frequency (%)
First lactation	411,291	8,886	2.16
Second lactation	238,432	8,994	3.77
Third lactation	130,983	6,578	5.02
Over all lactations (1^{st} to 3^{rd})	126,690	9,751	7.70

Occurrence of clinical mastitis by lactation

Data set	No. of cows	No. of cases	Occurrence (%)
First lactation	411,291	9,136	2.22
Second lactation	238,432	9,328	3.91
Third lactation	130,983	6,853	5.23
Over all lactations (1^{st} to 3^{rd})	126,690	10,481	8.27

Frequency of culling due to mastitis by lactation

Data set	No. of cows	No. of culled cows	Culling due to mastitis (%)
First lactation	411,291	3,505	0.85
Second lactation	238,432	3,879	1.63
Third lactation	130,983	3,177	2.43

Mean of SCC and SCS by lactation

Data set	No. of cows	SCC (*1000) (mean, SD)	SCS (mean, SD)
First lactation	411,291	161.04 (328.7)	2.74 (1.48)
Second lactation	238,432	219 (334.44)	3.27 (1.52)
Third lactation	130,983	285.56 (407.23)	3.67 (1.54)

With regard to incidence of clinical mastitis, as mentioned in the section 3.3.2 (Material and Methods), it was defined as a binary trait depending on the presence or absence of at least one test-day report of clinical mastitis during the lactation. Each lactation record had six fields where CAR could be stored. If a cow had at least one CAR indicating presence of clinical mastitis (CAR = 16) then the cow received a code of 1; if a cow had not CAR = 16 in any of the fields for CAR, then the cow received a code of 0. The mastitis incidence indicated in table 4.5 was calculated as the percentage of cows with clinical mastitis coded as 1 (this is, all cows with at least one report of clinical mastitis) considering all the cows by each parity number. The frequency of clinical mastitis in this study (2.16% to 5.02%) is low compared to values reported in other studies. Heringstad *et al* (1999) reported that the frequency of clinical mastitis was 21% in Norwegian cattle; Nash *et al* (2000) found 25% of clinical mastitis in first and second lactation Holstein cows in a study performed on eight farms in US. Other studies have reported incidence ranging from 1.7% to 54.6%, with an average of 14.2% (Kelton *et al*, 1998). In Canada, Uribe *et al* (1995) reported incidence rates of 12.5% and 16.7 % for first lactation and all lactations (no indication of the number of lactations used was provided), respectively, using information from a project carried out in Ontario, involving 7416 cows in 98 herds.

In the PATLQ, the frequency of recording of cases of CM was once per month, which may lead to an underestimation of the incidence of clinical mastitis.

In another study in Canada, Van Dorp *et al* (1996) found an incidence of mastitis of 3.9% in first lactation cows belonging to 20 herds (3176 cows) in British Columbia. Those herds were participating in an on-farm program for recording of data and management of information. In this case, the incidence reported in the present study is similar to the report by Van Dorp et al (1996); the reason may be that the recording of cases of clinical mastitis could be similar to the system applied in Québec from where the information of mastitis used in the present study was obtained (*i.e.* reports of clinical mastitis cases in on-farm basis by producers on the test-day). The frequency found in this study may be considered normal in milk recording programs that record presence of mastitis cases on a voluntary basis. Differences between systems of recording health information make almost impossible to compare incidence across studies. The incidence of mastitis reported by Heringstad *et al* (1999) is based on mandatory reports of each case of clinical mastitis receiving treatment. In the Nordic countries, veterinarians are the only personnel allowed to apply the treatments

against mastitis. In that recording system, because cases are closely followed is expected higher accuracy in the report of the disease.

The 25% of frequency of clinical mastitis reported by Nash *et al* (2000) is based on a study carried out on eight herds (1860 cows) in the US; in that study research technicians made weekly visits to the herds to ensure the correct application of the protocol of the study that included the identification and classification of clinical mastitis cases.

With respect to the occurrence of clinical mastitis by lactation, the percentage of occurrence reported in the second part of Table 4.3 was obtained by adding up all the reports of clinical mastitis during the lactation and taking each of them as an independent case of clinical mastitis (*i.e.* each CAR = 16 in any of the six fields available per record).

Occurrence of clinical mastitis, defined as the number of cases of mastitis during the lactation is a trait that has not been thoroughly analyzed in other studies. This can be explained by the difficulty in identifying independent clinical cases that occur with few days of difference between each other. For instance, Nash *et al* (2000) decided to classify consecutive clinical mastitis cases as independent each other, whether they had occurred within 30 days of each other. In the present study 8,886 cows had 9,136 cases of clinical mastitis in first lactation; this represents an average of 1.03 cases per cow; second lactation cows had 1.04 cases of mastitis (9,328 cases/8,994 cows), and 3rd lactation cows showed 1.04 cases (6,853 cases/6,578 cows) These averages are lower than the 1.8 cases per 1st lactation cows (864 cases/479 cows) and the 1.7 cases for 2nd lactation cows reported by Nash *et al* (2000).

However, weekly follow up by trained personnel, such as in the case of the study by Nash *et al* (2001) of clinical cases of mastitis within milk recording schemes is more the exception than the rule, thus difficult to replicate. In any case, the incidence and occurrence of clinical mastitis seem to agree with the fact that cows in 2nd and 3rd parity are more often affected by mastitis than first lactation cows.

The frequency of culling due to mastitis was 0.85%, 1.63%, and 2.43% for first, second, and third lactation, respectively. In this study the culling due to mastitis was defined as a binary trait depending on whether the cow was culled (1) or not (0); also this definition was made for culling due to mastitis in each parity. With regard to culling due to mastitis, in previous studies performed in Holsteins in Québec it was concluded that the rates of the main reasons of involuntary culling, such as mastitis and high somatic cell counts, increased with

the parity number (Dürr *et al*, 1997; 1997b). Table 4.3 shows results that suggest the same trend, which is expected given that this study used information that was also included in the studies by Dürr *et al*. Those authors reported that 35,595 cows (from first lactation to 14th parity) out of 1,558,080 were culled due to mastitis, representing a proportion of culling due to mastitis of 2.3%. Information is not available elsewhere regarding the rate of culling due to mastitis in the Holstein population of Quebec.

A similar effect of parity was observed on the expression of SCC, or SCS; in this study cows in third lactation showed higher levels of SCC and SCS, as expected. Values for SCS from this study are similar to those estimated by Nash *et al* (2000) of 2.73, and 2.85 in 810 first-lactation cows, and 348 second-lactation cows, respectively. The values reported by Van Dorp *et al* (1996) of 1.63 SCS for first lactation cows (27 herds, 2503 cows) in Canada are slightly lower than the result found in this study, although it is necessary to emphasize that that study was based on 27 herds and 2503 cows. In US, Castillo-Juarez *et al* (2002) calculated an average of 2.73 SCS, with a standard deviation of 1.63, for 248,230 first-lactation cows which is similar to the values for the 1st lactation cows of the present study. In Norway, Norwegian first-lactation cows (77,110 cows) were reported to have an average SCS of 4.18 with a standard deviation of 1.17 (Ødergard *et al*, 2002). Pösö and Mäantysaari (1996) reported Finnish Ayrshire cattle with means of SCS of 4.42, 4.28, and 4.27, for first, second and third lactation; the standard deviations reported were around 0.88, 0.90, and 0.91, respectively, slightly lower than the variation found in the present study.

The coherence of the descriptive statistics of this study with respect to other values reported by other researchers suggests that no significant bias was introduced by the chosen editing criteria. The following section continues with the descriptive analysis of the data used in this study.

4.2.1 Incidence of clinical mastitis

The incidence of clinical mastitis by year of calving is shown in Figure 4.1. The incidence of clinical mastitis was estimated as the number of cows with at least one case of clinical mastitis during the lactation over the total number of cows by year of calving. Figure 4.1 shows the incidence of clinical mastitis for 1st, 2nd, and 3rd lactation cows.

It is convenient to point out that in this study the term incidence is not taken with the connotation that currently is used within veterinary epidemiology. In epidemiology of mastitis *incidence* is the number of new cases of intramammary infections or clinical mastitis in the population at risk during a given period. In the same context, *prevalence* of mastitis is the number of cows (or quarters) that are diagnosed as infected divided by the total number of cows (or quarters) currently at risk (Erskine, 2001).

The definition of clinical mastitis incidence of this study agrees with the Lactational Incidence Risk (cumulative incidence) proposed by Kelton *et al* (1998) defined as the number of lactations with one or more cases of clinical mastitis divided by the number of lactations.

Figure 4.1 shows that the incidence of clinical mastitis is higher as animals grow older. Normally, it is expected that older cows have a higher frequency of mastitis; more time of exposure to the risks factors may result in more cases of mastitis. This could explain why 1st lactation cows have lower incidence of mastitis, as shown in Table 4.1.

Also, an increasing trend in the incidence of mastitis is observed through years in 1st, 2nd, and 3rd lactations. This trend may be explained by the increased milk yield potential that has taken place in the Holstein cows as a result of selection. The negative (unfavorable) genetic correlation between milk yield and mastitis may generate cows with more susceptibility to mastitis.

It has been suggested that if emphasis of selection for production is privileged over selection for mastitis, it might be expected to increase the incidence of CM as a result of the genetic correlation between more milk yield and clinical mastitis incidence (Stradberg and Shook, 1989). However, studies on immune function traits of cows selected for high and average milk production have yielded conflicting results with respect to whether indeed high yield negatively affects immune response (Detilleux *et al*, 1995).

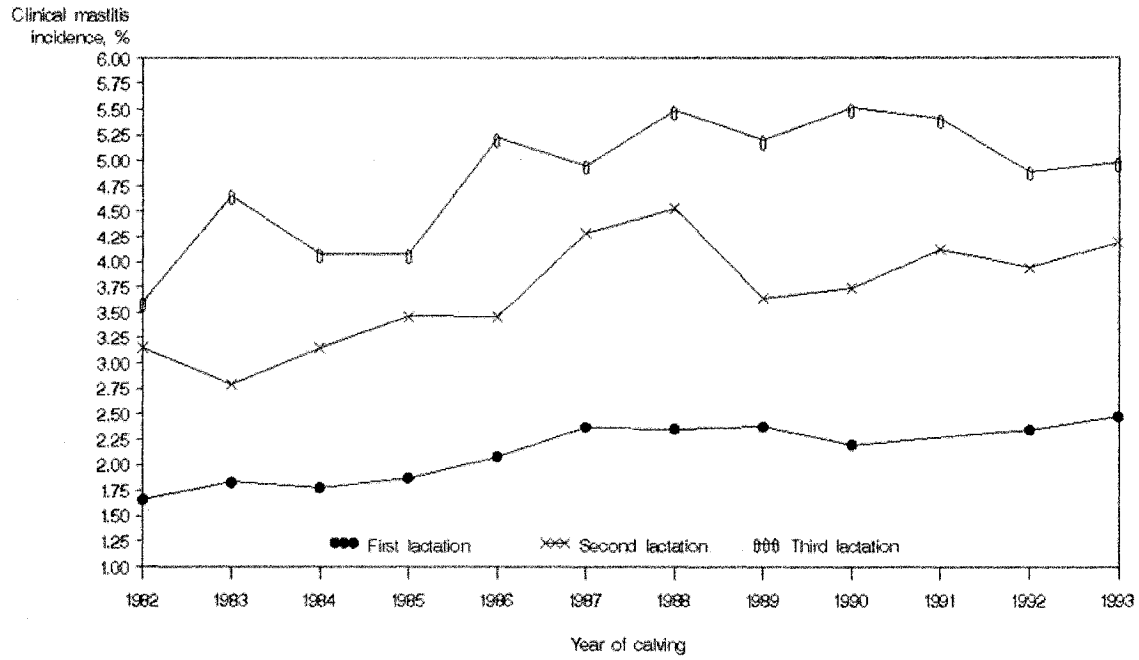


Figure 4. 1 Incidence of clinical mastitis by year of calving of the cows (number of cows with mastitis over total number of cows by year of calving) in first, second and third lactations

Another perspective has been used to explain the increased incidence of mastitis. For instance, Heringstad *et al* (1999) have reported an increased trend in mastitis incidence in first lactation cows (Norwegian cattle) from 1978 onwards. In 1978 the incidence was 13%, and in 1994 it was almost 30%. These authors ascribed that increase to a strengthened system of quality to pay for milk that made producers put more attention to improving the recording of clinical mastitis. The Norwegian health recording system was introduced in 1975 (Heringstad *et al*, 1999).

The higher incidence of mastitis shown in Table 4.1, as well as the trend in mastitis incidence in Figure 4.1 are consistent with an increase of incidence of mastitis as a result of selection on milk yield. The reasons will be explained in section 4.2.4 which describes the changes on SCS observed during the same period. On the other hand, no information is available to explain that, in Québec, between 1982 and 1993, there was an increasing interest to improve mastitis recording and that as a result more cases of clinical mastitis were

recorded; however, next section shows that, in the population under study, indeed there was an increase in the number of cases reported of clinical mastitis.

4.2.2 Occurrence (total number of cases) of clinical mastitis

The occurrence of clinical mastitis by year of calving is shown in the figure 4.2. Occurrence of mastitis was defined as the total number of reports of clinical mastitis during the lactation.

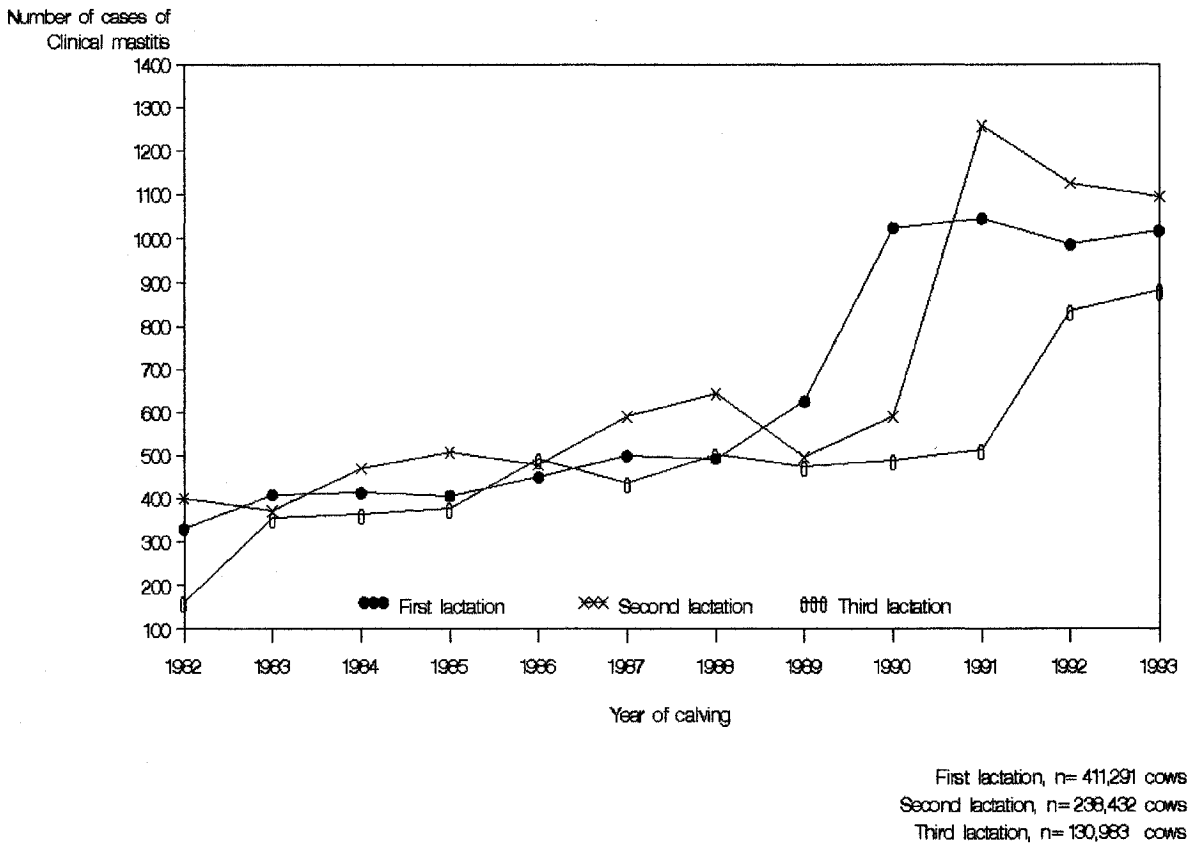


Figure 4. 2 Occurrence of clinical mastitis by year of calving of the cows in first, second and third lactations. The total number of cases clinical mastitis is plotted in this figure.

The occurrence of clinical mastitis shows an increasing trend through the years. Third lactations cows show less occurrence of clinical mastitis than first and second lactation cows.

Figure 4.2 shows the increasing of number of cases of CM reported every year since 1982 to 1993; the increase may be a result of the interest of producers to report cases of clinical mastitis. There was not evidence published elsewhere regarding total number of cases of mastitis through years to compare the results of the present study.

4.2.3 Culling due to mastitis

Figure 4.3 displays percentage of cows culled due to mastitis by year of calving. As was shown in Table 4.3 the proportion of cows culled due to mastitis in third lactation is higher than in first and second lactation cows (2.46% versus 0.85% and 1.65%, respectively). This is in agreement with findings by Dürr *et al* (1997, 1997b). These authors analyzed the rates of culling in Québec Holstein cows using a logistic regression model. They found that culling rate for some major involuntary reasons increased with parity number; in the case of mastitis, rates increased after first parity.

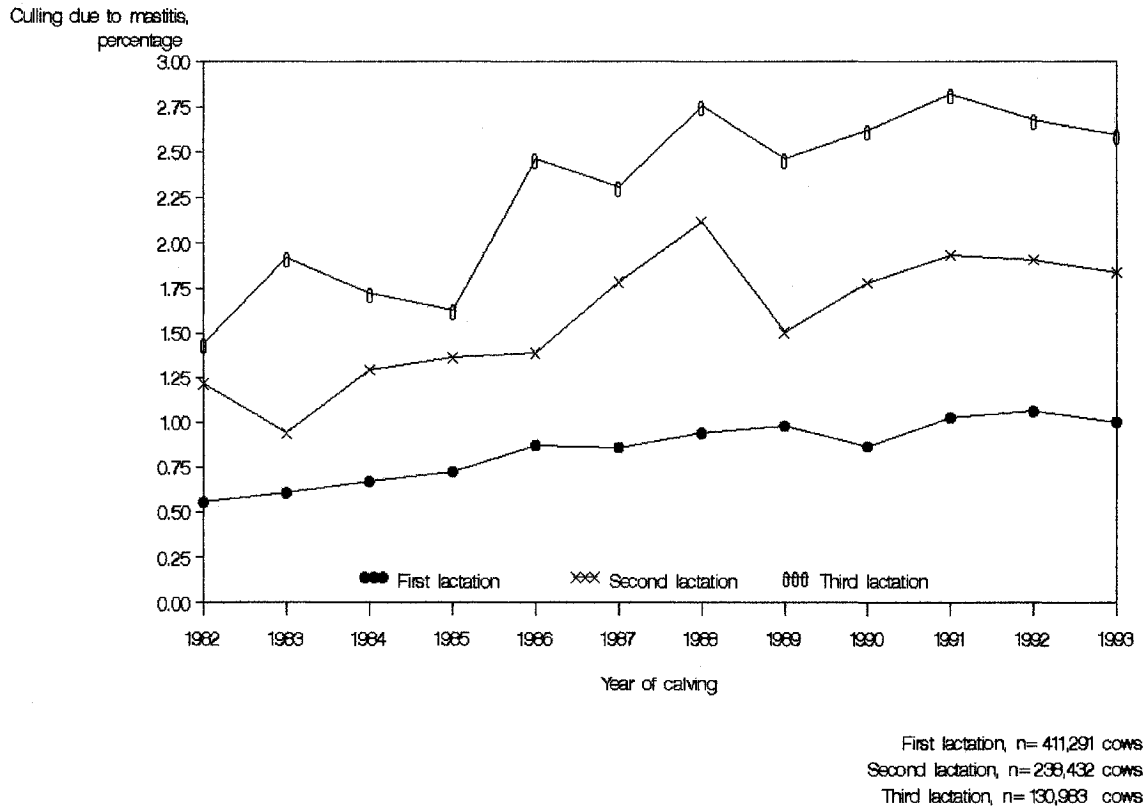


Figure 4. 3 Rate of culling due to mastitis by year of calving in first, second and third lactation

Some of the results displayed in Table 4.3 may help to explain this trend. Higher incidence of mastitis and more cases of mastitis are found in third parity, hence a cow with more parities, and thus with more chances of being affected by mastitis is more likely to be culled than a heifer.

Also, the higher rate of culling due to mastitis in 3rd lactation may be explained by the interest of producers in using selection against clinical mastitis by culling those cows that express more frequently this pathology.

From the results of this study, it can be observed that not only more cows were culled at third parity; additionally, an increasing trend in culling due to mastitis is observed from 1982 to 1993 as shown in Figure 4.3. This is another of the conclusions arrived at by Durr *et al* (1997).

If the increasing rate of culling through the years reflects the interest of producers for diminishing the impact of mastitis in their herds, then the increase in the number of reported cases of clinical mastitis may be consequence of the need for information to base those culling decisions.

However, when involuntary culling increases, such as culling due to mastitis, producers are left with few opportunities to eliminate animals on the base of their performance (*i.e.* production levels of milk, fat, or protein), in other words, there will be less voluntary culling. A review by Erskine (2001) shows that culling due to mastitis represented from 8 to 22% of the total culls; in order to put those values in perspective, voluntary culling represented 27 to 49% of the total culls.

In the case of Québec, recent information from the milk recording program (PATLQ, 1999, 2000, 2001, 2002, and 2003) indicates that culling due to mastitis is the 2nd major cause of culling, only after reproductive problems. In 1999 and 2000, mastitis represented around 15% of all the declared reasons of culling; from 2001 to 2003 mastitis represented a steady 13% of the culling.

The previous evidence encourages the realization of efforts to obtain new tools that aim to reduce the susceptibility to mastitis and thus reduce this important source of involuntary culling.

4.2.4 Average of SCS by year of calving

Figure 4.4 plots the average of SCS by year of calving. Consistently, 1st lactation cows show lower level of SCS than 2nd and 3rd lactation cows. Also, there was a decline in SCS between 1983 and 1990, however an increase is observed between 1991 and 1993. A decline in the average of SCS has been reported in other countries as result of the application of several prophylactic and therapeutic measures.

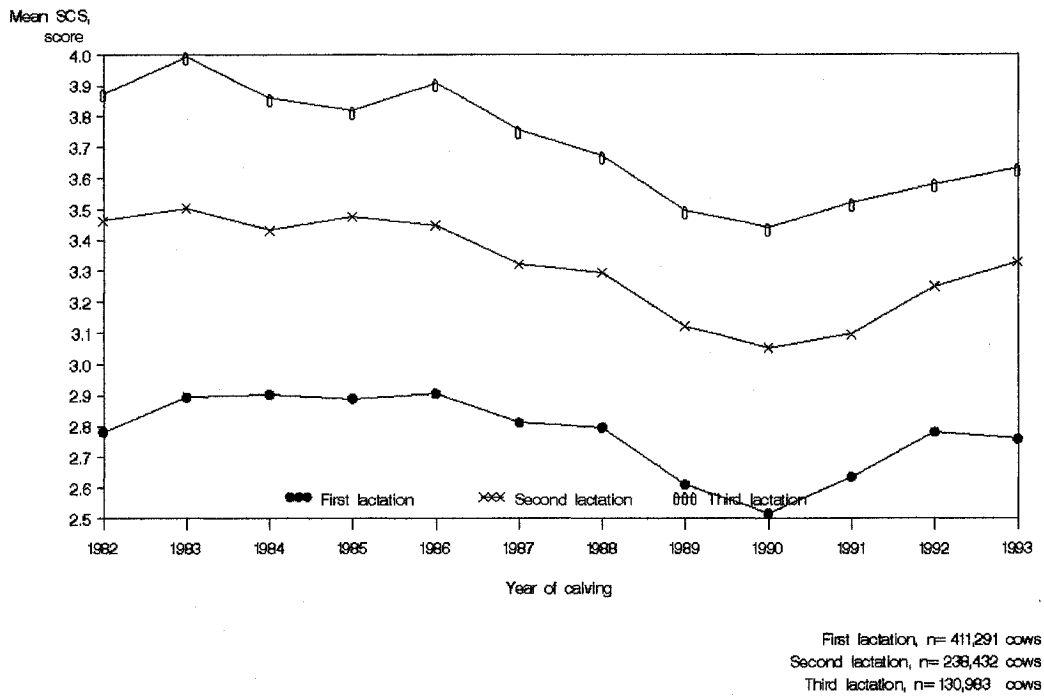


Figure 4. 4 Mean of somatic cell score (SCS) by year of calving in first, second and third lactation

A possible explanation for the trend of SCS shown in Figure 4.4 may be attempted by using the results discussed so far. Although there is not direct evidence that show the effect of culling due to mastitis on SCS, a possibility is that the increasing culling due to mastitis (as observed in Figure 4.3) may have eliminated cows with higher level of SCS, thus affecting the average of SCS in the subsequent years. It has to be noticed that culling due to mastitis (Figure 4.3) shows a peak from 1986. In Figure 4.4 it can be appreciated that from 1987 onwards there is a reduction of SCS.

Another factor in play in Canada is the selection for conformation traits that has been placed as one of the breeding objectives in the Canadian dairy industry. Monardes *et al* (1990) estimated heritabilities and genetic correlations for SCC and conformation traits. Some udder conformation traits had negative genetic correlations with SCC: fore udder (-0.22), rear udder (-0.16), udder texture (-0.17). Therefore, better udder conformation results in less SCC. However, in a more recent study (Van Dorp *et al*, 1996) the genetic relationship between udder conformation traits and SCC was less conclusive. Further studies are required to decipher the genetic relationship between mastitis and conformation.

In that sense, evidence regarding attempts to map QTL in dairy cattle may give additional hints to explore the genetic basis between mastitis and conformation; for instance, a QTL affecting rear udder height has been mapped on BTA 11 (Ashwell *et al*, 1998) 1.3 and 17 cM apart from two QTL that affect clinical mastitis (Holmberg and Andersson-Eklund *et al*, 2004).

In Canada, the average annual genetic trend for SCS was 8 times higher during 1994-1999 (0.004 EBV SCS) than during 1989-1999 (0.0005 EBV SCS) (*Canadian Dairy Network, 2003. http://cdn.ca/Articles/0305/trends/genetictrends.html; accessed August 2, 2003*). These trends, as well as the increased susceptibility to mastitis discussed in the previous sections encourage analyze genetic factors that may have a role in improving mastitis resistance.

4.3 GENERATION INTERVAL

Generation intervals (GI) are a key player in genetic improvement. There are four paths of selection in dairy cattle: Sires to breed cows, sires to breed bulls, dams to breed cows, and dams to breed bulls. Along the years selection strategies change and as a consequence the generation interval for each path also changes. Animals with no ancestors in a pedigree may be mistakenly taken as belonging to the same genetic group, when in reality they may belong to different generations, where the selection intensity may has been different. That is why it has been customary in the genetic evaluation process to assign phantom parents to these animals and in that way alleviate the lack of pedigree information. In order to correctly assign phantom parents to animals the average generation interval for each path of selection is essential. Table 4.4 shows the average generation interval by each path of selection calculated for the present study.

