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**Towards a better understanding of using breeding to control
mastitis in sheep and cattle**

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June 2005



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1. Background, introduction and aetiology of mastitis

Mastitis is the biggest single reason for culling Texel sheep prematurely in the UK (S. McLean, pers. comm.) For this reason, a main priority of the Texel Sheep Society is to initiate and support the development of long-term strategies to breed mastitis-resistant sheep. This is particularly important, as long-term approaches to control disease in farm livestock are urgently needed, as there is growing evidence for resistance to the use of antibiotics in farm livestock.

This report reviews the disease both in sheep and cattle, methods of diagnosis, the current control measures in use, and the extent to which breeding (including our molecular genetic knowledge) for resistance to mastitis is an effective tool to avoid premature culling for mastitis, to extend the productive life of Texel sheep. In addition, using Markov-chain dynamic programming, it includes results from modelling the economic impact of the disease at farm level.

Most of the information available on mastitis in the literature relates to dairy cattle, with well-documented evidence of the causative bacteria, prevalence and incidence of disease, economic costs and control measures, including quantitative and molecular methods of breeding for resistance to mastitis. Much less information on mastitis is reported for sheep, which in turn, is dominated by the dairy sheep sector in Mediterranean countries. There is almost no reported data on mastitis in terminal sire lines. For this reason, this review includes literature on mastitis on both dairy cattle and sheep, with the premise that such information will also be relevant to Texel and other meat sheep breeds.

Introduction

Mastitis is considered to be one of the most important health problems in dairy cattle and sheep (Heringstad *et al.*, 2005; Leitner *et al.*, 2004). It can be defined as an inflammation of the mammary gland resulting from the introduction and multiplication of pathogenic microorganisms in the mammary gland. The main causative bacteria include *Staphylococcus aureus* and *Streptococcus agalactiae*, (both of which are contagious) and coliforms, streptococci and enterococci. All of these pathogens are found in animals' environment (bedding, manure and soil). These major pathogens can cause **clinical mastitis**, which can lead to swelling or pain in the udder, changes in milk composition and appearance, increased rectal temperature, lethargy, anorexia and even death. Other, minor pathogens are also responsible for inflammation of the udder and a rise in Somatic Cell Count (SCC), although they rarely lead to changes in milk composition. **Sub-clinical mastitis** does not lead to visible changes in milk or the udder, although it is characterised by reduced milk yield, altered milk composition and the presence of inflammatory components and bacteria in milk. Both forms of mastitis can have serious economic consequences, due to loss of production and premature culling of affected animals.

1. Aetiology and diagnosis of mastitis

There are many different strains of bacteria that have been implicated in mastitis in sheep. A total of 447 *Micrococcaceae* strains (389 of which were identifiable) were isolated in a total of 497 sheep and goat milk samples (Deinhofer & Pernthaner, 1993). As well as the most common mastitis causing bacteria (*S. aureus* and *Streptococci*), several other pathogens have been isolated in mastitic sheep mammary glands. These include *Actinomyces pyrogenes* (Saratsis *et al.*, 1998), *Clostridium perfringens*, *Escherichia-Coli* and *Pasteurella haemolytica*, amongst others. However, most serious teat lesions are generally associated with *S. aureus* (Onnash *et al.*, 2005) with approximately 35% of clinical cases due to this bacteria alone. Other coliforms such as *Pseudomonas aeruginosa* and *Klebsiella pneumonia* have also been implicated in outbreaks of acute mastitis with high levels of mortality in lactating dairy sheep (Menzies, 2000).

The diagnosis of mastitis is conventionally undertaken using clinical examination and if necessary, by bacterial culture using laboratory tests. These include for example ATB 32 STAPH tests. However to the farmer and practitioner, the causative organism is not as important as the total amount of all mastitis-causing pathogens. For this reason, diagnostic tools exist that allow farmers to monitor bacterial counts of the milk, as a way of managing mastitis in their flock (or herd). All techniques have been developed for **dairy** cattle and sheep, and hence rely on using milk samples for undertaking the tests.

The most common method for the diagnosis of high bacterial counts in milk due to mammary infections is the use of Somatic Cell Count (SCC). This test is done in the laboratory where counts of epithelial and inflammatory cells (per ml) are made from a milk sample (usually taken on 'test' days, i.e. at regular intervals during lactation). The levels of cell counts indicate the presence or absence of organisms that cause intramammary infections. SCC can be regarded as a surrogate measure of mastitis and is a good gauge of the existence of subclinical mastitis (Barillet *et al.*, 2001). It is important to know at what levels of SCC milk production and the health of sheep are affected. In dairy sheep, current estimates in the range of 600,000 to 800,000 per ml indicate that infections are present (Billon & Decremoux, 2005) and ewes with less than 500,000 per ml are considered to be 'healthy' (Bergonier *et al.*, 1994), subsequently confirmed by Barillet *et al.*, 2001. In dairy cattle recording schemes it is generally accepted that SCC is a more accurate assessment of udder health than records of clinical mastitis (CM) because (i) CM field data are routinely lacking completeness, accuracy and standardisation, (ii) SCC has a higher heritability than CM (Mrode *et al.*, 1998) and therefore is more suitable for use in breeding programmes, and (iii) it reflects incidence of subclinical intramammary infections (Rupp & Boichard, 1999).

The California Mastitis Test (CMT) as the name suggests, is another diagnostic tool used in the USA to detect mastitis. It reacts with DNA present in epithelial and inflammatory cells to form a gel. The greater the DNA present in the milk then the greater the amounts of gel present in the test and the greater the bacterial count. Even though the end result is essentially a subjective assessment of the amount of gel present, it correlates well with somatic cell counts (Hueston *et al.*, 1986.; Menzies, 2000). However, there are contrasting reports on the efficacy of the CMT. In a study comparing different methods to quantify mastitis, the CMT method proved to be a less reliable method as it only explained 33% of the variation in SCC scores, Keisler *et al.*, 1992. By comparison, in a study of Pampinta dairy sheep in Argentina, the correlation between CMT and SCC was 0.64, with the CMT scores (in brackets) corresponding well to the SCC levels 223,576 (0), 245,248 (1), 1,159,109(3), and 2,460,833(4) cells/ml (Suarez *et al.*, 2002).

Flow cytometry (FC) is a method by which physical and chemical characteristics of cells or particles can be measured as they travel in suspension past a sensing point. This method has been developed recently to quantify Somatic Cell Counts in milk, and is particularly good for detecting subclinical mastitis (e.g. Tian *et al.*, 2005; Holm *et al.*, 2004).

