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1 **A longitudinal study of factors associated with acute and chronic mastitis**

2 **and their impact on lamb growth rate in 10 suckler sheep flocks in Great**

3 **Britain**

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8 **ABSTRACT**

9 A 2-year prospective, longitudinal study of 10 suckler sheep flocks in Great Britain was run

10 to identify factors associated with acute mastitis (AM) and chronic mastitis, and their impact

11 on lamb growth rate. Data were collected on AM, intramammary masses (IMM; a marker for

12 chronic mastitis), udder and teat conformation, teat lesions, body condition, ewe nutrition,

13 litter size, lamb weight and general flock management. Each flock was visited twice each

14 year, approximately 4 weeks before lambing and 9 weeks into lactation, for two years and all

15 ewes present at a visit were examined. There were 7021 examinations in total. AM was

16 reported in 2.1 - 3.0% of ewes / year; this ranged from 0.0% to 37.1% by flock. IMM were

17 detected in 4.7% of ewes in pregnancy and 10.9% of ewes in lactation. Once an IMM had

18 been detected there was an increased risk of future IMM although IMM were not consistently

19 present. The majority of ewes had good udder conformation to suckle lambs. Factors

20 associated with AM, IMM in pregnant and lactating ewes, udder conformation and lamb

21 daily live weight gain were explored using mixed effect multivariable models. An increased

22 risk of AM was associated with underfeeding protein in pregnancy (OR 4.05), forward

23   pointing teats (OR 2.54), downward pointing teats (OR 4.68), rearing ≥ 2 lambs (OR 2.65),

24   non-traumatic teat lesions (OR 2.09); and marginally associated with the presence of IMM.

25   An increased risk of IMM in lactation was associated with AM during lactation (OR 12.39),

26   IMM in pregnancy (OR 4.79), IMM in the previous lactation (OR 4.77), underfeeding energy

27   in pregnancy (OR 6.66) and traumatic teat lesions (OR 2.48). An increased risk of IMM in

28   pregnancy was associated with IMM in the previous pregnancy, IMM in the previous

29   lactation and underfeeding energy in the previous lactation (OR 2.95). Lower lamb daily live

30   weight gain was associated with traumatic teat lesions, IMM in lactation (-0.01 kg / day) and

31   AM (-0.04 kg / day). We conclude that inadequate nutrition is an important cause of mastitis

32   in suckler ewes which farmers could address in part using current nutritional guidelines but

33   further work is needed. The relationship between AM and IMM indicates that separating or

34   culling ewes with IMM would help reduce AM.

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36   **Key words:** Longitudinal study; Suckler ewe; Mastitis; Lamb daily live weight gain; Mixed

37   effect models.

38   **1. Introduction**

39   In ewes, acute mastitis (AM) can lead to sudden death, loss of an affected udder half, chronic

40   intramammary infection detected as masses (abscesses) in the mammary gland, raised

41   somatic cell count (SCC), or full recovery. Farmers have reported a flock incidence of AM of

42   0 - 5% per year in England and Ireland (Cooper et al., 2016; Onnasch et al., 2002), although

43   the true figures might be higher. It has been suggested that farmers under-report AM, even in

44   dairy ewes that are observed more frequently than suckler ewes (Lafi et al., 1998). Anecdotal

45   reports from farmers indicate that 20 – 30% of ewes culled from the flock at weaning have

46   udder damage from AM or chronic mastitis with palpable intramammary masses (IMM).

47 Given that the average replacement rate in suckler flocks in the UK is 20%, this amounts to

48 approximately 8% of the national flock removed because of mastitis each year.

49 The economic costs of mastitis for the farmer therefore come from treatment costs, costs of

50 replacement ewes when ewes die or are prematurely culled (due to losing the function of one

51 or both glands or other udder damage such as IMM), reduced income from loss of lambs and

52 for ewes with a SCC > 400,000 cells / ml milk, reduced milk production that causes slower

53 growth rates in lambs (Arsenault et al., 2008; Huntley et al., 2012). AM is also a significant

54 welfare concern; it is a painful disease that can lead to death while ewes with IMM are often

55 prematurely culled by farmers. AM and IMM both affect milk production (Arsenault et al.,

56 2008; Huntley et al., 2012) which impacts negatively on lamb health and welfare.

