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A longitudinal study of factors associated with acute and chronic mastitis and their impact on lamb growth rate in 10 suckler sheep flocks in Great Britain

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ABSTRACT

A 2-year prospective, longitudinal study of 10 suckler sheep flocks in Great Britain was run to identify factors associated with acute mastitis (AM) and chronic mastitis, and their impact on lamb growth rate. Data were collected on AM, intramammary masses (IMM; a marker for chronic mastitis), udder and teat conformation, teat lesions, body condition, ewe nutrition, litter size, lamb weight and general flock management. Each flock was visited twice each year, approximately 4 weeks before lambing and 9 weeks into lactation, for two years and all ewes present at a visit were examined. There were 7021 examinations in total. AM was reported in 2.1–3.0% of ewes/year; this ranged from 0.0% to 37.1% by flock. IMM were detected in 4.7% of ewes in pregnancy and 10.9% of ewes in lactation. Once an IMM had been detected there was an increased risk of future IMM although IMM were not consistently present. The majority of ewes had good udder conformation to suckle lambs. Factors associated with AM, IMM in pregnant and lactating ewes, udder conformation and lamb daily live weight gain were explored using mixed effect multivariable models. An increased risk of AM was associated with underfeeding protein in pregnancy (OR 4.05), forward pointing teats (OR 2.54), downward pointing teats (OR 4.68), rearing $\geq$ 2 lambs (OR 2.65), non-traumatic teat lesions (OR 2.09); and marginally associated with the presence of IMM. An increased risk of IMM in lactation was associated with AM during lactation (OR 12.39), IMM in pregnancy (OR 4.79), IMM in the previous lactation (OR 4.77), underfeeding energy in pregnancy (OR 6.66) and traumatic teat lesions (OR 2.48). An increased risk of IMM in pregnancy was associated with IMM in the previous pregnancy, IMM in the previous lactation and underfeeding energy in the previous lactation (OR 2.95). Lower lamb daily live weight gain was associated with traumatic teat lesions, IMM in lactation (−0.01 kg/day) and AM (−0.04 kg/day). We conclude that inadequate nutrition is an important cause of mastitis in suckler ewes which farmers could address in part using current nutritional guidelines but further work is needed. The relationship between AM and IMM indicates that separating or culling ewes with IMM would help reduce AM.

1. Introduction

In ewes, acute mastitis (AM) can lead to sudden death, loss of an affected udder half, chronic intramammary infection detected as masses (abscesses) in the mammary gland, raised somatic cell count (SCC), or full recovery. Farmers have reported a flock incidence of AM of 0–5% per year in England and Ireland (Cooper et al., 2016; Onnasch et al., 2002), although the true figures might be higher. It has been suggested that farmers under-report AM, even in dairy ewes that are observed more frequently than suckler ewes (Lafi et al., 1998). Anecdotal reports from farmers indicate that 20–30% of ewes culled from the flock at weaning have udder damage from AM or chronic mastitis with palpable intramammary masses (IMM). Given that the average replacement rate in suckler flocks in the UK is 20%, this amounts to approximately 8% of the national flock removed because of mastitis each year.

The economic costs of mastitis for the farmer therefore come from treatment costs, costs of replacement ewes when ewes die or are prematurely culled (due to losing the function of one or both glands or other udder damage such as IMM), reduced income

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from loss of lambs and for ewes with a SCC > 400,000 cells/ml milk, reduced milk production that causes slower growth rates in lambs (Arsenault et al., 2008; Huntley et al., 2012). AM is also a significant welfare concern; it is a painful disease that can lead to death while ewes with IMM are often prematurely culled by farmers. AM and IMM both affect milk production (Arsenault et al., 2008; Huntley et al., 2012) which impacts negatively on lamb health and welfare.

Larger litter size, older age, a previous case of mastitis, breed, management systems and geographical region are all reported risk factors for AM (Arsenault et al., 2008; Larsgard and Vaabenoe, 1993; Pereira et al., 2014; Waage and Vatn, 2008) indicating that both individual ewe and environmental factors are involved in disease pathogenesis. Poor body condition has been linked to increased risk of subclinical mastitis (Arsenault et al., 2008; Huntley et al., 2012), clinical mastitis (Onnasch et al., 2002) and traumatic teat lesions (Cooper et al., 2013) and so poor nutrition is also likely to be an important risk for mastitis.

In dairy sheep, good udder conformation is associated with a decreased risk of mastitis (Casu et al., 2010). A number of linear scoring systems of udder traits have been developed in European dairy sheep to assess udder conformation (de la Fuente et al., 1996; Marie-Etancelin et al., 2005; Casu et al., 2006). In some dairy breeds udder traits, such as vertically aligned teats (Labussière, 1988), have been included in breeding programmes with the aim of improving machine milking ability (de la Fuente et al., 1996; Marie-Etancelin et al., 2005; Casu et al., 2006). In suckler ewes an optimum teat angle of 45° downwards to the horizontal (score 5 in Casu et al., 2006) was associated with greater weight gain in lambs (Huntley et al., 2012) and decreased risk of traumatic teat lesions caused by lambs (Cooper et al., 2013) than other teat angles. This indicates that suckler and dairy ewe 'ideal' udder conformation varies for some traits. Other traits are uniformly consistent, for example, dairy ewes with pendulous udders and teats placed high on the udder are more prone to poor udder health (Casu et al., 2010) and in suckler ewes pendulous udders are associated with higher milk SCC (Huntley et al., 2012).