The use of FC is now being offered as a service to Nevada sheep farmers to test for subclinical mastitis (Melanie McFarland *et al.*, 2000, Holcombe, 2005). Importantly, it also relies on the collection and analysis of milk samples.

2. Incidence and timing of mastitis

Sheep

There are several reports of the prevalence of mastitis in sheep from different countries and inevitably, in different breeds. These vary greatly, and according to how they were recorded. Some studies were conducted in abattoirs on cull ewes, and others using farmer surveys. Notably, there is not a general consensus among the reports. However, culling due to mastitis in Rambouillet sheep in the USA was reported to be 46% (Holcombe, 2005), and between 13% and 50% in the UK, following inspection at the abattoir (Bocklisch & Wetzstein, 1994b). Mastitis in dairy sheep in the USA, as identified by a positive milk culture, was reported to be up to 35% of ewes, and by positive SCC and milk culture, of 4 to 17% (Menzie, 2000). In a study on 6500 ewes of a large German sheep breeding unit over two lambings, an average of 7% of ewes suffered from clinical mastitis, and 84% of all ewes had udder-pathogenic bacteria Bocklisch & Wetzstein, 1994a. In a study of Irish ewes, acute clinical mastitis incidence was reported to be 0.53% although chronic sub-clinical mastitis was 4.5 times higher than this at 2.8% of all ewes (Onnash *et al.*, 2005). The incidence, risk and the aetiology of mammary abnormalities during the dry-period were examined in a study of Greek dairy ewes. Abnormal secretion, lumps, nodules, diffuse hardness, abscesses and cysts were the abnormalities detected. The cumulative incidence of mammary abnormalities during the dry-period was 5.1%. Forty seven percent of the cases detected developed during the first three weeks after cessation of lactation (Saratsis *et al.*, 1998). Little relevant information is published for the UK sheep situation although in 1991, Watkins *et al.* reported results from 2092 milk samples that had been collected at 3-weekly intervals from 358 ewes. The period prevalence of subclinical mastitis in this study was 11.7% and the prevalence remained relatively constant over the course of lactation (5.5-7.0%). The prevalence of subclinical mastitis increased with age of ewe but was not influenced by the presence of teat lesions. Also, there was a significant association between the development of clinical mastitis and antecedent subclinical mastitis caused by the same organism.

The timing for the onset of mastitis is important if future research into breeding for mastitis-resistant sheep necessitate the collection of milk samples. In a review by (Bergonier *et al.*, 2003) the majority of mastitis cases observed in 5 studies occurred at the beginning of machine milking up to the first third of lactation. Other studies reported mastitis occurrence from the first week post-partum (Onnash *et al.*, 2005) to three weeks after the cessation of lactation (Saratsis *et al.*, 1998), although according to Bergonier *et al.*, 2003) the mastitis occurrence at drying off is unusual, and mainly caused by different pathogens related to poor environmental hygiene practice.

Cattle

In French dairy cattle, clinical mastitis incidence is reported to be 12.6% in the first lactation cows, and 20% for all parities (Rupp & Boichard, 1999). In the USA, 27% of first lactation, and 22% in the second lactation had at least one clinical mastitis episode (Nash *et al.*, 2002). Other European studies report lower incidence, being 10.9 % in Finland and 11.1% in Sweden for a lactation period from -10 to 150 d, and 21% in Denmark for -10 to 180 d. This may well be due to the inclusion of resistance to mastitis in their breeding programmes for the past two decades (Stein & Sehested, 1999). Another Swedish study showed the number of veterinary-treated cases of mastitis per 100 lactations to be 18.3 in year 2000–2001, and udder diseases, together with high SCC, were the second leading reason for culling in year 2001, accounting for nearly 24% of culled cows (Svensk Mjöl, 2002).

Several studies on the prevalence and incidence of mastitis (Bennett *et al.*, 1999a,b) were used to derive a series of assumptions to estimate the economic impact of mastitis in the UK. For subclinical mastitis, the estimate was between 15-20% of all quarters affected in a 12-month period, and the incidence rate of 32-71 cases of clinical mastitis treated per 100 cows per year.

3. Economics of mastitis

Economic loss due to mastitis is of major concern to most dairy industries. Such losses are consequences of lower milk production, poor milk quality, discarded milk, costs of veterinary care and involuntary culling. In addition, there is emerging evidence on resistance to antibiotics by some of the mastitis-causing bacterial strains (Saratsis *et al.*, 1998). These two issues alone support the case for long-term, sustainable strategies to control mastitis in sheep. However, there is sparse evidence to support the extent of losses incurred that are directly due to mastitis.

Cattle

The most comprehensive study of the economic costs associated with mastitis in the UK dairy herd is that of Bennett (Bennett *et al.*, 1999a,b). These studies included estimates of output losses due to mastitis, to reflect differences in the incidences of both clinical and subclinical mastitis as well as variations in the reported impact of the disease on milk loss and premature culling. In these studies, the total annual costs of clinical mastitis to the UK dairy industry, not including penalties on subclinical mastitis were reported to be £187m. Another more recent study on the opportunities for incorporating genetic elements into the management of farm animal diseases (Bishop *et al.*, 2005) reported that additional benefits from improved milk quality through decreased SCC depend critically upon the current mean SCC. At the national average SCC, the benefit of reducing SCC by 1% is £0.50/cow. At a national penetration of 50% this translates to a cumulative annual benefit of £0.6m resulting in total benefits of £1.5m/year to the UK dairy industry.

Sheep

The economic consequences of mastitis in sheep are manifested in low weaning weights, lamb deaths and the culling of affected ewes. Losses in milk production have been reported to be between 20 and 37% leading to up to 4kg difference in lamb weights at weaning between lambs from mastitic vs. non-mastitic ewes (Menzies, 2000). Differences in milk production estimated between affected and non-affected udders in sheep resulted in differences up to 50% Keisler *et al.*, 1992, 55% (Saratsis *et al.*, 1999) and 37% (Fthenakis & Jones, 1990). In the latter study, the reduction in milk quality as a result of both clinical and subclinical mastitis lead to a reduction in lamb growth of 66g/day. The implications for having high somatic cell count (SCC) milk in French dairy sheep breeds is similar to that for dairy cattle, with economic penalties of around 10% Barillet *et al.*, 2001. In the UK, sheep milk has to conform to standards as recommended by the British Sheep Dairying Association, although payments according to milk SCC quality are variable and are not set at a national level.

In order to justify expenditure on tackling disease, it is important to understand the effects of mastitis on the flock to bridge the existing gap in quantifying economic costs to the UK sheep industry. For this reason, the study below quantifies the costs of mastitis tailored to pedigree Texel flocks.