57 Larger litter size, older age, a previous case of mastitis, breed, management systems and

58 geographical region are all reported risk factors for AM (Arsenault et al., 2008; Larsgard and

59 Vaabenoe, 1993; Pereira et al., 2014; Waage and Vatn, 2008) indicating that both individual

60 ewe and environmental factors are involved in disease pathogenesis. Poor body condition has

61 been linked to increased risk of subclinical mastitis (Arsenault et al., 2008; Huntley et al.,

62 2012), clinical mastitis (Onnasch et al., 2002) and traumatic teat lesions (Cooper et al., 2013)

63 and so poor nutrition is also likely to be an important risk for mastitis.

64 In dairy sheep, good udder conformation is associated with a decreased risk of mastitis (Casu

65 et al., 2010). A number of linear scoring systems of udder traits have been developed in

66 European dairy sheep to assess udder conformation (de la Fuente et al., 1996; Marie-

67 Etancelin et al., 2005; Casu et al., 2006). In some dairy breeds udder traits, such as vertically

68 aligned teats (Labussière 1988), have been included in breeding programmes with the aim of

69 improving machine milking ability (de la Fuente et al., 1996; Marie-Etancelin et al., 2005;

70 Casu et al., 2006) . In suckler ewes an optimum teat angle of 45˚ downwards to the horizontal

71   (score 5 in Casu et al., 2006) was associated with greater weight gain in lambs (Huntley et al.,

72   2012) and decreased risk of traumatic teat lesions caused by lambs (Cooper et al., 2013) than

73   other teat angles. This indicates that suckler and dairy ewe 'ideal' udder conformation varies

74   for some traits. Other traits are uniformly consistent, for example, dairy ewes with pendulous

75   udders and teats placed high on the udder are more prone to poor udder health (Casu et al.,

76   2010) and in suckler ewes pendulous udders are associated with higher milk SCC (Huntley et

77   al., 2012).

78   A common practice among suckler sheep farmers is to check the udder of each ewe at the end

79   of lactation or 6 weeks before the start of the breeding season. Ewes with udder damage or

80   IMM are often, but not always, culled. The impact of this practice is unknown; possible

81   hypotheses include that it reduces onward transmission of bacterial strains causing mastitis,

82   reduces the number of slow growing lambs in a flock, reduces the selection of replacement

83   lambs from ewes with chronic mastitis and slows down the selection of more susceptible

84   offspring.

85   The aims of this study were to examine the hypotheses above, by investigating ewe risks for,

86   and inter-relationships between, AM, IMM and udder conformation and their impact on lamb

87   growth rate, in approximately 4000 ewes observed prospectively for two years.

88

2. Materials and methods

2.1. Selection of study farms

91   Study farms were identified from farmers with existing relationships with the University of

92   Warwick and from a list of farmers interested in participating in research on mastitis provided

93   by AHDB Beef & Lamb. Farmers who expressed an interest were visited by Edward Smith

94　(EMS) and Laura Green (LEG) and the project was explained in full. Once farmers agreed to

95　participate, informed consent was obtained; participants were free to withdraw from the

96　project at any stage. We aimed to recruit 4000 ewes, assuming that 8% of ewes would have

97　udder abnormalities, this sample size had a power of 80% with 95% significance to detect

98　factors that double the risk of disease, assuming a minimum exposure of 10%.

99　*2.2. Data collection*

100　Data collection occurred from November 2012 to July 2014. Each flock was visited twice

101　each year, once when ewes were in late pregnancy and once when ewes were in mid - late

102　lactation. Farmers were interviewed to gather information on flock management and

103　nutrition. Data on number of lambs in pregnant ewes at scanning, lambing dates, litter size

104　and lamb birth and 8-week weights were obtained from farm records. Farmers were asked to

105　record all cases of AM treated during each lactation; this was part of their routine prior to

106　participation in the study. In addition, researchers took note of any ewe they observed with

107　AM during the examination in lactation. If that ewe was missing from the farmer's records it

108　was added to the list of ewes with AM used in the analysis.

109　Every ewe was inspected at each visit. Sheep were examined upright in the narrowest portion

110　of a race, while held by a clamp, or while restrained by an assistant. Udder conformation

111　scores were assessed from a kneeling / crouched position behind the ewe using sight and

112　touch. One of two trained researchers (EMS or CG (Claire Grant)) examined the ewes. An

113　assistant recorded data into a handheld data-logger (Agrident APR500) using custom-

114　designed software (Border Software Ltd).

