STUDIES ON MASTITIS IN SHEEP AT THE ROYAL VETERINARY COLLEGE

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Mastitis is an important constraint on sheep production in many parts of the world and most studies on the disease have been in countries where sheep are kept as a source of milk and milk products. Despite having a population of upwards of 20 million ewes, mastitis has been little studied in Britain. The only systematic study of the disease in the UK was that of Leyshon in 1929 who described cases in East Anglia and drew attention to the importance of staphylococci and mentioned a bipolar organism, probably *Pasteurella haemolytica*.

Our work on mastitis, initiated in 1985, was reported to the Society that year where we described the aims and form of our bacteriological survey on mastitis in lowland flocks in England and Wales. In the present paper we summarise the results of that survey, experiments on *P. haemolytica* mastitis, observations on subclinical mastitis and briefly discuss epidemiological, preventive and control aspects of the disease.

A bacteriological survey of acute mastitis

Incidence

In 1985-87 we had under surveillance approximately 30,000 ewes among some 70 lowland flocks, the numbers fluctuating between the three years. The incidence of acute mastitis varied between flocks but,

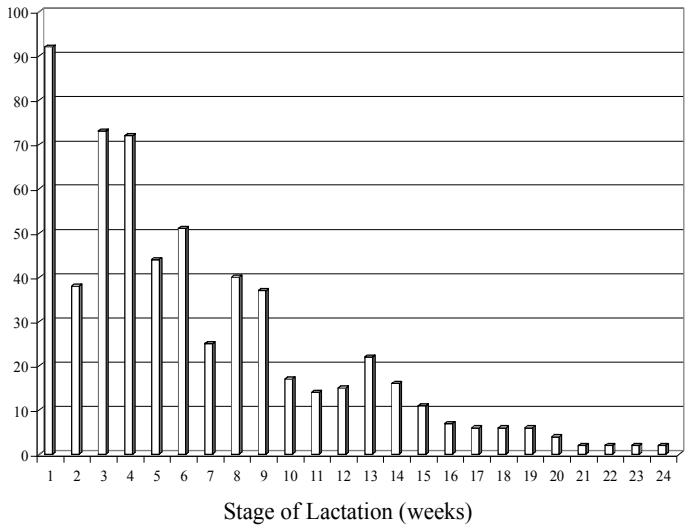

Figure 1. The number of milk samples submitted during each week of lactation.

Table 1.
The results of the bacteriological examination of milk samples from 730 ewes with acute mastitis (1985 - 1987)

	1985		1986		1987		Total	
	n	%	**n**	%	**n**	%	**n**	%
Bacteriologically positive samples	197	88	286	91	164	87	647	89
Bacteriologically negative samples	26	12	30	9	27	13	83	11
Total examined (100%)	223	100	316	100	191	100	730	

Table 2.
The results of bacteriological examination of 647 'positive' milk samples

	1985		1986		1987		TOTAL	
Bacteria isolated	**n**	%	**n**	%	**n**	%	**n**	%
In pure culture								
Staphylococcus aureus	55	27.9	127	44.4	54	32.9	236	36.5
Pasteurella haemolytica	97	49.2	64	22.4	51	31.1	212	32.8
Coagulase -ve staphylococci	8	4.1	10	3.5	7	4.3	25	3.9
Streptococcus spp.	5	2.5	9	3.2	5	3.1	19	2.9
Escherichia coli	2	1.0	8	2.8	4	2.4	14	2.2
Actinomyces pyogenes	3	1.5	2	0.7	4	2.4	9	1.4
Others	2	1.0	14	4.9	5	3.1	21	3.2
	172	87.2	234	81.9	130	79.3	536	82.9
In mixed culture								
Staph. aureus + other bacteria	6	3.1	17	5.9	9	5.5	32	4.9
P. haemolytica + other bacteria	8	4.1	12	4.2	9	5.5	29	4.5
P. haemolytica + *Staph. Aureus*	6	3.1	8	2.8	6	3.6	20	3.1
Other combinations	5	2.5	15	5.2	10	6.1	30	4.6
	25	12.8	52	18.1	34	20.7	111	17.1
All isolates	**197**	**100.0**	**286**	**100.0**	**164**	**100.0**	**647**	**100.0**

Table 3.
The results of the examination of bacteriologically positive milk samples from 647 ewes with acute mastitis.

