In the large farm animal species, particularly in cattle, blood typing is routinely used for parentage control. In France, the national Cattle Blood Typing Service, established by I.N.R.A. in 1958, performs, at present, about 9,000 parentage checks annually.

The blood type of an animal is established using a battery of monospecific reagents, which means that they cannot be further fractionated by absorption. The antigenic character detected on the red cell membrane by a monospecific reagent is called an "antigenic factor" (or "factor"). These definitions of a monospecific reagent and of an antigenic factor are purely operational. In cattle, a total of about 80 different antigenic factors have already been identified. All of them, except perhaps the J factor (STONE, 1962) are transmitted as simple mendelian characters. However, they are not all genetically independent, but belong to only 11 genetic systems which are of an unequal complexity. In the most complex systems, especially the B and C systems, but also in the S, F and A systems, an "allele" may control more than one, and sometimes up to some 15 factors. Such groups of factors, called phenogroups (STORMONT, 1955) were initially considered as indissociable (STORMONT et al., 1951).

The basic rules in parentage control are known as the two "exclusion rules". These rules define the situations in which the parentage of an offspring has to be excluded. They are: 1) when the offspring has received an allele absent in the genotype of both of its presumed parents (First exclusion rule); 2) when the offspring has not received an allele it should have received from one of its presumed parents (Second exclusion rule).

In practice, as each factor behaves as a simple mendelian character, parentage verification may be carried out considering only the presence or absence of factors. However, taking phenogroups into account is more discriminant, in that it makes possible the detection of wrong parentages that would have escaped a verification based on the comparison of factors only. In fact, as long as they are considered as indissociable genetic units, phenogroups and not factors, should logically be used first in parentage testing.

The difficulty lies in the fact that genetic recombination occurs within complex blood group systems, at least within the B and C cattle systems, in such a way that phenogroups cannot be considered as entirely indissociable units (for detailed references see GROSCLAUDE et al., 1979, for the B system, and GUERIN et al., 1981, for the C system). One then has to take...
this phenomenon into account in parentage testing. This implies an extensive
analysis of the problem, leading to guidelines usable in blood typing services.
The present report is the first attempt to provide such an analysis. To aid
clarity, the discussion will be confined to the B system, but it is easily tran-
posable to any other complex system with internal recombination, such as the C
system.

CURRENT STATUS OF THE GENETIC MAP OF THE B SYSTEM.

As will be seen later, the knowledge of the genetic map of the B
system (mainly deduced itself from observations on cases of recombination) as
well as the knowledge of the probabilities of recombination between the ge-
etic determinants of the various factors in the map, are of first importan-
ce for approaching the problem considered here. Figure 1 shows the current sta-
tus of the map, that is being established in our Laboratory. As compared to the
map proposed in 1979 (GROSCLAUDE et al., 1979) this present version includes
information derived from additional cases of recombination, that will be listed
in a forthcoming paper (GROSCLAUDE et al., 1982) : factors A" and B", not men-
tioned in 1979, have now been introduced ; in addition, factors 0₁, P₁, P', P₁
and the E'₃ factor close to T are more precisely located. As already discussed
by GROSCLAUDE et al. (1979) this map shows interesting features. Note for exam-
ple that factors, members of a particular subtype series may be scattered along
the map of the system (E' and J' series). This very likely indicates that trans-
position of duplicated segments occurred within the DNA sequence coding for the
B system. Factors I' and I" which are related (I' and I" form with I₂ a non
linear subtype system) may also derive from a duplication. Interestingly, a broa-
der homology is suggested between the map at the left (G₂, E'₃G", B', B") and at
the right (BG₂K, E'₃, G₃T) of E₁-Q' (remembering that T and B" form a non linear
subtype system with T₂).

The operational length of the DNA sequence coding for the B system,
d₀₁', that is the probability of recombination between the genetic determinants
of the terminal factors, Q and I', was estimated to be 7.10⁻³, or 0.7 centimor-
gan (GROSCLAUDE et al., 1981). For the moment the distances of only two subdi-
visions have been estimated : between the determinants of Q and I₁ (d₀₁' = 4.10⁻³)
and between those of K' and I' (dₓ₁' = 2.8.10⁻³).