Cost of Sheep Mastitis in Texel Flocks

Costing farm animal disease is not a simple exercise. Inappropriate calculation may be misleading and could lead to poor and ultimately expensive decision making (Stott, 2005). Unfortunately, few published estimates of the costs of specific diseases follow good practice as laid out by McInerney (1996). The reason for this is usually lack of appropriate data (Bennett, 2003). All too often the average total cost (output losses plus control costs) of a disease is quoted which is meaningless (McInerney, 1996). The main reason for this is that such costs represent the average balance between the disease and the investments made in controlling it, and *not* its impact on individual farm profitability, which is particularly important for endemic diseases like mastitis. Such diseases cannot be eradicated overnight at no cost to the farmer as is implied by the average total cost figure. What farmers really need to know is how much more or less should they be spending on mastitis in order to minimise its total cost to them? This is known as the 'avoidable loss' (McInerney, 1996) and is the amount by which farm profits would increase if the farmer were to deal with mastitis in the best possible way. The approach described here aims to assess the cost of mastitis in this way within the constraints of knowledge of the disease.

Modelling

To overcome the problem of data shortage, the Epidemiology Unit at SAC have pioneered the use of spreadsheet models to represent the epidemiology and economics of endemic disease in Scottish farm livestock (see for example Gunn *et al.*, 2004). A similar approach is taken here. Aspects of the epidemiology of mastitis in sheep flocks likely to affect farm profit were represented using a Markov chain (Agrawal & Heady, 1972). The flock is described in terms of 'states' through which ewes may pass during a sequence of annual production cycles. In this case the states were parity (1 to 5), mastitis status (yes or no) and replaced, giving a total of 11 states. This is of course, only a rough approximation of practical realities, designed to fit the constraints and objectives of this study.

The progression of the ewes through the states is determined by the risk of contracting mastitis and by replacement rates. The latter are shown in Table 3.1.

Table 3.1. Replacement rates by parity in the Markov chain

Parity	Proportion replaced
1	0.20
2	0.17
3	0.17
4	0.19
5	1.00

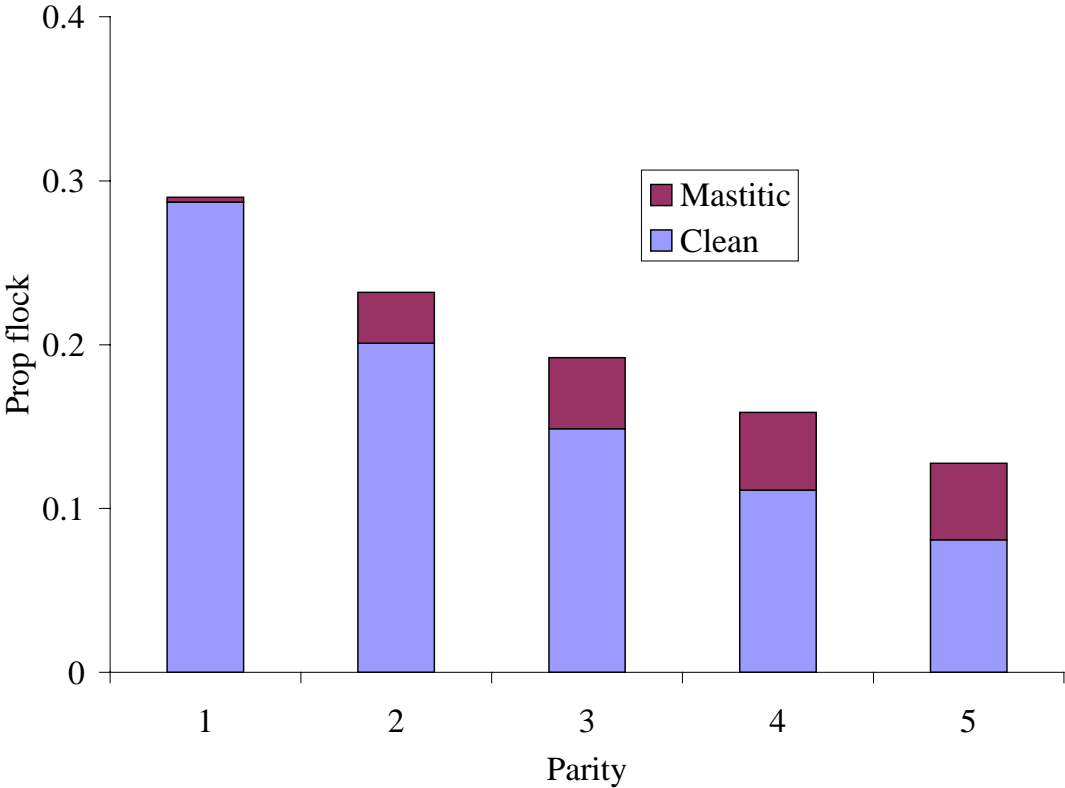
Replacement rates for mastitic ewes were assumed to be 0.10 greater than those shown in Table 3.1. The risks of mastitis by parity are shown in Table 3.2.

Table 3.2. Mastitis risks in the Markov chain

Risk	Probability
Of mastitis in parity 1	0.10
Of mastitis in parity $n+1$ if free in parity n	0.10
Of mastitis in parity $n+1$ if mastitic in parity n	0.75

As all these assumptions are fixed over time, the flock structure is stable in the long run and independent of the flock structure assumed at the start of the model. The long-run flock structure is shown in Figure 1.

Figure 1: Long run flock structure predicted by the Markov Chain



The long run flock structure reflects the assumptions in Tables 3.1 and 3.2, which represent the impact of mastitis on flock dynamics. This flock structure was converted into an enterprise gross margin using the physical and financial performance assumptions shown in Table 3.3. For the basis of gross margin calculations in this context see SAC (2004) page 212-213, lowground breeding ewes - early finished lamb production. Notice that we have assumed that mastitis reduces the proportion of lambs fit for sale as breeding animals by a factor of 0.33. However, it is assumed to have no other effect on physical output or on output prices.

The difference in gross margin obtained by changing appropriate assumptions in tables 3.1 to 3.3 gives an indication of the main costs of mastitis. If the assumptions associated with current and best practice in mastitis control are known then the avoidable loss can be calculated as demonstrated by McInerney (1996). In the absence of such knowledge, sensitivity analysis can be performed on the assumptions to indicate the likely magnitude of the avoidable loss or the most important missing information needed to assess the avoidable loss.