Bacterial isolates	n	%
Staph. aureus	236	36.5
Staph. aureus + others	32	4.9
P. haemolytica	212	32.8
P. Haemolytica + others	29	4.5
P. Haemolytica + *Staph. aureus*	20	3.1
Other genera (in pure culture)	88	13.6
Other combinations of genera (in mixed culture)	30	4.6
Total	647	100.0

on average, was 4 - 5% within the three years. The range among individual flocks was between 0 - 24%.More than 10% of ewes were affected in 10% of flocks. The death rate was 0.3%. (In a different survey among mountain flocks we found the incidence to be between 1 - 2%).

Bacteriological results

During the three year period milk samples were obtained from 730 ewes with mastitis. Of these, 647 (88.6%) were bacteriologically positive (Table 1); 536 (82.8%) contained bacteria in pure culture and 111 (17.2%) in mixed culture. The identity of the bacteria isolated from the positive samples are shown in Tables 2 and 3. Two bacteria predominated: *Staphylococcus aureus* and *P. haemolytica* (present in 44.5% and 40.3% of the bacteriologically positive samples respectively). Of 261 isolates of *P. haemolytica*, 233 (89.2%) were typed serologically. The results are shown in Table 4. The most frequently isolated serotype was A2 (30.5%).

Table 4.
Serotype of 233 isolates of *Pasteurella haemolytica* from ewes with acute mastitis

Serotype	A2	A6	A11	A7	A1	A9	A13	A5	A8	A12	UT
N	71	24	24	16	14	14	14	11	8	8	29
%	30.5	10.3	10.3	6.9	6.0	6.0	6.0	4.7	4.4	3.4	12.5

UT = untypable

The number of milk samples submitted for laboratory examination during each week of lactation is shown in Figure 1. Submission rates were highest during the first week and between the third and fourth week of lactation. Most bacteriologically negative samples and those from which bacteria other

than *Staph. aureus* and *P. haemolytica* were isolated came from ewes in the first week of lactation. By contrast, the peak period prevalence for both these bacteria (which together constituted 81.8% of bacteriologically positive samples) was the third, fourth, fifth and sixth week of lactation.

The results clearly demonstrated that *P. haemolytica* is a major mastitis pathogen, a fact not previously appreciated because the traditional view had been that most ovine mastitis was caused by *Staph. aureus*.

Experimental *P. haemolytica* mastitis

Our experimental work concentrated on mastitis caused by *P. haemolytica* because this bacterium had previously been regarded as primarily a respiratory pathogen and its role as a major cause of mastitis in sheep was a new finding.

Severe, acute mastitis, clinically and pathologically identical to the natural disease, is produced by inoculating as few as 10 cfu of certain pathogenic isolates into the lactating mammary gland. Neutrophils invade the gland in large numbers about nine hours after inoculation, which coincides with the rapid multiplication of bacteria in the milk. The gland is obviously inflamed by 24 hours after inoculation and gross necrotic changes may be visible at 3 days. The early lesion of hyperaemia and haemorrhage of mammary tissue is at either the cranial or caudal pole, furthest from the teat, and the lesion subsequently spreads to the remainder of the gland. The reason for this paradoxical direction of spread of infection is not known.

Mastitis is only produced when the bacteria are inoculated into the teat sinus and the gland is not infected when inoculation is into the distal teat canal or when the teat is immersed in a broth culture of *P. haemolytica*. This finding demonstrates that, although very small numbers of *P. haemolytica* may cause severe mastitis, for a ewe to develop the disease they must fully penetrate the teat canal. How and when this occurs under natural conditions is not known but is worthy of further investigation.

There is considerable variation between isolates of *P. haemolytica* in their experimental pathogenicity for the ovine mammary gland. Isolates from ewes with subclinical *P. haemolytica* mastitis inoculated into glands of experimental ewes either colonise the gland giving rise to only a slight increase in cell count with no macroscopic changes to the milk or the gland or are eliminated within hours of inoculation. The factors which give rise to this variation in mammary pathogenicity are not known but there is no direct association between virulence and serotype, cytotoxin production *in vitro* or with growth in milk *in vitro*.

In addition to *P. haemolytica* isolates from the mammary gland, small numbers of *P. haemolytica* (A2) isolated from the nasal cavity of lambs produce severe mastitis when inoculated into the lactating gland of ewes. This suggests that the *P. haemolytica* that cause mastitis in ewes originate as commensals of the lamb (see below).