115　At the examination during pregnancy, ewe identification, body condition score (BCS: 0 – 5 in

116　0.5 increments; Defra PB1875) and the presence / absence of IMM in each udder half were

117　recorded. Masses were defined as a physically detectable mass of abnormal consistency

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118   compared with the rest of the glandular tissue. At the examination during lactation, ewe

119   identification, BCS and the presence / absence of IMM in each udder half were also recorded.

120   In addition, udder conformation, including teat position, teat angle, udder drop and degree of

121   separation of udder halves; was recorded using a linear scoring system of udder traits adapted

122   from Casu et al. (2006) and similar to that reported in Cooper et al. (2013) (Figure S1). Udder

123   width was measured at the widest point of the udder (1 cm increments) and teat length was

124   recorded by measuring the left teat in 0.5 cm increments. The presence of wool on the udder

125   was recorded, as were any teat lesions, recorded as traumatic (broken skin) or non-traumatic

126   (e.g. warts, spots, orf-like lesions).

127   Two researchers carried out the examinations, so an inter-rater reliability study was

128   conducted to test between observer variability. Both researchers (EMS and CG) carried out

129   the examination during lactation on the same 137 ewes at different times on the same day

130   supported by different assistants.

131   Nutrition was assessed by taking representative samples of forage and concentrates and

132   submitting them to Sciantec Analytical Services (Selby, Yorkshire, England) for analysis.

133   The metabolisable energy (ME; MJ/kg), crude protein (CP; %), moisture (%), ash (%), oil-b

134   (%) and dry matter (DM; %) content of the concentrates; and the DM (g/kg), CP (g/kg), oil-b

135   (g/kg), ash (g/kg), neutral detergent fibre (NDF; g/kg), acid detergent fibre (ADF; g/kg),

136   sugar (g/kg), D value (digestibility of the dry matter)(%), ME (MJ/kg) and digestible energy

137   (DE; MJ/kg) of the forages were determined. Silage samples were analysed for intake and

138   fermentation characteristics, effective rumen degradable protein (ERDP; g/kg), digestible

139   undegraded protein (DUP; g/kg) and nitrogen solubility. Spring and winter grass (nutrition

140   value assumed to be 12.3MJ/kgDM and 19% CP and 10.8 MJ/kgDM and 17% CP

141 respectively) was assumed to be in sufficient supply to meet the appetite of the ewes in

142 combination with any supplementary feeds offered, unless otherwise advised.

143 ADAS UK Ltd. were contracted to carry out analysis of each farm's nutritional data using the

144 ADAS Sheepfeed rationing program (a computer program based on the Agricultural and

145 Food Research Council (Great Britain) 1993 advisory manual on the energy and protein

146 requirements of ruminants (AFRC, 1993)) and Microsoft Excel 2010 (Microsoft Corp.,

147 Redmond, WA) for grass based diets. Adequacies of energy and protein levels were assessed

148 and within each flock, ewes were categorised by scanning results / number of lambs reared as

149 'OVERFED', 'UNDERFED' or 'ADEQUATE' for dietary energy during pregnancy, dietary protein

150 during pregnancy, dietary energy during lactation and dietary protein during lactation.

151 *2.3. Data management*

152 Data were downloaded from the datalogger as text files and converted to Microsoft Excel

153 2010 (Microsoft Corp., Redmond, WA) format. All the animal, nutrition and management

154 data from each farm, for each year, were combined into a single dataset per farm, which was

155 imported into Microsoft Access 2010 (Microsoft Corp., Redmond, WA) to create a single

156 database of all farms. Data checks and corrections were carried out at each stage. Queries

157 were written to extract information as required for analysis. A single spreadsheet containing

158 all the required data from all farms and both years was produced. Data for all ewes were kept

159 in the dataset regardless of any missing data, which may have occurred due to incomplete

160 examinations, incomplete records sent by the farmer and / or ewes being absent at an

161 examination.

162 The annual cumulative incidence rate of acute mastitis was calculated from farmer records

163 and researcher observations. The point prevalence of intramammary masses was calculated

164 per farm per visit. A variable "Intramammary mass detected in the previous lactation" was

165 created where ewes were categorised as "No" (no IMM detected in the previous lactation),

166 "Yes" (at least 1 IMM detected in the previous lactation) or "Don't know" (ewe was not

167 examined). All ewes in the dataset were categorised as "Don't know" in year 1.