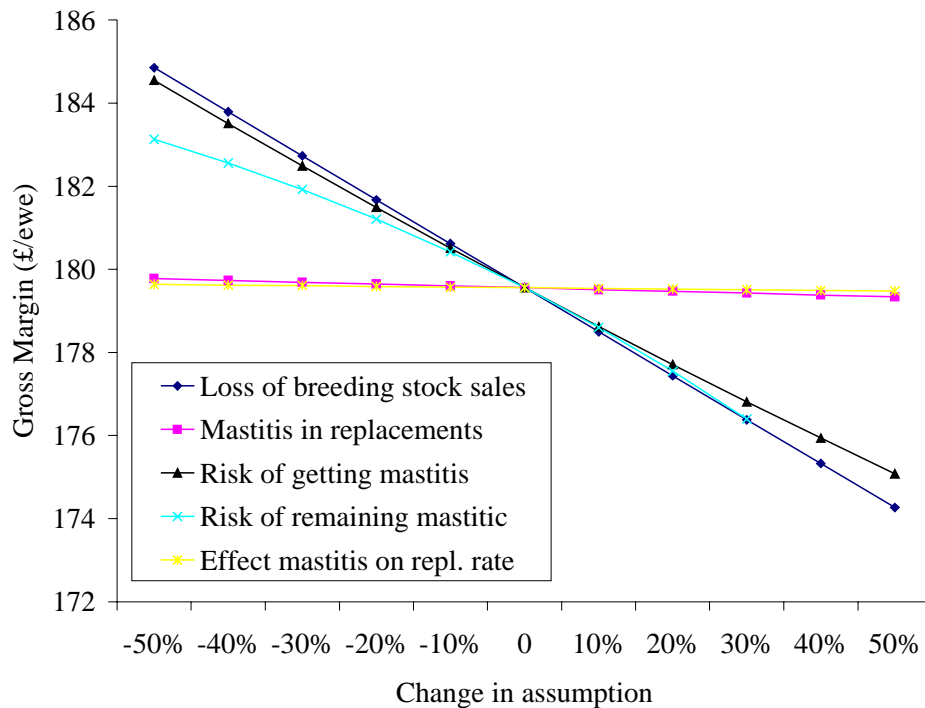
Table 3.3 Physical and financial assumptions for enterprise gross margin calculation

Parity:	Universal	Clean ewes					Mastitic ewes				
	Assumps.	1	2	3	4	5	1	2	3	4	5
Lambs sold /ewe		1.3	1.5	1.5	1.5	1.4	1.3	1.5	1.5	1.5	1.4
<i>Proportion sold:</i>											
Store		0	0	0	0	0	0	0	0	0	0
Fat		0.6	0.6	0.6	0.6	0.6	0.74	0.74	0.74	0.74	0.74
Breeding – male		0.2	0.2	0.2	0.2	0.2	0.14	0.14	0.14	0.14	0.14
Breeding – female		0.2	0.2	0.2	0.2	0.2	0.14	0.14	0.14	0.14	0.14
<i>Prices (£/lamb)</i>											
Fat		56	56	56	56	56	56	56	56	56	56
Breeding – male		600	600	600	600	600	600	600	600	600	600
Breeding – female		150	150	150	150	150	150	150	150	150	150
Cull ewe value (£)		35	35	35	35	35	35	35	35	35	35
Wool sales (£/ewe)	1.85										
Repl. Ewe cost (£)	150										
Ram repl (£/ewe)	7										
<i>Variable Costs (£/ewe):</i>											
Purchased feed		11	11	11	11	11	11	11	11	11	11
Forage	7										
Vet & Med	4										
Other	11.41										
<i>Extra costs to treat mastitis (£/treated ewe per year)</i>											
Antibiotics	18										
Labour	5										
Propn ewes treated	0.1										
Cost per mastitic ewe	2.3										

Results

The gross margin based on the above assumptions but with no mastitis losses was £191/ewe. When mastitis was included the gross margin fell to approximately £180/ewe, i.e. a drop of £11/ewe (6%). Effects of changing key assumptions related to mastitis are shown in Figure 2:

Figure 2: Sensitivity of gross margin to key assumptions related to mastitis



The proportion of replacement ewes with mastitis (parity 1, Table 3.2) and the effect of mastitis on replacement rate had little impact on gross margin and hence on the estimate of mastitis cost. The risk of remaining mastitic in the next parity if currently mastitic (Table 3.2) gave intermediate sensitivity, with rather more impact if the assumption was increased rather than decreased. Results were more sensitive to the risk of getting mastitis in the next parity if currently free (Table 3.2). Of greatest sensitivity of the assumptions tested was the reduced proportion of breeding stock sales if the ewe was mastitic (default setting was 0.33). If this setting was reduced by 50% to 0.165 then gross margin rose to £185/ewe, corresponding to a mastitis cost of £6/ewe. However, a 50% increase in this assumption reduced gross margin to £174/ewe (£17/ewe for mastitis).

Given the sensitivity of the results to the impact of mastitis on breeding stock sales, the sensitivity to breeding stock prices was also tested. It was found that a 10% change in breeding male lamb prices caused a change of approximately £17/ewe in gross margin. If mastitis did have an impact on the prices received for breeding stock then this would clearly be a very important effect.

Summary and Conclusions

Lack of information about the impact of mastitis on a sheep flock, its prevalence or its response to treatment made it impossible yet to conduct a proper economic analysis of this disease. In addition, lack of knowledge about levels of involuntary culling due to mastitis in purebred Texel flocks may well mean that the estimates here are conservative. However, a dynamic computer model of a lowground sheep flock selling pure-bred lambs for breeding was constructed and used to estimate the burden mastitis places on such a flock. This was found to be about £11/ewe or 6% of gross margin. However, this figure is sensitive to the assumptions made. In particular, the loss of breeding stock sales due to mastitis will greatly influence its importance in this type of flock. Of the epidemiological assumptions tested, the risk of mastitis free ewes contracting mastitis in subsequent parities was most important.

4. Genetic basis of mastitis

Benefits of using breeding strategies to select mastitis resistant animals

Selective breeding for mastitis resistance requires at least a suitable selection trait or molecular genetic marker, and that the additive genetic variance for this trait is of sufficient magnitude. In addition, effective breeding requires knowledge of its genetic relationship with other traits of economic importance.