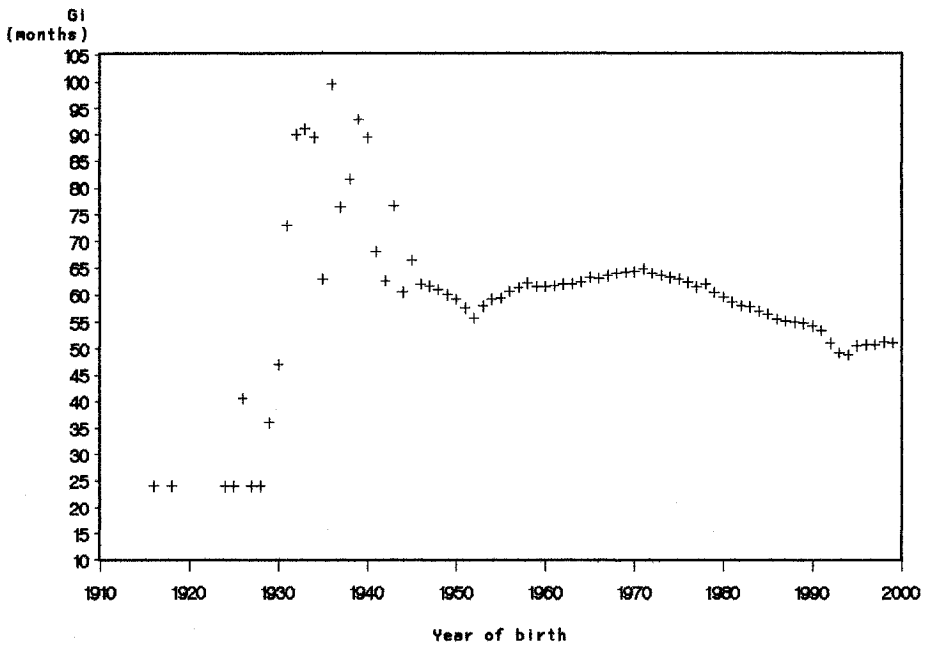
Table 4. 4 Generation interval for each selection path in Holstein cattle in Canada

	Dam to breed cows	Dam to breed bulls	Sires to breed cows	Sires to breed bulls
Pairs of animals	4,375,495	11,528	5,626,251	118,103
Months (years)	56 (4.7)	75 (6.3)	82 (7)	102 (8.5)
SD, Months (years)	28 (2.3)	35 (2.9)	40 (3.3)	43 (3.6)

The averages of generation interval shown in Table 4.6 were obtained with information from animals born between 1940 and 1994. Few references are available with regard to generation intervals in dairy cattle. Westell and Van Vleck (1987), and Miglior and Van Doormal (2002) have presented studies with reports of averages for the different paths of selection.

The paths dam to breed cows, sires to breed cows, and sires to breed bulls from the present study are similar to those reported by Westell and Van Vleck (1987): 4.86, 2.88, 8.47, and 9.73 years for dam to breed cows, sire to breed cows, and sire to breed bulls path of selection, respectively. However, the path Dams to breed bulls found in the present study (6.3 years) is longer than the 2.9 years reported by Westell and Van Vleck (1987).

The path sire to breed bulls of the present study lies within the range reported by Miglior and Van Doormal (2002). These authors analyzed the trend in generation interval of the path sires-to-breed-bulls, according different world geographic zones. They found that across world geographic zones the trend of generation interval for this path of selection has been drastically reduced from 1984 to 1997 (10 years *versus* 6.5 years, respectively). The explanation for the decreasing generation interval seems connected to the change in selection strategies to obtain new proven sires. Over time more emphasis has been placed on using new proven sires to breed bulls. Nowadays, an increasing number of new proven sires are tested with the first crop of daughters; in the past the sons of the sires were born when most of the sires had a second crop of daughters. In other words, sires are being proven faster. Miglior and Van Doormal (1987) did not study the other paths of selection. Figure 4.5 shows trends in generation intervals for the paths dams to produce cows, and dams to produce bulls. Figure 4.6 shows the paths sires to produce cows and sires to produce bulls.



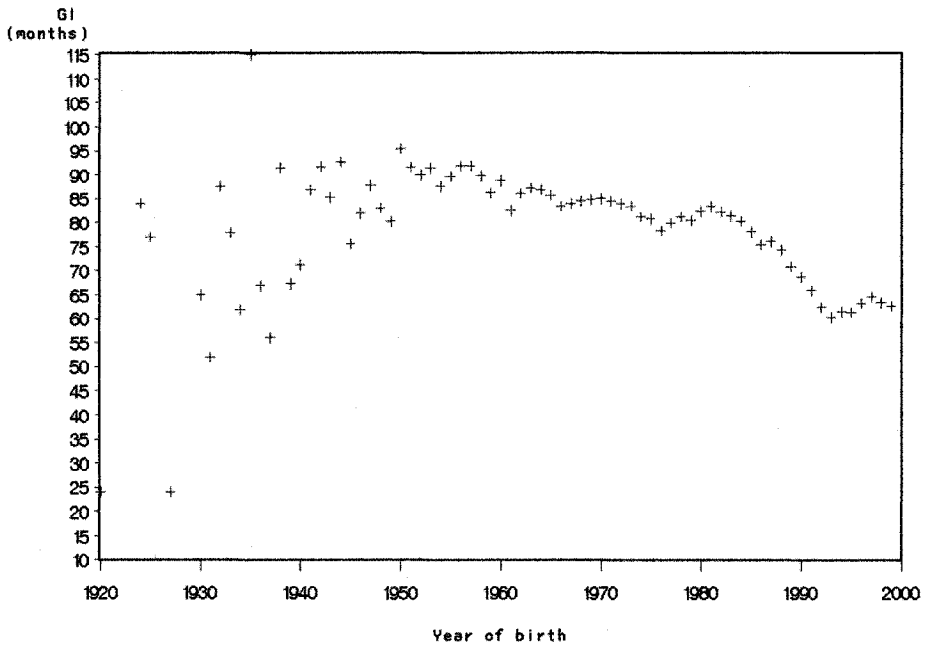
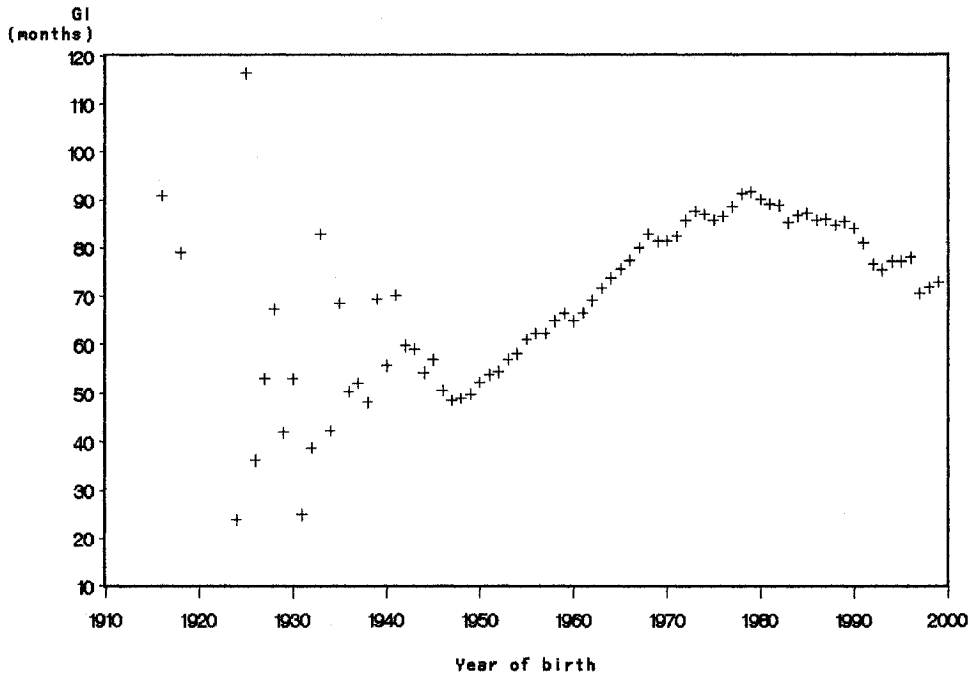


Figure 4. 5 Generation interval (GI) mean by each path of selection: dams to produce cows (dam to cows), dams to produce bulls (dam to bulls). The information spans from 1916 to 2000.



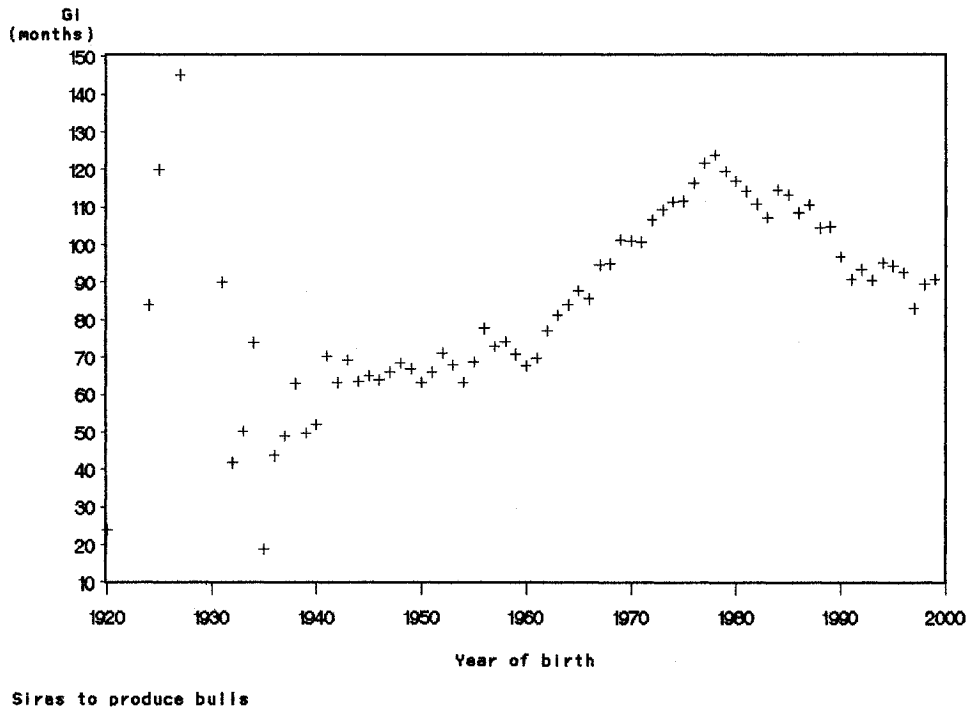


Figure 4. 6 Generation interval (GI) mean by each path of selection: sires to produce cows (sires to cows), sires to produce bulls (sires to bulls). The information spans from 1916 to 2000.

The trend observed in the present study for the path sires to breed bulls, shows a similar trend from 1989 to 1999 to the one reported by Miglior and Van Doormal (2002) for Canadian sires. They reported an average generation interval of 112 months for sires born in 1984, 100 months for sires born in 1989, 94 months for sires born in 1994, and 75 months for sires born in 1997. Additionally an historical trend (before 1984) not studied by those authors is given here. The generation intervals plotted corresponding to 1920 to 1940 show an erratic behavior resulting from the low number of pair of animals available to estimate the average generation interval for that period. From 1950 to 1960 the generation interval oscillated between 63 to 70 months (Table 7.2, appendix 1), followed by an increasing trend from 1960 to 1978 when generation interval ranged from 68 to 124 months. From 1980 to 1999 the trend is decreasing (from 117 months to 91) and matches the one described by Miglior and Van Doormal (2002). The trend observed for the generation interval of sires to breed cows is quite similar to the trend of sires to breed bulls. The main difference may be that the increase in generation interval is clearer from 1950 in the case of sires to breed cows; in the case of sires to breed bulls the GI increases from 1960 onwards.

The main differences between the GI of dams to breed cows or bulls with respect the path sires to produce cows or bulls is that the trend of the GI of Dams to bred cows is flat from 1945 (67 months) to 1975 (63 months), then it drops to 51 month in 1999. In the case of the path dams to bred bulls, it goes from 96 months in 1950 to 89, 85, 82, 69, and 62 months in 1960, 1970, 1980, 1990, and 1999, respectively.

The generation interval for dams to breed sires and dams to breed cows show a declining trend almost since 1950. This may be the result of improvements in reproductive efficiency. In contrast, the trends of sires to breed cows and sires to breed bulls appear to be the result of the strategies to select and prove sires; as a result these practices appear to have delayed the testing of bulls until late seventies, and being changed to accelerate the testing of bulls from late seventies to the present.

4.4 VARIANCE COMPONENTS

The objective of this part of the study was to estimate variance components and to generate Estimated Transmitting Abilities (ETA) to be included in analyses of associations of genetic markers with mastitis traits. Table 4.5 shows variance components and heritabilities for the four traits under study by parity. Heritabilities for binary traits (clinical mastitis incidence and culling due to mastitis) are shown without transformation to the normal scale.

Table 4. 5 Variance components for clinical mastitis incidence, occurrence of clinical mastitis, culling due to mastitis, and somatic cell scores by lactation number

Lactation	Trait	V_s	V_p	h^2	SE
1		1.37984	209.88856	0.007	0.000
2	Clinical mastitis	2.81556	355.41076	0.008	0.000
3	incidence	4.24512	463.33829	0.009	0.000
Over all lactations (1^{st} to 3^{rd})		12.65472	686.44277	0.018	0.000
1		0.00016	0.02287	0.007	0.000
2	Occurrence of	0.00032	0.03983	0.008	0.000
3	clinical mastitis	0.00048	0.05282	0.009	0.000
Over all lactations (1^{st} to 3^{rd})		0.00168	0.08535	0.020	0.000
1		0.3138	84.22473	0.004	0.000
2	Culling due to	0.93872	159.09438	0.006	0.000
3	mastitis	1.78216	232.78175	0.008	0.000
1		0.17012	1.89154	0.09	0.002
2	SCS	0.2072	1.87663	0.11	0.002
3		0.26976	1.93094	0.14	0.003

In general, the estimates of h^2 increased with the parity number as expected; in addition, those h^2 of traits directly related to resistance to clinical mastitis, such as incidence

of clinical mastitis, occurrence of clinical mastitis, culling due to mastitis, are quite low. Few studies have been carried out to obtain genetic parameters for mastitis resistance traits.

The h^2 for clinical mastitis incidence from 0.007 to 0.009 from this study is lower than the report by Van Dorp *et al* (1996) of 0.04 obtained with an animal model that included fixed effects of herd, year-season, and age. Nash *et al* (2000) reported estimates of h^2 for clinical mastitis incidence according to causal microorganism in first and second lactations. The estimates were within the range of 0.03 (coagulase-negative staphylococci) to 0.25 (Streptococci other than *Streptococcus agalactiae*) in first lactations, and from 0.01 (all organisms) to 0.19 (coliform species and streptococci other than *Streptococcus agalactiae*). The model used by Nash *et al* (2000) included the effect of the sire, age at first calving, and lactation length.

The estimates of h^2 for occurrence of clinical mastitis are lower than other estimates reported, for instance Nash *et al* (2000) estimated h^2 for total number of clinical episodes considering all the causal microorganisms in first and second lactations as 0.42, 0.15, respectively. These were the only estimates of number of cases of clinical mastitis found published elsewhere.

The estimates of h^2 for culling due to mastitis have been found to be lower than the 0.011 reported by Heringstad *et al* (2003) with an animal model with effects of age at calving, month of calving, and herd-year of calving. In that study the authors found a genetic correlation between culling due to mastitis and clinical mastitis of 0.48.

The h^2 of SCS from this study are in agreement with the average of 0.11 ± 0.04 cited by Ødergard *et al* (2003) obtained with a sire model including the effects of age at calving, month of first calving, and herd-year of calving. Other estimates are: 0.15 by Pösö and Mantysaari (1996) who used a sire model with age at calving, year-season of calving, and herd-year of first calving. Other similar results are the h^2 of 0.12 to 0.14 from Sander-Nielsen *et al* (1997); 0.09 to 0.12 (Mrode *et al*, 2001), and 0.09-0.11 (Castillo-Juarez *et al*, 2000). Canadian estimates of h^2 for SCS in lactations 1 to 3 were 0.09, 0.09, and 0.11, respectively (Reents *et al*, 1995). These estimates are also similar to those reported by Monardes *et al* (1990). Similar h^2 (0.123) were found by Da *et al* (1992) in the US.

4.5 ESTIMATED TRANSMITTING ABILITIES FOR MASTITIS RESISTANCE TRAIT AND SCS

In this study ETA for mastitis resistance traits and SCS were obtained. Table 4.7 shows the means for ETA and Standard Error of Prediction (SEP) for the traits under study by parity.

Table 4. 6 Means for Estimated Transmitting Abilities (ETA) and standard error of prediction (SEP) for clinical mastitis incidence, occurrence of clinical mastitis, culling due to mastitis, and SCS by lactation

Clinical mastitis incidence	Mean ETA	Mean SEP
First lactation	0.09576	0.693
Second lactation	-0.098	0.832
Third lactation	2.131	1.128
Over all lactations (1^{st} to 3^{rd})	-1.687	1.805

Occurrence of clinical mastitis	Mean ETA	Mean SEP
First lactation	0.00191	0.010
Second lactation	-0.0009	0.010
Third lactation	0.021	0.011
Over all lactations (1^{st} to 3^{rd})	-0.0186	0.0208

Culling due to mastitis	Mean ETA	Mean SEP
First lactation	0.01967	0.358
Second lactation	-0.047	0.498
Third lactation	0.579	0.753

SCS	Mean ETA	Mean SEP
First lactation	0.3347	0.182
Second lactation	0.046	0.168
Third lactation	-0.152	0.216

Because recording clinical mastitis episodes is more difficult than recording SCC, this latter trait has been used as selection criterion to reduce mastitis incidence. However, evidence shows that SCC is not the best measure to evaluate the capacity of the animals to engage an effective immune response against mastitis. Genetic correlation between CM and SCS has been estimated within the range of 0.60 to 0.72.

With the ETA obtained in this study Pearson correlation estimates were calculated between ETA of clinical mastitis resistance traits (incidence of clinical mastitis, occurrence of clinical mastitis and culling due to mastitis) and ETA of SCS. Table 4.6 shows correlations from 0.20 to 0.42 between mastitis resistance traits and SCS. On the other hand, as expected, correlations between mastitis incidence, occurrence of clinical mastitis or culling due to mastitis were higher showing values above 0.70.

Table 4. 7 Pearson correlation estimates between ETA of sires (n=1913) for SCS, clinical mastitis incidence, culling due to mastitis, and occurrence of clinical mastitis by lactation

First lactation	Clinical mastitis incidence	Culling due to mastitis	Occurrence of clinical mastitis
SCS	0.38	0.36	0.36
Clinical mastitis incidence		0.83	0.99
Culling due to mastitis			0.82

Second lactation	Clinical mastitis incidence	Culling due to mastitis	Occurrence of clinical mastitis
SCS	0.41	0.36	0.42
Clinical mastitis incidence		0.73	0.97
Culling due to mastitis			0.68

Third lactation	Clinical mastitis incidence	Culling due to mastitis	Occurrence of clinical mastitis
SCS	0.20	0.43	0.27
Clinical mastitis incidence		0.70	0.96
Culling due to mastitis			0.77

Additionally, linear regression coefficients were estimated using the ETA for SCS and clinical mastitis incidence, occurrence of clinical mastitis, and culling due to mastitis for 1st, 2nd, 3rd lactations.

Relationships between the ETA for mastitis resistance traits (clinical mastitis, culling due to mastitis, occurrence of clinical mastitis) in first, second and third lactation are illustrated in Figure 4.6, 4.7, and 4.7. The regression analyses considered 1913 sires which obtained genetic evaluations from the analysis of first lactations, 1638 sires which obtained genetic evaluations from the analysis for second lactations, and 1320 sires which obtained genetic evaluations from the analysis of third lactations, respectively.

In general it can be seen that as SCS increases incidence of clinical mastitis increases, there are more cases of clinical mastitis, and more culling due to mastitis occurs. This is in agreement with results obtained by Philipsson *et al* (1995). They analyzed the relationships between breeding values for clinical mastitis and breeding values for SCC of bulls of two Swedish breeds of dairy cattle (Red and White and Friesian). They found that for Red and White bulls breeding values for CM increased 0.35 per unit change of breeding value for SCC.

In this study ETA for CM increased 0.9189 (s.e. ± 0.05178), 1.08114 (s.e. ± 0.05871), and 0.9557 (s.e. ± 0.1276) per unit of change of ETA for SCS, in first, second and third lactation, respectively. The coefficients of regression of ETA on mastitis resistance traits and SCS suggest that although weak, there is a linear relationship between mastitis resistance traits and SCS. The coefficients of regression of ETA for occurrence of clinical mastitis on SCS were 0.0096 (s.e. ± 0.00057), 0.0118 (s.e. ± 0.0006), and 0.01242 (s.e. ± 0.00121) for first, second, and third lactation, respectively. The coefficient of regression of ETA for culling due to mastitis on SCS were 0.4445 (s.e. ± 0.02609), 0.5021 (.s.e. ± 0.03179), and 0.848 (s.e. ± 0.0493) for first, second, and third lactation, respectively.

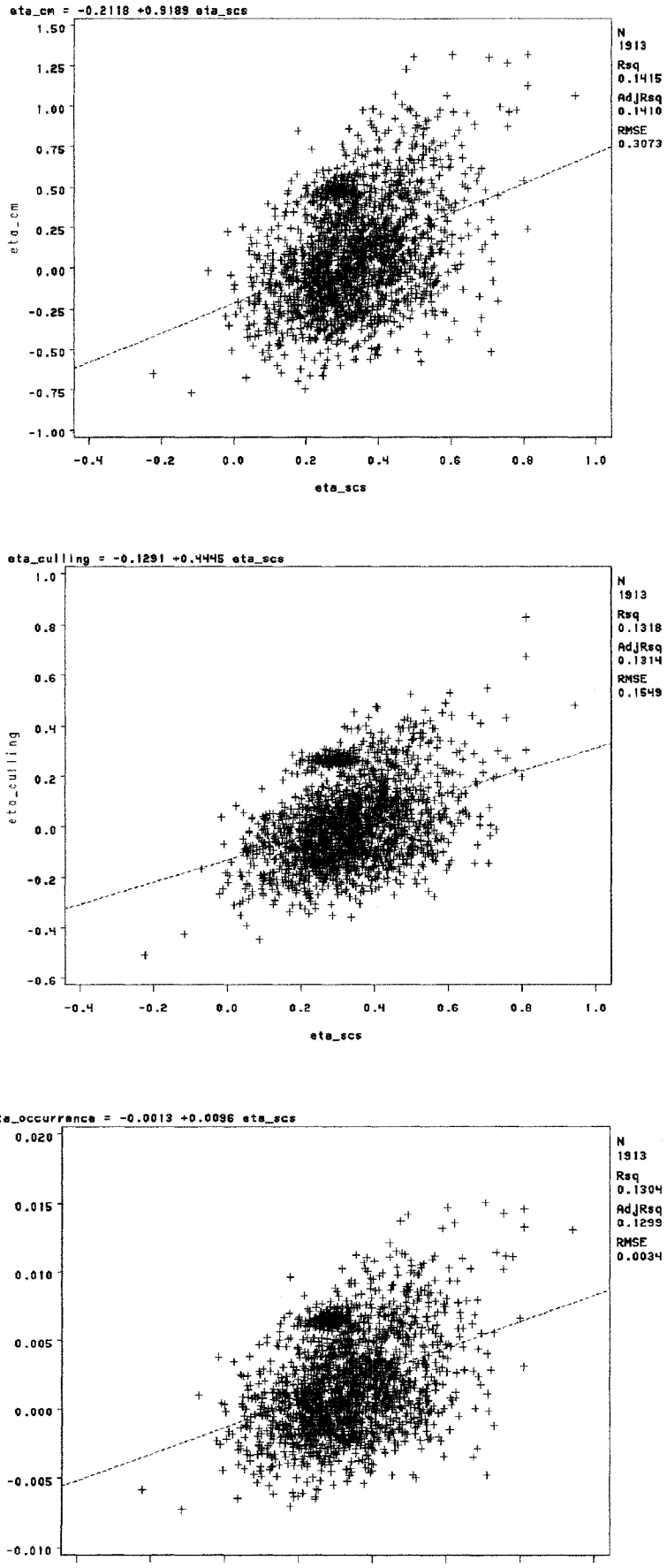


Figure 4. 7 Regression of ETA for Clinical mastitis, Culling due to mastitis and occurrence of clinical mastitis on ETA for somatic cell score (First lactation). ETA = Estimated transmitting ability, CM = clinical mastitis, SCS = somatic cell score, culling = culling due to mastitis, ncases = occurrence of clinical mastitis.

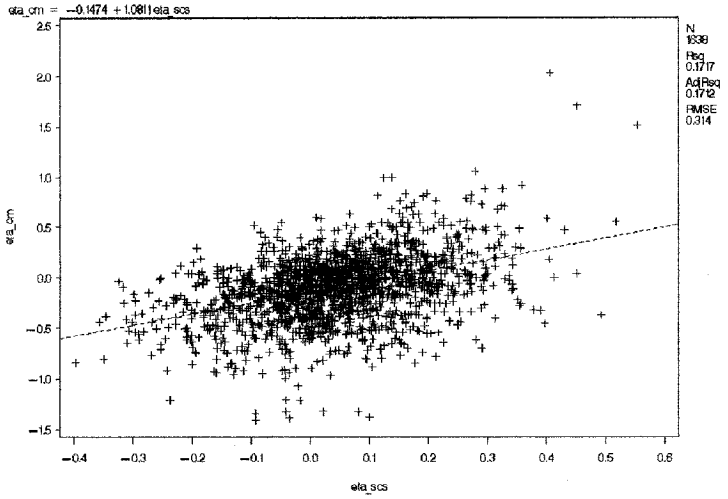
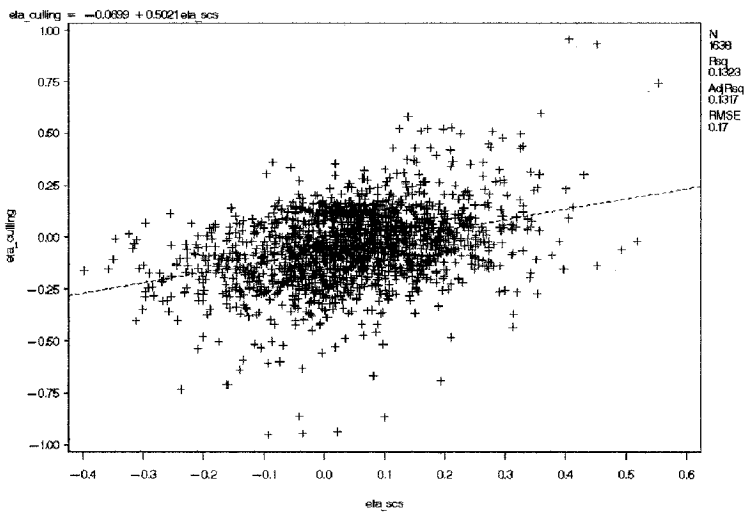
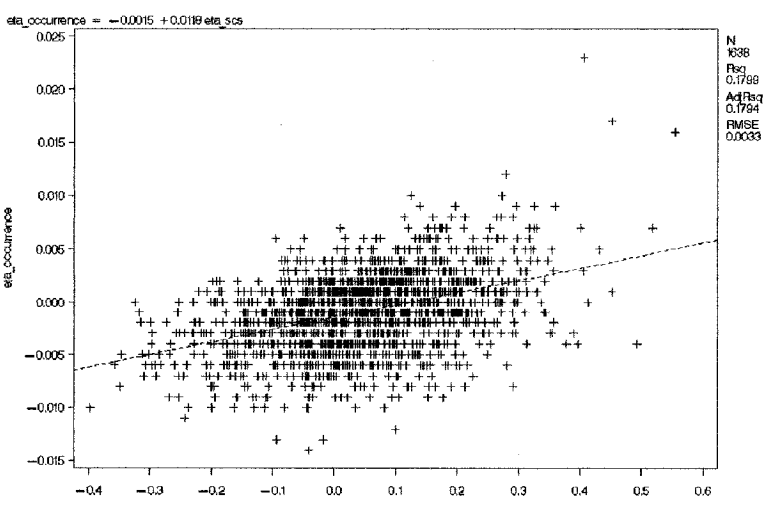


Figure 4. 8 Regression of ETA for Clinical mastitis, Culling due to mastitis, and occurrence of clinical mastitis on ETA for somatic cell score (Second lactation). ETA = Estimated transmitting ability, CM = clinical mastitis, SCS = somatic cell score, culling = culling due to mastitis, ncases = occurrence of clinical mastitis.





