SUSCEPTIBILITY TO MASTITIS : A REVIEW OF FACTORS RELATED TO THE COW

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LA SENSIBILITÉ AUX MAMMITES : REVUE DES FACTEURS LIÉS A LA VACHE

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General conclusions

Broadly, speaking, three interdependent elements are involved in mammary infections of the dairy cow : microorganisms, environment and the cow itself.

Microorganisms are directly responsible for the disease. Streptococci and staphylococci are the cause of about 90 % of infections. Other species of bacteria such as *Escherichia coli,* Pseudomonas and *Corynebacterium pyogenes* are sometimes isolated. Most infections are latent or sub-clinical and are consequently not apparent to the dairy farmer who has difficulty in appreciating their importance.

Environmental factors, nutrition, climate, housing, bedding, milking machine, etc., influence the living conditions of bacteria the opportunity they are given to penetrate the quarters and, to a certain extent, the susceptibility of the animal. The dairy farmer may have a beneficial or unbeneficial influence, modifying the environment according to his conception of herd management, the attention he gives to the maintenance and use of his milking equipment and to level of hygiene.

The cow is often seen as a passive « element » although a certain number of studies have reported differences between animals where susceptibility to infections is concerned.

The complex nature of this pathology makes progress in the fight against the disease slow and limited ; all in all, the situation today seems little different to that of 30 years ago.

It is nevertheless possible to obtain and maintain a low level of infection by applying a mastitis control system with the aim of reducing the duration of infections and incidence of new infection. This system, perfected by the English researchers at the National Institute for Research in Dairying at Reading in the 1960's, is based on the application of the following measures : good general husbandry and milking practice, including regular testing and maintenance of the milking machine, teat-disinfection by dipping the whole teat in an appropriate solution immediately after milking, systematic treatment of quarters at drying-off with an effective long acting antibiotic, culling of animals which do not respond to treatment.

Mastitis control, however, has both conceptual and material limitations and the more long-term aim of research is to prevent mammary infections by reducing the susceptibility of the cow.

There exists a group of anatomical and physiological natural defence mechanisms, for the most part non-specific, which are involved at three successive stages of the infection process : penetration, establishment, then multiplication of bacteria. These defence mechanisms have their effect at two levels, the teat and the udder itself. The differences in susceptibility to infections observed between cows, as well as the existence of different types of infection — latent, sub-clinical, chronic, or clinical — indicate that a difference also exists in the ability of the host to respond to agression, according to the efficiency of its defence mechanisms.

This review proposes to examine the different factors, located at teat and udder level, which could be involved in resistance to infections, as well as the possible means of stimulating and/or inducing this resistance.

Factors of susceptibility or resistance to mammary infections

1. *Physiological factors of the cow influencing susceptibility to infection*

1.1. Age and stage of lactation

Both quarter infections and clinical mastitis occur increasingly with the age of lactation of the animal (Oliver *et al.,* 1956 ; Wilton *et al.,*

1972 ; Rainard and Poutrel, soumis pour publication). According to Oliver *et al.* (1956) the increase in the number of cases of clinical mastitis in previously healthy animals is closely connected with age of lactation, up to the fifth lactation. This does not necessarily imply a greater susceptibility to infection, but rather to inflammation. A greater predisposition to infection could be the consequence of a number of characteristics associated with age of lactation, in particular lengthening of teat, of more precisely of distance between udder and floor, lesions present on the teat (Hebel *et al.*, 1979) and loss of sphincter patency (Little, 1937).

The results of Oliver *et al.* (1956) indicate that on the whole occurrence of new infections and mastitis decrease according to stage of lactation and that this decrease is greatest between the first and second month of lactation. In an experiment conducted on three herds, we observed (Rainard and Poutrel, 1982) from bacteriological analyses carried out every three weeks on all quarters that 50 % of infections are established in the first three months of lactation. On the other hand, the infection status remains practically unchanged in the last two months of lactation. Reasons for a greater susceptibility of animals at the start of lactation remain unknown. It has been suggested that the important physiological changes, particularly hormonal ones, which take place « *post partum* » may reduce udder resistance (Aström, 1972 ; Guidry *et al.*, 1975).

It is worth noting that in the particular case of experimental staphyloccal infections carried out in the udder cistern, the most favourable period for the establishment of persistant infections was found to be between the second and fourth month of lactation (Poutrel and Lerondelle, 1980).

1.2. Milk yield, ease and milking rate

Mammary infections are caused, with a few exceptions, by the penetration of microorganisms through the teat canal. Hence the importance of the sphincter which ensures the closing of the end of the teat canal between milkings. Sphincter patency is usually estimated by the ease with which the quarter can be milked.