Cattle

Using breeding as a measure to combat mastitis in dairy cattle has been the subject of considerable research effort over the past 20 years or so. Genetic evaluations for somatic cell count (SCC) in dairy cattle have become available only in the last decade in most countries. As SCC is genetically correlated with mastitis, selection for reduced SCC will lower the incidence of mastitis. Considerable evidence now exists that has established unfavourable genetic correlations between resistance to mastitis and milk production traits. Beilharz *et al.* (1993) and subsequently Rauw *et al.* (1998) and Bakken *et al.* (1998) used resource allocation theory to explain the negative genetic correlations that many, but not all, researchers have observed between performance traits and those important for fitness and health. Several countries have included mastitis into breeding programmes alongside conventional traits to stem the deterioration in genetic susceptibility as a result of selection for increased productivity. Mark *et al.* (2002) list 12 such countries. Veerkamp *et al.* (1998) estimated that selection to decrease mastitis or limit the rate of increase of mastitis, with a cumulative impact of 1% per year and a national penetration of 50% would result in a national benefit of £0.9m/year and Conington *et al.*, 2003) reported that increases in mastitis incidence would be halted by the inclusion of SCC into dairy cow genetic evaluations.

Sheep

Selection for resistance to mastitis in sheep is currently underway in the French dairy sheep breed *Lacaune* by the use of SCC as a proxy trait for mastitis, and incorporating it into sheep dairy breeding programmes Rupp *et al.*, 2002).

Genetic parameters

There are several estimates of heritabilities for somatic cell count and other milk traits (Table 4.1). They h^2 values for SCC are relatively low and range from 0.04 to 0.24, depending on the breed and stage of lactation when they were estimated. All estimates are from dairy sheep breeds. Genetic correlations with other production traits are shown in Table 4.2. For comparison, similar tables for cattle are shown for heritabilities (Table 4.3) and genetic correlations (Table 4.4). From Table 4.2, it is clear that there is no consensus among the literature estimates for the genetic relationships among milk yield and SCC, unlike those for dairy cattle. It may well be that the genetic improvement in sheep dairy breeds has not been implemented to the same degree as that for dairy cattle and therefore any susceptibility among high-producing sheep to mastitis is not yet apparent. It could also mean that there simply are no antagonisms between selection for increased milk production and SCC in sheep. What these estimates do imply, is that any attempt to introduce milk sampling as a way to detect mastitis in the Texel breed will need relevant, robust estimates of genetic parameters in the relevant population in particular in relation to key production traits.

Table 4.1: Heritabilities estimates of milk production traits in sheep

Traits	Heritability	Population (breed)	References
SCC	0.15 (0.04-0.12)		
Milk yield	0.34	French Lacaune dairy sheep	Barillet <i>et al.</i> , 2001
Milk fat	0.50		
Milk protein	0.63		
SCC	0.12		
milk yield	0.24	Spanish Churra ewes	El Saied <i>et al.</i> , 1999
Protein percentage	0.17		

SCC	0.13	French Lacaune dairy sheep	Rupp <i>et al.</i> , 2003
SCC	0.11	Churra ewes	Othmane <i>et al.</i> , 2002
SCC (1st lactation)	0.12	Manchega ewes	(Serrano <i>et al.</i> , 2005)
SCC (2 nd lactation)	0.19		
SCC (3 rd lactation)	0.24		
SCC (all lactations)	0.04		

Table 4.2: Genetic correlation between mastitis (or SCC) and other production traits in sheep

Traits	Genetic correlation	Population	References
SCC-milk yield	0.15	French Lacaune dairy sheep	Barillet <i>et al.</i> , 2001
SCC-milk yield	-0.15	Spanish Churra ewes	El Saied <i>et al.</i> , 1999
SCC-protein %	-0.47		
SCC-milk yield	0.18	French Lacaune Dairy sheep	Rupp <i>et al.</i> , 2003
SCC-fat content	0.04		
SCC-protein content	0.03		
SCC-milk yield (ml/d)	-0.36		
SCC-Fat (g/L)	0.04		
SCC-Protein (g/L)	0.13		
SCC-Casein (g/L)	0.09	Churra ewes	Othmane <i>et al.</i> , 2002
SCC-Serum protein (g/L)	0.20		
SCC-cheese yield (kg/100 L)	0.33		
SCC-120d Milk Yield (1 st lactation)	-0.12	Manchega ewes	Serrano <i>et al.</i> , 2005)
SCC-120d Protein % (1 st lactation)	0.23		
SCC-120d %Dry Matter (1 st lactation)	-0,04		
SCC-120d Milk Yield (2 nd lactation)	-0.14		
SCC-120d %Protein (2 nd lactation)	0.09		
SCC-120d %Dry matter (2 nd lactation)	0.02		
SCC-120d Milk Yield (3 rd lactation)	-0.15		
LSCS-120d %Protein (3 rd lactation)	0.08		
SCC-120d %Dry Matter (3 rd lactation)	-0.00		
SCC-120d Milk Yield (1 st , 2 nd and 3 rd lactation)	-0.16		
SCC-120d %Protein (1 st , 2 nd and 3 rd lactation)	0.22		
SCC-120d %Dry Matter (1 st , 2 nd and 3 rd lactation)	0.04		

Table 4.3: Heritabilities of mastitis and SCC in cattle

Traits	Heritability	Methods	Population	References
Clinical Mastitis (CM)	0.08±0.004	threshold-liability model	Norwegian Dairy Cattle	Heringstad <i>et al.</i> , 2005
305-d protein yield	0.19±0.007	a linear Gaussian model	Norwegian Dairy Cattle	
CM (1 st lact.)	0.03	a mixed linear animal model	Swedish Holstein cows	Carlen <i>et al.</i> , 2004
CM (2 nd lact.)	0.012			
CM (3 rd lact.)	0.012			
SCC (1 st lact.)	0.14			
Scc (2 nd lact.)	0.13			
SCC (3 rd lact.)	0.10			
CM	0.001-0.06 (from a review)		Nordic data	Heringstad <i>et al.</i> , 2000
SCC	0.08-0.19 (from a review)		Nordic data	Heringstad <i>et al.</i> , 2000
CM	0.024	Animal-REML	French Holstein cows	Rupp & Boichard, 1999
SCC	0.17			
CM	0.02	multitrait REML with a sire model	Ayrshire cows	Koivula <i>et al.</i> , 2005
CM	0.035	AI-REML with a linear sire model	Danish Holstein Cattle	Hansen <i>et al.</i> , 2002
CM	0.04		Holstein Cows	Van Dorp <i>et al.</i> , 1998
SCC	0.14(first lactation), 0.16(second lactation)	REML and a sire model	Canadian Dairy cattles	Boettcher <i>et al.</i> , 1998
SCS	0.09(first lactation); 0.09 (second lactation); 0.11 (third lactation)	multiple-trait animal model	Ontario Holstein cows	Reents <i>et al.</i> , 1995
CM	0.014			Emanuelson <i>et al.</i> , 1988
SCC	0.08			

Table 4.4: Genetic correlation between mastitis (or SCC) and other production traits in cattle.