A common practice among suckler sheep farmers is to check the udder of each ewe at the end of lactation or 6 weeks before the start of the breeding season. Ewes with udder damage or IMM are often, but not always, culled. The impact of this practice is unknown; possible hypotheses include that it reduces onward transmission of bacterial strains causing mastitis, reduces the number of slow growing lambs in a flock, reduces the selection of replacement lambs from ewes with chronic mastitis and slows down the selection of more susceptible offspring.

The aims of this study were to examine the hypotheses above, by investigating ewe risks for, and inter-relationships between, AM, IMM and udder conformation and their impact on lamb growth rate, in approximately 4000 ewes observed prospectively for two years.

2. Materials and methods

2.1. Selection of study farms

Study farms were identified from farmers with existing relationships with the University of Warwick and from a list of farmers interested in participating in research on mastitis provided by AHDB Beef & Lamb. Farmers who expressed an interest were visited by Edward Smith (EMS) and Laura Green (LEG) and the project was explained in full. Once farmers agreed to participate, informed consent was obtained; participants were free to withdraw from the project at any stage. We aimed to recruit 4000 ewes, assuming that 8% of ewes would have udder abnormalities, this sample size had a power of 80% with 95% significance to detect factors that double the risk of disease, assuming a minimum exposure of 10%.

2.2. Data collection

Data collection occurred from November 2012 to July 2014. Each flock was visited twice each year, once when ewes were in late pregnancy and once when ewes were in mid-late lactation. Farmers were interviewed to gather information on flock management and nutrition. Data on number of lambs in pregnant ewes at scanning, lambing dates, litter size and lamb birth and 8-week weights were obtained from farm records. Farmers were asked to record all cases of AM treated during each lactation; this was part of their routine prior to participation in the study. In addition, researchers took note of any ewe they observed with AM during the examination in lactation. If that ewe was missing from the farmer's records it was added to the list of ewes with AM used in the analysis.

Every ewe was inspected at each visit. Sheep were examined upright in the narrowest portion of a race, while held by a clamp, or while restrained by an assistant. Udder conformation scores were assessed from a kneeling/crouched position behind the ewe using sight and touch. One of two trained researchers (EMS or CG (Claire Grant)) examined the ewes. An assistant recorded data into a handheld data-logger (Agrident APR500) using custom-designed software (Border Software Ltd).

At the examination during pregnancy, ewe identification, body condition score (BCS: 0–5 in 0.5 increments; Defra PB1875) and the presence/absence of IMM in each udder half were recorded. Masses were defined as a physically detectable mass of abnormal consistency compared with the rest of the glandular tissue. At the examination during lactation, ewe identification, BCS and the presence/absence of IMM in each udder half were also recorded. In addition, udder conformation, including teat position, teat angle, udder drop and degree of separation of udder halves; was recorded using a linear scoring system of udder traits adapted from Casu et al. (2006) and similar to that reported in Cooper et al. (2013) (Fig. S1). Udder width was measured at the widest point of the udder (1 cm increments) and teat length was recorded by measuring the left teat in 0.5 cm increments. The presence of wool on the udder was recorded, as were any teat lesions, recorded as traumatic (broken skin) or non-traumatic (e.g. warts, spots, orf-like lesions).

Two researchers carried out the examinations, so an inter-rater reliability study was conducted to test between observer variability. Both researchers (EMS and CG) carried out the examination during lactation on the same 137 ewes at different times on the same day supported by different assistants.

Nutrition was assessed by taking representative samples of forage and concentrates and submitting them to Sciantec Analytical Services (Selby, Yorkshire, England) for analysis. The metabolisable energy (ME; MJ/kg), crude protein (CP; %), moisture (%), ash (%), oil-b (%) and dry matter (DM; %) content of the concentrates; and the DM (g/kg), CP (g/kg), oil-b (g/kg), ash (g/kg), neutral detergent fibre (NDF; g/kg), acid detergent fibre (ADF; g/kg), sugar (g/kg), D value (digestibility of the dry matter) (%), ME (MJ/kg) and digestible energy (DE; MJ/kg) of the forages were determined. Silage samples were analysed for intake and fermentation characteristics, effective rumen degradable protein (ERDP; g/kg), digestible undegraded protein (DUP; g/kg) and nitrogen solubility. Spring and winter grass (nutrition value assumed to be 12.3 MJ/kgDM and 19% CP and 10.8 MJ/kgDM and 17% CP respectively) was assumed to be in sufficient supply to meet the appetite of the ewes in combination with any supplementary feeds offered, unless otherwise advised.

ADAS UK Ltd., were contracted to carry out analysis of each farm's nutritional data using the ADAS Sheepfeed rationing program (a computer program based on the Agricultural and Food Research Council (Great Britain) 1993 advisory manual on the energy and protein requirements of ruminants (AFRC, 1993)) and Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) for grass based diets. Adequacies of energy and protein levels were assessed and within each flock, ewes were categorised by scanning results/number of lambs reared as 'Overfed', 'Underfed' or 'Adequate' for dietary energy during pregnancy, dietary protein during pregnancy, dietary energy during lactation and dietary protein during lactation.

2.3. Data management

Data were downloaded from the datalogger as text files and converted to Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) format. All the animal, nutrition and management data from each farm, for each year, were combined into a single dataset per farm, which was imported into Microsoft Access 2010 (Microsoft Corp., Redmond, WA) to create a single database of all farms. Data checks and corrections were carried out at each stage. Queries were written to extract information as required for analysis. A single spreadsheet containing all the required data from all farms and both years was produced. Data for all ewes were kept in the dataset regardless of any missing data, which may have occurred due to incomplete examinations, incomplete records sent by the farmer and/or ewes being absent at an examination.