According to McEwen and Cooper (1947) incidence of mastitis is higher in easy milking quarters than in hard milking quarters. In order to investigate a possible relationship between sphincter patency and rate of infection, Dodd and Neave (1951) preferred to use a less subjective method, classifying first lactation cows into five groups according to their milking rates. They thus found a very strong positive correlation between milking rate and incidence of infection during lactation and at the start of drying-off, *Staphylococcus aureus* being the bacterial species most often isolated. Using this species implanted 3 mm inside the teat canal, Prasad and Newbould (1968) confirmed the preceeding results. They, in fact, established correlations between intramammary rate of infection and length of teat canal, milk yield and milking rate. As Dodd and Neave (1951) point out, over-milking, more frequent with fast-milking cows, cannot be offered as a valid explanation for these results, as in such a case front quarters would be expected to have a higher incidence of infection, whereas the opposite is in fact observed — hind quarters are more frequently infected. They thus admit that a larger and slacker sphincter, characteristics associated with fast milking facilitate the penetration of bacteria.

Other authors have found no significant correlation between yield and incidence of infection and mastitis (Legates and Grinnells, 1952 ; Schmidt and Van Vleck, 1965 ; Smith and Schultze, 1970 ; Wilton *et al.*, 1972 ; Geer *et al.*, 1979). On the other hand, Grootenhuis *et al.* (1979) report a higher resistance to infection in fast-milking animals with a high yield.

Relationships between milking characteristics and incidence of mammary infections remain, therefore much debated. These conflicting results may be explained by the different methods used, notably criteria taken into account for the diagnosis of mastitis, which are, according to each particular case, results of bacteriological examinations, cell counts or California Mastitis Test, or clinical symptoms. Moreover the assessment of this type of relation may be biased since production from infected quarters is in any case reduced. Finally, as Smith and Schultze (1970) emphasise, genetic differences between cows concerning ease with which bacteria, particularly staphylococci, may colonize the teat skin, may also influence results.

Milking characteristics, rate and production are highly heritable (Markos and Touchberry, 1970) and first lactation yield is generally used as a selection criterion. No present data permits us to affirm whether this selection increases susceptibility to mammary infection or not.

2. *Morphological characteristics of the udder and teat influencing susceptibility to infection*

According to Young *et al.* (1960), cows with the most pendular-shaped quarters appear to be

the most susceptible to mammary infections. Similarly, long teats increase the risk of accidental trauma (Grommers *et al.,* 1971) and these lesions constitute potential sources of microorganisms which increase the probability of quarter infection (Neave *et al.,* 1969).

Whether it be due to the shape of the udder or the length of the teat (in particular the rear teats), the distance from the tip of the teats to the floor, an important source of potential contamination, is considered an important parameter (Geer *et al.,* 1979 ; Hebel *et al.,* 1979 ; Higgins *et al.,* 1980). Forbes' review (1969) shows that the relationship between shape of teat end and of the teat itself and incidence of infection remains uncertain. Recently Geer *et al.* (1979), found no significant relationship either between susceptibility to infections and shape of tip of teat, or between the latter and hyperkeratosis, which is however found to occur more frequently in animals in the high infection rate group. Quarters with cylindrical-shaped teats were found by Hickman (1964) and Rathore (1976) to be more often infected than those with funnel-shaped teats. Higgins *et al.* (1980) found bottle shape to be the least favourable. It is widely admitted that funnel shape is the one which should be aimed at, particularly because it is, to a large extent, less susceptible to teat-cup crawl, repeated occurrence of which damage the tissues. Discrimination often practised against long, cylindrical teats and teats which are not plumb-shaped may therefore be justified, even though it appears important above all to avoid extreme shapes which are not adapted to machine milking.

Observations on the relationship between morphological characteristics and mastitis all emphasize the importance of age of lactation. This, together with low heritability of teat conformation traits, makes a selection programme for cows on this basis highly implausible. However, length of teat and distance from tip of teat to floor, with a high heritability index ($h^2 = 0.40$) (Higgins *et al.,* 1980) are the exception and certainly deserve future consideration.

3. *Teat resistance mechanisms*

The overall « barrier » effect of the teat may be demonstrated experimentally. The same inoculum (about 100 bacteria) of the two same strains of *Staphylococcus aureus,* gave different results according to whether the bacteria were deposited in the teat canal or in the mammary cistern (Poutrel and Lerondelle, 1980). The number of persistant infections established was lower in the first case, 58 % as opposed to 82 %, whereas the number of transitory infections was higher, 25 % as opposed to 8 %.

