Genetic resistance and diseases

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Abstract

Selecting cattle most resistant to the development of infectious diseases will decrease costs of production and should therefore be included in the overall breeding objective. Such selection goals may include resistance to infection with a pathogen (absence of susceptibility), resistance to disease (no-development of disease), tolerance (capability of untreated individuals to maintain a reasonable level of productivity in the face of disease), and resilience (ability of affected individuals to require minimal treatment to maintain acceptable performance). Given those definitions, selected questions raised by the complexity of the interrelations between a pathogen and its host are discussed with examples from bovine mastitis.

Background

So far, genetic selection of animals placed emphasis on productivity and efficiency and has potentially reduced natural disease resistance. Indeed, studies have shown that as animal production increased, resistance to disease decreased (Boettcher et al., 1992, Rogers et al., 1998, Lund et al., 1994, Luttinen et al., 1997). But the whole context of production in Europe is changing dramatically with globalization, reduction in production subsidies, decreasing commodity prices, and increased emphasis on health, food safety, animal welfare and the environment. Many of the potential animal breeding strategies proposed to approach problems for future sustainable production systems refer to a broader definition of breeding goals balancing animal higher productivity with improved functional traits such as health, fertility, and feed intake capacity. Thus, in dairy cows, the need is to improve fertility, disease resistance, fitness and longevity of cows, to reduce metabolic stress and increase the quality, safety and health qualities of milk (Olesen et al., 2000)

Animal resistance is the quality that deters or controls disease formation. Genetic resistance may be directed at non-infectious diseases such as BLAD (Shuster et al., 1992), bovine chondrodysplastic dwarfism (Takeda et al., 2002), or dermatosparaxis (Tajima et al., 1999) but here we will discuss only issues related to genetic resistance to infectious diseases, i.e., diseases resulting from the presence and activity of a microbial agent. In this context, resistance exists in the intimate relationship between the animal and the pathogen in their environment and there are several types of disease resistance in terms of the effects of genes on the animal and the pathogen.

The components of genetic resistance

ANIMAL - Resistance to pathogens may refer to all mechanisms contributing to a decrease in the detrimental effect of the pathogen, such as acquisition of avoidance behaviour (escaping infection by maturing before the epidemic develops), expression of inducible defences and modification of the life history traits. A more specific definition of resistance refers to the biochemical and physiological changes preventing proper parasite establishment, survival, and/or development. This may further be divided into resistance and tolerance to pathogens.

- True resistance or resistance to infection reduces or prevents infection. It is sometimes called qualitative resistance because animals are either resistant or susceptible, without

intermediate levels. Complete resistance is rare, is usually specific to an individual pathogen and is usually receptor-related. Examples of such complete resistance are K88 *E. coli* receptor in swine or the gene coding for receptor to avian sarcoma and leucosis viruses. This type of resistance is also called "major-gene" or "single-gene" resistance because animals with this type of resistance usually have one or a few specific, well-defined genes that confer a high level of resistance to the specific pathogen. Often, the gene gives the animal resistance to only one specific pathogen. If other pathogens are present, the animal needs different "major genes" to resist each parasite.

- Tolerance or resistance to disease describes the reaction of an animal to an infection. It is often called quantitative because there are intermediate levels ranging from resistant to susceptible. It is the resistance to disease development and may be classified in 3 categories: It may refer to a complete level of tolerance (no-development of disease), to the ability of an animal to maintain a reasonable level of productivity when it is diseased (true tolerance), and to the ability of affected individuals to require minimal treatment to maintain acceptable performance (resilience). For example, it has been shown that 20.7% of holsteins experimentally challenged with the same dose of *S. aureus* Newbould 305 did not establish infection in any of the quarters and that 20% had all quarters infected (Schukken et al., 1999). Usually, tolerance involves several or many genes ("polygenic" resistance) but exactly which genes are involved may be unknown. It usually is effective against several pathogens and does not give an animal as high a level of resistance as major-gene resistance.
- Another level of resistance is the clearance, or the ability of the infected host to get rid of the pathogen. It is well known that duration of infection may be quite different across different animals infected with the same infectious doses. For example, geometric mean duration of environmental streptococcal infection is 12 days with a range from 1 to 370 days (Todhunter et al., 1995).