The annual cumulative incidence rate of acute mastitis was calculated from farmer records and researcher observations. The point prevalence of intramammary masses was calculated per farm per visit. A variable "Intramammary mass detected in the previous lactation" was created where ewes were categorised as "No" (no IMM detected in the previous lactation), "Yes" (at least 1 IMM detected in the previous lactation) or "Don't know" (ewe was not examined). All ewes in the dataset were categorised as "Don't know" in year 1.

Lamb daily live weight gain (DLWG) was calculated by subtracting the lamb birth weight from the lamb 8-week weight and dividing by the lamb's age in days at the 8-week weighing. Where lambs were not weighed at birth, but lambs of the same breed were weighed (on the same or another farm), the average of this weight (for lambs born as singles, twins or triplets) was used to calculate DLWG based on lambing dates and litter size. Birth weights and DLWGs of litter mates were summed to give litter birth weights and litter DLWGs for each ewe.

2.4. Statistical analyses

Minitab 17 (Minitab Inc., 2013) was used for preliminary data analysis. Frequency distributions of explanatory variables were explored and where a category contained low numbers (in most cases <50) of observations it was merged with the neighbouring category where appropriate. Ewes rearing ≥3 lambs were merged with ewes rearing 2 lambs because only 119 ewes reared ≥3 lambs over the two years. IMM in an udder half was re-categorised as at least one IMM in the whole udder because there were very few explanatory variables at udder half level.

Data from the inter-rater reliability study was analysed using percentages of exact agreement/1-2-3 point disagreements, Cohen's Kappa, Kendall's coefficient of concordance, intra-class correlation coefficients and tests for correlation and bias. Latent class analysis in Mplus version 7 (Muthén and Muthén, 1998–2012) was used to elucidate whether ewes could be sub-grouped by the teat and udder conformation variables.

The following were investigated in mixed effect multivariable models: factors associated with AM, IMM in pregnancy, IMM in lactation, and lamb daily live weight gain. In addition, factors associated with traumatic teat lesions, non-traumatic teat lesions, BCS, and each udder conformation variable were explored. Longitudinal analyses were restricted to variables that had been recorded at an earlier visit or at the same time as the outcome variable. Where explanatory variables were recorded at the same time as the outcome variable and cause and effect were not differentiated, excluding the variable was investigated.

Two three-level binary logistic models were used to explore the factors associated with AM and IMM in lactating ewes. These models took the form:

$$\mathrm{Logit}(\pi_{ijk}) = \beta_0 + \beta x_k + \beta x_{jk} + \beta x_{ijk} + v_k + u_{jk}$$

where $\mathrm{Logit}(\pi_{ijk})$ is the log odds of the probability that IMM or AM is present; β_0 is the constant, βx is a series of vectors of fixed effects that vary at k (farm), j (ewe) and i (observation), with residual variance estimates at farm (v_k) and ewe (u_{jk}). Level 1 variance followed a binomial error distribution.

One two-level binary logistic model was used to explore factors associated with IMM in pregnant ewes in year 2. This model took the form:

$$\mathrm{Logit}(\pi_{ij}) = \beta_0 + \beta x_j + \beta x_{ij} + u_j$$

where $\mathrm{Logit}(\pi_{ij})$ is the log odds of the probability that IMM are present, β_0 is the constant, βx is a series of vectors of fixed effects that vary at j (farm) and i (ewe), with residual variance estimates at u_j and level 1 variance followed a binomial error distribution.

A three-level continuous outcome model was used to explore the factors associated with lamb DLWG. This model took the form:

$$y_{ijk} = \beta_0 + \beta x_k + \beta x_{jk} + \beta x_{ijk} + v_k + u_{jk} + e_{ijk}$$

where y is the continuous outcome variable DLWG, β_0 is the intercept, and βx is a series of vectors of fixed effects that vary at k (farm), j (ewe) and i (lamb), with residual variance estimates at v_k, u_{jk} and at level 1 e_{ijk} with a mean of zero and standard deviation of 1.

All models were run in MLwiN version 2.31 (Rasbash et al., 2014) with iterative generalised squares for sample estimation. Forward manual stepwise model building was used to identify the variables that had a significant association ($P < 0.05$) with the outcome variable. Variables were considered significant when the 95% confidence intervals did not include unity for binomial models, 0 for continuous outcome models (Wald's test). All non-significant variables were retested in the final model to investigate residual confounding (Cox and Wermuth, 1996). Where two variables were highly correlated the most biologically plausible variable was retained in the model. The models were also run with farm as a fixed effect as well as a random term. The model fits were tested by examining plots of the residuals (continuous outcome models) and by the Hosmer – Lemeshow test (binary logistic models).

3. Results

3.1. Summary statistics

Data from 10 farms were included in the final dataset. Six farms participated in both years of the study, three farms participated for year 1 only (one farm provided management data for year 2) and one farm participated for year 2 only. The farms were located throughout Great Britain in Cheshire, Devon, Gloucestershire, Gwynedd, Herefordshire, Northumberland, Perth and Kinross, Powys, Shropshire and West Sussex. They included both pedigree and commercial flocks and indoor and outdoor lambing flocks (Table 1).

Data were collected on 3650 ewes in year 1 and 3371 in year 2, giving a total of 7021 examinations of 4721 ewes over the two years. A total of 1604 ewes were present over the two years of the study, and 1307 of those were examined at all four visits. Summary statistics are presented in Tables S1 and S2. The inter-rater reliability study showed good agreement between the two researchers on all measures (data not shown).

Table 1
Summary of the ten study farms.