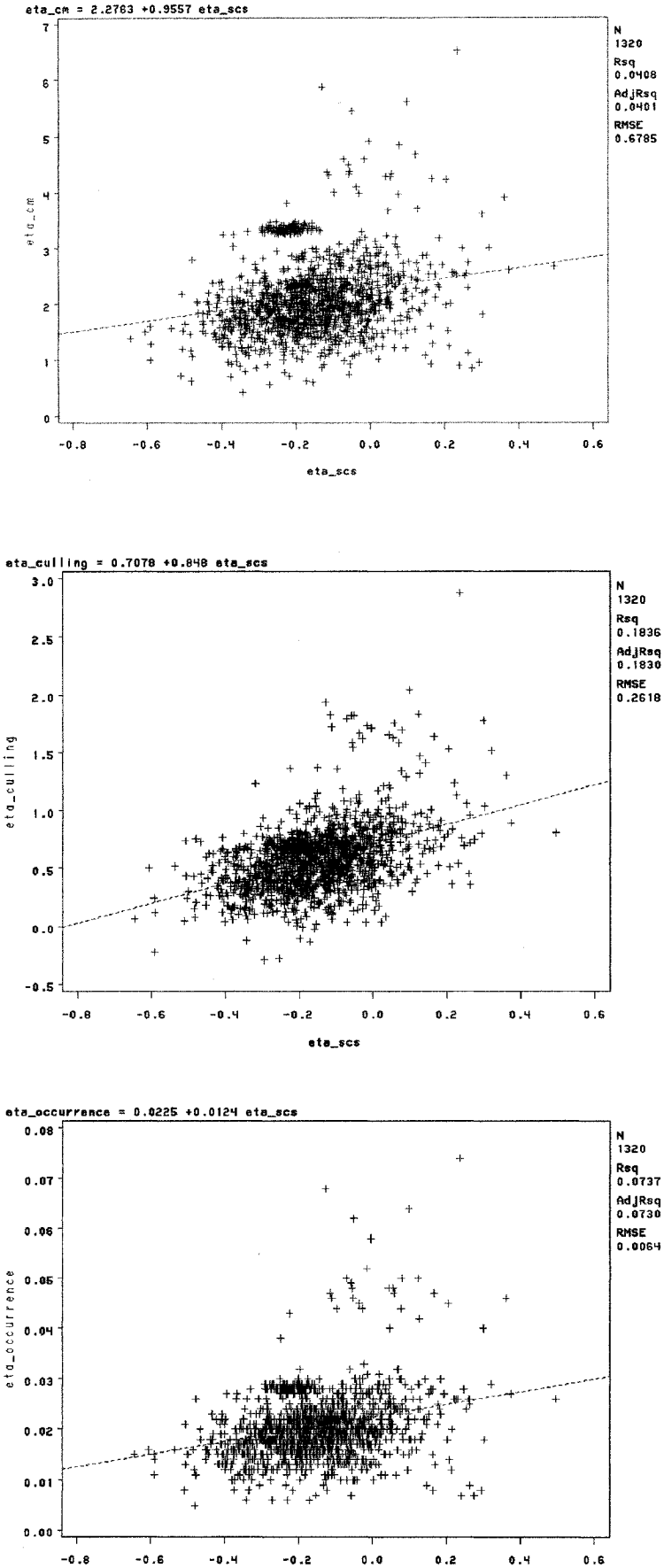


Figure 4. 9 Regression of ETA for Clinical mastitis, Culling due to mastitis, and occurrence of clinical mastitis on ETA for somatic cell score (Third lactation). ETA = Estimated transmitting ability, CM = clinical mastitis, SCS = somatic cell score, culling = culling due to mastitis, ncases = occurrence of clinical mastitis.

In order to detect QTL, variation must be present in the trait under study. In the case of the ETA generated in this study Figures 4.9 to 4.11 show the distribution for the ETA of the traits under study for each parity number.

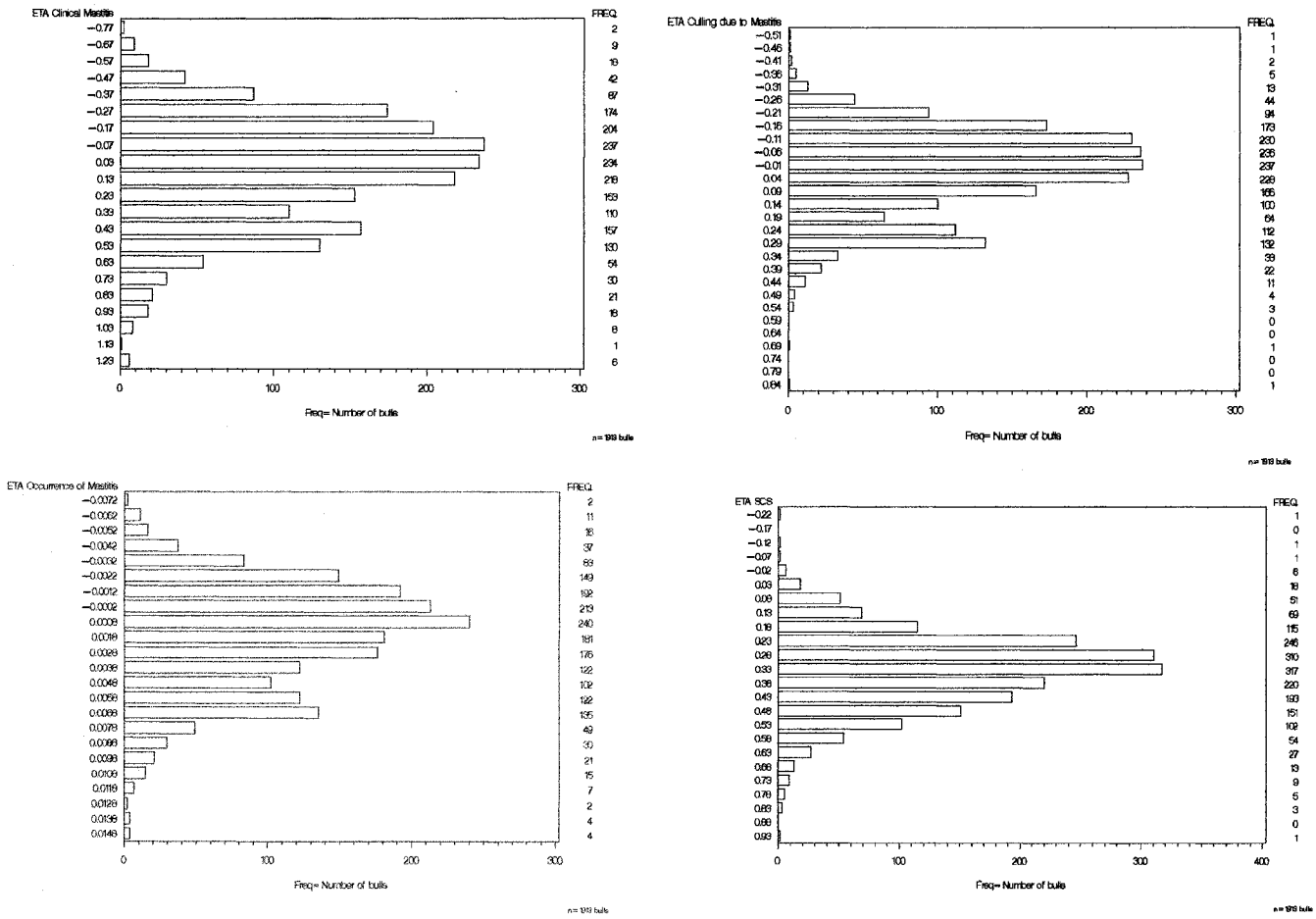


Figure 4. 10 Distribution of ETA for Clinical Mastitis, culling due to mastitis, occurrence of clinical mastitis and SCS (first lactation)

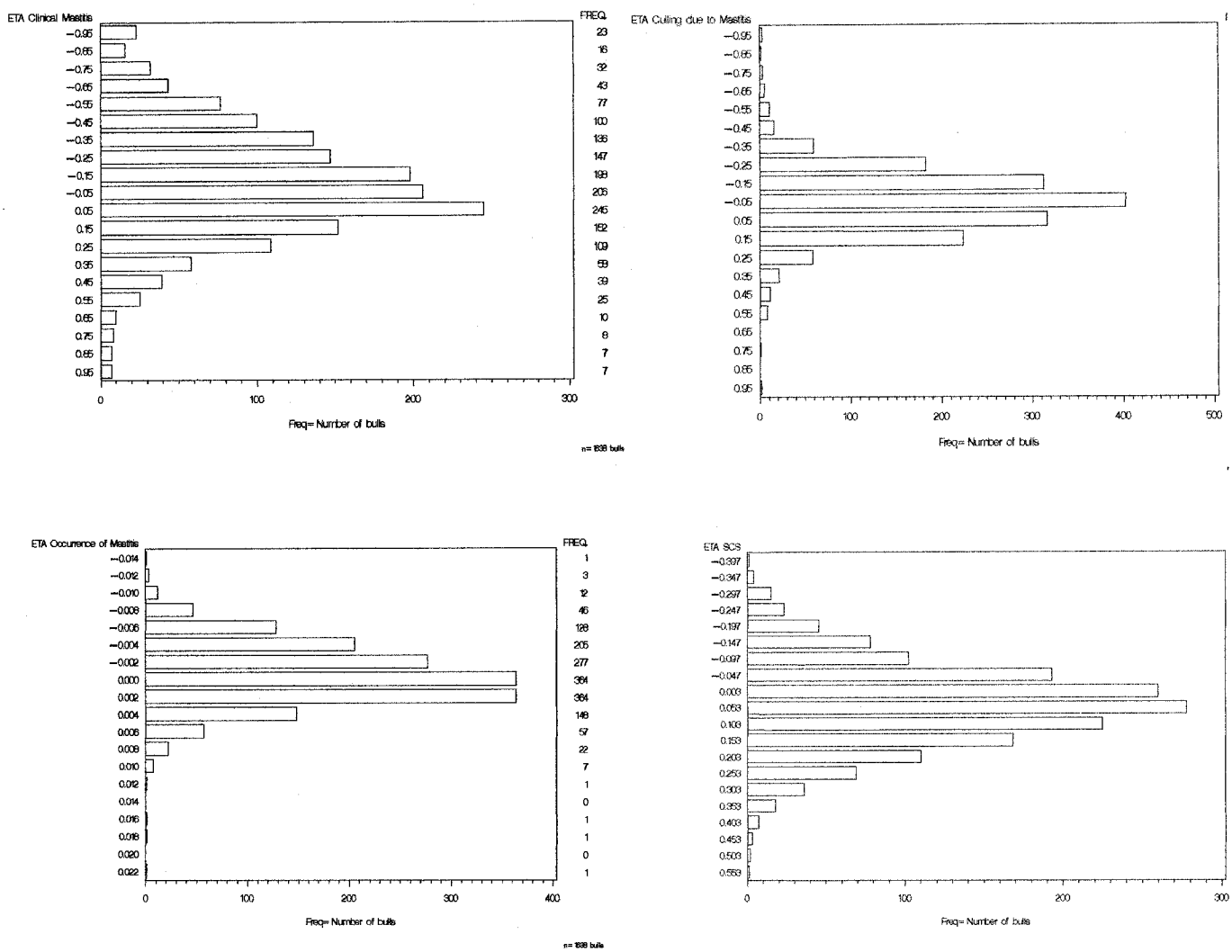


Figure 4. 11 Distribution of ETA for Clinical Mastitis, culling due to mastitis, occurrence of clinical mastitis and SCS (second lactation)

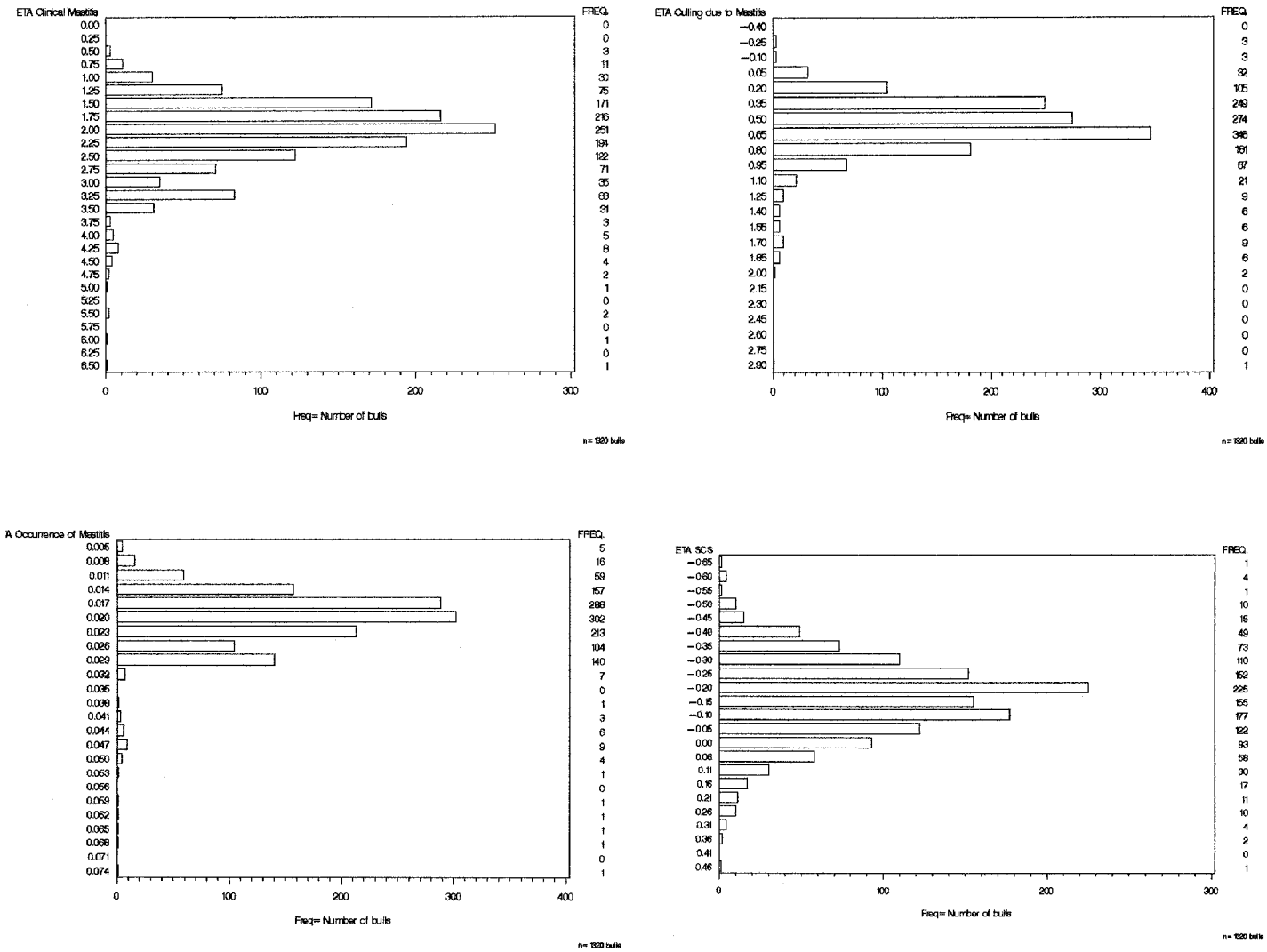


Figure 4. 12 Distribution of ETA for Clinical Mastitis, culling due to mastitis, occurrence of clinical mastitis and SCS (third lactation)

4.6 GENETIC TRENDS

Mastitis continues to be a significant economic problem for the dairy industry. Analysis of genetic trends allows one to evaluate how the use of genetic programs has had an impact on the population of interest. In the case of clinical mastitis if there is susceptibility to mastitis it justifies the efforts to map QTL that may affect mastitis resistance. The Figure 4.12 plots the average ETA for each trait considering the information for each parity number by year of birth of the sires. In each case, although erratic, the trends seem to be increasing over the time.

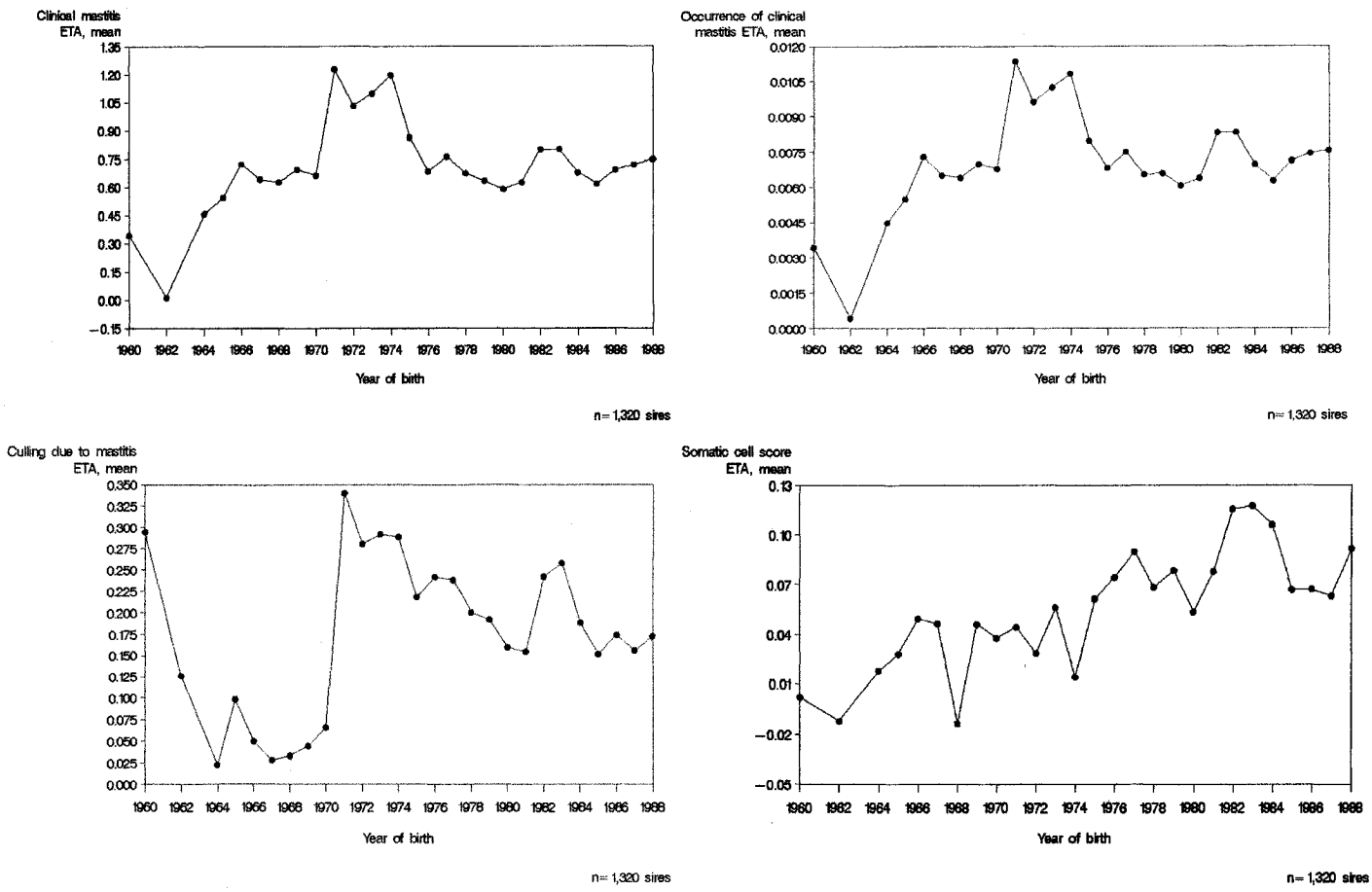


Figure 4. 13 Mean of ETA for clinical mastitis, culling due to mastitis, occurrence of clinical mastitis and SCS by year of birth of the sires. Only sires with ETA for clinical mastitis in first, second and third lactations were considered.

The erratic pattern may be a result of a lack of a systematic selection for these traits. Indeed, in Canada only since 1995 the LPI includes a weight for udder health through the incorporation of SCS.

The trends shown in Figure 4.12 suggest that genetic susceptibility to mastitis was increased during the period from 1960 to 1998. This may be a consequence of the genetic correlation between milk yield and mastitis. During the same period there was an increase in milk yield from 6500 to 8000 kg per cow/year. A comprehensive body of evidence suggests that increased genetic potential for milk yield is correlated to increased disease susceptibility.

In general, the previous results suggest that, in the population under study:

- There is higher incidence of mastitis, higher occurrence of mastitis cases, and more culling due to mastitis as parity increases, and through years

- The trend for SCS decreased from 1982 to 1990, but from there it shows an increasing trend, and it is higher in older cows.

As a consequence, two statements of relevance for this study seem to be supported with the evidence discussed so far: i) the incidence of clinical mastitis, culling due to mastitis, number of cases of clinical mastitis, and SCS shown in Figures 4.1 to 4.4 suggest that the susceptibility to mastitis has increased with years, especially in older cows, and ii) the h^2 for clinical mastitis incidence, number of cases of clinical mastitis, culling due to mastitis and SCS, though low, but nevertheless within ranges reported in other studies, in addition to the distribution of ETA displayed in Figures 4.10 to 4.11, and the genetic trends displayed in Figure 4.12 suggest that there is genetic variability for mastitis resistance in the population under study.

Despite the heritabilities for mastitis resistance traits are low, especially for incidence of clinical mastitis, occurrence of clinical mastitis and culling due to mastitis, it can be said in terms of herd health, that genetic variation can not be neglected. The genetic standard deviation of incidence of clinical mastitis ranged from 1.17 (first lactation) to 2.1 (third lactation); the genetic standard deviation of occurrence of clinical mastitis ranged from 0.013 (first lactation) to 0.022 (third lactation); the genetic standard deviation of culling due to

mastitis ranged from 0.41 (first lactation) to 0.52 (third lactation); the genetic standard deviation of SCS ranged from 0.013 (first lactation) to 0.022 (third lactation); the genetic standard deviation of SCS ranged from 0.41 (first lactation) to 0.52 (third lactation). In a recent review on the genetics of resistance to mastitis in dairy cattle, Rupp and Boichard (2003) discussed the importance of the variability of mastitis resistance; they speculated that if the genetic standard deviation of frequency of clinical mastitis is of about 5%, in an environment with 20% average frequency of clinical mastitis, the frequencies for extreme genotypes may range from 10 to 30%.

Because of the discussion above, analysis of associations between genetic polymorphisms and mastitis resistance traits in the population of Holsteins in Quebec is justified. One of the approaches that will help understand the genetics of mastitis resistance is the analysis of the associations between genetic polymorphisms with quantitative traits that express in a certain degree the capacity of the animals to resist the onset of mastitis. The following sections present the results from the main objective of the present study.

4.7 ASSOCIATION ANALYSES

The following sections describe the results obtained from analyses performed across-population and within-family.

4.7.1 Across population analyses

The use of genetic markers to detect QTL is based on the presence of LD between the genetic markers and the QTL. In this study no significant effects of the markers were detected in any of across population analyses. The complete results obtained with the across population model are displayed in tables 7.2 to 7.15 (appendix 3). The *p-values* obtained in the across-population analyses were within the range of 0.8240 to 0.9985.

There are a number of possible causes of these non-significant results. On the one hand, it may be that there is no QTL affecting any of the mastitis resistance traits in the population under study. However, for reasons that will be clear later, in the case of this study, this is not the most likely cause. On the other hand, assuming that there is at least one QTL with effect on mastitis resistance in the population under study, a possible explanation is that the genetic

markers and the QTL are in linkage equilibrium in the population even if linked, and therefore no particular association between any of the marker alleles and any of the QTL alleles became apparent.

An assessment of the least-squares estimates of the means (presented in tables 7.2 to 7.15) allowed verification that indeed there were no major differences between groups of sires according to the allele inherited from the grandsire (allele + vs allele -). Linkage equilibrium across population is common in outbred populations such as dairy cattle and it has been referenced elsewhere (Dekkers and Hospital, 2002).

The estimates of ETA for the various mastitis resistance traits analyzed across population (Appendix 3, Tables 7.2 to 7.15) shows that in some cases there was a large difference between the average genotypic value of genotypes +/+ and -/-, as for example in the case of genotypes ODC1 +/+ (0.1679 ± 2.9) and ODC1 -/- (0.08796 ± 0.7218) in clinical mastitis (see table 7.2 for this particular example); however, the large standard error, associated with the reduced number of sires available to perform the analyses, may have prevented the detection of statistically significant effects.

It has been shown that selection may reduce the difference between the phenotypes of animals carrying alternative QTL genotypes, thus reducing the power to detect QTL (Mackinnon and Georges, 1992). In the case of the population used in this study, selection was more likely placed on milk yield and not on clinical mastitis incidence or SCS.

Despite the lack of significant associations across population, this study provides insight within an area that lacked information. There is no documented evidence of QTL mapping studies using the genetic markers described in this thesis. As will be commented in the next section, all the main studies performed between the period 1997-2004 to map QTL affecting mastitis or SCS have reported effects of some markers (mainly anonymous markers) within some grandsire families, but no significant effects have been reported across population.

A solution for the limitation of across population analysis in outbred populations has been proposed (Weller, 1990), and it consists in performing association analyses on a family-basis (*i.e.*: within-family analysis).

4.7.2 Within-family analysis

Table 4.8 displays the results from the within-family analyses by marker and trait using genetic evaluations for first lactation. The analyses produced significant results for the marker nested within grandsire for culling due to mastitis (ODC1) and SCS (GH61, GH1300). The values displayed correspond to the comparison wise *p-values* (obtained from the analysis with the original data, before permutations) and in parenthesis the empirical trait-wise *p-values* (obtained after permuting the data sets). This threshold value obtained from permutation tests minimizes the probability of error type I. A comparison wise value was considered statistically significant if it was smaller than the empirical trait-wise value, otherwise it was not. As described in the section Material and Methods, the usefulness of the *p-values* obtained from the permutation tests is that it allows one to reject or not a comparison-wise *p-value* as significant if it is lower than the empirical threshold represented by the *p-value* from permutation tests, this is, the trait-wise *p-value*. For instance, for clinical mastitis, two markers were significant when considering the comparison wise *p-value*: GH61 (p value=0.0489) and PRL361 (*p-value*=0.0451), however after permutation tests the empirical trait-wise *p-values* were still lower: GH61 (empirical *p-value*=0.0242) and PRL361 (empirical *p-value*=0.0342). In that case the decision is that the markers are not statistically significant ($P< 0.05$). In another example for culling due to mastitis (Table 4.8), ODC1 was significant with a comparisonwise *p-value* of 0.0172, and after permutations, the trait-wise *p-value* was 0.0401, thus ODC1 was significant in the analysis for culling due to mastitis. If a *p-value* after permutations happens to be lower than the comparisonwise *p-value*, the decision would be that the marker is not statistically significant, even though that the comparisonwise *p-value* was apparently low.

This is the same context for tables 4.9 to 4.12 that display results from within-family analyses for second, third, and over all lactations (from 1st to 3rd) lactations.

Table 4. 8 Within family analysis (1st lactation) for clinical mastitis incidence, occurrence of clinical mastitis, culling due to mastitis and somatic cell score. For the genetic markers statistically significant with comparisonwise value, the empirical traitwise *p-value* is also displayed (in parenthesis)

Chromosomal position	Marker	Clinical mastitis	Occurrence	Culling	SCS
BTA11	ODC1	NS	NS	0.0172 (0.0401)	NS
	ODC2	NS	NS	NS	NS
BTA20	GHRAL	NS	NS	NS	NS
	GHRAC	NS	NS	NS	NS
	GHRST	NS	NS	NS	NS
BTA19	GH1	NS	NS	NS	NS
	GH41	NS	NS	NS	NS
	GH42	NS	NS	NS	NS
	GH61	0.0489 (0.0242)	NS	NS	0.0407 (0.056)
	GH62	NS	NS	NS	NS
	GH1258	NS	NS	NS	NS
	GH1300	NS	NS	NS	0.0308 (0.0587)
	GH5183	NS	NS	NS	NS
	GH5255	NS	NS	NS	NS
BTA23	PRL152	0.0451 (0.0342)	NS	NS	NS
	PRL361	NS	NS	NS	NS
BTA14	CRH291	NS	NS	NS	NS
BTA5	IGF390	NS	NS	NS	NS
BTA11	ACTH341	NS	NS	NS	NS
	ACTH388	NS	NS	NS	NS

The analyses of associations in first lactations between markers and occurrence of clinical mastitis yielded no significant results; a result difficult to explain because significant results for occurrence of clinical mastitis were observed in the rest of analyses for second, third, and over all lactations. The analyses of ETA for first lactation produced less significant results than second and third lactations. This may be explained by the greater expression of clinical mastitis late in the life of cows; as a cow stays longer in production, it will be more exposed to mastitis risks factors.

Analyses for second lactations, presented in table 4.9, produced significant results for the marker nested within grandsire for culling due to mastitis (GH61), clinical mastitis (GH61, PRL152), and occurrence of clinical mastitis (GH61).