3.1. Influence of length and diameter of teat canal in susceptibility to mastitis

According to Murphy and Stuart (1965), length of teat canal does not influence susceptibility to infections by *Streptococcus agalactiae.* This conclusion is shared by Hickman (1964) and Mac Donald (1975), who consider, however, that diameter of teat canal is an important parameter. Thus Mac Donald (1975) established infections by *Aerobacter aerogenes* and *Streptococcus agalactiae* in 25 % of quarters with a diameter of less than 0.2 mm against 75 % for quarters with a diameter of more than 0.5 mm. If one accepts a positive correlation between diameter of teat canal and milking rate, fast-milking cows should be considered the most susceptible to infection. We have seen that this conclusion is not unanimously accepted.

3.2. Bactericidal activity of keratin

The keratin lining the teat canal is considered as an important factor in protection against infection. Murphy (1959) obtained a temporary decrease in resistance to infection after having partially removed the keratin by a « reaming » process. Non-esterified fatty acids (Adams and Rickard, 1963) or proteins (Hibbit *et al.,* 1969) are believed to be responsible for this bactericidal activity.

Other mechanisms may play an important role. Prasad and Newbould (1968) observed a greater incidence of infection when staphylococci were deposited at a distance of 4 mm inside the teat canal than when they were deposited at a distance of 3 mm. According to them, this difference is not due to a simple physical phenomenon, but perhaps reflects the anatomical differences linked to the structure or position of cells producing antibacterial substances.

Teat resistance mechanisms, the importance of which it is difficult to evaluate, have in any case a limited efficacy and bacteria may persist and even multiply in the column of residual milk present in the teat canal after milking. This led Philipps *et al.* (1969) and Roguinsky (1975) to recommend squirting after milking which gives a significant decrease in the incidence of infection, with results similar to those obtained by teat-dipping.

4. *Infection resistance mechanisms in the udder*

Bacteria having penetrated via the teat canal and escaped the different defence mechanisms functioning at this level can reach the udder cistern where other factors having an antibacterial activity may intervene. Among those elements capable of killing bacteria or inhibiting their growth two groups may be distinguished : cells (polymorphonuclear, leucocytes, macrophages, lymphocytes) and proteins. Certain of these proteins (lactoperoxidase, lysozyme, lactoferrin, complement) have a non-specific activity, whilst the immunoglobulins have a specific activity.

4.1. Proteins and enzymes in milk having a non specific antimicrobial activity

The main components having a non-specific bacterial activity, previously called lactenins, have been described and reviewed notably by Reiter and Oram (1967) Schanbacher and Smith (1975) and Hibbit and Hill (1977).

4.1.1. *The lactoperoxidase/thiocyanate/hydrogen peroxide system* inhibits the growth of certain species of streptococci such as *Streptococcus agalactiae* and *Streptococcus uberis* which produce lactic acid (Reiter *et al.,* 1963 ; Mickelson, 1966). In order to be efficient, the three elements which make up this system need to be present simultaneously. Peroxidase is always present in milk, but the concentration of SCN^- depends on the feeding regime. Certain streptococci produce their own H_2O_2 which leads to their self-destruction whereas some species such as *Escherichia coli* and *Staphylococcus aureus* are only killed by an exogenous supply of H_2O_2.

4.1.2. *Lysozyme.* The presence of this enzyme in cattle and its concentration in milk in relation to infection are the subject of much discussion. Certain authors have not been able to show the presence of lysozyme in bovine serum (Hill *et al.,* 1976), tears or leucocytes (Padgett and Hirsch, 1967). There seem in fact to exist important variations between animals, this apparently having a genetic basis (Lie, 1980). Whereas Goudswaard *et al.* (1978) maintain that lysozyme is found only in mastitis milk where it is present in high concentrations, Weaver and Kroger (1978) recorded no differences between concentrations in mastitic milk and milk from the healthy quarter. The use of different techniques, however, can only partially account for such contradictory results even if lysozyme concentrations in the milk are very likely to be low. Even though the lytic activity of lysozyme present in the milk, measured *in vitro* on a *Micrococcus lysodeikticus* strain, appears higher than that of the lysozyme present in human milk or egg (Vakil *et al.,* 1969), its efficiency *in vivo* against the major pathogens involved in mammary infections still needs to be demonstrated. Synergic lytic effect produced on *E. coli* with IgA and complement (Wilson and Spitznagel, 1968 ; Hill and Porter, 1974) is unlikely to occur given the very low concentration of these components in milk.