Farm	Main Breed	Lambing		*N* year 1	*N* year 2	*N* present year 1 & 2
A	Lleyn	Apr/May	Outdoor*	321	322	225
B	Charollais	Dec	Indoor	145	155	75
C	Charollais	Dec/Jan	Indoor	60	56	37
D	Charollais	Dec	Indoor	74	93	44
E	Texel	Feb/Mar	Indoor	116	89	72
F	Lleyn	Apr/May	Outdoor*	1522	1509	1151
G	Texel	Mar/Apr	Indoor	165	NV	*NA*
H	Texel	Feb	Indoor	87	NV	*NA*
I	Crossbreeds/Lleyn	Mar/Apr	Indoor	1160	NV (1113)†	(689)
J	Texel	Feb/Mar	Indoor	NV	34	*NA*
Total *N*				3650	3371	2293 (1307 PFE)

N: Number of ewes; *: Small number lambed indoors; NV: Not visited; NA: Data not available for this farm; PFE: Present at all four exams; †: Not visited year 2 but provided data.

Table 2
Number and percentage of acute mastitis and intramammary masses in lactation and pregnancy for 10 GB sheep flocks.

Farm	Acute mastitis				Lactation IMM				Pregnancy IMM			
	Year 1		Year 2		Year 1		Year 2		Year 1		Year 2	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A	5	1.6	5	1.6	14	4.4	40	12.4	11	3.4	21	6.5
B	3	2.1	13	8.4	34	23.4	21	13.5	17	11.7	8	5.2
C	3	5.0	1	1.8	11	18.3	9	16.1	4	6.7	4	7.1
D	6	8.1	8	8.6	22	29.7	19	20.4	10	13.5	3	3.2
E	43	37.1	11	12.4	18	15.5	18	20.2	11	9.5	4	4.5
F	15	1.0	18	1.2	71	4.7	164	10.9	22	1.4	81	5.4
G	9	5.5	NA	NA	13	7.9	NA	NA	16	9.7	NA	NA
H	0	0	NA	NA	7	8.0	NA	NA	8	9.2	NA	NA
I	25	2.2	14	1.3	81	7.0	NA	NA	38	3.3	NA	NA
J	NA	NA	2	5.9	NA	NA	14	41.2	NA	NA	6	17.6
Total no. & % affected	109	3.0	72	2.1	271	8.7	285	14.3	147	4.1	128	5.7
Total no. examined	3650		3371		3101		1992		3562		2238	

IMM: Intra-mammary mass; No.: number; %: percentage of flock; NA: Data not available.

Table 3
Number and percentage of intramammary masses (IMM) in pregnancy and lactation in 1294 ewes from 6 GB sheep flocks present for all 4 observations over 2 years.

Pregnancy Year 1 IMM present	Lactation Year 1 IMM present	Pregnancy Year 2 IMM present	Lactation Year 2 IMM present
No:1255 (97.0%)	No: 1202 (95.8%)	No: 1139 (94.8%)	No: 1001 (87.9%)
			Yes: 138 (12.1%)
		Yes: 63 (5.2%)	No: 42 (66.7%)
			Yes: 21 (33.3%)
	Yes: 53 (4.2%)	No: 44 (83.0%)	No: 28 (63.6%)
			Yes: 16 (36.4%)
		Yes: 9 (17.0%)	No: 1 (11.1%)
			Yes: 8 (88.9%)
Yes: 39 (3.0%)	No: 28 (71.8%)	No: 21 (75.0%)	No: 16 (76.2%)
			Yes: 5 (23.8%)
		Yes: 7 (25.0%)	No: 5 (71.4%)
			Yes: 2 (28.6%)
	Yes: 11 (28.2%)	No: 10 (90.9%)	No: 3 (30%)
			Yes: 7 (70%)
		Yes: 1 (9.1%)	No: 0 (0.00%)
			Yes: 1 (100%)

Acute mastitis affected 2–3% of all ewes per year, flock range 0%–37.1% (Table 2). Approximately 5% of pregnant ewes and 11% of lactating ewes had at least one IMM over the course of the study; flock range from 1.4 to 41.2% (Table 2). There were 1294 ewes examined for IMM at all 4 examinations. Ewes with an IMM at an examination were at increased risk of an IMM in future examinations although IMM were not consistently detected at subsequent examinations (Table 3).

Over 75% of ewes were fed adequate energy and protein during pregnancy in year 1 (Table S3). This did vary by flock: on farm E ewes bearing ≥2 lambs were underfed energy and all ewes were underfed protein; on farm B, single bearing ewes were underfed while twin and triplet bearing ewes were overfed; and on farm G single bearing ewes were overfed energy. During lactation in year 1, only 35% of ewes were fed adequate energy and 53% of ewes were fed adequate protein. Ewes rearing ≥2 lambs were underfed energy on all farms. Generally ewes rearing ≥2 lambs were also underfed protein.

In year 2, most ewes were fed adequate energy and protein during pregnancy, except on farm B, where all ewes were overfed both. There was, again, more underfeeding in lactation but slightly less than in year 1. In both years, dietary energy and protein tended to be correlated, especially during pregnancy, with ewes either receiving adequate amounts of both or neither.

Table 4
Three-level binary logistic model of factors associated with acute mastitis in 3847 observations of 3019 ewes.