168 Lamb daily live weight gain (DLWG) was calculated by subtracting the lamb birth weight

169 from the lamb 8-week weight and dividing by the lamb's age in days at the 8-week weighing.

170 Where lambs were not weighed at birth, but lambs of the same breed were weighed (on the

171 same or another farm), the average of this weight (for lambs born as singles, twins or triplets)

172 was used to calculate DLWG based on lambing dates and litter size. Birth weights and

173 DLWGs of litter mates were summed to give litter birth weights and litter DLWGs for each

174 ewe.

175 *2.4. Statistical analyses*

176 Minitab 17 (Minitab Inc. 2013) was used for preliminary data analysis. Frequency

177 distributions of explanatory variables were explored and where a category contained low

178 numbers (in most cases < 50) of observations it was merged with the neighbouring category

179 where appropriate. Ewes rearing ≥ 3 lambs were merged with ewes rearing 2 lambs because

180 only 119 ewes reared ≥ 3 lambs over the two years. IMM in an udder half was re-categorised

181 as at least one IMM in the whole udder because there were very few explanatory variables at

182 udder half level.

183 Data from the inter-rater reliability study was analysed using percentages of exact agreement

184 / 1-2-3 point disagreements, Cohen's Kappa, Kendall's coefficient of concordance, intra-class

185 correlation coefficients and tests for correlation and bias. Latent class analysis in Mplus

186 version 7 (Muthén and Muthén, 1998-2012) was used to elucidate whether ewes could be

187 sub-grouped by the teat and udder conformation variables.

188　The following were investigated in mixed effect multivariable models: factors associated

189　with AM, IMM in pregnancy, IMM in lactation, and lamb daily live weight gain. In addition,

190　factors associated with traumatic teat lesions, non-traumatic teat lesions, BCS, and each

191　udder conformation variable were explored. Longitudinal analyses were restricted to

192　variables that had been recorded at an earlier visit or at the same time as the outcome

193　variable. Where explanatory variables were recorded at the same time as the outcome

194　variable and cause and effect were not differentiated, excluding the variable was investigated.

195　Two three-level binary logistic models were used to explore the factors associated with AM

196　and IMM in lactating ewes. These models took the form:

197　$\text{Logit}(\pi_{ijk}) = \beta_0 + \beta x_k + \beta x_{jk} + \beta x_{ijk} + v_k + u_{jk}$

198　where $\text{Logit}(\pi_{ijk})$ is the log odds of the probability that IMM or AM is present; β_0 is the

199　constant, βx is a series of vectors of fixed effects that vary at k (farm), j (ewe) and i

200　(observation), with residual variance estimates at farm (v_k) and ewe (u_{jk}). Level 1 variance

201　followed a binomial error distribution.

202　One two-level binary logistic model was used to explore factors associated with IMM in

203　pregnant ewes in year 2. This model took the form:

204　$\text{Logit}(\pi_{ij}) = \beta_0 + \beta x_j + \beta x_{ij} + u_j$

205　where $\text{Logit}(\pi_{ij})$ is the log odds of the probability that IMM are present, β_0 is the constant, βx

206　is a series of vectors of fixed effects that vary at j (farm) and i (ewe), with residual variance

207　estimates at u_j and level 1 variance followed a binomial error distribution.

208　A three-level continuous outcome model was used to explore the factors associated with lamb

209　DLWG. This model took the form:

210　$y_{ijk} = \beta_0 + \beta x_k + \beta x_{jk} + \beta x_{ijk} + v_k + u_{jk} + e_{ijk}$

211 where y is the continuous outcome variable DLWG, β_0 is the intercept, and βx is a series of

212 vectors of fixed effects that vary at k (farm), j (ewe) and i (lamb), with residual variance

213 estimates at v_k, u_{jk} and at level 1 e_{ijk} with a mean of zero and standard deviation of 1.

214 All models were run in MLwiN version 2.31 (Rasbash et al., 2014) with iterative generalised

215 squares for sample estimation. Forward manual stepwise model building was used to identify

216 the variables that had a significant association ($P < 0.05$) with the outcome variable.