SOME OBSERVATIONS ON THE PROBABILITY OF RECOMBINATION.

In the following, the term "new" phenogroup will be used to designate
a recombined phenogroup different from both parental phenogroups, whether this
particular phenogroup has already been observed or not in the breed. "New" thus
refers to the parental phenogroups, and not to the phenogroups found in the
breed.

It is easy to see that recombination within the B system will only pro-
duce a "new" phenogroup if the parental phenogroups differ by at least 2 non-
allelic factors, i.e. when at least 2 non-allelic factors are in a heterozygous
state in the parental genotype, whenever they are in cis or in trans position.
Thus, recombination can neither produce a "new" phenogroup from a homozygous genotype, nor from a genotype with only one heterozygous factor (eg. \(B_{3G1TE}^{31}K'/B_{G1TE}^{13}K\) the most frequent genotype in the Montbéliarde, or \(B_{1E4}^{13}I'/B_{1E4}^{13}I\) common in the Charolaise, etc...).

The other rule is that the apparent probability of recombination of an individual genotype, i.e. the probability of a "new" phenogroup appearing in the gametes of an animal with this genotype, depends on the distance between the two heterozygous factors when the genotype includes only two such factors, or between the two terminal heterozygous factors when the genotype includes more than two heterozygous factors. The shorter the distance, the lower the apparent probability of recombination. This probability may vary from nearly 0 (terminal factors very close on the map) to \(7 \times 10^{-3}\), the latter value being the estimate of \(d_{Q1}\) (GROSCLAUDE et al., 1979).

Because the apparent probability of recombination of a particular genotype, may vary from 0 to \(d_{Q1}\), the apparent mean probability of recombination in a breed \(R_{\text{breed}}\), i.e. the probability that a gamete randomly drawn from the breed transmits a "new" phenogroup, is lower than \(d_{Q1}\). \(R_{\text{breed}}\) can be estimated, under certain assumptions, knowing the phenogroup frequencies in the breed; it was found by GROSCLAUDE et al. (1982) to have a rather constant value, close to \(2 \times d_{Q1}/3\), in 3 different breeds (Charolaise, Normande, Montbéliarde) and under different assumptions regarding the relative distance of factors in the map of the system. With \(d_{Q1} = 7 \times 10^{-3}\), as estimated by GROSCLAUDE et al. (1979), \(R_{\text{breed}} = 4.5 \times 10^{-3}\).

Finally, it is interesting to consider the probability of an offspring receiving a "new" phenogroup from one of its parents. This probability is of course equivalent to \(2R_{\text{breed}} = 9 \times 10^{-3}\), thus close to \(10^{-2}\). However, in practice, the "new" phenogroup will not always be detected in the offspring blood type, due to a masking by the phenogroup transmitted by the other parent. The proportion of families in which a "new" phenogroup is expected to be directly observed is thus lower than \(9.10^{-3}\). An order of magnitude of \(5 \times 10^{-3}\) can be proposed as a first approximation. This value is not negligible, and cattle blood group specialists may consider it to be an overestimate. As a matter of fact it is derived from only one estimate of \(d_{Q1}\), and may thus need revision. Perhaps more importantly, extrapolation from this value, which was obtained in the descent of a few genotypes in one single breed, is questionable if the rate of recombination within a given interval can vary depending, for example, on the particular characteristics of the DNA structures controlling the different phenogroups.

TAKING RECOMBINATION INTO ACCOUNT IN PARENTAGE CONTROL.

One is faced with the problem of discriminating between erroneous parentage and occurrence of recombination at the B system in the following situation: 1) the phenogroup presumed to originate from one of the parents is different from both of its own phenogroups, but only includes factors
present in these phenogroups; 2) no incompatibility is detectable using any of the other blood group loci.