4.1.3. *Lactoferrin.* Lactoferrin concentration in colostrum and bovine milk is low compared to that observed in man (Schanbacher and Smith, 1975 ; Smith and Schanbacher, 1977). The concentration increases with stage of lactation (Senft *et al.,* 1976) infection and inflammation of quarter (Harmon *et al.,* 1975 ; Harmon *et al.,* 1976) and during mammary gland involution when its concentration can be multiplied 100 times to reach 30 to 100 mg/ml (Welty *et al.,* 1976). Its antibacterial activity is due to its ability to chelate iron molecules, thereby inhibiting growth of certain bacterial species for which iron is indispensable, such as *Escherichia coli* and *Staphylococcus aureus* (Bishop *et al.,* 1976 ; Bullen *et al.,* 1978). High concentrations of citrate inhibit the antibacterial activity of lactoferrin. The greatest susceptibility of cows to new infections at the beginning of drying-off and in the period near to calving, coliform infections in particular, as well as the high level of resistance observed during mammary involution (Neave *et al.,* 1950) are almost certainly related to the changes in concentrations of lactoferrin and citrate and to the citrate/lactoferrin ratio which exist during these periods (Smith and Schanbacher, 1977).

These results suggest that the antibacterial activity of lactoferrin is not insignificant and that it could play an even more important role in the prevention of mammary infections if it was possible to increase its concentration in milk and dry secretions. There has recently been a successful experimental attempt to do this by infusion at drying-off with colchicine and/or endotoxin which accelerate the mammary gland involution process at drying-off (Smith and Oliver, 1981).

4.1.4. *Complement.* The biological properties of the activated complement, notably cytolysis, attraction of polymorphonuclear leucocytes by chemotactic factors derived from C_3 and C_5 and its participation in the ingestion and intracellular killing of bacteria indicate that it could play an

important role in the prevention of mammary infections.

Using the conglutination test, Reiter and Oram (1967) detected complement frequently in bulk milk and regularly in milk from individual cows, at the end of lactation, dry secretions and colostrum. In actual fact, this test only requires participation of the first components of the complement whose presence has been confirmed in milk from healthy quarters collected at mid-lactation (De Cueninck, 1979). The presence of all the components of the complement in colostrum has been demonstrated by bactericidal and/or hemolysis tests (Brock *et al.,* 1975a; Ecklad *et al.,* 1981). Complement activity diminished rapidly in samples collected at calving to practically nil 96 hours afterwards. Total complement activity seems variable (Reiter and Brock, 1975) if not absent (Ecklad *et al.,* 1981) in milk samples collected during lactation.

These results all suggest that the complement does not play an important role in the defence mechanisms against infection, at least during lactation. However, improvements in both preparation of milk samples and techniques for determination of complement in milk could possibly change these conclusions.

4.2. Immunoglobulins

Immunoglobulin concentration varies considerably ; it can exceed 100 mg/ml in colostrum, and falls to 0.5-1 mg/ml in milk collected during lactation (Lascelles, 1979). IgG_1 and IgG_2 immunoglobulins are blood derived either by a selective transfer mechanism for IgG_1 (Lascelles, 1979) or passively by adhesion to polymorphonuclear leucocytes (PMN) for IgG_2 (Watson, 1976). Selective transfer of IgG_1 is hyperactive during colostrum formation when they represent about 80 % of the total immunoglobulins, but it is greatly reduced during lactation. There are therefore about 50 times less immunoglobulins in milk than in blood. Although inflammation increases the permeability of the glandular mammary epithelium for serum proteins, selective transfer of IgG_1 is inhibited in the first hours after the acute phase of inflammation (McKenzie and Lascelles, 1968). IgA and IgM are quantitatively of little importance in milk (about 0.2 mg/ml) and are mainly locally synthesized.

There are several mechanisms possible for protection by immunoglobulins against mammary infections, these mechanisms intervening at different stages of the infection process. IgA and IgM can prevent adhesion of bacteria to the glandular mammary epithelium and thus facilitate their « flushing out » during milking. Cytophilic IgG_2 can act as opsonins making phagocytosis by PMN more effective. Immunoglobulins can also neutralize virulence factors such as toxins.