Variable	Category	*N* affected	% affected	OR	Lower 95% CI	Upper 95% CI
Age at lambing (yrs.)	1	6	1.37	Reference		
	2	63	4.40	4.19	0.71	24.60
	3	36	2.54	1.71	0.28	10.67
	4	23	2.49	3.52	0.55	22.40
	5–7	34	2.60	3.07	0.50	18.84
	>7	3	6.00	**13.00**	**1.24**	**136.33**
Number of lambs rearing	1	59	2.49	Reference		
	≥2	112	3.17	**2.65**	**1.49**	**4.72**
Breed	Lleyn	62	1.40	Reference		
	Crossbreeds	26	1.74	1.09	0.34	3.50
	Charollais	31	6.04	**6.67**	**2.20**	**20.23**
	Texel	62	13.60	**18.75**	**6.04**	**58.20**
Teat angle	5	30	1.46	Reference		
	7–9	13	3.85	1.18	0.49	2.86
	6	34	3.41	1.76	0.89	3.47
	4	38	2.63	**3.99**	**2.05**	**7.79**
	3–1	9	4.89	**4.68**	**1.36**	**16.16**
Teat position	3	47	1.81	Reference		
	1–2	61	4.10	**2.54**	**1.51**	**4.28**
	4–5	17	1.79	0.82	0.40	1.69
Non-traumatic teat lesions	None	111	2.32	Reference		
	At least 1 teat	21	7.09	**2.09**	**1.07**	**4.09**
IMM when pregnant	No	138	2.50	Reference		
	Yes	26	9.49	1.82	0.90	3.70
IMM in the previous lactation	No	27	1.76	Reference		
	Yes	8	11.27	3.16	0.82	12.15
	Don't know	146	2.70	1.52	0.73	3.18
Pregnancy protein	Adequate	115	1.93	Reference		
	Overfed	16	7.31	2.65	0.82	8.58
	Underfed	43	35.83	**4.05**	**1.44**	**11.35**
		Overall mean	*Affected mean*			
Litter DLWG (kg)		0.52	0.43	**0.03**	**0.01**	**0.18**
		Variance	SE			
Random effects	Farm	1.22	1.18			
	Ewe	1.00	1.00			
	Year					

N: Number; OR: Odds ratio; CI: Confidence interval; DLWG: Daily live weight gain; SD: Standard deviation. Reference: baseline category for comparison.
Where categories are in bold they are statistically different from the reference category at $P < 0.05$.

3.2. Multivariable analyses

3.2.1. Factors associated with acute mastitis in lactating ewes

Data from 3847 examinations (3019 ewes) were included in the model investigating AM (Table 4). Key results were that underfeeding protein in pregnancy was associated with an increased risk of AM. In addition, older ewes, those rearing ≥2 lambs, terminal sire producing pedigree ewes, teat angle and position and non-traumatic teat lesions had significant associations with AM. IMM during lactation the previous year and IMM when pregnant were moderately associated with a higher risk of AM although they were not significant at $P < 0.05$. With farm added as a fixed effect, Farms C, D, E and G had a significantly higher risk of AM than Farm A and age at lambing >7, non-traumatic teat lesions and underfeeding protein in pregnancy were no longer significant (data not shown), indicating flock level differences in nutrition, teat health and age of ewes.

3.2.2. Factors associated with intramammary masses in lactating ewes

Data from 3735 examinations (2916 ewes) were included in the model investigating IMM in lactating ewes in years 1 and 2 (Table 5). There was a greater than 12-fold odds of IMM in lactation when a ewe had AM and greater than 4-fold odds if the ewe had previous IMMs in pregnancy or lactation. In addition, a higher flock percentage of IMM in pregnancy was associated with a significant increased risk of IMM in lactation. All these risk factors highlight the strong role of prior infection in the ewe and flock for current IMM. Underfeeding energy in lactation was a significant risk for IMM (>6-fold odds), again highlighting the role of poor nutrition in mastitis in these suckler ewes. Teat lesions and udder conformation was also associated with IMM.

3.2.3. Factors associated with intramammary masses in pregnant ewes

Data from 1427 ewes were included in the model investigating intramammary masses in pregnant ewes in year 2 (Table 6). As with IMM in lactation, previous infection and poor nutrition were the key risks: ewes with IMM at previous examinations in year 1 were significantly more likely to have IMM when pregnant in year 2 than those without IMM at previous examinations and underfeeding energy in lactation in year 1 was associated with an increased risk of IMM while underfeeding protein in lactation in year 1 was associated with a decreased risk of IMM when pregnant in year 2. No other variables were significantly associated with an IMM in pregnancy in year 2.

Table 5
Three-level binary logistic model of factors associated with intramammary masses in lactation in year 1 and 2 in 3735 observations of 2916 ewes.