Table 4. 9 Within family analysis (2nd lactation) for clinical mastitis incidence, occurrence of clinical mastitis, culling due to mastitis and somatic cell score. For the genetic markers statistically significant with comparisonwise value, the empirical traitwise *p-value* is also displayed (between brackets)

Chromosomal position	Marker	Clinical mastitis	Occurrence	Culling	SCS
BTA11	ODC1	NS	NS	NS	NS
	ODC2	NS	NS	NS	NS
BTA20	GHRAL	NS	NS	NS	NS
	GHRAC	NS	NS	NS	NS
	GHRST	NS	NS	NS	NS
BTA19	GH1	NS	NS	NS	NS
	GH41	NS	NS	NS	NS
	GH42	NS	NS	NS	NS
	GH61	***0.0002(0.0141)****	***0.0001 (0.0135) ****	***0.0039(0.0053)****	NS
	GH62	NS	NS	NS	NS
	GH1258	NS	NS	NS	NS
	GH1300	0.0380 (0.0167)	0.0384 (0.0185)	NS	NS
	GH5183	NS	NS	NS	NS
	GH5255	NS	NS	NS	NS
BTA23	PRL152	***0.0161(0.0227)****	0.0171 (0.0123)	NS	NS
	PRL361	NS	NS	NS	NS
BTA14	CRH291	NS	NS	NS	NS
BTA5	IGF390	NS	NS	***0.0322(0.0352)****	NS
BTA11	ACTH341	NS	NS	NS	NS
	ACTH388	NS	NS	NS	NS

In these analyses, no association between markers and SCS was found. As was pointed out previously, more significant effects were found in second lactation than in first lactations. In addition, it can be observed that some of the significant results were produced by the same markers (markers for GH at BTA19 and a marker for PRL at BTA23, IGF390 at BTA5).

Two markers (GH61 and IGF390) that did produce non-significant results in the analysis for culling due to mastitis in first lactations were found affecting culling due to mastitis in second lactations.

Table 4.10 presents the results of analyses for third lactations, which showed significant results for the marker nested within grandsire for culling due to mastitis (GHRAC, GH61, IGF390), clinical mastitis (GHRAC, GH61, IGF390), occurrence of clinical mastitis (GHRAC, GH61, IGF390), and SCS (GH61, GH1300).

Table 4. 10 Within family analysis (3rd lactation) for clinical mastitis incidence, occurrence of clinical mastitis, culling due to mastitis and somatic cell score. For the genetic markers statistically significant with comparisonwise value, the empirical traitwise *p-value* is also displayed (between brackets)

Chromosomal position	Marker	Clinical mastitis	Occurrence	Culling	SCS
BTA11	ODC1	NS	NS	NS	NS
	ODC2	NS	NS	NS	NS
BTA20	GHRAL	NS	NS	NS	NS
	GHRAC	0.0145(0.0214)*	0.0028 (0.0073)*	0.0092(0.0197)*	NS
	GHRST	NS	NS	NS	NS
BTA19	GH1	NS	NS	NS	NS
	GH41	NS	NS	NS	NS
	GH42	NS	NS	NS	NS
	GH61	***0.0114(0.0161)****	***0.0058 (0.0062) ****	***0.0060(0.0127)****	0.0080(0.0610)*
	GH62	NS	NS	NS	NS
	GH1258	NS	NS	NS	NS
	GH1300	0.0397 (0.0283)	0.0493 (0.0164)	NS	0.0409(0.0622)*
	GH5183	NS	NS	NS	NS
	GH5255	NS	NS	NS	NS
BTA23	PRL152	NS	NS	NS	NS
	PRL361	NS	NS	NS	NS
BTA14	CRH291	NS	NS	NS	NS
BTA5	IGF390	0.0029(0.0244)*	0.0038 (0.0125)*	***0.0055(0.0146)****	NS
BTA11	ACTH341	NS	NS	NS	NS
	ACTH388	NS	NS	NS	NS

A difference in the analyses of ETA for mastitis resistance traits in third lactation by comparison with those of first and second lactations is that all traits were found to have a significant association with markers.

A marker in BTA20 was found to be in association with all the traits but one (SCS); that marker was not in association with mastitis resistance in first and second lactations.

Table 4.11 shows analyses for mastitis resistance (incidence and occurrence of clinical mastitis) over the first three lactations produced significant results for the marker nested within grandsire for culling due to mastitis, and occurrence of clinical mastitis (GH61 and IGF390, in both cases).

Table 4. 11 Within family analyses across first three lactations. For the genetic markers statistically significant with comparisonwise value, the empirical traitwise *p-value* is also displayed (between brackets)

Chromosomal position	Marker	Clinical mastitis	Occurrence
BTA11	ODC1	NS	NS
	ODC2	NS	NS
BTA20	GHRAL	NS	NS
	GHRAC	NS	NS
	GHRST	NS	NS
BTA19	GH1	NS	NS
	GH41	NS	NS
	GH42	NS	NS
	GH61	0.0019 (0.0514)*	0.0009 (0.0433) *
	GH62	NS	NS
	GH1258	NS	NS
	GH1300	NS	NS
	GH5183	NS	NS
	GH5255	NS	NS
BTA23	PRL152	NS	NS
	PRL361	NS	NS
BTA14	CRH291	NS	NS
BTA5	IGF390	0.0015 (0.0628)*	0.0004 (0.0618)*
BTA11	ACTH341	NS	NS
	ACTH388	NS	NS

For all the previous results, it has to be pointed out that at 5% level of significance, from 80 tests in a lactation (*i.e.* four traits by twenty markers per lactation) one may expect 4 significant results by chance. In first lactations, initially five marker effects were found statistically significant, but after permutations tests, two of them (GH61 and PRL361) were found not to surpass the empirical threshold obtained by shuffling the data sat. In second lactations initially eight significant results were found; after permutation tests three of those significant results were rejected, hence only five significant results were considered truly statistically significant. In third lactations as well two significant results with the nominal significance level (*i.e.* with no adjustment with permutation tests) were found (GH1300 in CM and occurrence of clinical mastitis). As a result from the initial 13 significant results using the comparisonwise significance level, eleven were still significant based on the permutation tests. Globally, from 26 significant results, seven were rejected after the statistical adjustment obtained by permutations tests (*i.e.* 27 % of the initially detected).

More significant results were found when analyses were performed using genetic evaluations of third lactation than first or second lactations. It is impossible to establish from the results of this study whether different gene expression at different parity is responsible for the pattern of the significant results. The evidence that it is available from this study is that the mastitis resistance traits studied here (incidence of clinical mastitis, occurrence of clinical mastitis, culling due to mastitis and SCS) showed variation and the trend was to have increased expression of clinical mastitis, culling due to mastitis and SCS as parity increased. Further studies may be required to dissect the differential gene expression of genes related to mastitis resistance.

That more significant effects were found in third lactation seems to be in contradiction with the fact that more selection is applied to third lactation cows. It has to be emphasized that not all third lactation cows were kept (*i.e.* there was culling due to mastitis) as the proportion of cows culled due to mastitis in third lactation (2.6 %) was higher that in first and second lactation (0.85 and 1.65%, respectively).

Additional analyses within grandsire families might help to clarify the effect of these markers on the quantitative traits under study. Table 4.12 presents the output from within-family analysis from those markers that showed significant effects in the pooled analyses described in tables 4.12 to 4.15. The real identification of the grandsire family (*i.e.* grandsire's registration number) is not displayed. Further fine mapping studies are required to

ensure the location and true practical value of the putative QTL, before publicly disclosing the identities of the grandsires involved in the present study.

The information displayed in table 4.12 includes the number of the chromosome where the genetic marker is located, the trait analyzed, and the marker allele difference obtained from the mixed model analyses using a contrast between genotypes +/+ and -/- within family, as well as the standard error of the corresponding contrast and the *p-value*. The information displayed corresponds to the significant results from the analyses of the first, second, third and over all lactation data sets. The markers with significant effect were widespread on five BTA (5, 11, 19, 20, and 23).

Table 4. 12 Within-family significant marker allele difference for first lactation

BTA	Marker	Trait	Parity	Family	Marker allele difference	SE	P
5	IGF390	Clinical mastitis	3	1	-0.7499	0.3376	0.0271
		Clinical mastitis	3	8	2.5520	0.5900	<0.0001
		Clinical mastitis	3	9	2.4910	0.6219	<0.0001
		Clinical mastitis	Over lactations	1	-0.9043	0.4744	0.0576
		Clinical mastitis	Over lactations	8	2.5309	0.8291	0.0025
		Clinical mastitis	Over lactations	11	1.7223	0.8740	0.0497
		Clinical mastitis	Over lactations	12	2.0250	0.9270	0.0297
		Clinical mastitis	Over lactations	9	2.8824	0.8740	0.0011
		Occurrence of mastitis	3	1	-0.00726	0.00366	0.0479
		Occurrence of mastitis	3	8	0.028995	0.00639	<0.0001
		Occurrence of mastitis	3	9	0.02858	0.00674	<0.0001
		Occurrence of mastitis	Over lactations	1	-0.01108	0.00553	0.0459
		Occurrence of mastitis	Over lactations	8	0.02936	0.00966	0.0026
		Occurrence of mastitis	Over lactations	11	0.01980	0.01018	0.0527
		Occurrence of mastitis	Over lactations	12	0.02716	0.01080	0.0124
		Occurrence of mastitis	Over lactations	9	0.03366	0.01018	0.0011
		Culling	2	8	0.4665	0.2003	0.0206
		Culling	2	9	0.8357	0.2112	<0.0001
		Culling	2	5	0.2508	0.1191	0.0359
		Culling	3	1	-0.3599	0.1695	0.0346
		Culling	3	8	1.2703	0.2963	<0.0001
5	GH1300	Occurrence of mastitis	Over lactations	7	-0.03596	0.01302	0.0062
1	ODC1	Culling	1	3	0.2809	0.1306	0.0319
	GH1300	SCS	1	4	0.3611	0.1509	0.0172
		SCS	1	5	-0.2188	0.0950	0.0220
		SCS	1	6	-0.3631	0.1521	0.0175
19	GH1300	Clinical mastitis	2	7	-1.2302	0.5322	0.0215
		Occurrence of mastitis	2	7	-0.01188	0.0056	0.0360
		Clinical mastitis	3	7	-2.1906	0.8861	0.0094
		Occurrence of mastitis	3	7	-0.02628	0.00921	0.0047
		SCS	3	7	-0.5347	0.2075	0.0106
20	GHRAC	Clinical mastitis	3	1	1.8330	0.4345	<0.0001
		Clinical mastitis	3	10	0.7665	0.3198	0.0170
		Occurrence of mastitis	3	1	0.02229	0.00462	<0.0001
		Occurrence of mastitis	3	10	0.008643	0.00340	0.0115
		Culling	3	1	0.8406	0.2204	0.0002
		Culling	3	10	0.4397	0.1622	0.0070
23	PRL361	Clinical mastitis	1	2	-0.6315	0.2016	0.0734

Over lactations refers to the definition of the trait considering 1st to 3rd lactations (Section 3.3.2, Material and Methods).

From Table 4.12 it can be noticed that three genetic polymorphisms (IGF390, GH1300, and GHRAC) showed significant effects on more than one trait. Two markers (ODC1 and PRL361) showed effects only on one trait. IGF390 had significant effects across 2nd, 3rd and over all lactations. GH1300 was significant across 2nd and third lactations.

More significant effects were found in traits expressed in 3rd lactations than in 1st or 2nd lactations. This may be expected as a result of the late expression of CM as discussed in section 4.2 and its subsections.

Published evidence of QTL affecting mastitis using markers similar to the ones used in the present study was not found. However, there are several QTL affecting SCS, CM, and udder conformation that have been reported by using mainly anonymous markers.

With regard to CM, the definition that has been widely used is CM as a binary trait (assigning it a value either of 1 or 0); if a cow showed CM in at least a test day along the lactation, the binary trait received a value of 1; otherwise it received a value of 0. Only three reports of QTL affecting CM were found (Klungland *et al*, 2001; Holmberg and Andersson-Eklund, 2004; Schulman *et al*, 2004). Klungland *et al* (2001) reported QTL on BTA 6 (position 37 cM), BTA 8 (position 54 cM), and BTA 14 (position 93 cM). Holmberg and Andersson-Eklund (2004) reported QTL on BTA11 (position 25.7 and 41 cM, respectively); Schulman *et al* (2004) found a QTL on BTA 14 (position 40 cM). None of the QTL above mentioned was detected on the same chromosomes that harbor the QTL found in this study.

With respect to occurrence of clinical mastitis and culling due to mastitis, no evidence of QTL had been described so far.

SCS is the trait that more widely has been studied with regard to QTL for mastitis resistance. This is comprehensible as SCC and SCS are easier to record than any other mastitis resistance trait. Literature available on the topic shows that the following chromosomes may harbor QTL affecting SCS: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 18 to 23, 26, and 27. The higher density of reports lies on BTA 7, where QTL have been mapped at 61.6 cM (Ashwell *et al*, 2004), 75-97 cM (Kühn *et al*, 2003), and 124.4 cM (Heyen *et al*, 1999). On BTA 5 a QTL at 6.7 cM (Zhang *et al*, 1998) and another one at 100 cM (Heyen *et al*, 1999), practically at both extremes of the chromosome, have been found. BTA8 harbors two QTL, one at 16.7 cM (Reinsch *et al*, 1998) and at 54 cM (Klungkand *et al*, 2001). On BTA 11 within a section of 40 cM, three QTL have been reported; one at 25.7 cM and other at 41

cM (Holmberg and Andersson-Eklund, 2004), the third QTL was mapped at 63 cM (Schulman *et al*, 2004). On BTA 22 three findings are documented: at 43.7 and 44.5 cM (Heyen, *et al*, 1999) and at 80 cM (Ashwell *et al*, 2004). In this case, due to the short distance between the two QTL located around 40 cM, the possibility of the presence of a single QTL within the region can not be ruled out. At BTA 23 four QTL have been found: at 17.3 cM (Reinsch *et al*, 1998), at 52 cM, 61 cM (Holmberg and Andersson-Eklund, 2004), and at 80 cM (Ashwell *et al*, 2004). BTA 26 is reported with two QTL at 0 cM (Ashwell *et al*, 2004) and other one within a segment located between 50.6 cM and 59.8 cM (Zhang *et al*, 1998).

A summary of the results from the studies that have reported QTL for CM, SCS and udder conformation is given in table 7.16 in appendix 4.

How do the results of the present study collaborate with others to dissect the genetic control of mastitis resistance? To evaluate the importance of the results obtained in this study it may be appropriate to look at them within the context of how deeply mastitis resistance traits have been explored through the detection of QTL in other studies. Table 4.13 shows an extract of those studies that have chromosomal locations in common with the results from the present study.

In particular, some of the QTL locations found in this study are located on chromosomes where other QTL have been detected by other research groups affecting traits such as CM and SCS (Heyen *et al*, 1999; Rodriguez-Zas *et al*, 2002; Ashwell *et al*, 2004; Bennewitz *et al*, 2004; Holmberg and Andersson-Eklund, 2004; Schulman *et al*, 2004), or udder conformation (Van Tassell *et al*, 2000). However, as was mentioned, those studies used only anonymous markers. Those markers are in most of cases far from the polymorphisms used in this study. The significant effects reported in the literature were also obtained from within family analyses, as was the case of the significant results obtained in this study; a summary of the main findings related to QTL affecting SCS and clinical mastitis is given in Table 4.13. This table shows the number of the chromosome where QTL have been reported, as well as the traits that they affect, additionally the last column displays the identity of the markers used in this study that resulted significant as well their position on the chromosome.

Table 4. 13 Significant results from the several research groups that have mapped QTL affecting SCS or mastitis

BTA	Trait (other study)	Marker, position	Reference	Markers in this study
5	SCS	BM6026, 6.7 cM	1	IGF-1, 74 cM
	Rear udder width	3P1, 18.8 cM	2	
	SCS	BM315, 100.1 cM	3	
11	SCS	N/E, 63 cM	4	ODC, 8.97 cM
	SCS	INRA177, 67.1 cM	1	
		BM7169, 41 cM		
19	SCS	N/E, 32 cM	5	GH, 65.7 cM
20	SCS	N/E, 82 cM	6	GHR, 59 cM
23	SCS	BM1443, 67.1 cM	1	PRL, 43.2 cM
	SCS	BB705-BM1818, 80 cM	7	
	SCS	D2355, 52 cM	3	
	SCS	RM033, 17.3 cM	8	

References: 1= Holmberg and Andersson-Eklund (2004); 2=Van Tasell *et al* (2000); 3=Heyen *et al* (1999); 4=Schulman *et al* (2004); 5=Bennewitz *et al* (2003); 6=Rodriguez-Zas (2002); 7= Ashwell *et al* (2004); 8=Reinsch *et al* (1998)

The present study found associations between some markers and mastitis resistance traits. The location of the markers under study provides more insight into the genetic control of mastitis resistance; the location of QTL affecting mastitis resistance traits from this study with respect to other QTL reported by other studies is clearer when the chromosomal regions associated with mastitis resistance are displayed graphically. Figure 4.15 shows the set of BTA, as well as the position where QTL have been found.

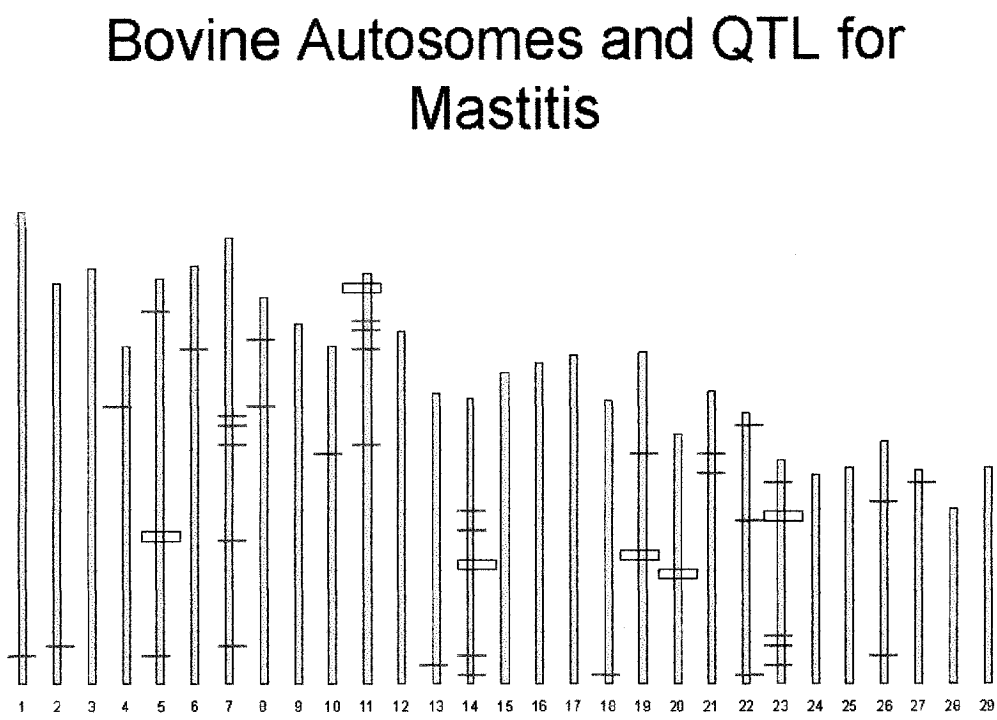


Figure 4. 14 Graphical representations of the 29 bovine autosomes, and locations where QTL have been mapped. Transverse lines represent sites where QTL have been mapped and open boxes sites where the polymorphisms used in this study lie.

Some BTA do not have QTL affecting mastitis such as BTA 3, 9, 12, 15, 16, 17, 24, 25, 28, and 29. Some BTA show only one QTL such as 1, 2, 4, 6, 10, 13, 18, and 27. The BTA that show more QTL affecting mastitis are: 5, 7, 8, 11, 14, 19, 20, 21, 22, 23, and 26. The chromosomes where QTL were detected in this study are 5, 11, 14, 19, 20, and 23. In Figure 4.14 an open box points out the positions of the polymorphisms that yielded significant results in this study. BTA 20 had no report of QTL affecting mastitis before this study was performed; in this study polymorphisms of GHR were detected in association with mastitis resistance traits. Other polymorphisms from this study on BTA 11, 14 and 23 were detected in association with mastitis resistance. As shown in Table 4.13 the distance between QTL from other studies and those detected in this study varies, as is the case of the polymorphisms of GHR that are located at 23 cM from one of the QTL detected by Rodriguez-Zas et al (2002), or the polymorphisms of PRL that are located at only 8.8 cM

from the QTL detected by Heyen et al (1999). On BTA 11 the polymorphisms of ODC are the ones more distant from other QTL reported in the literature.

4.8 PERSPECTIVES

The main question to be answered by this study was: are there associations between genetic markers of genes related to immune response (GH, GHR, ODC, ACTH, PRL, CRH, IGF-1) and mastitis resistance traits (incidence of clinical mastitis, occurrence of clinical mastitis, culling due to mastitis, and SCS) in the Holstein population of Canada? The answer is positive. However, more questions might be raised as a consequence of the results of this study. On the basis of the present findings and results from other research groups, it is likely that regions located on BTA 5, 11, 19, 20, and 23 harbor QTL affecting resistance to mastitis. Do those regions indeed harbor QTL that affect *per se* mastitis resistance?

In order to clarify the molecular basis for mastitis resistance further research must be focused on fine mapping the regions where significant results from this study agreed with other findings from other groups. The regions to genotype may be segments of BTA 5, 11, 19, 20, and 23. To do so, several markers publicly available may be used along with the markers already used in the present study. In general, fine mapping studies use a marker density of at least 10 cM. With a new data base with such features new association analysis, such as those described in this study may be performed. Several studies have performed fine mapping for QTL for productive traits, such as milk yield, fat percentage, fat yield, protein percentage, and protein yield (Riquet *et al*, 1999; Blott *et al*, 2003; Farnir *et al*, 2003; Olsen *et al*, 2004), however there is no report of fine mapping of QTL for mastitis.

An accurate estimation of QTL effects is a requirement before any attempt is made to establish MAS. Once accurate detection of QTL has taken place, and MAS has been established, association analysis must routinely performed each generation in order to monitor whether the QTL is still in linkage disequilibrium with the marker.

A number of modifications may be adopted to perform association analysis in the population under study. With regard to the phenotypic traits recorded, it is necessary take into account that the most reliable measures of udder health are SCC, udder conformation, milking speed, and incidence of clinical mastitis. In Canada there are already genetic evaluations for SCS, conformation, and milking speed. However, incidence of clinical

mastitis continues to be a problematic trait to be recorded. It is recommended to increase the frequency and accuracy of mastitis recording. In this matter, it is relevant the inclusion of veterinary diagnostics of mastitis, as well as the information of the veterinary treatments into the lactation records; these modifications to the recording can have a direct impact on the sensitivity of association analyses. If the etiology of mastitis is recorded (not only when the case occurs on the test-day, but any time during the lactation), association analysis may provide evidence of different QTL that may affect different type of mastitis (i.e. according the pathogen involved). In Québec there is an attempt to combine a health data bank from veterinaries practicing in the province and information from the PATLQ (*Le producteur de lait québécois*, March 2005, p. 16-17). The potential use of these combined sources of information is promising in terms of QTL mapping studies. It has already been proposed for the Canadian Holstein population that analysis of profile of test-day SCC through the lactation would provide with information regarding individual differences to deal with external factors, such as bacteria, climate stress, and management (Monardes, 1984). The importance of SCC patterns has been also emphasized in a study of the Dutch dairy population (Holstein-Friesian, Dutch-Friesian and Meuse-Rhine-Yssel breeds) (de Hass, 2003). Different pathogens have different pathogenesis therefore the response of the cows against infections might be monitored through a differentially expressed pattern of SCC. Analysis of SCC patterns and their relationship with etiology of CM would provide with trait indicators that may be included in QTL mapping studies such as the one performed in this thesis; thus, association analyses will provide insight into the molecular basis for immune response for specific mastitis-causing pathogens.

Another modification for performing association studies consists in identifying sires according to their EBV for SCS (*i.e.* top and low) and obtain their genotypes (for the markers of interest) and perform association analysis, both by across-population analysis and by comparing both groups of sires within grandsires (contrasting the two groups of sons of a grandsire, provided the sons are homozygous and the grandsire heterozygous).

Other approach that has been proposed is selective pooling, that has been applied to map QTL for milk protein percentage (Lipkin *et al*, 1998); this method has the advantage of reducing costs of genotyping because is based in screening marker alleles among pooled DNA samples of the extreme (high and low) phenotypic groups of offspring of sires.

It is recommended that future application of the methodology to map QTL for mastitis resistance in the population under study includes not only the candidate genes analyzed in this study. One promising approach to detect new candidate genes for diseases is the application of comparative genome analysis. The availability of DNA sequences for several species (human, mouse, rat, swine, bovine) offers the possibility of detect genetic similarities among species that may be used in the application of studies of host response to infections and therefore obtain insight into metabolic pathways involved in disease resistance. Evidence shows that *E. coli* is gaining importance in the profile of infections that cause bovine mastitis; therefore, a possible application of comparative genome analysis could be the genetic dissection of host response to infections caused by *E. coli* in model organisms, such as mouse models. Once such genes responsible of resistance against infections will be detected by using animal models, their bovine orthologues may be used to expand DNA data banks (such as the one used in this study); genetic markers for those candidate genes may be used in association analyses to determine their effect on resistance against mastitis in bovines.

May the chromosomal regions harboring QTL for mastitis resistance contain factors, in addition to the QTL themselves, that interrelate with the QTL to produce the effect on mastitis resistance? Evidence has shown that several other molecular factors may have a determinant role in the expression (or not expression) of genes. The results from association analyses give narrow limits when one tries to make inferences with regard to the molecular basis of the mechanism of action of the QTL. Indeed, none of the studies that have mapped QTL for mastitis resistance in livestock, discussed in previous section, has attempted to elaborate on hypotheses to clarify the role of the detected QTL. However, with the growing body of knowledge about gene expression in other species some hypotheses may be offered on how mastitis resistance is genetically determined in domestic livestock. Thus, is it likely that those detected QTL affecting mastitis resistance are in fact signaling not only genes whose products may affect mastitis resistance, but also regions where more complex interrelationships occur to determine in greater or lesser extent mastitis resistance?

Several genetic disorders in several species have been mapped by tracking regions identical by descent (Hungtindon's disease in humans, malignant hyperthermia in pigs, chondrodysplastic dwarfism in bovines). The principle is that if a single mutation is responsible for the expression of a disease, the mutation can be traced through the inheritance

of segments that were originated in the ancestors. In the case of dairy cattle the use of AI has allowed the generation of large families paternal half-sibs. Studies using techniques to identify identical by descent (IBD) chromosomal segments may help to reduce the extent of fine mapping using genetic markers. Also, if there are factors other than QTL affecting gene expression the more likely place where they may occur is within regions that are shared by the descendants (regions identical by descent). Thus, if a QTL is affecting a trait in some of the sons of a GS, it would be interesting to analyze the possible existence of factors such as DNA methylation that may occur in some of their sons. A technique is available, Direct IBD Mapping (Smirnov et al, 2004), that allows isolation of the regions IBD from the progeny of an individual.

The possibility of obtaining IBD segments offers help in the determination of the molecular basis for the detected QTL. For instance, epigenetic changes, such as methylation of cytosines may occur with more frequency than thought before. Segments IBD may be processed to obtain epigenetic methylation patterns by using techniques such as the Bisulfite technique (Laird et al, 2004). This would allow study patterns of methylation in the bulls used as sires within AI programs.

Also, not only mRNA (originated from the exons) has a role in the expression of genes (through the translation of proteins); recent evidence has suggested that RNA from intronic origin has a role in gene regulation by affecting the expression of genes with a mechanism of gene-silencing called RNA interference (RNAi) (Mattick, 2002). Moreover, there is evidence of links between microRNAs (miRNAs; RNA produced by enzymatic processing of non coding RNAs) (Mattick, 2002) and epigenetic imprinting.

For the above discussed, further research on the dissection of the complexity lying in the chromosomal regions that harbor QTL affecting mastitis resistance must be encouraged. Although a complete dissection of the factors affecting mastitis resistance will take years this study may help in dissecting the genetic basis for mastitis resistance in Holstein cattle.