Mastitis can be produced experimentally in cattle, in addition to sheep, by the inoculation of small numbers of both bovine and ovine isolates. However, non-ruminant animals do not appear to be susceptible to *P. haemolytica* mastitis experimentally, suggesting that the cytotoxin of *P. haemolytica*, which is specific for neutrophils of ruminant animals, has a role in pathogenesis.

Subclinical mastitis

The prevalence, causes and possible effects of subclinical mastitis (SCM) in the UK have not been widely studied. Our investigations included field work, bacteriological cultures, estimation of somatic cell count and experiments on infection caused by coagulase negative staphylococci (cns).

Bacteriology and somatic cell counts

The principal causes of SCM are cns, streptococcal species, *Staph. aureus* and *P. haemolytica*. Cns are the predominant isolates from milk samples in the absence of high somatic cell counts because they may be present as commensals in teat canal and sinus without infecting the mammary gland itself.

Somatic cell counts from milk samples of clinically healthy ewes show that the adoption of an upper limit of 1.0×10^6/ml for apparently normal milk is a satisfactory working standard. Counts above that number indicate the need for bacteriological examination of the milk. Studies of the relationship between cell counts and the scores of the Whiteside test (WST) and the California mastitis test (CMT) show that when a value of 1.0×10^6 cells/ml is taken as the upper limit for normal milk samples, 91.6% and 93.0% of samples of + or more in the WST and CMT respectively were above this limit. Thus these two tests are suitable simple techniques for the detection of subclinical mastitis under field conditions.

In the flocks we investigated the prevalence of SCM was 10-30%. In one survey, over 2,000 milk samples from 358 ewes in seven lowland flocks in southern England were examined. Bacteria were isolated from 20.4% of the ewes. Positive WST scores were recorded from 78 (21.8%) of ewes. Of 169 bacteriological positive and 127 WST positive samples, 82 were both bacteriologically and WST positive. Using the definition of SCM as the presence of both bacteriologically positive and WST positive results, 11.7% of ewes were affected during lactation. The period prevalence in flocks ranged from 6.4% to 18.9%. SCM occurred more frequently in ewes that were multiparous and older than 2 years.

The most important causes of acute mastitis in the UK are *Staph. aureus* and *P. haemolytica*. The importance of subclinical infection caused by these bacteria merits further consideration. There is evidence that in some circumstances clinical mastitis often precedes subclinical infection.

Experiments on SCM caused by cns

Subclinical mastitis was induced by inoculating the mammary glands of Welsh Mountain (WM) and Dorset Horn (DH) ewes with cns (in this case *Staph. simulans*). Two groups were inoculated 6 days after birth and one group 16 days after birth. Cns were isolated from all inoculated glands for at least 20 days after inoculation and in the majority of ewes for at least 35 days.

The somatic cell counts of all milk samples from the inoculated glands increased and were greater than 10^6/ml throughout the experiment. The mean volume of milk collected from ewes inoculated 6 days after lambing was 27.3% less (WM) and 22.8% less from ewes inoculated 16 days (WM) than that from control ewes. For DH ewes the difference in milk yield from the ewes inoculated at 6 days was 37.3% less. The growth rate over the period of the experiment was significantly lower, e.g. in the WM ewes inoculated at 6 days old, the weight of the lambs 7 weeks later was 3.2 kg less than the weight of a lamb in the control group. Lambs not only had a reduced weight at weaning, they also consumed more food. Most of the growth retardation occurred between the 5th and the 20th days after lambing, an age at which lambs are highly dependent on milk.

These findings show that subclinical mastitis results in decreased milk yield and consequent growth retardation of lambs especially in early life when the major determinant of growth rate is the amount of milk consumed. The practicalities of management and husbandry make it unlikely that subclinical mastitis will ever be much investigated in commercial meat flocks. However, the importance of subclinical mastitis needs to be considered in at least two circumstances. First, any nutritional research on milk production in ewes or on growth rate of lambs should take into account the possible presence of subclinical mastitis, because these experiments have shown how milk yield and lamb growth may be significantly diminished when ewes are affected. Secondly, subclinical mastitis in ewes could undoubtedly affect the productivity of dairy flocks in the UK and, for this reason, it requires investigation. Thirdly, subclinical mastitis should be considered as a differential diagnosis of poor growth rates in lambs, especially where there is uneven growth of lambs in the flock. Unilateral subclinical mastitis may even give rise to uneven growth of twin lambs as each lamb consistently sucks the same udder half.