Theoretically, it would be possible to make a decision on some of such cases (hereafter designated as "dubious" cases) if all possible parents of an offspring were known and their blood types available. In fact, under present farming conditions and use of blood typing, such conditions are rarely fulfilled, at least in France. Consequently, examination of a dubious case can only be based on the probabilities of the two alternative possibilities about the parentage, i.e. to be either right or wrong.

Suppose for example a dubious case in which, the phenogroup in question in the B system, called \( \phi \), was received by the offspring from the sire. The calculation of the a posteriori probabilities of this parentage being right \( (P_{R/\phi}) \) or wrong \( (P_{W/\phi}) \) is possible, following a Bayesian approach.

Let \( w \) be the a priori probability of the parentage being wrong, hence \( 1-w \) the a priori probability of the parentage being right.

- \( f \) the frequency of the phenogroup \( \phi \) in the "population" (breed, herd or group of AI bulls),
- \( r \) the probability of the particular recombination that would produce the phenogroup \( \phi \) from the genotype of the presumed sire,
- \( e \) the probability of exclusion of a wrong sire, drawn from the "population", using blood group systems other than the B system, hence \( 1-e \) is the probability of non exclusion.

It can be seen that the probability of observing the supposed case with the hypothesis that the parentage is right, is \( r \), and with the hypothesis that the parentage is wrong, is \( f \) \( (1-e) \).

Then, applying the Bayes theorem gives:

\[
P_{R/\phi} = \frac{(1-w) \cdot r}{(1-w) \cdot r + w \cdot f \cdot (1-e)} = \frac{1}{w \cdot f(1-e) + (1-w) \cdot r}
\]

and

\[
P_{W/\phi} = \frac{1}{1 + \frac{(1-w) \cdot r}{f(1-e)}}
\]

It seems valid to retain for \( w \) the value of the observed mean rate of parentage errors in the breed, or in the herd, although in a similar situation (estimation of the probability of paternity in man), such an option was rejected by SALMON-BONNEROT (1977). Assuming for example the values \( w = 0.04 \) and \( 1-e = 0.5 \), then
Fig. 1. Tentative partial map of the B system of cattle blood groups, according to GROSCLAUDE et al. (1982). The linear order of 17 genetic determinants, or groups of genetic determinants, is given in the median line. In brackets: the order of the enclosed determinants could not be established. Groups of factors not enclosed in brackets (YY', E'3G", E'3G"F18, O3K") correspond to subtype or subtype-like associations.

The other genetic determinants shown are located according to the arrows: <— > defines the region in which a particular determinant occurs (e.g. the determinant for B" occurs between those for G' and for E'1); (or — >), indicates that this genetic determinant is located to the left (or to the right) of another determinant (e.g. the determinant for E'1 is to the left of that for B). * BG2K is very likely close to GjT. The operational distance between the determinants for Q and I' was estimated to be 0.7 centimorgan (GROSCLAUDE et al., 1979).

Fig. 2. Diagrammatic representation of the transmission of the B system factors of the Normande bull "Bail" to 342 offspring showing the occurrence of 5 cases of recombination. The genotype of the bull in this system is Bg1A"I"/Bg1O'P'1A"B"I". •: an offspring whose dam did not possess factors O', P', and B" in her genotype; these matings are all conclusive and the bull transmitted either GjA"I" or GjO'P'1A"B"I"; o: an offspring whose dam possessed part, or all, of the above 3 factors; part of these matings are not conclusive; 1: an offspring having received the recombinant phenogroup GjP'1A"B"I"; 2: an offspring having received the recombinant phenogroup GjO'"A"I".

In all five cases of recombination the matings were conclusive. Note that 3 offspring tested consecutively have received a recombinant phenogroup, an event with a very low probability.
As could be intuitively expected, the values of $P_{R/\phi}$ and $P_{W/\phi}$ will depend on the relative values of $f$ and $r$. For example, $P_{R/\phi}$ has low values ($\leq 0.05$) when the phenogroup $\phi$ is frequent ($f = 10^{-1}$) and when $r \leq 10^{-4}$, the latter range of values corresponding to very short intervals in the map, or to genetic events more complex, thus much rarer than single crossing-over (see below). Conversely $P_{W/\phi}$ has low values ($\leq 0.02$) when $\phi$ is rare ($\leq 10^{-3}$) and when the value of $r$ corresponds to the occurrence of single crossing-over within a longer interval ($10^{-3} < r < 7.10^{-3}$).