CONCLUSIONS

In this study mastitis resistance was defined through several measurements using an available data set from a milk recording organization. Increased susceptibility to mastitis (higher incidence of clinical mastitis, higher cases of clinical mastitis, higher culling due to mastitis, and higher SCS) was found through years and parity. This result emphasizes the need for detecting genetic markers that due to their association with mastitis resistance may be used in genetic selection.

Heritabilities for incidence of clinical mastitis, occurrence of clinical mastitis, culling due to mastitis, and SCS were low but within ranges reported elsewhere. Genetic variation was found for mastitis resistance traits in the population under study.

The results from the across-population analyses suggest that the polymorphisms used as genetic markers are in population-wide linkage equilibrium with the genes controlling the traits used as indicator of mastitis resistance.

Associations between various genetic polymorphisms in candidate genes affecting immune response and various mastitis resistance traits were detected through the application of within-family analyzes, using mixed models methodology and controlling error Type I. Several association studies were performed and QTL affecting mastitis resistance on Canadian Holsteins were found to be physically linked to genetic markers for IGF-1 (BTA 5), ODC (BTA 11), GH (BTA 19), GHR (BTA20), PRL (BTA 23).

REFERENCES

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

Aggrey S. E., J. Yao, M. P. Sabour, C. Y. Lin, D. Zadworny, J. F. Hayes, and U. Kuhnlein. 1999. Markers within the regulatory region of the growth hormone receptor gene and their association with milk-related traits in Holsteins. The Journal of Heredity 90:148-151.

Agriculture and Agri-Food Canada. 2003. Dairy animal improvement statistics, p. 31. http://dairyinfo.gc.ca/statsbook2003.pdf, Accessed January 5, 2004.

Almeida, R. 1996. Study of abnormal Test-Days in Quebec Holstein cows. M.Sc. Thesis McGill University.

Amin, A. A., and G. Tibor. 2001. Sample test-day heritability estimates for somatic cell score for Hungarian Holstein-Friesian crossbreds. Proc. British Society of Animal Science, p. 96.

Andersson, L. and M. Georges. 2004. Domestic-animal genomics: deciphering the genetics of complex traits. Nature Review Genetics 5:202-212.

Ashwell, M. S., Y. Da, C. P. Van Tassell, P. M. Vanraden, R. H. Miller, and C. E. Rexroad Jr. 1998. Detection of putative loci affecting milk production and composition, health, and type traits in a United States Holstein population. J. Dairy Sci. 81:3309-3314.

Ashwell, M. S., C. P. Van Tassell, and T. S. Sonsgtegard. 2001. A genome scan to identify quantitative trait loci affecting economically important traits in a US Holstein population. J. Dairy Sci. 84:2535-2542.

Ashwell, M. S., D. W. Heyen, T. S. Sonstegard, C. P. Van Tassell, Y. Da, P. M. VanRaden, M. Ron, J. I. Weller, and H. A. Lewin. 2004. Detection of quantitative trait loci affecting milk production, health, and reproductive traits in Holstein cattle. J. Dairy Sci. 87:468-475.

Auldist, M. J., S. Coats, B. J.Sutherland, J. J. Mayes, and G. H. McDowell. 1996. Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of cheddar cheese. Journal of Dairy Research 63:269-280.

Barkema, H. W., Y. H. Schukken, T. J. Lam, M. L. Biboer, G. Benedictus and A. Brand. 1999. Management practices associated with the incidence rate of clinical mastitis. J. Dairy Sci. 82:1643-1654.

Barkema, H. W., J. D. Van der Ploeg, Y. H. Schukken, T. J. Lam, G. Benedictus, and A. Brand. 1999b. Management style and its association with bulk milk somatic cell count and incidence rate of clinical mastitis. J. Dairy Sci. 82:1655-1663.

Barker, A. R., F. N. Schrick, M. J. Lewis, H. H. Dowlen, and S. P. Oliver. 1998. Influence of clinical mastitis during early lactation on reproductive performance of Jersey cows. J. Dairy Sci. 81:1285-1290.

Barrow, P. A., and J. S. Soothill. 1997. Bacteriophage therapy and prophylaxis: rediscovery and renewed assessment of potential. Trends in microbiology 5: 268-271.

Bennewitz, J., N. Reinsch, C. Grohs, H. Levéziel, A. Malafosee, H. Thomsen, N. Xu, C. Looft, C. Kühn, G. A. Brockmanm, M. Schwerin, C, Weimann, S. Hiendleder, G. Erhardt, I. Medjugorac, I. Russ, M. Förster, B. Brenig, F. Reinhardt, R. Reents, G. Averdunk, J. Blümel, D. Boichard, and E. Kalm. 2003. Combined analysis of dta from two granddaughter designs: a simple strategy for QTL confirmation and increasing experimental power in dairy cattle. Gen. Sel. Evol. 35:319-338.

Bennewitz, J., N. Reinsch, S. Paul, C. Looft, B. Kaupe, C. Weimann, G. Erhardt, G. Thaller, Ch. Kühn, M. Schwerin, H. Thomsen, F. Reinhardt, R. Reents, and E. Kalm. 2004. The DGTA1 K232A mutation is not solely responsible for the milk production quantitative trait locus on the bovine chromosome 14. J. Dairy Sci. 87:431-442.

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

Blosser, T. H. 1979. Economic losses from and the National Research Program on mastitis in the United States. J. Dairy Sci. 62:119-127.

Blott, S., J. J. Kim, S. Moisio, A. Schimidt-Küntzel, A. Cornet, P. Berzi, N. Cambisano, C. Ford, B. Grisart, D. Johnson, L. Karim, P. Simon, R. Snell, R. Spelman, J. Wong, J. Vilkki, M. Georges, Frédéric Farnir, and W. Coppieters. 2003. Molecular dissection of a quantitative trait locus: a phenylalanine-to-tyrosine substitutioin in the membrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. Genetics 163: 253-266.

Boichard, D., S. Fritz, M. N. Rossignol, M. Y. Boscher, A. Malafosse, and J. J. Colleau. 2002. Implementation of marker-assisted selection in French dairy cattle. Communication 22-03. 7th World Congress on Genetics Applied to Livestock Production.

Boldman, K. G., L. A. Kriese, L. D. Van Vleck, C. P. Van Tassell, and S. D. Kachman. 1995. A manual for use of MTDFREML. A set of programs to obtain estimates of variances and covariances. USDA, Agric. Res. Service, Beltsville, MD.

Bouchard, L., S. Blais, C. Desrosiers, X. Zhao, and P. Lacasse. 1999. Nitric oxide production during endotoxin-induced mastitis in the cow. J Dairy Sci. 82:2574-2581.

Bradley, A. J. 2002. Bovine mastitis: an evolving disease. The Veterinary Journal 164:116-128.

Brinkmann V., U. Reichard, C. Goosman, B. Fauler, Y. Uhlemann, D. S. Weiss, Y. Weinrauch, and A. Zychlinsky. 2004. Neutrophil extracellular traps kill bacteria. Science 303:1532-1535.

Burton, J. L., S. A. Madsen, J. Yao, S. S. Sipkovsky, and P. M. Coussens. 2001. An immunogenics approach to understanding periparturient immunosuppression and mastitis susceptibility in dairy cows. Acta Vet. Scand 42:407-424.

Carr, D. J. J., B. R. Acosta, A. E. Jacobson, and J. E. Blalock. 1990. Corticotropin-releasing hormone augments natural killer cell activity through a naloxone-sensitive pathway. J. Neuroimmunology 28:53-61.

Carter-Su, C., J. Schwartz, and L. S. Smit. 1996. Molecular mechanism of growth hormone action. Ann Rev Physiol 58:187-207.

Castillo-Juarez, H., P. A. Oltenacu, R. W. Blake, C. E. Mcculloch, and E. G. Cienfuegos. 2000. Effect of herd environment on the genotypic and phenotypic relationships among milk yield, conception rate, and somatic cell score in Holstein cattle. J. Dairy Sci. 83:807-814.

Churchill G. A., and R. W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138:963-971.

Clark, R. 1997. The somatogenic hormones and insulin-like growth factor-1: stimulators of lymphopoiesis and immune function. Endocrine reviews 18:157-179.

Coppieters W., J. Riquet, J. J. Arrantz, P. Bertzi, N. Cambisano, B. Grisart, L. Karim, F. Marcq, L. Moreau, C. Nezer, P. Simon, P. Vanmanshoven, D. Wagenaar, M. Georges. 1998. A QTL with major effect on milk yield and composition maps to bovine Chromosome 14. Mammalian Genome 9:540-544.

Da, Y., M. Grossman, I. Misztal, G. R. Wiggans. 1992. Estimation of genetic parameters for somatic cell score in Holsteins. J. Dairy Sci. 75:2265-2271.

De Hass, Y. 2003. Somatic cell count patterns. Improvement of udder health by genetics and management. Ph. D. thesis Wageningen University.

DeGroot, B. J., J. F.Keown, L. D.Van Vleck, and E. L.Marotz. 2002. Genetic parameters and responses of linear type, yield traits, and somatic cell scores to divergent selection for predicted transmitting ability for type in Holsteins. J. Dairy Sci. 85:1578-1585.

Dekkers, J. C. M. 1992. Structure of breeding programs to capitalize on reproductive technology for genetic improvement. J. Dairy Sci. 75: 2880-2891.

Dekkers, J. C. M. and F. Hospital. 2002. The use of molecular genetics in the improvement of agricultural populations. Nature Review Genetics 3:22-32.

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

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

Dürr, J. W., H. G. Monardes, R. I. Cue and J. C Philpot. 1997. Culling in Quebec Holsteins herds. 1. Study of phenotypic trends in herd life. Can. J. Anim. Sci. 77:593-600.

Dürr, J. W., H. G. Monardes, R. I. Cue and J. C Philpot. 1997b. Culling in Quebec Holsteins herds. 2. Study of phenotypic trends in reasons for disposal. Can. J. Anim. Sci. 77:601-608.

Elston, R. C., and J. Stewart. 1971. A general model for the genetic analysis of pedigree data. Hum. Hered. 21:523-542.

Eppard, P. J., J. J. Veenhuizen, and W. J. Cole. 1996. Effect of bovine somatotropin administered to periparturient dairy cows on the incidence of metabolic disease. J. Dairy Sci. 79:2170-2181.

Erskine R. J., R. J. Eberhart, L. J. Hutchinson, S. B. Spencer, M. A. Campbell. 1988. Incidence and types of clinical mastitis in dairy herds with high and low somatic cell counts. JAVMA 192:761-765.

Erskine, R. J. 2001. Mastitis control in dairy herds. Chapter 10, p. 397. In Heard health: food animal production medicine. Ed. Radostits O.M. 3rd ed. W.B. Saunders Co.

Falaki, M., N. Gengler, M. Sneyers, A. Prandi, S. Massart, A. Formigoni, A. Burny, D. Portetelle, and R. Renaville. 1996. Relationships of polymorphisms for growth hormone and growth hormone receptor genes with milk production traits for Italian Holstein-Friesian Bulls. J. Dairy Sci. 79:1446-1453.

Falconer, D. S. and T. S. Mackay. 1996. An introduction to quantitative genetics. 4th ed. Longman.

Fan W., K. Plaut, A. J. Bramley, J. W. Barlow, S. A. Mischler, and D. E. Kerr. 2004. Persistency of adenoviral-mediated lysostaphin expression in goat mammary glands. J. Dairy Sci. 87:602-608.

Fang, W., and S. Pysörälä. 1996. Mastitis-causing Eschericha coli: serum sensitivity and susceptibility to selected antibacterials in milk. J. Dairy Sci. 79:76-82.

Farnir, F., W. Coppieters, JJ. Arranz, P. Berzi, N. Cambisano, B. Grisart, L. Karim, F. Marcq, L. Moreau, M. Mni, C. Nezer, P. Simon, P. Vanmanshoven, D. Wagenaar, and M. Georges. 2000. Extensive genome-wide linkage disequilibrium in cattle. Genome Research 10:220-227.

Farnir, F., B. Grisart, W. Coippetiers, J. Riquet, P. Berzi, N. Cambisano, L. Karim, Myriam Mni, S. Moisio, P. Simon, D. Wagenaar, J. Vilkki, and M. Georges. 2002. Simultaneous mining of linkage and linkage disequilibrium to fine map quantitative trait loci in outbred half-sib pedigrees: revisiting the location of a quantitative trait locus with major effect on milk production on bovine chromosome 14. Genetics 161: 275-287.

Fox, P.P. and P.L.H. McSweeney. 1998. Dairy Chemistry and biochemistry, Chapter 8: enzymology of milk and milk products. Blackie Academic and Professional. Thompson Publishing.

FPLQ. 1983. Rapport Annuel de la Fédération des Producteur du lait du Québec, p. 16-17.

FPLQ. 1993. Rapport Annuel de la Fédération des Producteur du lait du Québec, p. 20-21.

FPLQ. 2002. Rapport Annuel Fédération des Producteur du lait du Québec, p28-29.

Geldermann, H. 1975. Investigations in inheritance of quantitative characters in animals by gene markers. I. Methods. Theor.Appl. Genet. 46: 319-330.

Georges, M., D. Nielsen, and M. MacKinnon. 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. Genetics 139:907-920.

Gibson, G., and S.V.Muse. 2002. A primer of genome science. Sinauer Associates, Inc. Publishers. 1st edition.

Gill, R., W.H. Howard, K.E. Leslie, and K. Lissemore. 1990. Economics of mastitis control. J. Dairy Sci. 73:3340-3348.

Goldammer T., H. Zerbe, A, Molenaar, H.J. Shuberth, R.M. Brunner, S.R. Kata, and H.M. Seyfert. 2004. Mastitis increases mammary mRNA abundance of β-defensin 5, Toll-like-receptor 2 (TLR2), and TLR4 but not TLR9 in cattle. Clinical and diagnostic laboratory immunology 11(1):174-185.

Goldberg, J.J., E.E. Wildman, J.W. Pankey, J.R. Kunkel, D.B. Howard, and B.M. Murphy. 1992. The influence of intensively managed rotational grazing, traditional continuous grazing, and confinement housing on bulk tank milk quality and udder health. J. Dairy Sci. 75:96-104.

Gouilleux, F., D. Moritz, M. Humar, R. Morigal, S. Berchtold and B. Grumer. 1995. Prolactin and interleukin-2 receptors in T-lymphocytes signal through a MGF-STAT5-like transcription factor. Endocrinology 136:5700-5708.

Griffiths, A.J.F., and J. Miller. 1999. Modern genetic analysis. W.H. Freeman & Co.

Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford, P.Berzi, N. Cambisano, M. Mni, S. Reid, P. Simon, R. Spelman, M. Georges, and R. Snell. 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGTA1 gene with a major effect on milk yield and composition. Genome Research 12:222-231.

Grisart, B. F. Farnir, L. Karim, N. Cambisano, J.J. Kim, A. Kvasz M. Mni, P. Simon, J.M. Frère, W. Coppietters, and M. Georges. 2004. Genetic and functional confirmation of the causality of the DGTA1 K232A quantitative trait nucleotide in affecting milk yield and composition. Proc. Natl. Acad. Sci. USA 101:2398-2403.

Gruet, P., P.Maincent, X.Berthelot, and V.Kaltsatos. 2001. Bovine mastitis and intrammamary drug delivery: review and perspectives. Advanced Drug Delivery Reviews 50:245-259.

Guérin-Faublée, V., F. Tardy, C. Bouveron, and G. Carret. 2002. Antimicrobial susceptibility of Streptococcus species isolated from clinical mastitis in dairy cows. International Journal of Antimicrobial Agents 19:219-226.

Gutierrez-Espeleta, G.A., P.W. Hedrick, S.T. Kalinowski, D. Garrigan, and W.M. Boyce. 2001. Is the decline of desert bighorn sheep from infectious disease result of low MHC variation. Heredity 86:439-450.

Haile-Mariam, M., M.E. Goddard, and P.J. Bowman. 2001. Estimates of genetic parameters for daily somatic cell count of Australian dairy cattle. J. Dairy Sci. 84:1255-1264.

Hansen, M., M.S. Lund, M.K. Sorensen, and L.G. Christensen. 2002. Genetic parameters of dairy cattle, protein yield, clinical mastitis, and other diseases in the Danish Holstein cattle. J Dairy Sci 85:445-452.

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

Harmon, R. J. 1996. Controlling contagious mastitis. Proceedings of the 1996 National Mastitis Council Regional Meeting. National Mastitis Council, p. 10.

Heringstad, B., G. Klemetsdal, and J. Ruane. 1999. Clinical mastitis in Norwegian cattle: frequency, variance components, and genetic correlation with protein yield. J. Dairy Sci. 82:1325-1330.

Heringstad, B., G. Klemetsdal, and J. Ruane. 2000. Selection for mastitis resistance in dairy cattle –a review with focus on the situation in the Nordic countries. Livest. Prod. Sci. 64:95-106.

Heringstad, B., G. Klemetsdal, and J. Ruane. 2001. Responses to selection against clinical mastitis in the Norwegian cattle population. Acta Agriculturae Scandinavica, Section A - Animal Science. 51:15-20.

Heringstad, B., G. Klemetsdal, and J. Ruane. 2001b. Variance components of clinical mastitis in dairy cattle –effects of trait definition and culling. Livest. Prod. Sci. 67:265-272.

Heringstad, B., G. Klemetsdal, and T. Steine. 2003. Selection responses for clinical mastitis and protein yield in two Norwegian dairy cattle selection experiments. J. Dairy Sci. 86:2990-2999.

Heyen, D. W., J. I. Weller, M. Ron, M. Band, J. E. Beever, E. Feldmesser, Y. Da, G. R. Wiggans, P. M. VanRaden, and H. A. Lewin. 1999. A genome scan for QTL influencing milk production and health traits in dairy cattle. Physiological Genomics 1:165-178.

Hill, W. G., and S. Knott. 1990. Identification of genes with large effects. In D. Gianola and K. Hammond (eds.), Advances of statistical methods for genetic improvement of livestock, p. 477-494. Springer-Verlag, Berlin.

Hillerton, J.E., A.J. Bramley, and R.T. Staker. 1995. Patterns of intramammary infection and clinical mastitis over a 5 year period in a closely monitored herd applying mastitis control measures. J. Dairy Research 62:39-50.

Hoblet, K.H., G.D. Schnitkey, D. Arbaugh, J.S. Hogan, K.L. Smith, P.S. Schoenberger, D.A. Todhunter, W.D. Hueston, D.E. Pritchard, G.L.Bowman, L.E. Heider, B.L. Brockett, and H.R. Conrad. 1991. Costs associated with selected preventive practices and with episodes of clinical mastitis in nine herds with low somatic cell counts. J. Am Vet Med Assoc. 199:190-196.

Hockett, M. E. 2002. Influence of experimentally-induced clinical mastitis on reproductive function of dairy cows. Ph. D. Thesis, University of Tennesse, USA.

Hogan, J. and K.L.Smith. 2003. Coliform mastitis. Vet. Res. 34:507-519.

Holmberg, M, and L. Andersson-Eklund. 2004. Quantitative trait loci affecting health traits in Swedish dairy cattle. J. Dairy Sci. 87:2653-2659.

ICAR (International Committee on Animal Recording). 2002. International agreement of recording practices. Section 7, guidelines for recording and evaluation of udder health, p.85-110.

Interbull (International Bull evaluation Service). 2002. Information on evaluations for production, conformation and udder health traits. Available in www-interbull.slu.se/udder/framesida-udder.htm

Judge, L.J., R.J. Erskine, and P.C. Bartlett. 1997. Recombinant bovine somatotropin and clinical mastitis: incidence, discarded milk following therapy, and culling. J. Dairy Sci. 80:3212-3218.

Kadarmideen, H.N., and J.E. Pryce. 2000. Effect of relationship between mastitis and somatic cell count on genetic selection for mastitis resistance in dairy cattle. Proc. British Society of Animal Science, p. 113.

Kehrli, M.E. Jr, and D.E. Shuster. 1994. Factor affecting milk somatic cell and their role in health of the bovine mammary gland. J. Dairy Sci. 77:619-627.

Kelm, S.C., J.C. Detilleux, and G. Freeman. 1997. Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. J. Dairy Sci. 80:1767-1775.

Kelton, D. F., K. D. Lissemore, and R. E. Martin. 1998. Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. J. Dairy Cattle. 81:2502-2508.

Kennedy, B.W., M. Quinton, and J.A.M. Van Arendonk. 1992. Estimation of effects of single genes on quantitative traits. J. Anim. Sci. 70:2000-2012.

Khatkar, M.S. P.C. Thomson, I. Tammen, H.W. Raadsma. 2004. Quantitative trait loci mapping in dairy cattle: review and meta-analysis. Genetics, Selection, Evolution 36:163-190.

Kirk, J.A., F. DeGraves, and J. Tyler. 1994. Recent progress in treatment and control of mastitis in cattle. J. Am Vet Med Assoc 204:1152-1158.

Kitchen, B.J. 1981. Review of the progress of dairy science: bovine mastitis: milk compositional changes and related diagnostic tests. Journal or Dairy Research 48:167-188.

Klungland H., A. Sabry, B. Heringstad, H. G. Olsen, L. Gomez-Raya, D. I. Våge, I. Olsaker, J. Ødergård, G. Klemetsdal, N. Schulman, J. Vilkki, J. Ruane, M. Aasland, K. Ronningen, S. Lien. 2001. Quantitative trait loci affecting clinical mastitis and somatic cell count in dairy cattle. Mammalian Genome 12:837-842.

Kossaibati, M.A., M. Hovi, and R.J. Esslemont. 1998. Incidence of clinical mastitis in dairy herds in England. Vet Rec. 143:649-653.

Kossaibati M. A., and R. J. Esslemont. 2000. The costs of clinical mastitis in UK dairy herds. Cattle Practice 8:323-328.

Kühn, Ch, G. Freyer, R. Weikard, T. Goldammer, and M. Shwerin. 1999. Detection of QTL for milk production traits in cattle by application of a specifically developed marker map of BTA6. Animal Genetics 30:333-340.

Kühn, Ch, J. Bennewitz, N. Reinsch, N. Xu, H. Thomsen, C. Looft, G. A. Brockmann, M. Schwerin, C. Weimann, S. Hiendleder, G. Erhardt, I. Medjugorac, M. Förster, B. Brenig, F. Reinhardt, R. Reents, I. Russ, G. Averdunk, J. Blümel, and E. Kalm. 2003. Quantitative trait loci mapping of functional traits in the German Holstein cattle population. J. Dairy Sci. 86: 360-368.

Kulberg, S., A.K. Storset, B. Heringstad, and H.J.S. Larsen. 2002. Reduced levels of total leukocytes and neutrophils in Norwegian cattle selected for decreased mastitis incidence. J. Dairy Sci. 85:3470-3475.

Laird, C. D., N. D. Pleasant, A. D. Clark, J. L. Sneeden, K. M. Anwarul Hassan, N. C. Manley, J. C. Vary Jr., T. Morgan, R. S. Hansen, and R. Stöger. 2004. Hairpin-bisulfite PCR: assessing epigenetic methylation patterns on complementary strands of individual DNA molecules. Proc. Natl. Acad. Sci. USA 101 (1): 204-209.

Leitner, G., E. Shoshani, O. Krikucks, M. Chaffer, and A. Saran. 2000. Milk leukocyte population patterns in bovine udder infection of different aetiology. J. Vet. Med B. 47:581-589.

Lien, S., L. Gomez-Raya, T. Steine, E. Fimland, and S. Rogne. 1995. Associations between casein haplotypes and milk yield traits. J. Dairy Sci. 78:2047-2056.

Lipkin, E., M. O. Mosig, A. Darvasi, E. Ezra, A. Shalom, A. Friedmann, and M. Soler. 1998. Quantitative trait locus mapping in dairy cattle by means of selective milk DNA pooling using dinucleotide microsatellite markers: Analysis of milk protein percentage. Genetics 149: 1557-1567.

Lund, M.S., J. Jensen, and P.H. Petersen. 1999. Estimation of genetic and phenotypic parameters for clinical mastitis, somatic cell production deviance, and protein yield in dairy cattle using Gibbs sampling. J. Dairy Sci. 82:1045-1051.

Lynch, M and B. Walsh. 1998. Genetics and analysis of quantitative traits. 1st. Ed. Sinanuer.

Ma, Y., D.M. Barbano, and M. Santos. 2003. Effect of CO2 addition to raw milk on proteolysis and lipolysis at 4oc. J. Dairy Sci. 86:1616-1631.

Mackay, T.F.C. 2001. The genetic architecture of quantitative traits. Annu. Rev. Genet. 35:303-339.

Mackinnon, M.J., and M.A.J. Georges. 1992. The effects of selection on linkage analysis for quantitative traits. Genetics 132: 1177-1185.

Mallard, B.A., J.C. Dekkers, M.J. Ireland, K.E. Leslie, S. Sharif, C. L. Vankampen, L. Wagter, and B.N. Wilkie. 1998. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. J Dairy Sci. 81:585-595.

Mattick, J. S. 2002. Challenging the dogma: the hidden layer of non-protein-coding RNAs in complex organisms. Bioessays 25: 930-939.

Meuwissen T.H.E., and M.E. Goddard. 2000. Fine mapping of quantitative trait loci using linkage disequilibrium with closely linked marker loci. Genetics 155:421-430.

Miglior, F, and Van Doormal, B. 2002. Global trends in international selection strategies of Holstein bulls. www.cdn.ca/committees/archives/Apr2002/Globalselectionstrategies-GEB.pdf.

Monardes, HG. 1980. Measurements of Somatic Cell Count on a lactation basis and their relationship to mastitis. M.Sc. Thesis McGill University, Canada.

Monardes, HG. 1984. Genetic and phenotypic parameters of lactation cell counts in different lactations of Holsteins cows. Ph. D. Thesis McGill University, Canada.

Monardes H., R. I. Cue, and J.F. Hayes. 1990. Correlations between udder conformation traits and somatic cell count in Canadian Holsteins cows. J. Dairy Sci. 73:1337-1342.

Morton, N. E., and C. J. Maclean. 1974. Analysis of family resemblance. III. Complex segregation of quantitative traits. Am. J. Hum. Genet. 26:489-503.

Moussaoui, F., I. Michelutti, Y. Le Roux, and F. Laurent. 2002. Mechanisms involved in milk endogenous proteolysis induced by a Lipopolysaccharide experimental mastitis. J Dairy Sci 85:2562-2570.

Mrode, R.A., G.J.T. Swanson, and C.M. Lindberg. 2001. Estimates of genetic parameters for test day somatic cell count fitting orthogonal polynomials. Proc. British Society of Animal Science, p. 15.

Mulder, H., A.F. Groen, P. Bijma, and G. de Gong. 2002. Genotype by environment interaction between automatic milking and conventional milking in Dutch dairy cattle. Proc. Colloquium Animal Breeding and Genetics, Wageningen.

Nadesalingam, J. 1999. Mapping and detection of quantitative trait loci (QTL) for milk production traits based on linkage analysis using microsatellite markers in Canadian Holstein cattle. Ph. D. thesis. Guelph University.

Nadesalingam, J., Y. Plante, and J. P. Gibson. 2001. Detection of QTL for milk production on chromosomes 1 and 6 of Holstein cattle. Mamm. Genome 12:27-31

Nash, D.L., G.W. Rogers, J.B. Cooper, G.L. Hargrove, J.F. Keown, and L.B. Hansen. 2000. Heritability of clinical mastitis incidence and relationships with sire transmitting abilities for somatic cell score, udder type traits, productive life, and protein yield. J. Dairy Sci. 83:2350-2360.

Neimann-Sorensen, A., and A. Robertson. 1961. The association between blood groups and several production characteristics in three Danish Cattle Breeds. Acta Agriculture Scan. 11:163-196.

Nelson, D. L., and M. M. Cox. 2000. Lehninger principles of biochemistry. Worth Publishers, 3rd ed.

Nickerson, S. C., W. E. Owens, and R. L. Boddie. 1995. Mastitis in dairy heifers: initial studies on prevalence and control. J. Dairy Sci. 78: 1607-1618.

Nikolic, J.A., M. Kulcsar, L. Katai, O. Nedic, Sz. Janosi, and Gy. Huszenicza. 2003. Periparturient endocrine and metabolic changes in healthy cows. J. Vet. Med. A. 50:22-29.

Ødergård, J., J. Jensen, G. Klemetsdal, P. Madsen, and B. Heringstad. 2002. Genetic analyses of somatic cell scores in Norwegian cattle with a test-day sire model allowing for a time trend in genetic (co)variance. Communication 13-28. 7th World Congress on Genetics Applied to Livestock Production.