Variable	Category	*N* affected	% affected	OR	Lower 95% CI	Upper 95% CI
Acute mastitis	No	479	7.00	Reference		
	Yes	77	42.54	**12.39**	**6.57**	**23.38**
IMM when pregnant	No	456	8.25	Reference		
	Yes	88	32.00	**4.79**	**3.23**	**7.12**
IMM in the previous lactation	No	186	12.13	Reference		
	Yes	34	47.89	**4.77**	**2.52**	**9.03**
	Don't know	336	9.19	**0.52**	**0.39**	**0.69**
Pregnancy protein	Adequate	424	7.11	Reference		
	Overfed	34	15.53	1.64	0.60	4.48
	Underfed	26	21.67	**0.07**	**0.01**	**0.34**
Pregnancy energy	Adequate	417	7.04	Reference		
	Overfed	43	15.03	0.62	0.24	1.58
	Underfed	24	26.67	**6.66**	**1.46**	**30.48**
Traumatic teat lesions	None	508	10.40	Reference		
	At least 1 teat	48	24.00	**2.48**	**1.56**	**3.95**
Non-traumatic teat lesions	None	506	10.57	Reference		
	At least 1 teat	50	16.89	**1.83**	**1.20**	**2.78**
Age at lambing (yrs.)	1	16	3.66	Reference		
	2	110	7.68	1.48	0.73	2.98
	3	117	8.25	1.90	0.92	3.89
	4	96	10.39	**2.74**	**1.30**	**5.76**
	5–7	151	11.54	**2.32**	**1.13**	**4.78**
	>7	8	16.00	1.84	0.55	6.16
Degree of separation of udder halves	3	133	10.12	Reference		
	6–8	37	8.56	**0.43**	**0.26**	**0.72**
	5	59	6.88	**0.42**	**0.28**	**0.64**
	4	97	9.16	0.72	0.51	1.03
	2	114	13.36	1.10	0.78	1.55
	1	72	20.93	**1.72**	**1.13**	**2.61**
Udder drop	7	284	8.63	Reference		
	8–9	79	10.10	0.94	0.64	1.40
	6	130	16.31	**1.81**	**1.34**	**2.45**
	5–1	47	31.54	**4.35**	**2.59**	**7.31**
Teat position	3	261	10.01	Reference		
	1–2	186	12.50	**1.34**	**1.01**	**1.78**
	4–5	94	9.87	0.95	0.69	1.31
		Overall mean	*Affected mean*			
Flock% of IMM in pregnancy		4.47	5.85	**1.11**	**1.06**	**1.17**
Litter DLWG (kg)		0.52	0.51	**0.25**	**0.11**	**0.54**
Days in milk		69.79	68.75	**1.01**	**1.00**	**1.02**
		Variance	SE			
Random effects	Farm	1.00	1.00			
	Ewe	1.10	1.24			
	Year					

N: Number; OR: Odds ratio; CI: Confidence interval; IMM: Intramammary mass; DLWG: Daily live weight gain; SD: Standard deviation; Reference: baseline category for comparison.
Where categories are in bold they are statistically different from the reference category at $P < 0.05$.

3.2.4. Factors associated with lamb daily live weight gain

Data from 6453 lambs were included in the model investigating lamb DLWG (Table 7). Key results were that lambs reared by ewes that had AM, an IMM, a traumatic teat lesion or a non-traumatic teat lesion, had lower DLWG than lambs reared by ewes without these issues.

3.2.5. Factors associated with udder conformation and teat lesions in lactating ewes

Results from the mixed effect multivariable models of the udder conformation variables, traumatic and non-traumatic teat lesions are included in the supplementary material (Tables S4–S11). Key results were that generally across the models, udder and teat conformation were associated with increasing age (i.e. increasing parities) and the mastitis disease variables, suggesting that conformation is poorer with increasing age and disease. Latent class analysis indicated that there were four classes of ewe; young ewes with good teat and udder conformation; young-middle aged ewes with poorer conformation, young-middle aged ewes with good conformation and older ewes with poorer conformation (data not shown). IMM when lactating and rearing ≥2 lambs were associated with an increased risk for both types of teat lesions.

The model fits were good (Figs. S2.1–2.14).

4. Discussion

This is the first prospective, longitudinal study of suckler ewes on the risks and interrelationships between AM, IMM, udder and teat conformation and lamb growth rate. The associations between AM, IMM and udder conformation are complex with, for exam-

Table 6
Two-level binary logistic model of factors associated with intramammary masses in pregnancy in year 2 in 1427 ewes.

Variable	Category	*N* affected	% affected	OR	Lower 95% CI	Upper 95% CI
Year 1 IMM when lactating	No	80	5.73	Reference		
	Yes	13	18.31	**3.13**	**1.49**	**6.58**
Year 1 IMM when pregnant	No	88	5.73	Reference		
	Yes	9	20.45	**4.05**	**1.69**	**9.70**
Lactation energy year 1	Adequate	23	3.46	Reference		
	Overfed	1	11.11	1.80	0.17	18.88
	Underfed	71	9.14	**2.95**	**1.78**	**4.89**
Lactation protein year 1	Adequate	80	6.91	Reference		
	Overfed	0				
	Underfed	15	5.10	**0.32**	**0.16**	**0.62**
		Variance	SE			
Random effects	Farm	1.00	1.00			
	Ewe					

N: Number; OR: Odds ratio; CI: Confidence interval; IMM: Intramammary mass; SD: Standard deviation; Reference: baseline category for comparison.
Where categories are in bold they are statistically different from the reference category at $P < 0.05$.

ple, dependent variables in one model (e.g. AM) being explanatory variables in another. The aim of investigating each aspect of udder health and conformation in multivariable models was to elucidate associations and develop hypotheses for development of AM and IMM. Udder conformation was investigated because in previous papers (Casu et al., 2010; Huntley et al., 2012) some udder and teat conformations have been linked to intramammary infections.

One key result was that AM was strongly associated with IMM in lactation. This 12-fold odds is indicative of causality with IMM a result of an episode of AM. Not all IMM were associated with AM, this could indicate that farmers are not observing all cases of AM because some are mild and others are missed, as suggested in dairy ewes (Lafi et al., 1998) and as observed by researchers in the current study when AM had not been recorded by farmers in some instances. The risk of IMM in lactation was also associated with previous IMM and flock percentage of IMM. This suggests that IMM can be a source of infection to other ewes in the flock, as is commonly thought and given as a reason for culling ewes with IMM (Gelasakis et al., 2015).