In order for these mechanisms to come into play, immunoglobulins present in milk firstly need to function as antibodies against antigens involved in the infection process, and secondly they need to be present in a sufficient and stable concentration. As we will see in the section dealing with vaccinations, the identification and complexity of antigens together with the low transfer of immunoglobulins from blood to milk has up until now rendered ineffective all attempts to immunize by systemic route. The alternative is local immunization. Although the local IgA immunity system is dormant in ruminants, contrary to that of most mammals, significant responses have been obtained after immunization in ewes (Lascelles and McDowell, 1970 ; Watson and Lascelles, 1973) and in cows (Newby and Bourne, 1977). In any case, we should consider whether it is really of interest to develop a local IgA immunity in the udder since this class of immunoglobulins could have an antiphagocytic effect (Wilton, 1978 ; Magnusson *et al.,* 1979), phagocytosis being recognised as one of the essential defence mechanisms. Local immunization poses a certain number of practical problems : it must be carried out in strictly aseptic conditions and should not result in damage to the secretory tissue, thus reducing milk production, these being some of the inconveniences which have already been encountered (Watson, 1981).

4.3. Leucocytes

The different leucocyte cell types, polymorphonuclear leucocytes, eosinophils, lymphocytes, and monocytes or macrophages, may be found in milk. According to a recent study (Lee *et al.,* 1980) PMN and macrophages cells involved in the phagocytic process, constitute 80 % to 90 % of cells in normal milk and epithelial cells less than 2 %. The threshold of 500 000 cells/ml is the maximum acceptable concentration for milk collected from non-infected quarters. The number of PMN is generally notably increased in infected quarters, but a low cell count is sometimes observed. Definitions of severity of infection and pathogenicity of bacterial species are based on cell count. Latent infections, subclinical and clinical mastitis, minor and major pathogens are thus distinguished (IDF, 1971 ; Griffin *et al.,* 1977 ; Poutrel and Rainard, 1982).

When milk from a quarter contains more than 500 000 cells/ml, it appears to be protected against experimental infections with *Aerobacter aerogenes* (Schalm *et al.,* 1964) or with *Staphylococcus aureus* (Postle *et al.,* 1978). Jain *et al.* (1968), demonstrated the efficacy of leucocytes in the prevention of infection. In their experiment, incidence of quarter infection by *Aerobacter aerogenes* was significantly, increased after leucopenia induced in cows by injection with equine anti-bovine leucocyte serum. Large numbers of leucocytes therefore make an efficient barrier against infection.

Milk leucocytes compared to those in the blood have, in fact, reduced phagocytic and bactericidal activity. Several hypotheses have been put forward to explain this deficiency. According to Naidu and Newbould (1973) milk PMN, by way of their containing 38 % less glycogen than blood PMN, possess less energy. In addition, milk is poor in specific opsonins, an important factor which may limit the efficacy of phagocytosis (Wisniowski *et al.,* 1965). Finally, it has been shown in *in vitro* experiments that the ingestion by PMN of casein and fat globules partially inhibits phagocytosis and killing of bacteria (Russel and Reiter, 1975 ; Paape *et al.,* 1975 ; Paape and Guidry, 1977).

This reduced efficacy of PMN shows that they must be present in large numbers in order to prevent infection. It has been shown that they appear in the udder in high concentrations about 24 hours after experimental infection with a small number of bacteria (Murphy and Stuart, 1953 ; Newbould and Neave, 1965 ; Schalm *et al.,* 1967), most probably, therefore, too late in the infection process. The fact that PMN need to be present in large numbers in order to be effective goes against the efforts made to improve the quality of milk based on a reduction in cell count.

In theory three solutions are possible for increasing the efficacy of phagocytosis :

— increase and maintenance of a high opsonin concentration in the milk ; the immunization and vaccination problem already mentioned ;

— increase and maintenance of high PMN concentration in certain fractions of milk, e.g. residual milk, in such a way that the cell concentration of the total milk remains within reasonable limits. As we will see in the section dealing with the induction of a non-specific resistance, promising results in this area have been obtained by insertion of an intramammary polyethylene loop fitted in the quarter ;

— exploitation, based on genetic selection, of the significant differences observed between cows with regard to phagocytic ability of PMN isolated from milk (Newbould, 1967 ; Paape *et al.,* 1978) or blood (Williams and Bunch, 1981).

Induction and stimulation of resistance to mammary infections

Two different ways of inducing and/or stimulating resistance of dairy cows to mastitis are possible. The first consists of developing a specific immunity directed against each of the principal bacterial species involved in mastitis, the second in investigating a non-specific protection, effective against all bacterial species. *A priori* these two courses cannot be seen as mutually exclusive and on the contrary may complement one another to offer better protection.

1. *Specific resistance : vaccination*

Induction of immunity to prevent mammary infections by vaccination comes up against two types of obstacle :

— the diversity of bacterial species, strains, virulence factors and consequently antigens ;

— the difficulty of obtaining antibodies in sufficient quantities and maintenance of high concentration.