Traits	Genetic correlation	Population	References
CM-305-d protein yield	0.43	Norwegian Dairy Cattle	Heringstad <i>et al.</i> , 2005
CM-SCC (1 st lact.)	0.68	Swedish Holstein cows	Carlen <i>et al.</i> , 2004
CM-LSCS (2 nd lact.)	0.66		
CM-LSCS (3 rd lact.)	0.77		
CM-milk (1 st lact.)	-0.13		
CM -milk (2 nd lact.)	-0.07		
CM -milk (3 rd lact.)	-0.06		
CM -fat (1 st lact.)	-0.11		
CM -fat (2 nd lact.)	-0.07		
CM -fat (3 rd lact.)	-0.07		
CM -protein (1 st lact.)	-0.11		
CM -protein (2 nd lact.)	-0.06		
CM -protein (3 rd lact.)	-0.06		
SCC-milk (1 st lact.)	-0.22		
SCC-milk (2 nd lact.)	-0.15		
SCC-milk (3 rd lact.)	-0.13		
SCC-fat (1 st lact.)	-0.17		
SCC -fat (2 nd lact.)	-0.13		
SCC -fat (3 rd lact.)	-0.11		
SCC -protein (1 st lact.)	-0.19		
SCC -protein (2 nd lact.)	-0.14		
SCC -protein (3 rd lact.)	-0.11		
CM-SCC	0.3-0.8 (from a review)		Heringstad <i>et al.</i> , 2005
CM-SCC	0.72	French Holstein cows	Rupp & Boichard, 1999 [5]
Milk yield-SCC	0.11-0.27		
Milk yield-CM	0.15-0.45		
CM-milk yield	0.38-0.56	Ayrshire cows	Koivula <i>et al.</i> , 2005 [40]
Protein yield-CM	0.33	Danish Holstein Cattle	Hansen <i>et al.</i> , 2002 [41]
CM-305d milk yield	0.15	Holstein Cows	Van Dorp <i>et al.</i> , 1998 [42]
CM-SCC	0.06		Emanuelson <i>et al.</i> , 1988 [45]

Molecular technologies to quantify mastitis-resistance

The use of genetic markers to indicate resistance or susceptibility to mastitis is an attractive proposition particularly for meat sheep breeds, as it does not require milk sampling. However, all the literature published to date on molecular markers refers to dairy cattle, and only one (unpublished) study that is relevant to dairy sheep. A summary of the literature is presented in Table 4.5. This table categorises the chromosomes on which genetic markers have been found, the relevant phenotypic trait, the closest marker position (in centimorgans, cM) and the significance of the marker (e.g. P=0.01 means, generally, that we can be 99% sure of the result). The table also includes the location confidence interval (the distance (in cM) within which the marker lies) and the reference. Even though there are markers reported for 22/30 cattle chromosomes, the greatest number (6) are on chromosome 23, where the genes responsible for the major histocompatibility complex are located. These genes are responsible for the induction and regulation of immune response and are the focus for several other studies related to disease resistance. In a review on the genetics of mastitis in cattle, Rupp & Boichard, 2003, the immune mechanisms underlying mastitis resistance pointed towards

better functionality of neutrophils (white blood cells) although the associations with them and clinical mastitis were not straightforward and require further investigation. This review gives a comprehensive summary of the major markers and candidate genes that have significant relationships with mastitis resistance or susceptibility. Interestingly, in one study, the markers associated with resistance to clinical mastitis were not the same as those responsible for low SCC and that the use of SCC data in QTL studies aimed at reducing the incidence of mastitis should be carefully evaluated. This highlights the usefulness of markers for traits that are essentially polygenic i.e. expressed by many different genes and it is notable that to date, no published reports exist of using molecular techniques for resistance to mastitis in dairy breeding programmes, although most such knowledge stems from cattle.

Table 4.5: Molecular markers for mastitis resistance

Chromo	Trait	Closest marker (position cM)	P(%)	Location confidence interval (cM).2/	Reference
3 (putative)	CM				Klungland <i>et al.</i> , 2001
4 (putative)	CM				Klungland <i>et al.</i> , 2001
6	CM				Klungland <i>et al.</i> , 2001
9	CM	TGLA73	***(chromosome) †(genome)		Holmberg & Andersson-Eklund, 2004a
10	CM		0.02	1	Schulman <i>et al.</i> , 2004
11	CM	INRA177	*(chromosome) NS (genome)		Holmberg & Andersson-Eklund, 2004a
11	CM		<0.01	1	Schulman <i>et al.</i> , 2004
14 (putative)	CM				Klungland <i>et al.</i> , 2001
18	CM	TGLA227	0.02(P-value at chromosome-wise significance level)	120	Schulman <i>et al.</i> , 2004
25	CM	ILSTS102-RM404	*(chromosome) NS (genome)		Holmberg & Andersson-Eklund, 2004a
27 (putative)	CM				Klungland <i>et al.</i> , 2001
14	CM(cofactor analysis)	BMS1747-BMS740	<0.01(P-value at chromosome-wise significance level)	25	Schulman <i>et al.</i> , 2004
21	CM(cofactor analysis)	RM151-INRA103	0.01(P-value at chromosome-wise significance level)	23	Schulman <i>et al.</i> , 2004
6	Quality of udder		0.000/0.008	89	Hiendleder <i>et al.</i> , 2003
3	SCC	BMC5227	0.0317(chromosome-wise P-value)	171	Schrooten <i>et al.</i> , 2000
7	SCC (putative)	BMS2258-OarAE129	0.025(chromosome-wise)	107	Kuhn <i>et al.</i> , 2003
9	SCC	CSSM56	**(chromosome) NS(genome)		Holmberg & Andersson-Eklund, 2004a