There was a tendency for IMM in pregnancy the same year or lactation the previous year to be associated with a higher risk of AM in the subsequent lactation. However, ewes were culled, sold or died throughout the study for many reasons, including some ewes with IMM removed from some flocks between years 1 and 2, and this might have weakened the association detected between IMM and subsequent IMM and AM. We can hypothesise that IMM are a result of an acute disease event and are themselves chronic, persistent infection that may increase a ewe's risk of subsequent AM.

The percentage of IMM was very high in the smaller flocks in the current study. These were pedigree flocks where the farmers were less likely to cull ewes with IMM and so the flock prevalence of IMM would have increased each year. Consequently, a high percentage of IMM could be due to spread of disease being more rapid in these flocks where affected and unaffected ewes and their lambs were kept together, or because of management decisions to retain affected ewes.

In the current study, IMM were not detected at all subsequent examinations once first detected, however, ewes with previously detected IMM were approximately 3–5 times more likely to have IMM at a later date (Tables 5 and 6). IMM in suckler ewes are typically abscesses (Smith et al., 2015). Abscesses are thought to be polymicrobial (Brook, 2002); they develop and rupture as part of their maturation cycle. Rupture facilitates the spread of bacteria which can subsequently reform abscesses elsewhere within their environment (Cheng et al., 2011). This cycle of growth and rupture might explain why IMM were present at one examination and not at a second but then present again at the third or fourth examination.

Another key finding of this study was that dietary levels of energy and protein in pregnancy and lactation impacted significantly on AM and IMM. One Flock (E) underfed protein to all ewes in pregnancy in year 1 and many flocks underfed energy and protein in lactation by current guidelines. Whilst underfeeding protein in pregnancy was not common on study farms, it warrants discussion because of the risk should this occur on any farm; insufficient protein in pregnancy will lead to reduced mammary development and inadequate milk supply throughout lactation (Fthenakis et al., 2012). Underfeeding energy in pregnancy and lactation was also a risk for IMM and the latter was common on our study farms (Table S3), but underfeeding protein was apparently protective. One recent study reported that if energy in the diet is adequate, increasing protein beyond requirements has no benefit for lamb growth rate (Van Emon et al., 2014). Another study (Barbagianni et al., 2015) reported that experimental ewes fed insufficient energy during pregnancy (to bring on pregnancy toxaemia) had more mastitis after lambing, these authors suggested that this was due to an impaired immune response caused by the increased concentrations of β-hydroxybutyrate, but it could have been a direct effect of low energy in the diet. It is possible that current guidelines on the absolute amount of energy and protein required, or the ratio between the two, are wrong.

AM might occur from poor diet because an inadequate milk supply leads to hungry lambs that traumatise the teats and udder (Cooper et al., 2013). Although we did not find an association between traumatic teat lesions and AM (in univariable or multivariable analysis) this was probably because we examined ewes at approximately 9 weeks after lambing and so lesions would have healed: Cooper et al. (2013) found that the incidence of traumatic teat lesions was greatest 3–4 weeks after lambing and healed within two weeks.

We studied a small number of convenience-selected farms, but if inadequate diets are common on GB sheep farms, nutrition could be a large attributable risk to udder health and farmers could improve udder health considerably using current nutritional guidelines (Agricultural and Food Research Council (Great Britain), 1993). We did not detect associations between ewe body condition and AM or IMM as in other studies (Arsenault et al., 2008; Huntley et al., 2012; Onnasch et al., 2002). This may be because we measured BCS at two time points, once in pregnancy and once lactation, when most ewes had adequate body condition. Huntley et al. (2012) reported an association between low BC and high SCC when BCS was measured weekly.

Table 7
Three-level continuous outcome model of factors associated with lamb daily live weight gain (kg) in 6453 lambs from 9 farms over 2 years.

Variable	Category	*N*	Mean	Coefficient	Lower 95% CI	Upper 95% CI
Lamb gender	Male	4356	0.35	Reference		
	Female	4325	0.33	−**0.03**	−**0.03**	−**0.02**
Number of lambs reared	**1**	2194	0.38	Reference		
	≥**2**	6254	0.32	−**0.05**	−**0.06**	−**0.04**
Lamb breed	Lleyn	4456	0.32	Reference		
	Crossbreeds	3129	0.36	**0.04**	**0.03**	**0.04**
	Charollais	695	0.33	**0.08**	**0.01**	**0.15**
	Texel	329	0.35	**0.06**	**0.01**	**0.11**
Acute mastitis	No	8466	0.34	Reference		
	Yes	215	0.28	−**0.04**	−**0.05**	−**0.02**
IMM when lactating	No	6058	0.34	Reference		
	Yes	715	**0.33**	−**0.01**	−**0.02**	−**0.01**
Traumatic teat lesions	No	6485	0.34	Reference		
	Yes	288	0.31	−**0.02**	−**0.03**	−**0.01**
Non-traumatic teat lesions	No	6350	0.341	Reference		
	Yes	422	0.338	−**0.01**	−**0.020**	−**0.004**
BCS in pregnancy	3	3278	0.34	Reference		
	Below 3	2965	0.35	−**0.01**	−**0.009**	−**0.002**
	Above 3	2205	0.33	**0.01**	**0.002**	**0.010**
BCS in lactation	3	2023	0.34	Reference		
	Below 3	4633	0.34	**0.01**	**0.003**	**0.011**
	Above 3	1440	0.34	0.00	−0.004	0.008
Pregnancy protein	Adequate	7935	0.34	Reference		
	Overfed	316	0.32	−**0.12**	−**0.15**	−**0.09**
	Underfed	113	0.29	−**0.10**	−**0.13**	−**0.07**
Lactation protein	Adequate	6453	0.34	Reference		
	Overfed	24	0.56	**0.12**	**0.08**	**0.16**
	Underfed	1929	0.34	−**0.02**	−**0.02**	−**0.01**
Lactation energy	OK	3329	0.35	Reference		
	Overfed	70	0.43	−**0.03**	−**0.05**	−**0.01**
	Underfed	5007	0.33	−**0.01**	−**0.023**	−**0.001**
Teat position	3	3525	0.34	Reference		
	1–2	1987	0.35	0.000	−0.003	0.004
	4–5	1216	0.34	−**0.004**	−**0.0792**	−**0.0001**
Udder drop	7	4468	0.34	Reference		
	5	240	0.36	−0.01	−0.02	0.00
	6	1211	0.35	0.00	−0.01	0.00
	8	794	0.33	−**0.02**	−**0.02**	−**0.01**
Udder width		8681		**0.008**	**0.006**	**0.010**
Teat length		8681		**0.008**	**0.004**	**0.012**
Lamb BW (kg)		8667		−**0.003**	−**0.005**	−**0.001**
Lamb age when when weighed (days)	$\hat{\mathbf{1}}$	8681		**0.032**	**0.01**	**0.05**
	$\hat{\mathbf{2}}$			−**0.0009**	−**0.0015**	−**0.0004**
	$\hat{\mathbf{3}}$			**0.00001**	**0.000006**	**0.000018**
	$\hat{4}$			0.000	0.00	0.00
Age at lambing (yrs.)		7879		**0.004**	**0.002**	**0.006**
		Variance	SE			
Random effects	Farm	0.00215	0.00103			
	Ewe	0.00139	0.00008			
	Lamb	0.00282	0.00007			