Ødergård, J., B. Heringstad, and G. Klemetsdal. 2003. Short communication: bivariate genetic analysis of clinical mastitis and somatic cell count in Norwegian dairy cattle. J. Dairy Sci. 87:3515-3517.

Olsen, H. G., S. Lien, M. Svendsen, H. Nielsen, A. Roseth, M. Aasland Opsal, and T. H. E. Meuwissen. Fine mapping of milk production QTL on BTA6 by combined linkage and linkage disequilibrium analysis. J. Dairy Sci. 87:690-698.

Paape, M., J. Mehrzad, X. Zhao, J. Detilleux, and C. Burvenich. 2002. Defense of the bovine mammary gland by polymorphonuclear neutrophil leukocytes. J. Mamm. Gland. Biol. And Neoplasia 7:109-121.

Paape, M. J., D. D. Bannerman, X. Zhao, and J.W. Lee. 2003. The bovine neutrophil: Structure and function in blood and milk. Vet. Res. 34:597-627.

Pan, Y., P.J. Boettcher, and J.P. Gibson. 2001. Bayesian segregation analysis of somatic cell scores of Ontario Holstein cattle. J. Dairy Sci, 84:2796-2802.

PATLQ. 1997. Rapport de production du Programme d'analys troupeaux laitier du Québec, p. 48.

PATLQ. 1998. Rapport de production du Programme d'analys troupeaux laitier du Québec, p. 50.

PATLQ. 1999. Rapport de production du Programme d'analys troupeaux laitier du Québec, p. 55.

PATLQ. 2000. Rapport de production du Programme d'analys troupeaux laitier du Québec, p. 63.

PATLQ. 2001. Rapport de production du Programme d'analys troupeaux laitier du Québec, p. 62.

PATLQ. 2002. Rapport de production du Programme d'analys troupeaux laitier du Québec, p. 64.

PATLQ. 2003. Rapport de production du Programme d'analys troupeaux laitier du Québec, p. 67.

Peeler, E.J., Green M. J., Fitzpatrick J.L., Morgan K.L. and Green L.E. 2000. Risk factors associated with clinical mastitis in low somatic cell count British herds. J. Dairy Sci. 83:2464-2472.

Philipsson, J., G. Ral, and B. Berglund. 1995. Somatic cell count as a selection criterion for mastitis resistance in dairy cattle. Livest. Prod. Sci. 41:195-200.

Pösö, J., and E. A. Mäntysaari. 1996. Relationships between clinical mastitis, somatic cell score, and production for the first three lactations of Finnish Ayrshire. J. Dairy Sci. 79:1284-1291.

Postel-Vinay, M.C., V.M. Coelho, M.C. Gagnerault, and M. Dardenne. 1997. Growth hormone stimulates the proliferation of activated mouse T lymphocytes. Endocrinology 138:1816-820.

Pyörälä, S. 2003. Indicators of inflammation in the diagnosis of mastitis. Vet. Res. 34:565-578.

Rajala-Schultz, P.J., Y.T. Grohn, C.E. McCulloch, and C.L. Guard. 1999. Effects of clinical mastitis on milk yield in dairy cows. J Dairy Sci. 82:1213-1220.

Reents R., J. Jamrozik, L.R. Schaeffer, and J.C. M. Dekkers. 1995. Estimation of genetic parameters for test day records of somatic cell score. J. Dairy Sci. 78:2847-2857

Reinsch, N., N. Xu, H. Thomsen, C. Looft, E. Kalm, S. Grupe, C. Kühn, M. Shwerin, B. Leyhe, S. Hiendleder, G. Erhard, I. Medjugorac, I. Russ, M. Förster, B. Brenig, R. Reents, and G. Averdunk. 1998. First results on somatic cell count loci from the ADR bovine mapping project. 6th World Congress on Genetics Applied to Livestock Production.

Renaville, R., N. Gengler, E. Vrech, A. Prandi, S. Massart, C. Corradini, B. Bertozzi, F. Mortiaux, A. Burny, and D. Portelle. 1997. Pit-1 gene polymorphism, milk yield, and conformation traits for Italian Holstein-Friesian bulls. J. Dairy Sci. 80:3431-3438.

Richard, M. 2002. Fine-mapping of a QTL on chromosome 20 in Holsteins. M.Sc. Thesis McGill University, Canada.

Riquet, J., W. Coppieters, N. Cambisano, J.J. Arrantz, P. Berzi, S.K. Davis, B. Grisart, F. Farnir, L. Karim, M. Mni, P. Simon, J. F. Taylor, P. Vanmanshoven, D. Wagenaar, J. E. Womack, and M. Georges. 1999. Fine-mapping of quantitative trait loci by identity by descent in outbred populations: Application to milk production in dairy cattle. Proc. Natl. Acad. Sci. USA 96:9252-9257.

Rodriguez-Zas, S. L., B. R. Southey, D. W. Heyen, and H. A. Lewin. 2002. Interval and composite interval mapping of somatic cell score, yield, and components of milk in dairy cattle. J. Dairy Sci. 85:3081-3091.

Ron, M, E. Feldmesser, M. Golik, I. Tager-Cohen, D. Kliger, V. Reiss, R. Domochovsky, O. Alus, E. Seroussi, E. Ezra, and J. I. Weller. 2004. A complete genome scan of the Israeli Holstein population for quantitative trait loci by a daughter design. J. Dairy Sci. 87:476-490.

Ronin, Y.I., Korol, A.B., and E. Nevo. 1999. Single- and multiple-trait mapping analysis of linked quantitative trait loci: some asymptotic analytical approximations. Genetics 151:387-396.

Rupp, R., and D. Boichard. 1999. Genetic parameters for clinical mastitis, somatic cell score, production udder type traits, and milking ease in first lactation Holsteins. J. Dairy Sci. 82: 2198-2202.

Rupp, R., F. Beaudeau, and D. Boichard. 2000. Relationship between milk somatic-cell counts in the first lactation and clinical mastitis occurrence in the second lactation of French Holstein cows. Prev. Vet. Med. 46:99-111.

Rupp, R., and D. Boichard. 2003. Genetics of resistance to mastitis in dairy cattle. Vet. Res. 34:671-688.

Sabour, M.P., C.Y. Lin, and C. Smith. 1997. Association of genetic variants of bovine growth hormone with milk production traits in Holstein cattle. J. Anim. Breed. Genet. 114:435-442.

Sander-Nielsen, U., G. A. Pedersen, J. Pedersen, and J. Jensen. 1997. Genetic correlations among health traits in different lactations. Pages 68–77 *in* Proc. Int. Workshop Genet. Improvement Functional Traits Cattle; Health. INTERBULL Bull. No. 15. Int. Bull Eval. Serv., Uppsala, Sweden.

Sandholm M. T. Honkanen-Buzalski, and R. Kangasniemi. 1984. Milk trypsin-inhibitor capacity as an indicator of bovine mastitis –a novel principle which can be automated. Journal of Dairy Research 51:1-9.

Santos, M.V., Y. Ma, and Barbano D.M. 2003. Effect of somatic cell count on proteolysis and lipolysis in pasteurized fluid milk during shelf-life storage. J. Dairy Sci. 86:2491-2503.

Sax, K. 1923. The association of size differences with seed-coat pattern and pigmentation in Phaseolus vulgaris. Genetics 8:552.

Schukken, Y.H., K.E. Leslie, D.A. Barnum, B.A. Mallard, J.H. Lumsden, P.C. Dick, G.H. Vessie, and M.E. Kehrli. 1999. Experimental Staphylococcus aureus intramammary challenge in late lactation dairy cows: quarter and cow effects determining the probability of infection. J. Dairy Sci. 82:2393-2401.

Schulman, N. F., S. M. Viitala, D. J. de Koning, J. Virta, A. Mäki-Tanila, and J. H. Vilkki. 2004. Quantitative trait loci for health traits in Finís Ayrshire cattle. J. Dairy Sci. 87:443-449.

Sharif, S., B.A. Mallard, and B.N. Wilkie. 1998. Associations of the bovine major histocompatibility complex DRB3 (BoLA-DRB3) alleles with occurrence of disease and milk somatic cell score in Canadian Dairy Cattle. Animal Genetics 29:185-193.

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.

Smirnov, D., A.Bruzel, M. Morley, and V. G. Cheung. 2004. Direct IBD mapping: identical-by-descent mapping without genotyping. Genomics 83(2): 335-345.

Sordillo, L.M., K. Shaferweaver, and D. DeRosa. 1997. Immunobiology of the mammary gland. J. Dairy Sci. 80:1851-1865.

Spelman, R.J., and H. Bovenhuis. 1998. Moving from QTL experimental results to the utilization of QTL in breeding programs. Animal Genetics 29:77-84.

Springbett, A. J., K. MacKenzie, J.A. Williams, and S.C. Bishop. 2003. The contribution of genetic diversity to the spread of infectious diseases in livestock populations. Genetics 165:1465-1474.

Srinivasan, M. and J. A. Lucey. 2002. Effects of added plasmin on the formation and rheological properties of rennet-induced skim milk gels. J. Dairy Sci. 85:1070-1078.

Stradberg, E, and G. E. Shook. 1989. Genetic and economic responses to breeding programs that consider mastitis. J. Dairy Sci. 72:2136.

Sviland, S., and S. Waade. 2000. Diagnostic alternatives used by veterinary surgeons for the recording of udder diseases in the Norwegian health card system for cattle. Acta Vet. Scand. 41:261-271.

Swalve, H.H. 2000. Review of world selection indices used in dairy cattle. 51st annual meeting of the EAAP. Poster G2:15.

Thompson, R. 1971. Sire evaluation. Biometrics 35: 339-353.

Tizard, I. 1996. Veterinary immunology: an introduction. 5th ed. W.E. Saunders Co.

Tucker, H.A. 2000. Hormones, mammary growth, and lactation: a 41-year perspective. J. Dairy Sci. 83:874-884.

Urech, E., Z. Puhan, and M. Schällibaum. 1999. Changes in milk protein fraction as affected by subclinical mastitis. J. Dairy Sci. 82:2402-2411.

Uribe, H.A., B.W. Kennedy, S.W. Martin, and D.F. Kelton. 1995. Genetic parameters for common health disorders of Holstein cows. J. Dairy Sci. 78:421-430.

Van Dorp, T.E., J.C.M. Dekkers, S.W. Martin, J.P.T.M. Noordhuizen. 1996. Genetic parameters of health disorders, and relationships with 305-day milk yield and conformation traits of registered Holstein cows. J Dairy Sci 81:2264-2270.

Van Tassell, C. P., M. S. Ashwell, and T. S. Sonstegard. 2000. Detection of putative loci affecting milk, health, and conformation traits in a US Holstein population using 105 microsatellite markers. J. Dairy Sci. 83:1865-1872.

Verdi, R. J. and D. M. Barbano. 1991. Properties of proteases from milk somatic cells and blood leukocytes. J Dairy Sci 74:2077-2081.

Visscher, P.M., D. Smith, S.J.G. Hall, and J.L. Williams. 2001. A viable herd of genetically uniform cattle. Nature 409:303.

Waage, S, S. Sviland, and S.A. Odegaard. 1998. Identification of risk factors for clinical mastitis in dairy heifers. J. Dairy Sci. 81:1275-1284.

Wanner, J. M., G.W. Rogers, M.E. Kehrli, and J.B. Cooper. 1998. Intramammary infections in primiparous Holsteins: heritabilities and comparisons of bovine leukocyte adhesion deficiency carriers and noncarriers. J. Dairy Sci. 81:3293-3299.

Weigent, D.A., J.E. Blalock, and R.D. LeBoeuf. 1991. An antisense oligodeoxynucleotide to growth hormone messenger ribonucleic acid inhibits lymphocyte proliferation. Endocrinology 128:2053-2057.

Weller, J.I., Y. Kashi, and M. Soller. 1990. Power of "daughter" and "granddaughter" designs for genetic mapping of quantitative traits in dairy cattle using genetic markers. J. Dairy Sci. 73:2525-2537.

Weller, J. I. 2001. Quantitative trait loci analysis in animals. 1st edition. CABI Publishing.

Weller, J.I., M. Golik, E. Seroussi, E. Ezra, and M. Ron. 2003. Population-wide analysis of a QTL affecting milk-fat production in the Israeli Holstein population. J. Dairy Sci. 86:2219-2227.

Wells, S. J., and S. L. Ott. 1998. What is current milk quality in US?. 37th National Mastitis Council Annual Meeting, p. 10.

Wenz, J.R., G.M. Barrington, F.B. Garry, K.D. McSweeney, R.P. Dinsmore, G. Goodell, and R.J. Callan. 2001. Bacteremia associated with naturally occurring acute coliform mastitis dairy cows. J. Am Vet Med Assoc 219:976-981.

Westell, R. A., and L. D. Van Vleck. 1987. Simultaneous genetic evaluation of sires and cows for a large population of dairy cattle. J .Dairy Sci. 70:1006-1017.

Westell, R. A., R. L. Quass, and L. D. Van Vleck. 1988. Genetic groups in an animal model. J. Dairy Sci. 71:1310-1318.

White, D.G. and P.F. McDermott. 2001. Emergence and transfer of antibacterial resistance. J. Dairy Sci. 84 (E. suppl.):E151-E155.

Yao, J, S.E. Aggrey, D. Zadworny, F.J. Hayes, and U. Kuhnlein. 1996. Sequence variations in the bovine Growth hormone Gene characterized by Single-Strand conformation polymorphism (SSCP) analysis and their association with milk production traits in Holsteins. Genetics 144:1809-1816.

Yao J., S. E. Aggrey, D. Zadworny, U. Kuhlein, and J.F. Hayes. 1998. Restriction length polymorphisms at the ornithine decarboxylase locus associated with milk protein yield in Holsteins. DNA Sequence –The Journal of sequencing and mapping, 8(4):203-213.

Zadworny, D. and U. Kuhnlein. 1990. The identification of the kappa-casein genotype in Holstein dairy cattle using the polymerase chain reaction. Theor Appl Genet 80:631-634.

Zhang, Q., D. Boichard, I. Hoeschele, C. Erndt, A. Eggen, B. Murkve, M. Pfister-Genskow, L. A. Witte, F. E. Grignola, P. Uimari, G. Thaller, and M. Bishop. 1998. Mapping quantitative trait loci for milk production ad health of dairy cattle in a large outbred pedigree. Genetics 149: 1959-1973.

APPENDIXES

APPENDIX 1 GENERATIONS INTERVAL PER EACH PATH OF SELECTION

Table 7. 1 Mean of calving interval for each path of selection according year of birth of the parent (For each selection path, columns are: Year of birth, number of animals, generation interval mean (months)

Dam to cows			Dam to bulls			Sire cows			Sire bull		
1916	1	24.0000	1920	1	24.000	1916	1	91.000	1920	1	24.000
1918	1	24.0000	1924	1	84.000	1918	1	79.000	1924	1	84.000
1924	1	24.0000	1925	1	77.000	1924	1	24.000	1925	1	120.000
1925	1	24.0000	1927	1	24.000	1925	2	116.500	1927	1	145.000
1926	3	40.6667	1930	1	65.000	1926	4	36.250	1931	2	90.000
1927	1	24.0000	1931	3	52.000	1927	1	53.000	1932	5	41.800
1928	3	24.0000	1932	4	87.750	1928	3	67.333	1933	6	50.333
1929	2	36.0000	1933	7	78.000	1929	2	42.000	1934	8	73.875
1930	2	47.0000	1934	10	61.900	1930	2	53.000	1935	2	19.000
1931	1	73.0000	1935	3	115.000	1931	1	25.000	1936	12	43.667
1932	6	90.0000	1936	16	66.938	1932	5	38.800	1937	8	49.000
1933	6	91.0000	1937	8	56.000	1933	6	83.000	1938	12	63.000
1934	7	89.4286	1938	13	91.462	1934	9	42.333	1939	12	49.750
1935	8	63.0000	1939	15	67.333	1935	13	68.692	1940	28	52.071
1936	12	99.5000	1940	32	71.219	1936	16	50.438	1941	29	70.379
1937	12	76.3333	1941	33	86.818	1937	21	52.143	1942	21	63.190
1938	10	81.6000	1942	25	91.600	1938	41	48.073	1943	30	69.333
1939	8	92.8750	1943	30	85.300	1939	21	69.571	1944	34	63.588
1940	17	89.4118	1944	38	92.658	1940	41	55.707	1945	44	65.227
1941	22	68.0000	1945	48	75.646	1941	42	70.357	1946	58	64.017
1942	40	62.6500	1946	58	81.931	1942	98	59.765	1947	84	66.024
1943	64	76.5781	1947	93	87.882	1943	83	59.084	1948	115	68.600
1944	141	60.6241	1948	118	83.085	1944	200	54.215	1949	94	66.979
1945	272	66.5368	1949	98	80.255	1945	323	56.950	1950	188	63.330
1946	521	62.1228	1950	163	95.546	1946	572	50.731	1951	253	66.067
1947	930	61.6527	1951	224	91.625	1947	999	48.649	1952	262	71.164
1948	1414	61.0481	1952	234	89.991	1948	1450	49.053	1953	267	68.067
1949	2187	60.1166	1953	244	91.373	1949	2263	49.877	1954	330	63.391
1950	3159	59.2830	1954	289	87.678	1950	3234	52.338	1955	372	68.758
1951	4312	57.6264	1955	338	89.657	1951	4345	53.823	1956	390	77.867
1952	6190	55.7514	1956	347	91.818	1952	6249	54.457	1957	431	72.991

1953	8093	57.9545	1957	378	91.825	1953	8148	57.001	1958	481	74.258
1954	10736	59.1909	1958	434	89.836	1954	10772	58.181	1959	591	70.821
1955	14242	59.4654	1959	574	86.263	1955	14332	61.107	1960	641	67.827
1956	17542	60.6909	1960	631	88.853	1956	17654	62.383	1961	688	69.795
1957	19157	61.4485	1961	693	82.556	1957	19284	62.390	1962	745	77.200
1958	21217	62.1861	1962	755	86.175	1958	21458	64.963	1963	795	81.370
1959	22274	61.5754	1963	800	87.333	1959	22684	66.570	1964	953	84.030
1960	23403	61.5710	1964	965	86.989	1960	24039	64.889	1965	1060	87.890
1961	25533	61.7877	1965	1068	85.810	1961	26511	66.638	1966	1111	85.680
1962	25968	62.0303	1966	1127	83.447	1962	27247	69.217	1967	1247	94.735
1963	24985	62.1569	1967	1268	84.024	1963	27168	71.783	1968	1315	94.981
1964	24430	62.5106	1968	1337	84.475	1964	30340	73.728	1969	1541	101.369
1965	22090	63.3416	1969	1557	84.924	1965	31330	75.639	1970	1841	101.179
1966	28071	63.1380	1970	1874	85.123	1966	33562	77.526	1971	1872	100.698
1967	32869	63.5823	1971	1917	84.556	1967	36802	80.175	1972	2223	106.704
1968	34446	64.0993	1972	2255	83.951	1968	39446	83.059	1973	2327	109.320
1969	35470	64.1985	1973	2375	83.398	1969	41270	81.542	1974	2274	111.558
1970	39591	64.3349	1974	2356	81.239	1970	47156	81.570	1975	2157	111.652
1971	42970	64.8129	1975	2273	80.8196	1971	53624	82.6318	1976	2317	116.514
1972	47881	64.0886	1976	2504	78.3367	1972	61921	85.8392	1977	2365	121.943
1973	49805	63.7235	1977	2617	79.9373	1973	67407	87.7672	1978	3080	124.040
1974	54188	63.2809	1978	3234	81.2959	1974	74865	87.1080	1979	4016	119.656
1975	56159	62.9562	1979	4008	80.4721	1975	78928	85.8603	1980	4203	117.029
1976	64400	62.3509	1980	4199	82.3365	1976	87619	86.6958	1981	4301	114.320
1977	76659	61.5230	1981	4286	83.3689	1977	102999	88.6639	1982	4378	110.940
1978	106668	62.1015	1982	4362	82.2315	1978	139764	91.3822	1983	4338	107.387
1979	116875	60.4833	1983	4314	81.3414	1979	158568	91.7793	1984	3900	114.718
1980	121846	59.6797	1984	3884	80.2760	1980	169855	90.1610	1985	3782	113.389
1981	134025	58.6460	1985	3764	78.0635	1981	188218	89.1463	1986	3804	108.533
1982	142577	58.0483	1986	3789	75.4605	1982	200108	88.8745	1987	4001	110.798
1983	144525	57.7578	1987	3977	76.0981	1983	203871	85.3486	1988	3866	104.596
1984	148126	56.9811	1988	3851	74.3770	1984	211054	86.7949	1989	3520	104.971
1985	149079	56.4485	1989	3504	70.9332	1985	213070	87.2837	1990	3356	96.934
1986	149438	55.5312	1990	3327	68.8073	1986	214152	85.9122	1991	3389	90.853
1987	151223	55.2044	1991	3366	65.9480	1987	216457	85.9890	1992	3483	93.417
1988	155128	54.9658	1992	3447	62.4352	1988	220244	84.8949	1993	3754	90.668
1989	153878	54.7826	1993	3735	60.2578	1989	220593	85.5612	1994	5485	95.443
1990	157180	54.2165	1994	5463	61.3947	1990	238160	84.2820	1995	5233	94.529
1991	160097	53.4799	1995	5207	61.3248	1991	238067	81.0597	1996	4479	92.673
1992	167330	51.0185	1996	4472	63.2847	1992	227137	76.6066	1997	4078	83.072
1993	179357	49.1601	1997	4086	64.7093	1993	223829	75.3556	1998	3940	89.575
1994	196126	48.8408	1998	3946	63.3702	1994	231155	77.2479	1999	2171	91.028
1995	209807	50.4888	1999	2169	62.6713	1995	237349	77.1911			
1996	209845	50.7505				1996	231243	77.9958			

1997	220950	50.8357	1997	237383	70.6978
1998	217886	51.2323	1998	230541	71.8640
1999	142098	51.1332	1999	149009	72.9905

APPENDIX 2 GRAPHICAL REPRESENTATIONS OF THE POLYMORPHISMS

GH1 (Yao et al, 1996)

Figure 7. 1 Genetic map of BTA 19

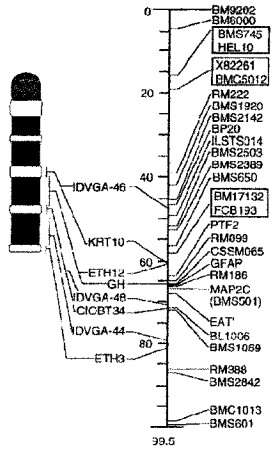
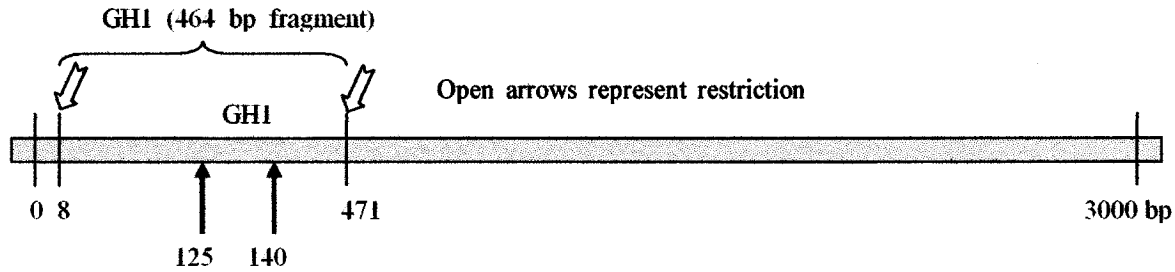


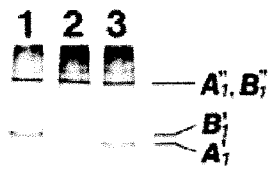
Figure 7. 2 Description of the localization of the polymorphisms on GH: GH1



Allele + = deletion of TGC repeat (5 repeats between position 125-140)

Allele - = insertion of TGC repeat (6 repeats between position 125-142)

Figure 7. 3 Polimorphisms on GH1



Polymorphisms GH1-258 and GH1-300 were obtained by direct sequencing. GH1-258 consists in a C → T in position 258; GH1-300 consists in a C → T in position 300.

GH41 and GH42 (Yao et al, 1996)

Figure 7. 4 Description of the localization of the polymorphisms on GH : GH4

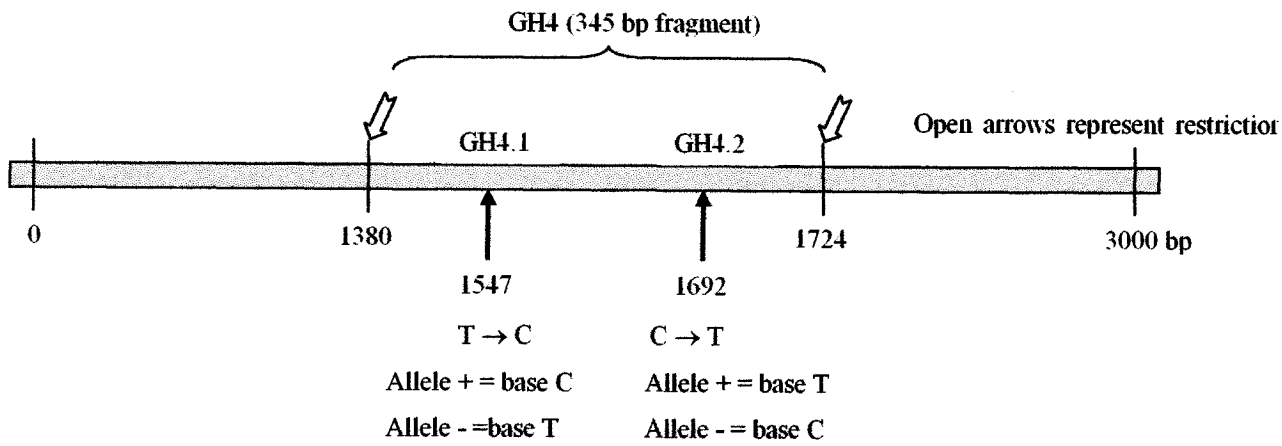
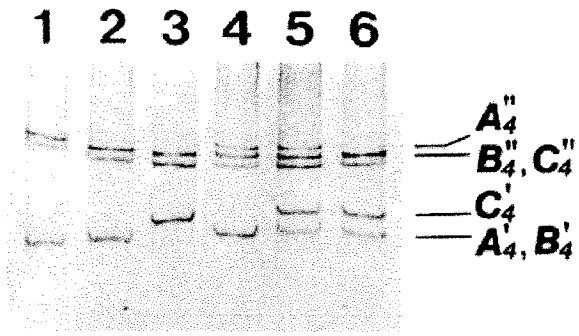
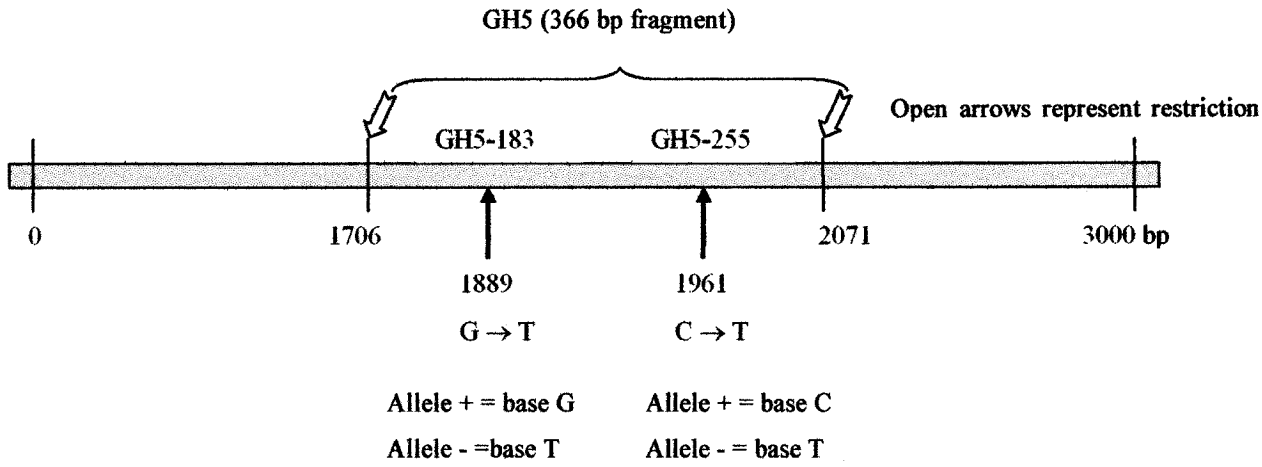


Figure 7. 5 Polymorphisms GH 4.1 and 4.2



GH5-183 and GH5-255

Figure 7. 6 Description of the localization of the polymorphisms on GH: GH5



Polymorphisms GH5-183 and GH5-255 were obtained by direct sequencing. GH5-183 consists in a G → T in position 183; GH5-255 consists in a C → T in position 255.