However, the main difficulty does not lie in the estimation of $P_{R/\phi}$ and $P_{W/\phi}$ but in the problem of which decision should be taken about the dubious parentage, knowing $P_{R/\phi}$ and $P_{W/\phi}$. This is an interesting problem of choice between two risks: exclude a right parentage ("a risk") or accept a wrong parentage ("b risk"). It would require a detailed discussion as in the analysis by SALMON-BONNEROT (1977) of paternity problems in man.

As far as cattle parentage control is concerned, a policy may be rather simply defined for practical use. This policy is based, as widely accepted, on the necessity of minimizing the a risk, if not of annuling this risk.

In these conditions the guidelines regarding dubious parentages could be as follows:

- If the phenogroup in question apparently derives from single crossing-over and if this particular phenogroup is absent or very rare in the breed, the parentage can be accepted, with a reasonably low b risk.

- If the phenogroup in question could only derive from a genetic event more complex than single crossing-over and if this particular phenogroup is frequent in the breed, the parentage should be rejected, with a reasonably low a risk.

- In any other situation, attempts should be made to get more information on the case: 1) by trying to identify with certainty all possible sires, 2) by making use of additional polymorphic systems, in order, if the parentage were wrong, to detect the incompatibility at a system other than the B system.

If no decisive conclusion can be drawn from these additional investigations, the parentage should be accepted. In this case, the greater the number of polymorphic systems considered, the lower will be the b risk.
Note the importance, for this procedure, of the information already available on the genetic map of the system, and on the rate of recombination.

Finally, it should be underlined that the problem of decision about dubious parentages is complicated by the fact that low probability, or even very low probability, does not mean certainty of non occurrence. In support of this, we have already demonstrated that genetic events other than single crossing-over actually occur within the B system (see "case Violon" in GROSCLAUDE et al., 1979). In addition, Figure 2 summarizes observations made on the progeny of a Normande bull "Bail": it can be seen that 3 recombinant phenogroups were successively observed in his progeny, although such an event had a probability of only about $10^{-6}$. In connection with this, one should be prepared for a case of simultaneous recombination within both the B and C systems.

In conclusion, although they do not, generally speaking, call in question the use of phenogroups rather than factors in parentage control, it is clear that recombination and the possible occurrence of other "accidents" within complex blood group systems should be considered seriously and that these phenomena are indeed a source of increased difficulties. Only a precise knowledge of the genetic map of the systems, including the estimation of the distances between the genetic determinants of the various factors, together with a clear vision of the different aspects of the problem, will help avoiding contestable conclusions.

ACKNOWLEDGEMENTS.

The author thanks Drs B. BONAITI and R.L. SPOONER for useful suggestions.