The second point having already been discussed in the section dealing with immunoglobulins, only the problem posed by antigens, will be dealt with here.

In the past, numerous more or less empirical attempts at vaccination mainly against staphylococcal mastitis, have been made. Whatever the type of immunity investigated, be it anti-bacterial, anti-toxin or anti-enzyme, no vaccination has up until now proved effective against staphylococcal infections (Anderson, 1978). At best, a limited protection against homologous strains was recorded (Blobel and Berman, 1962) or a reduction in the severity of the infection was possible with anti-toxin antibodies (Derbyshire, 1960). These results cannot be considered satisfactory on the one hand because there exists a great variety of serotypes including within a given herd (Plommet and Plommet, 1970), and on the other hand, because most of the staphylococcal infections are sub-clinical and chronic, it is important above all to prevent infection. The possibility of experimentally infecting several times the same quarter of a cow during the same lactation or during the following lactation, by the same strain of *Staphylococcus aureus* or by a different strain (Postle *et al.,*

1978 ; Poutrel and Lerondelle, 1978) illustrates the difficulty of developing an effective immunity. In order to prevent infection, it seems important to intervene in the first stages of the infection process, hence the interest in identifying the virulence factors associated with these stages. The identification of « protective antigens » is all the more difficult since certain characteristics of the strains are not constant. Thus it was possible to induce production of an extra-cellular capsule with an antiphagocytic activity and providing strains with greater virulence, by culture of *Staphylococcus aureus* in raw milk or in a media rich in salt and carbohydrate (Brock *et al.,* 1973 ; Brock and Reiter, 1976). Strains used for vaccination possessing this capsule seem to offer better protection against a challenge (Brock *et al.,* 1975b). An antigen produced by *Staphylococcus aureus* cultured under *in vivo* conditions and different to the capsule was recently identified (Watson and Prideaux, 1979).

These results offer without doubt interesting prospects for the future and for the perfecting of vaccines effective against gram positive bacteria, staphylococci and streptococci, for which it seems difficult to obtain specific immune responses in the udder (Norcross, 1979). The development of sensitive methods of the ELISA type (enzyme linked immunoadsorbent assays) together with the improvement of purification techniques for bovine immunoglobulins (Butler *et al.,* 1980 ; Srikumaran *et al.,* 1981) now allows easier identification and quantification of the class and/or sub-class of immunoglobulins with antibody activity. This will be of help in exploring and specifying the best conditions for the induction of a persistant immunity in the udder : nature and quantity of antigens, nature of adjuvant, route, time and interval of immunisations.

2. *Non-specific resistance*

2.1. Genetic selection

2.1.1. Heritability of susceptibility to mastitis. Because of the chronic character of most infections, the lack of an effective therapeutic treatment, and the constraints and limitations of mastitis control methods, the possibility of developing a genetic selection as a means of increasing resistance of animals to mammary infections has in the past few years aroused even more interest.

A certain number of works indicate that differences in susceptibility to mastitis exist between cows. Infections are not observed to be randomly distributed between cows, this distribution differing significantly from binominal distribution. Thus the proportion of cows with 0 and 4 quarters infected is higher than would be expected from a random distribution (Flock and Zeidler, 1969 ; Grootenhuis, 1975 ; Poutrel and Rainard, 1981). This bias can partly be attributed to age of animals, cross-infection and herd level of infection, but genetic factors are also implicated (Rainard and Poutrel, soumis pour publication).

To determine how much variability in susceptibility to mammary infections relies on genetic factors, three principal models have been used :

— Dam-daughter heritability (Lush, 1950 ; Legates and Grinnells, 1952 ; Young *et al.,* 1960 ; Wilton *et al.,* 1972).

— Influence of paternal factor in daughters or sisters (Young *et al.,* 1960 ; Schmidt and Van Vleck, 1965 ; Afifi, 1968 ; Wilton *et al.,* 1972 ; Grootenhuis, 1976 ; Alrawi, 1979).

— Comparison of groups of cows with high and low level of infection and investigation of correlation with anatomical characteristics or biochemical markers (Geer and Grommers, 1979).

The great number of models together with the large variety of methods of diagnosing infections (bacteriological tests, isolation of a determined bacterial species, cell counts, abnormal appearance of milk, clinical signs), probably explain the fact that results differ between authors. Thus genetic predisposition to infection was found important by Lush (1950), Legates and Grinnells (1952) with heritability indices of 0.38 and 0.27 respectively, but it was found unimportant, for example, by Wilton *et al.* (1972) and Hebel *et al.* (1979) with indices of 0.10 and 0.13.