N: Number; CI: Confidence interval; BW: Birth weight; BCS: Body condition score; SD: Standard deviation; Reference: baseline category for comparison. (Lamb age when weighed (days) was entered as a quadratic term).
Where categories are in bold they are statistically different from the reference category at $P < 0.05$.

The majority of ewes in our study had udders with good conformation (teat angle 5, teat position 3, udder drop 7; Fig. S1) to suckle lambs (Huntley et al., 2012). Udder conformation that differed from this optimum was associated with a small but significant increased risk of IMM and AM (Tables 4 and 5), as reported previously (Casu et al., 2010; Huntley et al., 2012). This association is likely to be causal in some instances, with increased exposure of the teat end to contamination or poor teat positioning making suckling difficult for lambs and so causing teat lesions. Udder conformation also changed in some ewes with IMM and after a severe case of AM (CG personal observation). These results, and the latent class analysis, suggest udder conformation is generally good but that age and udder disease can impact on udder and teat conformation. Breeders should be aware of avoiding selecting away from good udder conformation in any selection programme that inadvertently selects for altered phenotype. That said, in general, not selecting replacement ewes from ewes with poor udder conformation would be sensible.

Several other factors were associated with AM or IMM, including increasing ewe age and larger litter size. These risks are important and have been reported previously (Arsenault et al., 2008; Larsgard and Vaabenoe, 1993; Pereira et al., 2014; Waage and Vatn, 2008). We conclude that age needs to be managed through planned culling of older ewes. Where ewes are not able to feed their lambs adequately (due to age or large litter sizes/weights) then supplementing lambs' feed would reduce the risk of over-demand for milk from ewes.

Importantly for farmers, the presence of AM, IMM in lactation, traumatic teat lesions and non-traumatic teat lesions all impacted negatively on lamb daily live weight gain (Table 7). This is in line with previous findings (Arsenault et al., 2008; Huntley et al., 2012) and could be due to decreased milk production in the diseased gland or to ewes preventing lambs from suckling. Whatever the reason, this association adds to the economic cost of AM and IMM.

The study design was selected so that longitudinal data could be collected. This could only be done on a small number of farms and so the intention was not to identify representative farms but rather strengths of associations between factors to improve our understanding of the biology of AM, IMM and teat and udder conformation. Because of the need to visit farms on four occasions and to have extra data on AM, lamb weights and litter sizes between visits, we convenience-selected farms that were already collecting such data and where farmers were compliant with the study demands. Overall this was successful, although we aimed to follow 4000 ewes over 2 years but due to one large commercial farm dropping out of the study in year 2 and a high rate of attrition among the ewes, surprisingly only 1307 ewes were seen at all 4 examinations. Therefore we may not have had sufficient power to detect all risks, particularly when ewes were culled or sold at the end of year 1 due to AM and IMM.

Another potential weakness of this study is that 7 of the 10 farms were pedigree sheep breeding farms where management practices are different from large commercial farms, e.g. pedigree sheep breeders may be less likely to cull ewes for IMM as ewes are more valuable and replacement costs are higher, however, these flocks were selected because we could study the impact of retaining ewes with IMM.

Despite these weaknesses the results from this study are likely to be qualitatively generalisable to other flocks. Due consideration of diet, management of older ewes, ewes with large litters and ewes with IMM is necessary to reduce the risk of AM and IMM and to maintain growth rates of lambs.

5. Conclusions

We conclude that this study of 10 flocks indicates that IMM, diet and udder conformation all contribute to cases of acute and chronic mastitis in suckler ewes. The relationships are complex, but the pattern of events appears to be that ewes fed inadequate protein in pregnancy and inadequate energy in pregnancy and lactation are at greater risk of AM and IMM. AM leads to development of IMM, IMM persist and may cause more IMM and AM within a flock. We conclude that feeding appropriate levels of energy and protein both in pregnancy and lactation and managing IMM would increase udder health and consequently increase flock productivity and profitability.

Conflict of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2016.03.002.

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