SUMMARY

The occurrence of genetic recombination within the two most complex blood group systems of cattle, the B and C systems, complicates parentage control. The problem is discussed taking the B system as an example. The analysis is based on results already obtained on the genetic map of this system (Fig. 1, according to GROSCLAUDE et al., 1982) which is controlled by a chromosomal segment whose length, $d_{QI}$, was estimated to be 0.7 centimorgan (GROSCLAUDE et al., 1979). The probabilities of observing, due to a single crossing-over, a phenogroup different from both parental phenogroups varies, depending on the genotype, from 0 (homozygous genotypes, or genotypes with only one heterozygous factor) to $d_{QI}$, thus $7 \cdot 10^{-3}$. The order of magnitude of the probability of observing the occurrence of a recombination in a parentage was estimated to $5 \cdot 10^{-3}$, a value which is only preliminary.
In parentage verification, the existence of genetic recombination should be taken into consideration when, in a complex system, the phenogroup supposed to be transmitted to the offspring by one of the presumed parents is different from both phenogroups of this parent, but only includes factors present in these phenogroups, and when the parentage is compatible in other respects. The a posteriori probabilities for this parentage to be right \( (P_{R/\phi}) \) or wrong \( (P_{W/\phi}) \) can be estimated following the Bayesian method. Their values depend mainly on the relative values of \( r \) and \( f \), \( r \) being the probability of recombination producing the particular phenogroup, given the genotype of the presumed parent, and \( f \) being the frequency of the same phenogroup in the population. Knowing the values of \( P_{R/\phi} \) and \( P_{W/\phi} \), the decision to be taken depends on the choice between two risks, that of excluding a right parentage (\( \alpha \) risk) and that of accepting a wrong parentage (\( \beta \) risk). Guidelines are proposed for the usual situation where the \( \alpha \) risk has to be minimized.

After recalling that genetic events more complex than single crossing-over have been shown to occur in the B system (GROSCLAUDE et al., 1979), and after depicting an actually observed, although unexpected case, where 3 consecutive recombinants were found in the progeny of a bull (Fig. 2), it is underlined that, sooner or later, other events with a very low probability may be expected to occur, which complicates the problem considered here even more.

**RESUME**

L'incidence d'une recombinaison génétique au sein des deux systèmes de groupes sanguins bovins complexes, les systèmes B et C, rend plus délicate l'expertise des filiations. Le problème est discuté, à titre d'exemple, dans le cas du système B. L'analyse se base sur les résultats déjà acquis sur la carte génétique de ce système (Fig. 1, d'après GROSCLAUDE et al., 1982) qui est contrôlé par un segment chromosomique dont la longueur, \( d_{Q_1} \), a été estimée à 0,7 centimorgan (GROSCLAUDE et al., 1979). La probabilité d'apparition, sous l'effet d'une recombinaison simple, d'un phénogroupe différent des deux phénogroupes parentaux, varie, selon le génotype, de 0 (génotypes homozigotes, ainsi que génotypes hétérozygotes pour un seul facteur antigénique) à \( d_{Q_1} \), donc \( 7.10^{-3} \). L'ordre de grandeur de la probabilité d'observer la survenue d'une recombinaison dans une filiation a été estimé à \( 5.10^{-3} \), ce qui n'est toutefois qu'une première approximation.

En matière d'expertise de filiations, le problème lié à l'existence de la recombinaison se pose, quand, dans un système complexe, le phénogroupe considéré comme transmis au produit par l'un des parents présumés est différent des deux phénogroupes de ce parent, mais ne comporte que des facteurs constituant ces derniers, alors que, par ailleurs, la filiation est compatible. Les probabilités a posteriori que cette filiation soit vraie \( (P_{R/\phi}) \) ou fausse \( (P_{W/\phi}) \) peuvent être estimées par la méthode Bayesienne. Leur valeur dépend beaucoup des valeurs relatives de \( r \) et \( f \), \( r \) étant la probabilité d'apparition.
du phénogroupe par recombinaison connaissant le génotype du parent prémumé, et la fréquence de ce même phénogroupe dans la population. Connaissant les valeurs de \( P_{R/\phi} \) et \( P_{W/\phi} \), la décision à prendre dépend du choix entre deux risques, celui d'exclure une filiation vraie (risque \( \alpha \)), et celui d'accepter une filiation fausse (risque \( \beta \)). Une ligne de conduite est énoncée pour le cas habituel ou l'on cherche à minimiser le risque \( \alpha \).

En rappelant la mise en évidence, au sein du système B, d'événements génétiques plus complexes que la recombinaison simple (GROSCLAUDE et al., 1979), ainsi que l'observation d'un cas, a priori fort improbable, où 3 recombinaisons ont été trouvées consécutivement chez des produits d'un taureau (Fig. 2), il est souligné qu'à la longue, même des événements de faible probabilité en viennent à se produire, ce qui complique encore le problème considéré ici.

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