Chromo	Trait	Closest marker (position cM)	P(%)	Location confidence interval (cM).2/	Reference
11	SCC	BMS7169	****(chromosome)		Holmberg & Andersson-Eklund, 2004a
18	SCC	BM7109-ILSTS002	** (genome) 0.0103(chromosome-wise P-value)	70	Schrooten <i>et al.</i> , 2000
18	SCC	TGLA227	0.058(genome-wise)	117	Kuhn <i>et al.</i> , 2003
23	SCC	BM1443	*(chromosome) NS (genome)		Holmberg & Andersson-Eklund, 2004a
27	SCC (putative)	BM3507-TGLA179	0.004(chromosome-wise)	8	Kuhn <i>et al.</i> , 2003
10	SCC (putative)	TGLA378-TGLA102	0.027(chromosome-wise)	49	Kuhn <i>et al.</i> , 2003
1	SCS (cofactor analysis)		<0.01(P-value at chromosome-wise significance level)	59	Schulman <i>et al.</i> , 2004
3	SCS		0.01 (P-value at chromosome-wise significance level)	105	Schulman <i>et al.</i> , 2004
5	SCS	BL37-BM1819	9.1(F statistic) (chromosome-wise) (P<0.01)	54	Ashwell <i>et al.</i> , 2004
7	SCS	BM6117-BMS2258	8.8(F statistic) (chromosome-wise) (P<0.01)	61	Ashwell <i>et al.</i> , 2004
7	SCS	BM6117-BMS2258	3.2 (F statistic) (chromosome-wise) (P<0.01)	67	Ashwell <i>et al.</i> , 2004
11	SCS		0.03 (P-value at chromosome-wise significance level)	63	Schulman <i>et al.</i> , 2004
14	SCS		0.01 (P-value at chromosome-wise significance level)	63	Schulman <i>et al.</i> , 2004
15	SCS (genome-wise significant)	<i>BMS2684</i>	0.05	35_45 / 22_48	Boichard <i>et al.</i> , 2003
15	SCS	BMS2684-HBB	9.8(F statistic) (chromosome-wise) (P<0.01)	34	Ashwell <i>et al.</i> , 2004
18	SCS		0.02 (P-value at chromosome-wise significance level)	113	Schulman <i>et al.</i> , 2004
20	SCS	RM310-TGLA126	11.8(F statistic) (chromosome-wise) (P<0.01)	29	Ashwell <i>et al.</i> , 2004
21	SCS (cofactor analysis)		<0.01(P-value at chromosome-wise significance level)	51	Schulman <i>et al.</i> , 2004
22	SCS	BMS875-BM4102	3.32 (F statistic) (chromosome-wise) (P<0.01)	80	Ashwell <i>et al.</i> , 2004
23	SCS	BB705-BM1818	12.7(F statistic) (chromosome-wise) (P<0.01)	50	Ashwell <i>et al.</i> , 2004

Chromo	Trait	Closest marker (position cM)	P(%)	Location confidence interval (cM).2/	Reference
23	SCS	BB705-BM1818	3.02 (F statistic) (chromosome-wise) (P<0.01)	41	Ashwell <i>et al.</i> , 2004
23	SCS (cofactor analysis)		0.01(P-value at chromosome-wise significance level)	7	Schulman <i>et al.</i> , 2004
24	SCS (cofactor analysis)		<0.01(P-value at chromosome-wise significance level)	28	Schulman <i>et al.</i> , 2004
26	SCS	Centromere-BM1314	11.2(F statistic) (chromosome-wise) (P<0.01)	0	Ashwell <i>et al.</i> , 2004
26	SCS	Centromere-BM1314	11.0(F statistic) (chromosome-wise) (P<0.01)	0	Ashwell <i>et al.</i> , 2004
26	SCS	Centromere-BM1314	2.72 (F statistic) (chromosome-wise) (P<0.01)	0	Ashwell <i>et al.</i> , 2004
27	SCS		0.02 (P-value at chromosome-wise significance level)	3	Schulman <i>et al.</i> , 2004
29	SCS	BMC1206-BMS1948	8.0 (F statistic) (chromosome-wise) (P<0.01)	50	Ashwell <i>et al.</i> , 2004
29	SCS		0.01 (P-value at chromosome-wise significance level)	14	Schulman <i>et al.</i> , 2004
9	SCS (chromosome-wise significant)	<i>BMS1967</i>	1.3		Boichard <i>et al.</i> , 2003
21	SCS (chromosome-wise significant)	<i>TGLA122</i>	1.5		Boichard <i>et al.</i> , 2003
23	SCS (chromosome-wise significant)	<i>RM33</i>	1.7		Boichard <i>et al.</i> , 2003
10	SCS (genome-wise significant)	<i>DIK20</i>	0.9	66_90 / 6_92	Boichard <i>et al.</i> , 2003
5	Udder depth		0.046 (chromosome-/experiment-wise thresholds)	106 (location)	Hiendleder <i>et al.</i> , 2003
6	Udder depth		0.000/0.008	89	Hiendleder <i>et al.</i> , 2003

Candidate genes for mastitis resistance

The most extensively studied genes having significant associations with different indicators of mastitis are the MHC class II DRB3 alleles. The general consensus from the majority of the published literature

is that these genes may have potential usefulness as genetic markers of higher or lower risk of disease occurrence in cows. Specifically, the DRB3.2*23 has been associated with severe mastitis from which coliforms were the most commonly isolated bacteria (Sharif *et al.*, 1998). The presence of allele DRB3.2*16 was associated with higher EBV for SCC, and allele DRB3.2*8 was associated with increased EBV for clinical mastitis, as was the *IgG2b* allele and the normal CD18 allele. Alleles DRB3.2*11, *23, *IgG2a*, and the recessive allele for bovine leukocyte adhesion deficiency were associated with decreased clinical mastitis. A positive genetic association was found between allele DRB3.2*24 and EBV for intra mammary infections (IMI) by major pathogens and between DRB3.2*3 and IMI by minor pathogens. Several correlations between EBV for immunological assays and EBV for mastitis measures were significantly different from 0. Cows with low EBV for SCS tended to have neutrophils that had greater functional ability at maximal immunosuppression, low serum IgG1, and high numbers of circulating mononuclear cells. Immunological parameters, including physiological and molecular markers, are useful aids to understand the genetics of resistance to mastitis (Kelm *et al.*, 1997). It is also possible that the number of DQ genes that a cow actually has, and the ratio of certain T-cell subsets (CD4:CD8) affects their susceptibility or resistance to mastitis. Susceptibility to mastitis was associated with MHC haplotypes that have a single set of DQ genes, and animals with CD4:CD8 ratio of 0.42 compared to 3.2 for mastitis resistant animals (Park *et al.*, 2004).

Despite the evidence supporting the involvement of the DRB3 locus, there are inconsistent reports of whether or not they confer increased or reduced resistance to mastitis. In a review by Rupp and Boichard (2003), three authors showed significant association of allele DRB3.2*24 with susceptibility to mastitis, more intra mammary infections with major pathogens, more clinical mastitis and higher SCC. However, from other cited studies, allele DRB3.2*16 was associated with either higher or lower cell count. Similar inconsistent trends were reported for DRB3.2*23 and DRB3.2*8. Several explanations could be given to explain such trends. First, alleles may be related to resistance or susceptibility according to environmental conditions (present pathogens), which may be different in the five studied populations. More likely, the studied polymorphisms were not causal but linked to other MHC loci involved in mastitis resistance, which would lead to different associations according to families. Thus, analysis of effect of MHC haplotypes rather than single locus should be preferred to get a better handle on the links between genotype and resistance to mastitis.