PATHOGEN – Resistance and tolerance of an animal have their counterparts in the microbial agent : pathogenicity and virulence.

- Pathogenicity refers to the ability of an organism to cause disease (ie, harm the host). This ability represents a genetic component of the pathogen and the overt damage done to the host is a property of the host-pathogen interactions. Commensals and opportunistic pathogens lack this inherent ability to cause disease but microorganisms that are nonpathogens today may acquire some pathogenicity factors because of their rapid adaptability to the pressure factors such as radiation therapy, chemotherapy, or immunotherapy.
- The virulence of a pathogen is directly related to the ability of the organism to cause disease despite host resistance mechanisms. It is the rate at which parasites exploit host tissue and is affected by numerous variables such as the number of infecting bacteria, the route of entry into the body, specific and nonspecific host defense mechanisms, and virulence factors of the bacterium. It can be expressed by the number of organisms needed to provoke the disease stages or by the number of organisms needed to cause infection .

The above classification raises several questions that need to be addressed for genetic studies of infectious disease to advance (Lunney, 2005):

- Is there data indicating that disease resistance is heritable?
- Should research focus on resistance to any pathogen ?
- What disease phenotypes need to be targeted for best resistance or tolerance ?

- How much genetic data is needed?
- Which of tolerance or resistance is the most durable?
- How many animals should be selected?
- Would selection for faster recovery from disease be an advantage?
- How will production traits be affected by selection for disease resistance ?
- Should research focus on a single or multiple disease agents?
- Should selection intensity be equivalent for any pathogen ?
- …

Case study: bovine mastitis

Given the complex interaction between a pathogen and its host, we will only discuss selected issues from the above list with applications to bovine mastitis.

Is there data indicating that disease resistance is heritable?
Evidence from immunology and genetics studies demonstrate mastitis resistance is heritable. Indeed, selection based on clinical mastitis has lead to an annual decrease of 0.27% in Norway (Heringstad, 2003) even if heritability estimates for occurrence of clinical mastitis are less than 10% In many countries, somatic cell counts (SCC) are the primary traits used in breeding programs for improving udder health. This is because SCC are measured with automated devices, SCC increase in infected udders, SCC are genetically associated with clinical mastitis (r_g = 0.30 to 0.70) and SCC are more heritable (h^2 = 10 to 14%) than clinical cases (reviewed in Detilleux, 2002; Rupp and Boichard, 2003). Finally, evidence of genetic variation in the ability of cows to resist mastitis is brought by the fact that genetic markers have also been associated with changes in SCC (reviewed in Kerr and Wellnitz, 2003) but the markers are located on different chromosomes depending on the studied population.
Answer: YES.

What disease phenotypes need to be targeted?
There are concerns about the use of milk SCC as a measurement of resistance and in the methods used to estimate SCC genetic parameters. Indeed, SCC are collected on a monthly basis and the infection status of the udder is usually unknown which may lead to biased estimation of genotypic values. Although some statistical methods may help in refining the infection status based on SCC (Detilleux and Leroy, 2000; Ødegård et al., 2003), there are other traits available to identify animals more or less resistant to mammary infectious agents. For example, udder and teat conformation and milking speed are moderately associated with SCC and occurence of clinical mastitis. Teat end shape is highly heritable (h^2 = 0.60) and has been also associated with mastitis frequency (reviewed in Detilleux, 2002; Rupp and Boichard, 2003). Phenotypes measuring the immunological competence of the cows may also be of interest. Indeed, negative genetic correlations have been found between several functions of blood neutrophils on the one hand, and SCC or mammary infection caused by major mastitis pathogens on the other hand (Kelm et al., 1997), suggesting deficiency in neutrophil functions is associated with susceptibility and severity of mastitis (Paape et al. , 2003). Unfortunately, immunological tests are ususally done on blood samples which preclude their use on a large scale. More practicable assays, like measurements on milk samples (Mehrzad et al., 2001) should be developed for field studies. Experimental designs may also facilitate phenotype collection. For example, if a strong correlation exists between immunocompetence in sires and resistance to mastitis in their daughters, then only samples on bulls would be necessary for selecting resistant cows (Kelm et al., 1997; Fitzpatrick et al., 1999). Mathematical modeling may also help by