GH61 and GH62 (Yao et al, 1996)

Figure 7. 7 Description of the localization of the polymorphisms on GH

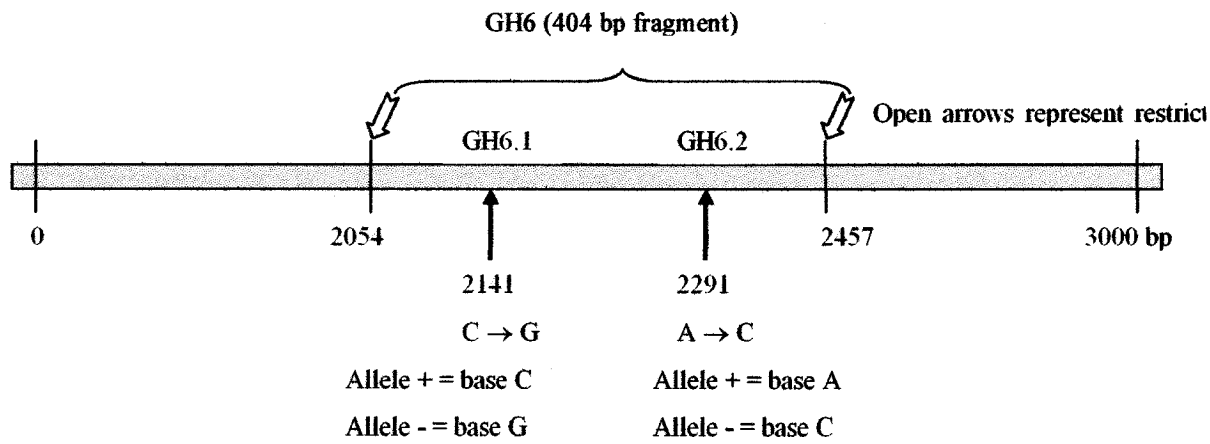
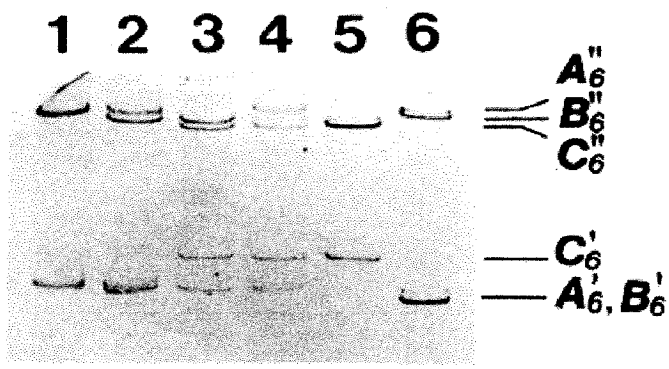


Figure 7. 8 Polymorphisms GH6.1 and GH6.2



GHR22-StuI, GHR21-AccI and GHR1-AluI (Aggrey et al, 1999)

Figure 7. 9 Genetic map BTA20

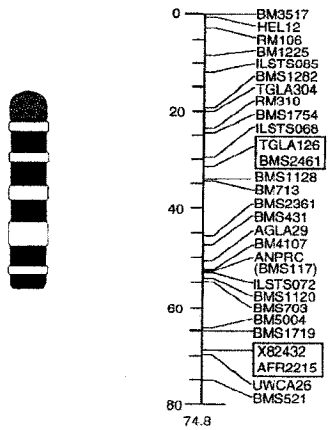


Figure 7. 10 Description of the localization of the polymorphisms on GHR

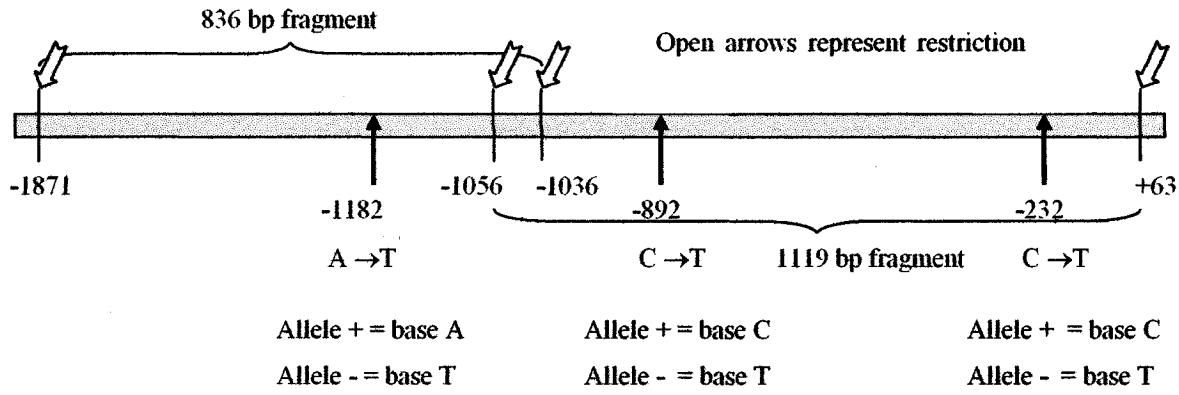
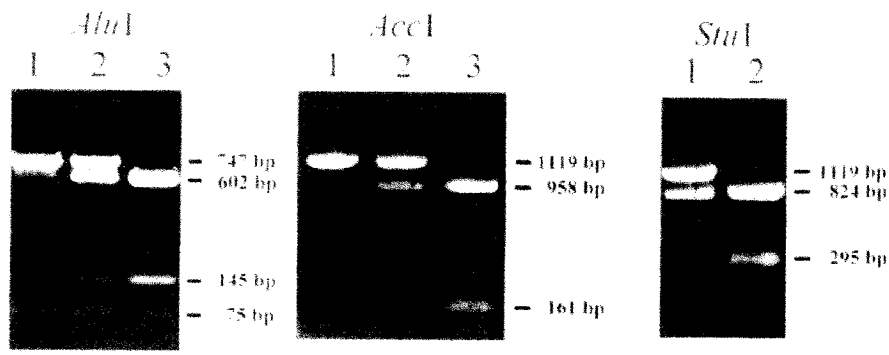


Figure 7. 11 Polymorphisms on GHR



ODC (Yao et al, 1998)

Figure 7. 12 Genetic map BTA 11

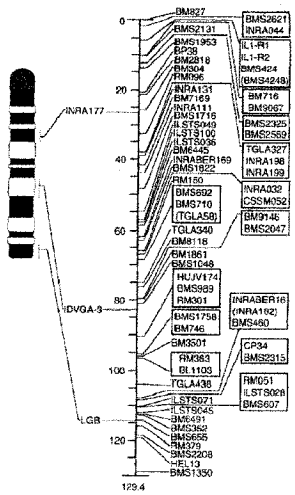


Figure 7. 13 Description of the localization of the polymorphisms on ODC

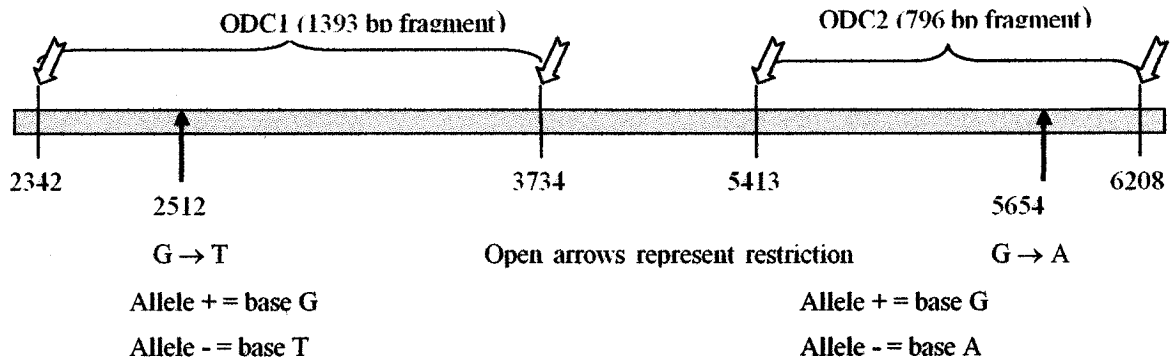


Figure 7. 14 Polymorphisms ODC

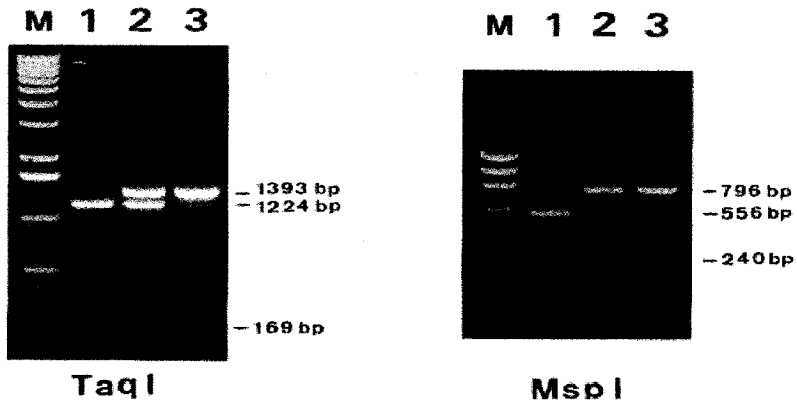


Figure 7. 15 Definition line for the FASTA files with the sequence used to obtain polymorphisms for ACTH, CRH, PRL and IGF-1. For each gene a segment of the sequence containing the polymorphisms (signaling the base substitution) is provided.

ACTH

```
>gi|163119|gb|M23813.1|BOVGROWP Bos taurus adrenocorticotropic hormone
(ACTH) mRNA, complete cds

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        560        570        580        590        600
ACTH gi|16      GGCGAGGAAG GAGGTTTCAA AAGGGGAAAA GGTTTCCCCT GGGTCAAGGC
                                      A
                                   ACTH-341

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        610        620        630        640        650
ACTH gi|16      TGGACTGCTA GAAAGAGGGA GAGAGCAGGG ATCTGTCTCC CCAAGCCCAC
                                                                  C
                                                              ACTH-388
```

CRH

```
>gi|15077524|gb|AF340152.1| Bos taurus corticotrophin-releasing hormone
precursor, gene, exon 2 and complete cds

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        410        420        430        440        450
CRH gi|150      GGAGAACGCC CTCGGCAGCC GCCAGGAGGC GCCGGCCGCC AGGAAGAGGC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        460        470        480        490        500
CRH gi|150      GATCCCAGGA ACCTCCCATC TCCCTGGATC TCACCTTCCA CCTCCTCCGA
                                                                     G
                                                                 CRH-291

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        510        520        530        540        550
CRH gi|150      GAAGTCTTGG AAATGACCAA GGCCGATCAG TTAGCACAGC AAGCTCATAR

                ....|....| ....|....| ....|....| ....
                        560        570        580
CRH gi|150      CAAYAGGAAA CTGTTGGACA TTGCTGGGAA ATGA
```

Figure 15. ...Continued

PRL

>gi|16755686|gb|AF426315.1|AF426315 Bos taurus prolactin precursor (PRL) gene, complete cds

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                        9010       9020       9030       9040       9050
PRL gi|167      TGGCTCCAAA ATCCAAGTGT AGAGACTTTC ATGTATCTTC CCTAATTTTT
                                 A
                               PRL-152

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        9060       9070       9080       9090       9100
PRL gi|167      AATTTGATAA ATAGAAAGAA CAAAGATGAG CTAATACTAC TAAAACTCAT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        9110       9120       9130       9140       9150
PRL gi|167      AATAACTCAT TATCTTTTGG ATGTTTAGGT TATTCCTGGA GCCAAAGAGA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        9160       9170       9180       9190       9200
PRL gi|167      CTGAGCCCTA CCCTGTGTGG TCAGGACTCC CGTCCCTGCA AACTAAGGAT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        9210       9220       9230       9240       9250
PRL gi|167      GAAGATGCAC GTTATTCTGC TTTTTATAAC CTGCTCCACT GCCTGCGCAG
                                 C
                               PRL-361
```

IGF1

>gi|8100788|gb|AF210383.1|AF210383S1 Bos taurus insulin-like growth factor I (IGF1) gene, exon 1

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                        1360       1370       1380       1390       1400
IGF1 gi|81      TCTTGTTTTT TAAATTTTGT GTTGGCTCTG GAATATAAAA TTGCTCGCCC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        1410       1420       1430       1440       1450
IGF1 gi|81      ATCCTCTACG AATATTCCTT TCATACGGGT AAGGTGTATT AGCAGATGTG
                      C
                    IGF-390

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        1460       1470       1480       1490       1500
IGF1 gi|81      TGTGTCTTCA CGCCCGGTAG AAAGTTAATC AGAGGACAGC ATCAGTATTT
```

APPENDIX 3 COMPLETE RESULTS FROM ACROSS POPULATION ANALYSES

Table 7. 2 Across population analysis for Clinical mastitis (first lactation). Estimates of ETA for incidence of clinical mastitis by each genotype (+/+, +/-, and -/-) (Estimate, S.E.). Degrees of freedom, F value and *P-value* for the contrast +/+ vs -/-.

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	0.1679 (2.8979)	0.1192 (0.9109)	0.08796 (0.7218)	1	678	0.00	0.9787
Odc2	0.1082 (0.6630)	0.08493 (0.9516)	0.05861 (5.4905)	1	717	0.00	0.9928
GHRal	0.08190 (0.9395)	0.1131 (0.7901)	0.1291 (1.3097)	1	697	0.00	0.9766
GHRac	0.1202 (0.7820)	0.08438 (0.8414)	0.1073 (1.9952)	1	693	0.00	0.9952
GHRst	0.1054 (0.5764)	0.06482 (1.8300)	0.4724 (10.2738)	1	697	0.00	0.9716
GH61	0.1020 (185.03)	0.08537 (463.64)	0.08537 (463.64)	1	706	0.00	0.9948
GH62	0.1076 (0.6840)	0.1076 (0.6840)	0.1076 (0.6840)	1	652	0.00	0.9862
GH1	0.1077 (2.3562)	0.07332 (1.0708)	0.09843 (1.5484)	1	307	0.00	0.9974
GH41	0.1081 (0.6729)	0.08733 (1.1821)	0.08234 (4.3796)	1	625	0.00	0.9954
GH42	0.09366 (0.7901)	0.1169 (1.4982)	0.2061 (4.0286)	1	442	0.00	0.9782
GH1258	0.1126 (1.8598)	0.1204 (0.9641)	0.1276 (1.2189)	1	427	0.00	0.9946
GH1300	0.1133 (1.2691)	0.1223 (0.9976)	0.1284 (1.6041)	1	422	0.00	0.9941
GH5183	0.1936 (5.1353)	0.07142 (1.1860)	0.1164 (0.6743)	1	619	0.00	0.9881
GH5255	0.09044 (1.1860)	0.1208 (0.8133)	0.09956 (1.2021)	1	612	0.00	0.9957
ACTH388	0.1214 (0.7062)	0.08591 (1.3205)	0.06247 (4.5934)	1	551	0.00	0.9899
ACTH341	0.1228 (1.3260)	0.1174 (0.9516)	0.06321 (1.3725)	1	462	0.00	0.9751
PRL152	0.1503 (10.2694)	0.07636 (1.4525)	0.1034 (0.6457)	1	605	0.00	0.9964
PRL361	0.09661 (0.7037)	0.08906 (1.1080)	0.1760 (4.3688)	1	603	0.00	0.9857
CRH291	0.08836 (0.8500)	0.1318 (1.0170)	0.01527 (2.6520)	1	523	0.00	0.9791
IGF390	0.1372 (1.3486)	0.07306 (0.8457)	0.1372 (1.1043)	1	581	0.00	1.0000

Table 7. 3 Across population analysis for Culling due to mastitis (first lactation). Estimate of ETA for incidence of clinical mastitis in first lactation by each genotype (Estimate ± s.e.). F value and *P-value* for the contrast +/+ *vs* -/-.

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	0.03118 (1.8356)	0.01584 (0.5770)	-0.00724 (0.457)	1	678	0.00	0.9838
Odc2	0.00854 (0.4195)	-0.01208 (0.602)	0.02357 (3.4740)	1	717	0.00	0.9966
GHRal	-0.00268 (0.5945)	0.005087 (0.4999)	0.01503 (0.8287)	1	697	0.00	0.9862
GHRac	0.004915(0.4948)	-0.00144 (0.5324)	0.005973 (1.2625)	1	693	0.00	0.9994
GHRst	0.000169 (0.3644)	0.02459 (1.1568)	0.08426 (6.4928)	1	697	0.00	0.9897
GH61	0.005376 (0.3724)	-0.01773 (0.9332)	-0.01798 (5.3070)	1	706	0.00	0.9965
GH62	0.005298 (0.4328)	-0.00401 (0.6794)	-0.03660 (2.0057)	1	652	0.00	0.9837
GH1	0.01419 (1.4909)	-0.00963 (0.6776)	-0.01156 (0.9798)	1	307	0.00	0.9885
GH41	0.004106 (0.4258)	-0.00237 (0.7480)	-0.02959 (2.7712)	1	625	0.00	0.9904
GH42	0.004298 (0.4999)	0.005069 (0.9480)	0.02421 (2.5491)	1	442	0.00	0.9939
GH1258	0.01147 (1.1768)	0.01298 (0.6100)	0.02720 (0.7713)	1	427	0.00	0.9911
GH1300	0.001947 (0.8030)	0.01803 (0.6312)	0.03695 (1.0150)	1	422	0.00	0.9784
GH5183	0.01613 (3.2494)	-0.00972 (0.7504)	0.007649 (0.4267)	1	619	0.00	0.9979
GH5255	-0.01222 (0.7504)	0.009278 (0.5146)	0.009084 (0.7607)	1	612	0.00	0.9841
ACTH388	0.01112 (0.4469)	-0.01427 (0.8355)	-0.07558 (2.9065)	1	551	0.00	0.9765
ACTH341	0.01655 (0.8390)	0.005192 (0.6021)	-0.02624 (0.8685)	1	462	0.00	0.9717
PRL152	0.04906 (6.4985)	0.002045 (0.9191)	-0.00012 (0.4086)	1	605	0.00	0.9940
PRL361	0.003010 (0.4463)	-0.00896 (0.7029)	0.008950 (2.7712)	1	603	0.00	0.9983
CRH291	-0.00700 (0.5379)	0.006442 (0.6435)	0.006508 (1.6780)	1	523	0.00	0.9939
IGF390	0.009513 (0.8534)	-0.00467 (0.5351)	0.01301 (0.6988)	1	581	0.00	0.9975

Table 7. 4 Across population analysis for Occurrence of clinical mastitis (first lactation). Estimate of ETA for incidence of clinical mastitis in first lactation by each genotype (Estimate± s.e.). F value and *P-value* for the contrast +/+ *vs* -/- .

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	0.002879 (0.03033)	0.002155(0.009534)	0.001840 (0.007554)	1	678	0.00	0.9735
Odc2	0.002042(0.006922)	0.001817(0.009935)	0.001709 (0.05732)	1	717	0.00	0.9954
GHRal	0.001761(0.009809)	0.002114(0.008248)	0.002249 (0.01367)	1	697	0.00	0.9769
GHRac	0.002166(0.008164)	0.001810(0.008785)	0.002039 (0.02083)	1	693	0.00	0.9955
GHRst	0.002021(0.006018)	0.001554 (0.01911)	0.006052 (0.1073)	1	697	0.00	0.9701
GH61	0.001991(0.006145)	0.001767 (0.01540)	0.001636 (0.08757)	1	706	0.00	0.9968
GH62	0.002033(0.007141)	0.001766 (0.01121)	0.001671 (0.03309)	1	652	0.00	0.9915
GH1	0.002036 (0.02460)	0.001708 (0.01118)	0.001971 (0.01617)	1	307	0.00	0.9982
GH41	0.002045(0.007025)	0.001797 (0.01234)	0.002054 (0.04572)	1	625	0.00	0.9998
GH42	0.001888(0.008248)	0.002112 (0.01564)	0.003240 (0.04206)	1	442	0.00	0.9749
GH1258	0.002091 (0.01942)	0.002153 (0.01006)	0.002239 (0.01273)	1	427	0.00	0.9949
GH1300	0.002090(0.01325)	0.002175(0.01042)	0.002258 (0.01675)	1	422	0.00	0.9937
GH5183	0.003094(0.05361)	0.001645(0.01238)	0.002138 (0.007040)	1	619	0.00	0.9859
GH5255	0.001880(0.01238)	0.002162(0.008491)	0.001992 (0.01255)	1	612	0.00	0.9949
ACTH388	0.002188(0.007373)	0.001788(0.01379)	0.001842 (0.04796)	1	551	0.00	0.9943
ACTH341	0.002230(0.01384)	0.002124(0.009935)	0.001583 (0.01433)	1	462	0.00	0.9741
PRL152	0.002322 (0.1072)	0.001644(0.01516)	0.002016 (0.006742)	1	605	0.00	0.9977
PRL361	0.001922(0.007365)	0.001856(0.01160)	0.002821 (0.04572)	1	603	0.00	0.9845
CRH291	0.001862(0.008874)	0.002301(0.01062)	0.001024 (0.02769)	1	523	0.00	0.9770
IGF390	0.002307(0.01408)	0.001695(0.008829)	0.002348 (0.01153)	1	581	0.00	0.9982

Table 7. 5 Across population analysis for Somatic Cell Scores (first lactation). Estimate of ETA for incidence of clinical mastitis in first lactation by each genotype (Estimate± s.e.). F value and *P-value* for the contrast +/+ *vs* -/- .

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	0.3367 (0.2847)	0.3573 (0.08948)	0.3347 (0.07091)	1	678	0.00	0.9947
Odc2	0.3480 (0.06497)	0.3334 (0.09326)	0.3865 (0.5386)	1	717	0.01	0.9434
GHRal	0.3343 (0.09206)	0.3459 (0.07742)	0.3655 (0.1284)	1	697	0.04	0.8433
GHRac	0.3476 (0.07663)	0.3414 (0.08246)	0.3376 (0.1956)	1	693	0.00	0.9623
GHRst	0.3430 (0.05648)	0.3484 (0.1794)	0.5046 (1.0103)	1	697	0.03	0.8732
GH61	0.3423 (0.05583)	0.3559 (0.1399)	0.3214 (0.7977)	1	706	0.00	0.9792
GH62	0.3426 (0.06702)	0.3378 (0.1052)	0.2868 (0.3108)	1	652	0.03	0.8607
GH1	0.3750 (0.2308)	0.3212 (0.1049)	0.3290 (0.1517)	1	307	0.03	0.8680
GH41	0.3463 (0.06593)	0.3332 (0.1159)	0.2855 (0.4293)	1	625	0.02	0.8889
GH42	0.3434 (0.07742)	0.3337 (0.1468)	0.3859 (0.3949)	1	442	0.01	0.9159
GH1258	0.3178 (0.1822)	0.3599 (0.09446)	0.3630 (0.1195)	1	427	0.04	0.8361
GH1300	0.3349 (0.1204)	0.3571 (0.09462)	0.3742 (0.1522)	1	422	0.04	0.8395
GH5183	0.3165 (0.5031)	0.3349 (0.1162)	0.3491 (0.06608)	1	619	0.00	0.9487
GH5255	0.3283 (0.1162)	0.3481 (0.07970)	0.3544 (0.1178)	1	612	0.02	0.8749
ACTH388	0.3492 (0.06921)	0.3236 (0.1294)	0.3091 (0.4503)	1	551	0.01	0.9300
ACTH341	0.3544 (0.1299)	0.3430 (0.09325)	0.3154 (0.1345)	1	462	0.04	0.8350
PRL152	0.4153 (1.0048)	0.3537 (0.1423)	0.3405 (0.06328)	1	605	0.01	0.9408
PRL361	0.3487 (0.06691)	0.3301 (0.1054)	0.3725 (0.4156)	1	603	0.00	0.9549
CRH291	0.3224 (0.08329)	0.3534 (0.09966)	0.3623 (0.2600)	1	523	0.02	0.8837
IGF390	0.3360 (0.1321)	0.3334 (0.08287)	0.3602 (0.1082)	1	581	0.02	0.8876

Table 7. 6 Across population analysis for Clinical mastitis (second lactation). Estimate of ETA for incidence of clinical mastitis in first lactation by each genotype (Estimate± s.e.). F value and *P-value* for the contrast +/+ *vs* -/-.

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	-0.00347 (4.2162)	-0.04136 (1.3042)	-0.07594 (1.0491)	1	549	0.00	0.9869
Odc2	-0.07315 (0.9816)	-0.04636 (1.2859)	-0.1092 (7.1271)	1	588	0.00	0.9960
GHRal	-0.08474 (1.8556)	-0.04652 (1.5325)	-0.04390 (2.5268)	1	571	0.00	0.9896
GHRac	-0.03574 (1.5549)	-0.07973 (1.6028)	-0.09285 (4.0547)	1	565	0.00	0.9895
GHRst	-0.04986 (1.1311)	-0.1837 (3.3673)	0.003220 (18.1334)	1	571	0.00	0.9977
GH61	-0.08240 (0.8475)	0.03415 (2.0215)	-0.3259 (13.3328)	1	581	0.00	0.9855
GH62	-0.06131 (1.2854)	-0.05838 (2.0800)	-0.1617 (9.0670)	1	555	0.00	0.9913
GH1	-0.07260 (4.4641)	-0.04485 (1.9904)	-0.05046 (2.9037)	1	274	0.00	0.9967
GH41	-0.06251 (1.3019)	-0.04452 (2.1910)	-0.1816 (9.0669)	1	530	0.00	0.9896
GH42	-0.06040 (1.4781)	-0.05291 (2.6736)	-0.2032 (7.4028)	1	402	0.00	0.9849
GH1258	-0.1838 (3.6267)	-0.04276 (1.8653)	-0.01526 (2.4341)	1	347	0.00	0.9692
GH1300	-0.09277 (1.7737)	-0.02785 (1.4591)	-0.04641 (2.3387)	1	342	0.00	0.9874
GH5183	-0.2799 (11.4683)	-0.04915 (2.2492)	-0.05956 (1.3208)	1	509	0.00	0.9898
GH5255	-0.1057 (2.2153)	-0.02396 (1.6028)	-0.07683 (2.3914)	1	502	0.00	0.9929
ACTH388	-0.05407 (1.3847)	-0.01616 (2.4676)	-0.3457 (9.6931)	1	445	0.00	0.9762
ACTH341	-0.08651 (2.6592)	-0.02158 (1.8654)	-0.09265 (2.6038)	1	376	0.00	0.9987
PRL152	0.001708 (0.1411)	-0.00095 (0.02140)	-0.00049 (0.009763)	1	504	0.00	0.9871
PRL361	-0.06706 (1.3478)	-0.08018 (2.1909)	0.01107 (9.6930)	1	503	0.00	0.9936
CRH291	-0.05768 (1.6553)	-0.05653 (1.9497)	-0.09470 (5.2346)	1	434	0.00	0.9946
IGF390	-0.02744 (1.9047)	-0.07454 (1.1973)	-0.07879 (1.6168)	1	479	0.00	0.9879

Table 7. 7 Across population analysis for Culling due to mastitis (second lactation). Estimate of ETA for incidence of clinical mastitis in first lactation by each genotype (Estimate± s.e.). F value and *P-value* for the contrast +/+ *vs* -/- .