Works which do not take into consideration the results of bacteriological examinations report on heritability of susceptibility to inflammation, i.e. the way in which the host responds to infection, rather than susceptibility to infection itself which can be different. Unfortunately, any methodology which is based on bacteriological tests is difficult to carry out on a large scale due to its cost and the technical skills it requires. Another criticism can be made against most of the works : the results were recorded from cows of different ages of lactation. We have seen that age of lactation greatly influences incidence of infection. When this is taken into account in statistical analyses, however, interpretation of the results is complicated by it. It also appears preferable to record results from first lactation animals, as is done with genetic selection for milk

production. Finally the different models used do not permit a quantitative measurement of resistance to infection. The following suggestion by Smith and Schultze (1970) therefore deserves consideration. Putting forward the hypothesis that the more susceptible a cow is, the more rapidly it becomes infected, they propose an index which takes into account the number of days of lactation it takes for 1, 2, 3 and 4 quarters to become infected.

2.1.2. *Genetic selection markers.* In order to proceed to a genetic selection we need to identify marker genes for resistance, which may be :

— major genes which have an important effect in the mechanisms of this resistance ;

— marker genes closely linked to the major gene ;

— adjacent genes, located on either side of major genes.

Different genes exhibiting biochemical polymorphism and which are potential markers have been studied : the blood serum groups (Koch *et al.,* 1968 ; Stur *et al.,* 1976), transferrin (Malik, 1970) and β lactoglobulin (Giesecke and Osteroff, 1975 ; Kriventsov *et al.,* 1975 ; Stur *et al.,* 1976). Generally speaking, most of the authors draw similar conclusions, i.e. heterozygous animals appear to be the most resistant. It is difficult to generalize from these partial results, and necessary to carry out such studies on a large number of herds in different regions in order to ensure that the conclusions drawn remain valid whatever the breed or environmental conditions.

Among those markers which may constitute components of resistance the leucocyte count has aroused much interest in recent years. Using a sire-daughter model, notably Afifi (1968), Grootenhuis (1976) and Alrawi *et al.* (1979) record a heritability of cell count which according to them can be used as a basis for genetic selection, cows with the highest cell concentrations being considered the most susceptible to mastitis. This last hypothesis contradicts the « cell-barrier » concept, and can be explained if one accepts that it is susceptibility to inflammation and not susceptibility to infection which is thus taken into consideration. Heritability of cell concentration was not confirmed by Sethar *et al.* (1979) and Higgins *et al.* (1980). These authors, on the contrary, found a correlation between cell concentration and distance between udder and floor, a characteristic which appears highly heritable. The possibility, thanks to automation of techniques, of performing counts on a large number of animals, should in the near future give a more precise idea of the interest the cell count holds as a marker of genetic selection.

Other markers associated with mechanisms directly involved in the infection process, such as adhesion of bacteria and phagocytic ability of leucocytes, would also be worth investigation. Frost *et al.* (1977) in an *in vitro* model allowing measurement of the adhesion of bacteria responsible for mastitis to the epithelial cells of the lactiferous sinus, found differences between cows but noted no differences between quarters of the same cow. Significant differences between cows have also been noted with regard to phagocytic capacity of leucocytes isolated from blood (Williams and Bunch, 1981) and from milk (Paape *et al.,* 1978). This reduction in the efficacy of milk leucocytes appears moreover, to correlate with the increase in incidence of clinical mastitis.

Another possible approach in the research into markers directly implicated in resistance to infection consists of measuring capacity and variability of animals' humoral immune response. The difficulty lies in finding a satisfactory model. Thus it appears difficult to say whether results obtained by Lie (1979) in serum of bulls after injection with human serum albumin are also valid for capacity for resistance to different bacterial species in the udder of the cow. In certain animal species, notably the mouse, and in man, it has been possible to demonstrate that genes belonging to the major histocompatibility complex (Ir genes) control antibody response, and that this complex has a major effect on resistance to diseases against which the immune system plays a protective role (Klein, 1980). A major histocompatibility complex also exists in cattle (Amorena and Stone, 1978) but present knowledge on this and particularly on the Ir genes (Adams and Brandon, 1981) is still not sufficient enough to be put to use. The development and application of such research for increasing resistance to mammary infections depend on a satisfactory immunisation model.