Transgenesis for mastitis resistance

Two studies have been reported that have developed transgenic animals to secrete antibacterial proteins. Kerr & Wellnitz (2003) produced 3 strains of mice that differed in their levels of *lysostaphin* in their milk. This protein has potent anti-*staphylococcal* activity and its secretion in milk confers substantial resistance to infection caused by intramammary challenge with *Staphylococcus aureus*. Additional antibacterial proteins are being sought that will complement *lysostaphin*. A potential benefit of transgenic application of antibacterial proteins is the concomitant sparing in the agricultural use of antibiotics currently used as human therapeutics. Following this initial study in mice, another study cited by Rainard (2005), created transgenic cows secrete *lysostaphin* in the cells of the mammary gland and just a single transgene expressed in the milk of dairy cows confers protection against *Staph. Aureus*. However, despite the enormity of this breakthrough, such is the acceptance by the general public in the UK of using genetic engineering in farm animals, it is unlikely that transgenic animals will be the route through which genetic resistance to mastitis is achieved.

5. Discussion and Conclusions

Mastitis in cattle costs the UK industry millions of pounds per annum in lost production and premature culling of affected animals. For sheep, our model predicts that mastitis results in losses of approximately £11 per ewe, (depending on key assumptions). With a Texel sheep population in UK of 326,279 (Pollot, 2005), this means that theoretically, mastitis costs the Texel sheep industry ca. £3.6M per annum.

Unexpectedly from its economic impact there is a dearth of information about the genetics, incidence and economical consequences of mastitis in meat sheep breeds. No information is published in such breeds about the potential to include mastitis resistance in meat sheep breeding programmes. Therefore the majority of all relevant literature reported is on dairy cattle, with some on dairy sheep. The implications of this are that fundamental research on mastitis in meat sheep breeds is urgently required. This needs to be done at the population level to understand the major contributing factors to mastitis, the timing, prevalence and ultimately genetics of the disease. This is important knowledge when choosing the right model for genetic evaluations for resistance to mastitis and this will enable us to develop robust recording protocols so that unbiased and objective records of familial susceptibility to mastitis can be understood, and appropriate breeding programmes can be instigated to breed mastitis-resistant sheep.

Selection against mastitis in dairy cattle is currently underway in several countries, although only in France for the Lacaune dairy sheep breed. One of the key components to select against clinical mastitis (CM) is the difficulty of recording the selection trait and hence the quality of the resulting data. For this reason the heritability of CM is lower than that of the proxy trait, Somatic Cell Count (SCC). Compared with clinical mastitis, SCC is routinely recorded in several countries and it has a higher heritability than clinical mastitis. There is a relatively high genetic correlation between clinical mastitis and SCC, so selection against high SCC will lead to a reduction in CM. However, to comprehensively evaluate the implications of selection, the genetic relationships among resistance to mastitis and other key performance traits need to be investigated before a breeding strategy can be employed.

As the continuous measurement of phenotypic indicator traits for mastitis resistance is time and labour intensive, the use of suitable molecular genetic markers could be more effective, and with the developments in molecular genetics, it is in principle, possible to find molecular genetic markers for some complicated disease traits. In cattle, many QTL for clinical mastitis and SCC were found by using whole genome scanning. For example, the QTL for clinical mastitis were localised to Chr. 9, 10, 11, 18 and 25 (Holmberg & Andersson-Eklund, 2004b; Schulman *et al.*, 2004). QTL for SCC were mapped to Chr. 3, 5, 7, 9, 11, 15, 18, 23, 26 and 29 (Boichard *et al.*, 2003; Holmberg & Andersson-Eklund, 2004b; Schrooten *et al.*, 2000; Schulman *et al.*, 2004; Kuhn *et al.*, 2003; Ashwell *et al.*, 2004), especially the QTL on Chromosomes 11, 15 and 18 reached the genome-wide significance level. Hence, chromosomes 9, 11 and 18 should be the candidate chromosomes for mastitis resistance in cattle as QTL for clinical mastitis and SCC are localised on the same chromosome. So more markers should be selected on these chromosomes to detect the fine location of QTL or genes of clinical mastitis and SCC until the real gene that controls the susceptibility of mastitis is known so that it can be used inbreeding for mastitis resistance. Due to the importance of mastitis affecting the Texel breed, and the costly, ineffective treatments used to control it a fresh approach to control the incidence of mastitis in sheep is required, potentially through the use of molecular technologies. The best route to achieve this would be to align the ovine genome with bovine genome and find the homozygous chromosomes or regions that are similar (9, 11 and 18) by using comparative genome mapping in sheep. The next stage would be to select sufficient markers, especially microsatellite markers, on the alignments with other species and detect the QTL for clinical mastitis or SCC, which then could be used in marker-assisted selection (MAS). Whole genome scanning also can be used to detect QTL for clinical mastitis and SCC if there is sufficient funding.

Detection of candidate genes for clinical mastitis or SCC is another way to improve the mastitis resistance in sheep. BoLA (bovine leucocyte antigen)-DBR3 gene of the bovine major histocompatibility complex (MHC), IgG2 gene and CD18 gene were studied as the candidate genes of clinical mastitis and SCC in cattle (Kelm *et al.*, 1997). BoLA-DRB3.2*16 was significantly associated with lower SCS in Holsteins ($P < 0.05$) (Sharif *et al.*, 1998). There was a significant association

between BoLA-DRB3.2*23 and occurrence of severe mastitis ($P < \text{or } = 0.05$) (Sharif *et al.*, 1998). To date, the corresponding studies in sheep haven't been reported. So, it is worthwhile to study the association between these genes and clinical mastitis in sheep. Single nucleotide polymorphisms (SNPs) can be detected by using PCR-RFLP or PCR-SSCP, and then the association between the genotype and phenotype needs to be analysed to finally select the favourable genotypes for mastitis resistance and their use in MAS.

The way forward

Due to the lack of data on mastitis in terminal sire sheep, and the need to urgently address the problem of mastitis in Texels, then it is possible to use the knowledge from other studies cited in this report, to direct us towards the most efficient route towards breeding mastitis-resistant sheep. Unfortunately the lack of conclusive evidence for robust genetic markers in this (or other terminal sire) sheep breeds necessitates the collection of field data and genetic material so that the recommendations of comparative mapping linked to field records of indicators of mastitis (SCC and CM) can be made. Once this work is completed, then it is likely that useful genetic markers and/or haplotype categories can be used for the selection of mastitis-resistant Texels.

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