Epidemiology

Ecology of bacteria

In our survey we found that *Staph. aureus* and *Pasteurella haemolytica* accounted for 80% of the cases of mastitis; most of the remaining 20% were due to environmental pathogens. *Staph. aureus* may inhabit the nose, mouth, skin, vagina, sometimes the normal teat skin of ewes and, rarely, the teat canal. *P. haemolytica* inhabits the nose, tonsil and mouth of ewes and lambs and, soon after lambing, the teat skin of ewes. *P. haemolytica* is not isolated from the teat skin of ewes before lambing or after weaning and is present on the teat skin only when lambs are sucking. The main source of infection from *P. haemolytica* for the teat of the ewe is the mouth of the lamb. In dairy sheep, where ewes are milked by hand or machine after lambs are removed, the incidence of *P. haemolytica* mastitis is dramatically reduced after lambs are weaned because *P. haemolytica* is no longer present on the teat of the ewe. The incidence of staphylococcal mastitis, however, is unaffected. Environmental pathogens that may infect the mammary gland include coliform bacteria, cns, various streptococci and *Pseudomonas* spp.

Predisposing factors

These are generally poorly understood, but there are several factors that may facilitate infection of the teat and mammary gland:

Behavioural. Vigorous sucking by strong healthy lambs, especially by twins or triplets striving for more milk, may result in trauma to mammary tissue by head butting or in abrasions of the teats by biting. Such sucking may be an indication of inadequate milk production by the ewe, which, in turn, may be the result of faulty nutrition or of pre-existing subclinical mastitis. In intensive husbandry systems, lambs may suck ewes other than their mother; such indiscriminate sucking could lead to transmission of pathogens from affected to unaffected glands and also increase the chance of teats being infected with *P. haemolytica*, which is carried in the throat of most lambs.

Environmental. In ewes that are housed for a few days after lambing, or when there is repeated use of lambing pens, faecal contamination of teats may predispose ewes to the development of coliform mastitis. It is for this reason that this form of mastitis is more common during the first week of lactation than at other times. When ewes lie on expanded metal floors they are more prone to teat injuries and mastitis than when on straw. Other environmental factors have been cited but are of uncertain significance and include cold wind and rain, lush pastures, and the presence of vectors.

Pathological. Lesions on the skin of the teats or glands, for example, those of staphylococcal dermatitis, orf, or abrasions caused by the teeth of lambs, may become foci of colonisation and multiplication of

bacteria that may be present on adjacent healthy skin. In our survey, the presence of teat lesions was a risk factor for staphylococcal but not for other forms of mastitis.

Morphological. There is much information on the possible relationship between anatomical features of the udder and teat and susceptibility to mastitis in cows but little about such relationships in sheep. In sheep, udder depth and circumference correlate positively with milk production. Teat length is highly correlated with teat diameter. Sphincter patency and speed of milking may influence infection. Some of these characteristics may be related to resistance to mastitis but more stringent investigation is required to establish their possible significance.

Studies on predisposition are probably the most important requirements to understand the development of mastitis. Predisposing factors, some of which are mentioned above, can be better investigated by veterinarians and shepherds working together on observational and experimental epidemiology.

Control of mastitis

Treatment of acute mastitis

This is generally ineffective in restoring function to an affected gland but the use of prompt appropriate antibiotics, given perenterally, may ensure survival of the ewe. *In vitro* tests showed that our mastitis isolates of *Staph. aureus* (232) and of *P. haemolytica* (166) were susceptible to penicillin and ampicillin (99% of isolates) and tetracyclines (95%of isolates). Affected ewes and their lambs should be penned and given individual attention and lambs may require artificial feeding.

Prevention

(a) Vaccination. Currently there are no commercial available vaccines against ovine mastitis.

(b) Application of techniques of husbandry. Improved techniques depend on a better understanding of the nature of predisposing factors.

(c) Breeding for resistance. There is no information on possible genetic influences on the occurrence of ovine mastitis in the UK, but in some countries there is increasing interest in the possibility of breeding for resistance to mastitis, for example, in Australia, Italy, Norway, Poland and the USA.

To consider whether genetic control of mastitis is feasible much more data is required on the incidence of mastitis in genetically defined populations. Such data would demonstrate whether the disease displays sufficient genetic variation to allow breeding programmes designed to enhance resistance.

An effective recording system to include a variety of breeds and flocks, accurate information on age and parity, number of lambs born and reared, milk yield, morphological features of the udder and teats and the incidence of the bacterial causes of mastitis would be essential. Furthermore, there would need to be investigations on possible interactions between environmental and genetic factors that influence resistance and susceptibility.

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