Genetic selection applied to animals for disease resistance can only be justified if the three following conditions are fulfilled (Spooner *et al.,* 1975) :

— the disease should be of economic importance ;

— there should exist in the animal population a recognized variation in susceptibility to the disease ;

— the genes responsible should be identifiable, allowing for substantial and rapid progress.

The first two conditions are satisfied as far as mastitis is concerned but the third is not yet satisfied as we have seen. Furthermore, once the genes were identified, detection of characteristics determined by them would have to be as inexpensive as possible and selection would not have to go against certain important characteristics of production.

Field observations show that the advantage of higher resistance to mammary infections possessed by some cows is partially cancelled out by bad hygiene and lack of good mastitis control methods (Rainard and Poutrel, soumis pour publication). This indicates that it is unrealistic to expect 100 % efficacy from genetic selection in the prevention of infection to which it can still, however, makes a valid contribution.

2.2. Inducing a cell-barrier to prevent the establishment of intra-mammary infection

We have seen that phagocytosis could be more effective if PMN pre-existed in sufficient numbers in the udder, within acceptable limits for the quality of milk.

Quarters infected by minor pathogens, coagulase-negative staphylococci and *Corynebacterium bovis,* have on average cell counts higher than those of healthy quarters, but distributions of numbers are very similar and, in addition, 87 % of quarters infected by these minor pathogens have less than 500 000 cells/ml (Poutrel and Rainard, 1982). It has been demonstrated experimentally that quarters pre-infected with coagulase-negative staphylococci show increased resistance to a superinfection with *Staphylococcus aureus* (Linde *et al.,* 1975 ; Poutrel and Lerondelle, 1980) or with *Streptococcus agalactiae* (Bramley, 1978). The protection thus provided by the minor pathogens seems to a large extent due to the increase in cell concentration, even though other mechanisms are very probably involved (Poutrel and Lerondelle, 1980). Results thus obtained in severe challenge conditions seem promising. However, this approach is incompatible with the present mastitis control scheme in which treatment at drying-off and teat-dipping lead to the elimination of major as well as minor pathogens.

Paape *et al.* (1981) recorded an increase in cell concentration of about 50 % in quarters into which an intramammary polyethylene device (IMD) was inserted, as compared to control quarters. In milk fractions collected before letdown, stripping milk and residual milk, cell concentration is higher than 1 000 000 cells/ml, whereas it remains lower than 500 000/ml in total milk. These high cell concentrations found in fractions collected before and after milking suggest the possibility of good protection against pathogens which infect the udder between milking. There is also, in response to the irritation provoked by the IMD, a passive transfer of certain proteins from serum to milk, especially among the immunoglobulins of IgG_2 which, as we have already seen, function as opsonins. However, protection offered by the IMD against natural infections (Kortum, 1980) and experimental infections (Paape *et al.,* 1981) seems relatively limited and other experiments appear necessary in order to draw conclusions upon its efficacy in the prevention of mammary infections.

General conclusions

1. For dairy cows in identical environmental conditions, differences in susceptibility to mammary infections and mastitis exist between animals.

2. The variation thus observed is the result of two phenomena :

— the ease with which bacteria can penetrate the teat canal ;

— the host's capacity to prevent bacteria having penetrated the mammary gland from persisting and multiplying.

3. It appears already possible to act effectively on the first phenomenon, particularly by culling animals for which certain anatomical characteristics of the udder and teat make them more or less unsuitable for machine milking. A genetic selection concerning the distance between tip of teat and floor could also have a rapid effect in decreasing the incidence of infection.

4. As the teat is the « gateway » for bacteria, it seems necessary to develop research which has a bearing on the resistance mechanisms which exist at this level. Data on patency of the sphincter and its connection with incidence of infection for example, or on the possibility of selecting animals which have a low susceptibility to loss of patency according to the number of lactation, are insufficient if not absent.

5. The influence of certain physiological characteristics, such as age and stage of lactation, on susceptibility to mastitis is unanimously accepted. On the other hand, the influence of milking rate production which up until now has been the basis of intense selection, remains debatable and needs to be precisely defined.

6. The induction and/or stimulation of a specific or non-specific resistance in the udder is not foreseeable in the near future. Progress in this area is linked to a better knowledge of both the infection process (antigens, virulence factors, adhesion) and resistance mechanisms able to act in the udder (humoral and cellular immune responses, phagocytosis).

7. There exists a heritability of susceptibility to mastitis which affects both the ease with which bacteria can penetrate and the udder's capacity for defence. The diversity of model and criteria used by different authors does not at present allow evaluation of this heratibility. Although difficult to work with bacteriological criteria seem the only ones which really allow assessment of susceptibility to infection.

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