quantifying the fate of neutrophil and bacteria during an inflammatory reaction and identifying the few essential events in the resistance mechanisms (Detilleux, 2004).
Answer: IT DEPENDS

How will production traits be affected by selection for disease resistance ?
In Holsteins, genetic correlations between disease traits and milk yield are positive, suggesting selection based solely on yield may increase incidence of disease (van Dorp et al., 1998). Genetic correlations are also positive between different health related traits (Lyons et al., 1991; Weigel et al., 2004) which suggests that cows less able to support the negative energy balance occuring after the part are more prone to develop diseases as if there were limited body resources for production and disease resistance. Existence of some costs associated with maintaining and operating resistance mechanisms has been demonstrated in plants and bees (Brown, 2003; Moret and Schmid-Hempel, 2000). In Holsteins, Kimura et al. (1999) observed also different distributions of leukocytes in mastectomized and normally-producing cows which illustrates the influence of metabolic-stresses associated with lactation on immune response profile. Therefore, diseases with detrimental effects on production will be indirectly selected against if artificial selection goals meet those of natural selection for maximum overall fitness (e.g., the presence of tumours due to avian leucosis decreases egg production). But when human-driven selection goals are not for overall fitness, negative r_g between productivity and disease resistance (e.g., selection for high milk peak associated with increased metabolic demands and occurrence of metabolic disorders) may occur because there is competition for resources among production- and fitness-related traits.
Answer: BADLY

Should selection intensity be equivalent for any pathogen ?
To answer part of this question, let's examine theories elaborated to explain the evolution and maintenance of pathogenicity and virulence in microorganisms. All are based on the concept of the fitness for the parasite (Levin, 1996). If transmissibility, virulence, and recovery – all components of the fitness - are independent, natural selection would favor highly transmissible, incurable, commensal or symbionts pathogens, so fitness is highest. In this situation, natural and artifical selection objectives are the same. However, the direction of natural selection may change according to the epidemiology and ecology of the microparasite: If virulence is positively associated with transmission (e.g., contagious pathogen such as *S. aureus*), natural selection will be towards an increased virulence. But if virulence is negatively associated with transmission (e.g., environmental pathogen such as *E. coli*), the parasite will not be transmitted and the evolution will be towards a decreased level of virulence. Given such observations, human-driven selection may be targetted to some specific type of pathogens.
Answer: NO

How many animals should be selected?
As with most contagious diseases, establishing 70% to 80% herd immunity will successfully limit contagious mastitis from spreading among susceptible animals (Detilleux, 2005) because the probability of a susceptible animal meeting an infected one is decreased so the spread of disease is slowed down or stopped altogether. This proportion will decrease even further in herds under control for mastitis (e.g., culling of chronically infected cows, dry cow therapy and antibiotic treatment). However, in the case of environmental disease, individual immunity is necessary due to fomite transmission.
Answer: 0 to 100%

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L'achondroplasie :