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	-0.1146 (2.8207)	-0.06260 (0.8726)	-0.06682 (0.7019)	1	549	0.00	0.9998
Odc2	-0.06402 (0.6567)	-0.06678 (0.8603)	-0.06617 (4.7681)	1	588	0.00	0.9996
GHRal	-0.07323 (0.9128)	-0.06385 (0.7539)	-0.04123 (1.2430)	1	571	0.00	0.9835
GHRac	-0.05326 (0.7649)	-0.06944 (0.7884)	-0.08844 (1.9946)	1	565	0.00	0.9869
GHRst	-0.05850 (0.5564)	-0.1089 (1.6564)	0.01346 (8.9200)	1	571	0.00	0.9936
GH61	-0.07318 (0.5670)	-0.02433 (1.3524)	-0.1364 (8.9200)	1	581	0.00	0.9944
GH62	-0.06028 (0.6323)	-0.06303 (1.0232)	-0.1313 (4.4602)	1	555	0.00	0.9875
GH1	-0.1076 (2.2008)	-0.07730 (0.9813)	-0.05962 (1.4315)	1	274	0.00	0.9854
GH41	-0.06061 (0.6404)	-0.05481 (1.0778)	-0.08407 (4.4602)	1	530	0.00	0.9959
GH42	-0.05774 (0.7287)	-0.07211 (1.3181)	-0.1743 (3.6495)	1	402	0.00	0.9750
GH1258	-0.1353 (1.7879)	-0.03116 (0.9196)	-0.03490 (1.2000)	1	357	0.00	0.9628
GH1300	-0.06672 (1.1867)	-0.03173 (0.9762)	-0.04774 (1.5647)	1	342	0.00	0.9923
GH5183	-0.1275 (5.6538)	-0.05246 (1.1088)	-0.06436 (0.6511)	1	509	0.00	0.9912
GH5255	-0.09065 (1.0921)	-0.03812 (0.7902)	-0.07719 (1.1789)	1	502	0.00	0.9933
ACTH388	-0.06333 (0.6826)	-0.05709 (1.2165)	-0.1646 (4.7787)	1	455	0.00	0.9833
ACTH341	-0.06958 (1.3110)	-0.04542 (0.9196)	-0.09280 (1.2837)	1	376	0.00	0.9899
PRL152	0.008391 (8.9388)	-0.06700 (1.3554)	-0.06765 (0.6184)	1	504	0.00	0.9932
PRL361	-0.06503 (0.6645)	-0.07299 (1.0801)	-0.05908 (4.7787)	1	503	0.00	0.9990
CRH291	-0.08085 (0.8161)	-0.04489 (0.9612)	-0.06406 (2.5806)	1	434	0.00	0.9951
IGF390	-0.05591 (1.2743)	-0.06493 (0.8010)	-0.07076 (1.0817)	1	479	0.00	0.9929

Table 7. 8 Across population analysis for Number of CASES of mastitis (second lactation). Estimates of ETA for incidence of clinical mastitis in first lactation by each genotype (Estimate ± s.e.). F value and *P-value* for the contrast +/+ *vs* -/- .

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	0.000281(0.04477)	-0.00029 (0.01385)	-0.00066 (0.01114)	1	549	0.00	0.9837
Odc2	-0.00059 (0.01042)	-0.00039 (0.01365)	-0.00122 (0.07568)	1	588	0.00	0.9935
GHRal	-0.00060 (0.01449)	-0.00040 (0.01196)	-0.00041 (0.01973)	1	571	0.00	0.9938
GHRac	-0.00030 (0.01214)	-0.00068 (0.01251)	-0.00047 (0.03166)	1	565	0.00	0.9959
GHRst	-00041(0.008831)	-0.00154 (0.02629)	0.000229 (0.1416)	1	571	0.00	0.9964
GH61	-0.00072 (0.008972)	0.000504(0.02140)	-0.00354 (0.1411)	1	581	0.00	0.9841
GH62	-0.00049 (0.01004)	-0.00049 (0.01624)	-0.00174 (0.07079)	1	555	0.00	0.9861
GH1	-0.00068 (0.03485)	-0.00030 (0.01554)	-0.00043 (0.02267)	1	274	0.00	0.9999
GH41	-0.00052 (0.01016)	-0.00035 (0.01710)	-0.00169 (0.07079)	1	530	0.00	0.9870
GH42	-0.00049 (0.01154)	-0.00042 (0.02087)	-0.00169 (0.05779)	1	402	0.00	0.9838
GH1258	-0.00180 (0.02831)	-0.00025 (0.01456)	0.000033 (0.01900)	1	347	0.00	0.9572
GH1300	-0.00088 (0.01878)	-7.93E-6 (0.01545)	-0.00038 (0.02476)	1	342	0.00	0.9871
GH5183	-0.00276 (0.08953)	-0.00035 (0.01756)	-0.00047 (0.01031)	1	509	0.00	0.9797
GH5255	-0.00101 (0.01730)	-0.00002 (0.01251)	-0.00073 (0.01867)	1	502	0.00	0.9911
ACTH388	-0.00040 (0.01081)	-0.00010 (0.01926)	-0.00370 (0.07568)	1	455	0.00	0.9656
ACTH341	-0.00078 (0.02076)	-0.00002 (0.01456)	-0.00094 (0.02033)	1	376	0.00	0.9958
PRL152	0.001708 (0.1411)	-0.00095 (0.02140)	-0.00049 (0.009763)	1	504	0.00	0.9876
PRL361	-0.00057 (0.01052)	-0.00062 (0.01710)	0.000376 (0.07568)	1	503	0.00	0.9901
CRH291	-0.00049 (0.01292)	-0.00049 (0.01522)	-0.0071 (0.04087)	1	434	0.00	0.9958
IGF390	-0.00013 (0.02022)	-0.00069 (0.01271)	-0.00066 (0.01717)	1	479	0.00	0.9840

Table 7. 9 Across population analysis for Somatic Cell Scores (second lactation). Estimate of ETA for incidence of clinical mastitis in first lactation by each genotype (Estimate± s.e.). F value and *P-value* for the contrast +/+ *vs* -/- .

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	0.02819 (0.3195)	0.05882 (0.09884)	0.04674 (0.07951)	1	549	0.00	0.9551
Odc2	0.04790 (0.07438)	0.05527 (0.09746)	0.04099 (0.5405)	1	588	0.00	0.9899
GHRal	0.04422 (0.09933)	0.05487 (0.08204)	0.06190 (0.1353)	1	571	0.01	0.9162
GHRac	0.05935 (0.08323)	0.04727 (0.08581)	0.02687 (0.2170)	1	565	0.02	0.8889
GHRst	0.05278 (0.06055)	0.03738 (0.1802)	0.1500 (0.9708)	1	571	0.01	0.9204
GH61	0.04561 (0.06170)	0.07320 (0.1472)	0.07470 (0.9708)	1	581	0.00	0.9762
GH62	0.05228 (0.06881)	0.04324 (0.1113)	0.008869 (0.4858)	1	555	0.01	0.9295
GH1	0.07345 (0.2389)	0.04664 (0.1066)	0.02713 (0.1555)	1	274	0.03	0.8710
GH41	0.05709 (0.06969)	0.04249 (0.1173)	-0.00139 (0.4857)	1	530	0.01	0.9052
GH42	0.05506 (0.07912)	0.05517 (0.1431)	0.007939 (0.3961)	1	402	0.01	0.9072
GH1258	0.01456 (0.1942)	0.05863 (0.09984)	0.07684 (0.1303)	1	347	0.07	0.7901
GH1300	0.03092 (0.1291)	0.07637 (0.1062)	0.05619 (0.1703)	1	342	0.01	0.9059
GH5183	-0.00199 (0.6137)	0.04771 (0.1204)	0.05527 (0.07070)	1	509	0.01	0.9262
GH5255	0.02063 (0.1186)	0.07167 (0.08580)	0.05663 (0.1280)	1	502	0.04	0.8366
ACTH388	0.05668 (0.07412)	0.03792 (0.1321)	0.04173 (0.5194)	1	455	0.00	0.9773
ACTH341	0.04866 (0.1423)	0.06434 (0.09986)	0.01680 (0.1394)	1	376	0.03	0.8731
PRL152	0.04020 (0.9691)	0.05604 (0.1472)	0.04966 (0.06714)	1	504	0.00	0.9922
PRL361	0.05201 (0.07215)	0.04867 (0.1173)	0.002127 (0.5193)	1	503	0.01	0.9242
CRH291	0.03575 (0.08860)	0.05454 (0.1044)	0.09901 (0.2802)	1	434	0.05	0.8297
IGF390	0.03909 (0.1387)	0.04629 (0.08717)	0.06432 (0.1177)	1	479	0.02	0.8898

Table 7. 10 Across population analysis for Clinical mastitis (third lactation). Estimate ETA for incidence of clinical mastitis in first lactation by each genotype (Estimate± s.e.). F value and *P-value* for the contrast +/+ *vs* -/- .

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	2.1439 (5.7539)	2.0285 (1.7520)	2.0647 (1.3616)	1	412	0.00	0.9893
Odc2	2.0240 (1.2685)	2.1025 (1.7182)	1.8118 (8.1369)	1	449	0.00	0.9795
GHRal	2.0225 (1.8456)	2.0820 (1.4836)	2.0559 (2.2898)	1	435	0.00	0.9910
GHRac	2.1133 (1.4785)	2.0045 (1.5377)	1.9087 (4.4890)	1	428	0.00	0.9656
GHRst	2.0778 (1.1038)	1.8623 (2.9673)	2.0238 (21.6030)	1	434	0.00	0.9980
GH61	2.0363 (1.1040)	2.1583 (2.6693)	1.8233 (21.5291)	1	442	0.00	0.9921
GH62	2.0461 (1.2349)	2.0901 (2.0144)	2.0412 (12.4720)	1	421	0.00	0.9997
GH1	2.0701 (4.1574)	2.0887 (1.9803)	2.0824 (3.0250)	1	194	0.00	1.0000
GH41	2.0494 (1.2514)	2.1047 (2.1286)	1.9691 (8.1648)	1	405	0.00	0.9922
GH42	2.0716 (1.4338)	2.0386 (2.6007)	2.0238 (7.2007)	1	302	0.00	0.9948
GH1258	1.9552 (3.3737)	2.0555 (1.7579)	2.0473 (2.2281)	1	283	0.00	0.9818
GH1300	2.0329 (2.1971)	2.0703 (1.8882)	2.0142 (2.9029)	1	278	0.00	0.9959
GH5183	1.8829 (9.6604)	2.0991 (2.1710)	2.0262 (1.2796)	1	386	0.00	0.9883
GH5255	1.9841 (2.1081)	2.1039 (1.5882)	2.0167 (2.2163)	1	382	0.00	0.9915
ACTH388	2.0454 (1.3423)	2.1699 (2.4003)	1.7278 (8.8190)	1	383	0.00	0.9716
ACTH341	2.0053 (2.4779)	2.1531 (1.8323)	1.9948 (2.6006)	1	281	0.00	0.9977
PRL152	2.1634 (15.2729)	1.9976 (2.6196)	2.0333 (1.2152)	1	384	0.00	0.9632
PRL361	2.0460 (1.2864)	1.9710 (2.1934)	2.3254 (10.8009)	1	380	0.00	0.9795
CRH291	2.0048 (1.6283)	2.0522 (1.8873)	2.1328 (4.7139)	1	325	0.00	0.9795
IGF390	2.1683 (2.4532)	2.0103 (1.5701)	2.0628 (2.0716)	1	370	0.00	0.9739

Table 7. 11 Across population analysis for Culling due to mastitis (third lactation). Estimate of ETA for incidence of clinical mastitis in first lactation by each genotype (Estimate± s.e.). F value and *P-value* for the contrast +/+ *vs* -/-.

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	0.5904 (4.0899)	0.5587 (1.2454)	0.5778 (0.9679)	1	412	0.00	0.9976
Odc2	0.5705 (0.9017)	0.5747 (1.2213)	0.4932 (5.7838)	1	449	0.00	0.9895
GHRal	0.5817 (1.3074)	0.5789 (1.0510)	0.5726 (1.6221)	1	435	0.00	0.9965
GHRac	0.5882 (1.0480)	0.5576 (1.0899)	0.5566 (3.1817)	1	428	0.00	0.9925
GHRst	0.5779 (0.7819)	0.5528 (2.1020)	0.3866 (15.3032)	1	434	0.00	0.9900
GH61	0.5648 (0.7838)	0.6125 (1.8926)	0.4836 (15.2595)	1	442	0.00	0.9958
GH62	0.5695 (0.8748)	0.5860 (1.4270)	0.5501 (8.8350)	1	421	0.00	0.9983
GH1	0.5806 (2.9450)	0.5803 (1.4028)	0.5520 (2.1428)	1	194	0.00	0.9937
GH41	0.5683 (0.8864)	0.5984 (1.5079)	0.4182 (5.7838)	1	405	0.00	0.9796
GH42	0.5884 (1.0157)	0.5329 (1.8423)	0.4661 (5.1009)	1	302	0.00	0.9813
GH1258	0.5272 (2.3899)	0.5679 (1.2453)	0.6218 (1.5783)	1	283	0.00	0.9737
GH1300	0.5523 (1.5618)	0.6024 (1.3421)	0.5850 (2.0634)	1	278	0.00	0.9899
GH5183	0.3540 (6.8434)	0.5891 (1.5379)	0.5651 (0.9065)	1	386	0.00	0.9756
GH5255	0.5210 (1.4933)	0.6010 (1.1251)	0.5659 (1.5700)	1	382	0.00	0.9835
ACTH388	0.5753 (0.9509)	0.6198 (1.7003)	0.2863 (6.2473)	1	343	0.00	0.9635
ACTH341	0.5767 (1.7553)	0.6232 (1.2980)	0.5233 (1.8422)	1	281	0.00	0.9833
PRL152	0.7604 (10.8194)	0.5702 (1.8557)	0.5572 (0.8608)	1	383	0.00	0.9851
PRL361	0.5790 (0.9113)	0.5099 (1.5538)	0.7348 (7.6512)	1	380	0.00	0.9839
CRH291	0.5331 (1.1535)	0.5850 (1.3370)	0.6331 (3.3393)	1	325	0.00	0.9774
IGF390	0.6093 (1.7438)	0.5742 (1.1161)	0.5711 (1.4725)	1	370	0.00	0.9866

Table 7. 12 Across population analysis for Number of CASES of mastitis (third lactation). Estimate of ETA for incidence of clinical mastitis in first lactation by each genotype (Estimate± s.e.). F value and *P-value* for the contrast +/+ *vs* -/- .

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	0.02070 (0.06164)	0.01967 (0.01877)	0.02028 (0.01459)	1	412	0.00	0.9948
Odc2	0.01987 (0.01359)	0.02045 (0.01841)	0.01766 (0.08717)	1	449	0.00	0.9800
GHRal	0.01977 (0.01970)	0.02033 (0.01584)	0.02029 (0.02445)	1	435	0.00	0.9867
GHRac	0.02078 (0.01579)	0.01951 (0.01642)	0.01866 (0.04793)	1	428	0.00	0.9666
GHRst	0.02034 (0.01179)	0.01823 (0.03168)	0.01891 (0.2306)	1	434	0.00	0.9950
GH61	0.01988 (0.01181)	0.02135 (0.02851)	0.01735 (0.2299)	1	442	0.00	0.9912
GH62	0.01999 (0.01318)	0.02049 (0.02151)	0.02148 (0.1332)	1	421	0.00	0.9911
GH1	0.02002 (0.04439)	0.02025 (0.02114)	0.02005 (0.03230)	1	194	0.00	0.9996
GH41	0.02006 (0.01336)	0.02066 (0.02273)	0.01982 (0.08717)	1	405	0.00	0.9979
GH42	0.02030 (0.01531)	0.01974 (0.02777)	0.01934 (0.07688)	1	302	0.00	0.9902
GH1258	0.01924 (0.03602)	0.02021 (0.01877)	0.02025 (0.02379)	1	283	0.00	0.9813
GH1300	0.01998 (0.02346)	0.02041 (0.02016)	0.01989 (0.03099)	1	278	0.00	0.9982
GH5183	0.01878 (0.1031)	0.02053 (0.02318)	0.01985 (0.01366)	1	386	0.00	0.9918
GH5255	0.01944 (0.02251)	0.02055 (0.01696)	0.01975 (0.02366)	1	382	0.00	0.9924
ACTH388	0.02002 (0.01433)	0.02125 (0.02563)	0.01740 (0.09416)	1	343	0.00	0.9781
ACTH341	0.01972 (0.02646)	0.02104 (0.01956)	0.01965 (0.02777)	1	281	0.00	0.9986
PRL152	0.02071 (0.1631)	0.01981 (0.02797)	0.01985 (0.01297)	1	383	0.00	0.9958
PRL361	0.02009 (0.01373)	0.01913 (0.02342)	0.02219 (0.1153)	1	380	0.00	0.9856
CRH291	0.01957 (0.01738)	0.02012 (0.02015)	0.02103 (0.05033)	1	325	0.00	0.9782
IGF390	0.02124 (0.02619)	0.01976 (0.01676)	0.02020 (0.02212)	1	370	0.00	0.9758

Table 7. 13 Across population analysis for Somatic Cell Score (third lactation). Estimates of ETA for incidence of clinical mastitis in first lactation by each genotype (Estimate± s.e.). F value and *P-value* for the contrast +/+ *vs* -/-.

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	-0.1566 (0.4007)	-0.1526 (0.1220)	-0.1335 (0.09483)	1	412	0.00	0.9553
Odc2	-0.1413 (0.08827)	-0.1402 (0.1196)	-0.1692 (0.5663)	1	449	0.00	0.9611
GHRal	-0.1267 (0.1280)	-0.1440 (0.1029)	-0.1386 (0.1588)	1	435	0.00	0.9535
GHRac	-0.1351 (0.1029)	-0.1452 (0.1070)	-0.1392 (0.3125)	1	428	0.00	0.9901
GHRst	-0.1421 (0.07656)	-0.1202 (0.2058)	-0.1883 (1.4992)	1	434	0.00	0.9755
GH61	-0.1457 (0.07152)	-0.1110 (0.1727)	-0.4382 (1.3994)	1	442	0.04	0.8340
GH62	-0.1434 (0.08564)	-0.1353 (0.1398)	-0.1567 (0.8652)	1	421	0.00	0.9878
GH1	-0.1396 (0.2884)	-0.1414 (0.1374)	-0.1477 (0.2099)	1	194	0.00	0.9819
GH41	-0.1375 (0.08678)	-0.1417 (0.1477)	-0.2228 (0.5663)	1	405	0.02	0.8818
GH42	-0.1369 (0.09944)	-0.1370 (0.1805)	-0.1502 (0.5093)	1	302	0.00	0.9792
GH1258	-0.1741 (0.2341)	-0.1340 (0.1219)	-0.1243 (0.1545)	1	283	0.03	0.8592
GH1300	-0.1601 (0.1420)	-0.1208 (0.1221)	-0.1324 (0.1878)	1	278	0.01	0.9065
GH5183	-0.2065 (0.6698)	-0.1386 (0.1505)	-0.1408 (0.08876)	1	386	0.01	0.9225
GH5255	-0.1538 (0.1462)	-0.1318 (0.1102)	-0.1352 (0.1538)	1	382	0.01	0.9300
ACTH388	-0.1364 (0.09309)	-0.1610 (0.1665)	-0.1351 (0.6118)	1	343	0.00	0.9983
ACTH341	-0.1325 (0.1718)	-0.1400 (0.1271)	-0.1677 (0.1804)	1	281	0.02	0.8876
PRL152	-0.00118 (1.0576)	-0.1404 (0.1816)	-0.1396 (0.08430)	1	383	0.02	0.8963
PRL361	-0.1397 (0.08921)	-0.1360 (0.1522)	-0.1052 (0.7491)	1	380	0.00	0.9636
CRH291	-0.1468 (0.1129)	-0.1476 (0.1309)	-0.1105 (0.3270)	1	325	0.01	0.9165
IGF390	-0.1210 (0.1706)	-0.1460 (0.1093)	-0.1445 (0.1442)	1	370	0.01	0.9163

Table 7. 14 Across population analysis for Clinical mastitis (over all lactations). Estimates of ETA for incidence of clinical mastitis in first lactation by each genotype (Estimates± s.e.). F value and *P-value* for the contrast +/+ *vs* -/-.

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	-1.0720 (7.0047)	-1.6604 (2.1329)	-1.6539 (1.6577)	1	412	0.01	0.9356
Odc2	-1.6951 (1.5442)	-1.5463 (2.0916)	-1.8179 (9.9053)	1	449	0.00	0.9902
GHRal	-1.6711 (2.2390)	-1.5589 (1.7999)	-1.6832 (2.7780)	1	435	0.00	0.9973
GHRac	-0.01734 (0.02007)	-0.01814 (0.02087)	-0.02169 (0.06094)	1	428	0.00	0.9459
GHRst	-1.6131 (1.3391)	-1.8735 (3.5999)	-1.6549 (26.2095)	1	434	0.00	0.9987
GH61	-1.6551 (1.3462)	-1.5463 (3.2506)	-2.5218 (26.9095)	1	442	0.00	0.9737
GH62	-1.6589 (1.4981)	-1.6003 (2.4439)	-1.0928 (15.1310)	1	421	0.00	0.9703
GH1	-1.5350 (5.0437)	-1.5583 (2.4024)	-1.4920 (3.6699)	1	194	0.00	0.9945
GH41	-1.6686 (1.5181)	-1.5779 (2.5824)	-1.5620 (9.9053)	1	405	0.00	0.9915
GH42	-1.6323 (1.7394)	-1.6210 (3.1552)	-1.6418 (8.7358)	1	302	0.00	0.9991
GH1258	-1.8462 (4.0930)	-1.6767 (2.1326)	-1.6917 (2.7030)	1	283	0.00	0.9749
GH1300	-1.7498 (2.6745)	-1.6575 (2.2985)	-1.7039 (3.5338)	1	278	0.00	0.9917
GH5183	-1.7725 (11.7194)	-1.6178 (2.6338)	-1.6701 (1.5524)	1	386	0.00	0.9931
GH5255	-1.7207 (2.5574)	-1.6006 (1.9268)	-1.6589 (2.6889)	1	382	0.00	0.9867
ACTH388	-1.6325 (1.7311)	-1.6192 (3.1397)	-1.6406 (8.6892)	1	302	0.00	0.9993
ACTH341	-1.6872 (3.0061)	-1.5288 (2.2229)	-1.7415 (3.1550)	1	281	0.00	0.9901
PRL152	-1.6153 (18.5262)	-1.7674 (3.1779)	-1.6475 (1.4743)	1	383	0.00	0.9986
PRL361	-1.6500 (1.5606)	-1.7519 (2.6610)	-0.7980 (13.1033)	1	380	0.00	0.9486
CRH291	-1.6909 (1.9754)	-1.6096 (2.2897)	-1.6700 (5.7188)	1	325	0.00	0.9972
IGF390	-1.4096 (2.9863)	-1.7016 (1.9114)	-1.7222 (2.5219)	1	370	0.01	0.9363

Table 7. 15 Across population analysis for Occurrence of Clinical mastitis (over all lactations). Estimates of ETA for incidence of clinical mastitis in first lactation by each genotype (Estimates± s.e.). F value and *P-value* for the contrast +/+ *vs* -/-.

Marker	+/+	+/-	-/-	Num DF	Den DF	F value	P value
Odc1	-0.01165 (0.07811)	-0.01819 (0.02378)	-0.01806 (0.01848)	1	412	0.01	0.9364
Odc2	-0.01864 (0.01722)	-0.01672 (0.02332)	-0.02045 (0.1105)	1	449	0.00	0.9870
GHRal	-0.01833 (0.02497)	-0.01705 (0.02007)	-0.01841 (0.03098)	1	435	0.00	0.9985
GHRac	-0.01734 (0.02007)	-0.01814 (0.02087)	-0.02169 (0.06094)	1	428	0.00	0.9459
GHRst	-0.01771 (0.01493)	-0.02019 (0.04014)	-0.01855 (0.2923)	1	434	0.00	0.9977
GH61	-0.01804 (0.01501)	-0.01722 (0.03625)	-0.02713 (0.2923)	1	442	0.00	0.9752
GH62	-0.01813 (0.01671)	-0.01771 (0.02725)	-0.01260 (0.1687)	1	421	0.00	0.9740
GH1	-0.01608 (0.05624)	-0.01682 (0.02679)	-0.01655 (0.04092)	1	194	0.00	0.9946
GH41	-0.01820 (0.01693)	-0.01772 (0.02880)	-0.01767 (0.1105)	1	405	0.00	0.9962
GH42	-0.01796 (0.01940)	-0.01759 (0.03518)	-0.01723 (0.09741)	1	302	0.00	0.9941
GH1258	-0.02007 (0.04564)	-0.01861 (0.02378)	-0.01840 (0.03014)	1	283	0.00	0.9756
GH1300	-0.01934 (0.02982)	-0.01833 (0.02563)	-0.01817 (0.03940)	1	278	0.00	0.9812
GH5183	-0.02078 (0.1307)	-0.01802 (0.02937)	-0.01815 (0.01731)	1	386	0.00	0.9841
GH5255	-0.01914 (0.02852)	-0.01751 (0.02149)	-0.01775 (0.02998)	1	382	0.00	0.9733
ACTH388	-0.01779 (0.01816)	-0.01694 (0.03247)	-0.02303 (0.1193)	1	343	0.00	0.9653
ACTH341	-0.01826 (0.03352)	-0.01673 (0.02479)	-0.01908 (0.03518)	1	281	0.00	0.9866
PRL152	-0.01779 (0.2066)	-0.01938 (0.03544)	-0.01801 (0.01644)	1	383	0.00	0.9992
PRL361	-0.01818 (0.01740)	-0.01898 (0.02967)	-0.00828 (0.1461)	1	380	0.00	0.9464
CRH291	-0.01843 (0.02203)	-0.01753 (0.02553)	-0.01911 (0.06377)	1	325	0.00	0.9920
IGF390	-0.01532 (0.03330)	-0.01874 (0.02131)	-0.01892 (0.02812)	1	370	0.01	0.9342

APPENDIX 4. QTL AFFECTING CLINICAL MASTITIS AND SCS IN DAIRY CATTLE

Table 7. 16 QTL affecting mastitis resistance traits reported in several studies.

BTA	Marker name, position (cM)	Trait	Reference
1	MAF46, 118.1	SCS	Reinsch et al (1998)
2	N/R, 99	SCS	Bennewitz et al (2003)
4	RM188-TGL116, 24.7-48.9	SCS	Zhang et al (1998)
5	BM6026, 6.7	SCS	Holmberg et al (2004)
	BM315, 100.1	SCS	Heyen et al (1999)
6	N/R, 37	CM	Klungland et al (2001)
7	BM6117, 61	SCS	Ashwell et al (2001)
	BM6117-BMS2258, 67	SCS	Ashwell et al (2001)
	BMS2258-OarAE129,75-96.6	SCS	Khun et al (2003)
	BMS1979, 124.4	SCC	Heyen et al (1999)
8	BM3419, 16.7	SCS	Reinsch et al, 1998
	TGLA10-INRA122, 54	SCS-CM	Klungland et al, 2001
10	TGLA378-TGLA102, 49	SCS	Khun et al, 2003
11	BM304, 24.4	Rear udder height	Ashwell et al (1998)
	INRA177, 25.7	CM	Holmberg et al, 2004
	BM7169, 41	CM	Holmberg et al, 2004
	N/R, 11, 63	SCS	Schulman et al, 2004
13	AGLA232, 79.5	SCS	Zhang et al (1998)
14	BMS1747-BMS740, 4.2-44.2	CM	Schulman et al (2004)
	BM302, 36.9	Udder conformation	Van Tassel et al (2000)
	ILSTS11-BM302, 10.6-36.9	SCS	Zhang et al (1998)
	N/R, 93	CM	Klungland et al (2001)
18	TGLA227, 117	SCS	Khun et al (2003)
19	N/R, 32	SCS	Bennewitz et al (2003)
21	N/R, 33 and 84	SCS	Rodriguez-Zas et al (2002)
22	BMS875-BM4102, 80	SCS	Ashwell et al (2004)
	BM3628, 44.5	SCS	Heyen et al (1999)
	CSSM026, 43.7	SCS	Heyen et al (1999)
23	BM1443, 67.1	SCS	Holmberg et al (2004)
	BB705-BM1818, 80	SCS	Ashwell et al (2004)
	RM033, 17.3	SCS	Reinsch et al (1998)
	D2355, 52	SCS	Heyen et al (1999)
26	BM1314, 0	SCS	Ashwell et al (2004)
	TGLA429-BM804, 50.6-59.6	SCS	Zhang et al (1998)
27	BM3507-TGLA179, 0-5.1	SCS	Khun et al (2003)