Une des formes de l'achondroplasie bovine est caractérisée par une croissance anormalement réduite de l'ensemble des os des membres et de la face chez le nouveau-né. Elle est toujours fatale. Les éleveurs la nomment "veau bouledogue" ou "veau tortue". Elle est décrite depuis longtemps dans de nombreuses races. Or en septembre 1999, des veaux anormaux sont apparus dans la descendance d'un même taureau Prim'Holstein appartenant à l'élite mondiale. Ces cas ont clairement démontré que ce taureau était porteur de l'anomalie. Sa transmission est autosomique récessive. En France, on a estimé à 1% la fréquence des veaux anormaux sur l'ensemble des produits de ce taureau. Une collaboration entre l'OGER (Ouest Génétique Elevage Reproduction), la SGQA et notre laboratoire a très rapidement mis en place un réseau de collecte d'informations et a ainsi permis, avec l'aide efficace des éleveurs, de prélever des échantillons de sang, et donc d'ADN, des veaux anormaux et de leur mère. Malheureusement l'état de conservation de ces veaux anormaux, à la mise bas, ne nous a pas permis, jusqu'à présent, d'isoler des ARN.
A partir du matériel biologique obtenu sur plus de 75 couples mère-veau, nous avons entrepris la cartographie du gène défectueux, à la fois par une approche de gènes candidats, sur la base des connaissances de malformations similaires chez l'Homme et la Souris, ainsi que par le balisage systématique de tout le génome à l'aide de marqueurs microsatellites. La combinaison de ces deux approches a permis de localiser en quelques semaines le gène défectueux dans une région contenant un gène candidat appartenant à la famille des collagènes (COL2A1). Un test diagnostique fondé sur 5 marqueurs - un de ces cinq marqueurs est situé dans le même BAC que le gène COL2A1 - a été mis au point et transféré au GIE LABOGENA à la mi-mars 2000 pour que les descendants directs du taureau porteur puissent être génotypés avant leur entrée en testage. Les porteurs de l'allèle " Bouledogue " sont ainsi actuellement écartés de la sélection. Ce test est le seul moyen d'éradiquer rapidement l'anomalie dans la population, tout en préservant l'apport génétique du reproducteur d'élite (Ducos et al, 2003, Ducos et al, 2003, Ducos et al, 2003, Eggen 2003, Eggen 2000).
Nos recherches se sont ensuite orientées vers l'identification de la mutation impliquée avec l'étude du gène candidat positionnel et fonctionnel (COL2A1). Toutefois, après le séquençage complet de ce gène et des comparaisons entre individus malades et sains, aucune mutation causale n'a pu être mise en évidence. Ceci est peut-être à rapprocher de l'absence de veaux anormaux parmi les 31 que nous avons produits à partir de taureaux et de vaches Prim'Holstein porteurs. La transmission de l'anomalie est non-dominante et un autre gène, localisé à distance sur le génome, pourrait intervenir dans son étiologie.

La syndactylie :

La syndactylie, appelée par les Anglo-saxons *mulefoot*, est une malformation caractérisée par la fusion des phalanges (Figure 2). Elle s'observe principalement dans la race Holstein. C'est une anomalie génétique à transmission autosomique récessive. Cette fusion des phalanges est soumise à deux gradients, un gradient antéro-postérieur et un gradient droite/gauche. L'anomalie apparaît pendant le développement embryonnaire des membres. Dès ce stade très précoce, le phénotype anormal est décelable.
En utilisant une stratégie de clonage positionnel, à partir d'une primo-localisation réalisée en 1996 , nous avons réduit l'intervalle de localisation à moins d'une mégabase et avons mis au point un test précis fondé sur l'identification de l'haplotype anormal à l'aide de marqueurs microsatellites et de SNP. Par ailleurs, ayant identifié dans un élevage une génisse homozygote porteuse de l'anomalie et importé de la semence de deux taureaux également homozygotes

porteurs, nous produisons actuellement des embryons homozygotes pour la syndactylie. Ce travail est réalisé au domaine expérimental animal du Pin (SGQA) et nous collaborons avec l'unité BDR pour l'étude des profils d'expression des gènes candidats lors de la mise en place des bourgeons embryonnaires des membres. Ces recherches, d'un point de vue fondamental, nous apporteront une meilleure compréhension du développement embryonnaire et, sur le plan pratique, devraient aboutir à la mise au point d'un test de détection des animaux porteurs. Nous vérifierons également si celle-ci existe chez d'autres races bovines (Eggen et al, 2003 (UNCEIA)).

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RESISTANCE AND TOLERANCE IN A HOST PLANT–HOLOPARASITIC PLANT INTERACTION: GENETIC VARIATION AND COSTS

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