217 Variables were considered significant when the 95% confidence intervals did not include

218 unity for binomial models, 0 for continuous outcome models (Wald's test). All non-

219 significant variables were retested in the final model to investigate residual confounding (Cox

220 and Wermuth, 1996). Where two variables were highly correlated the most biologically

221 plausible variable was retained in the model. The models were also run with farm as a fixed

222 effect as well as a random term. The model fits were tested by examining plots of the

223 residuals (continuous outcome models) and by the Hosmer - Lemeshow test (binary logistic

224 models).

225

226 **3. Results**

227 *3.1. Summary statistics*

228 Data from 10 farms were included in the final dataset. Six farms participated in both years of

229 the study, three farms participated for year 1 only (one farm provided management data for

230 year 2) and one farm participated for year 2 only. The farms were located throughout Great

231 Britain in Cheshire, Devon, Gloucestershire, Gwynedd, Herefordshire, Northumberland,

232 Perth and Kinross, Powys, Shropshire and West Sussex. They included both pedigree and

233 commercial flocks and indoor and outdoor lambing flocks (Table 1).

234 Data were collected on 3650 ewes in year 1 and 3371 in year 2, giving a total of 7021

235 examinations of 4721 ewes over the two years. A total of 1604 ewes were present over the

236 two years of the study, and 1307 of those were examined at all four visits. Summary statistics

237 are presented in Tables S1 and S2. The inter-rater reliability study showed good agreement

238 between the two researchers on all measures (data not shown).

239 Acute mastitis affected 2 - 3% of all ewes per year, flock range 0% to 37.1% (Table 2).

240 Approximately 5% of pregnant ewes and 11% of lactating ewes had at least one IMM over

241 the course of the study; flock range from 1.4 – 41.2% (Table 2). There were 1294 ewes

242 examined for IMM at all 4 examinations. Ewes with an IMM at an examination were at

243 increased risk of an IMM in future examinations although IMM were not consistently

244 detected at subsequent examinations (Table 3).

245 Over 75% of ewes were fed adequate energy and protein during pregnancy in year 1 (Table

246 S3). This did vary by flock: on farm E ewes bearing ≥ 2 lambs were underfed energy and all

247 ewes were underfed protein; on farm B, single bearing ewes were underfed while twin and

248 triplet bearing ewes were overfed; and on farm G single bearing ewes were overfed energy.

249 During lactation in year 1, only 35% of ewes were fed adequate energy and 53% of ewes

250 were fed adequate protein. Ewes rearing ≥ 2 lambs were underfed energy on all farms.

251 Generally ewes rearing ≥ 2 lambs were also underfed protein.

252 In year 2, most ewes were fed adequate energy and protein during pregnancy, except on farm

253 B, where all ewes were overfed both. There was, again, more underfeeding in lactation but

254 slightly less than in year 1. In both years, dietary energy and protein tended to be correlated,

255 especially during pregnancy, with ewes either receiving adequate amounts of both or neither.

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257   *3.2. Multivariable analyses*

258   *3.2.1. Factors associated with acute mastitis in lactating ewes*

259   Data from 3847 examinations (3019 ewes) were included in the model investigating AM

260   (Table 4). Key results were that underfeeding protein in pregnancy was associated with an

261   increased risk of AM. In addition, older ewes, those rearing ≥ 2 lambs, terminal sire

262   producing pedigree ewes, teat angle and position and non-traumatic teat lesions had

263   significant associations with AM. IMM during lactation the previous year and IMM when

264   pregnant were moderately associated with a higher risk of AM although they were not

265   significant at $P < 0.05$. With farm added as a fixed effect, Farms C, D, E and G had a

266   significantly higher risk of AM than Farm A and age at lambing > 7, non-traumatic teat

267   lesions and underfeeding protein in pregnancy were no longer significant (data not shown),

268   indicating flock level differences in nutrition, teat health and age of ewes.

269   *3.2.2. Factors associated with intramammary masses in lactating ewes*

270   Data from 3735 examinations (2916 ewes) were included in the model investigating IMM in

271   lactating ewes in years 1 and 2 (Table 5). There was a greater than 12-fold odds of IMM in

272   lactation when a ewe had AM and greater than 4-fold odds if the ewe had had previous IMMs

273   in pregnancy or lactation. In addition, a higher flock percentage of IMM in pregnancy was

274   associated with a significant increased risk of IMM in lactation. All these risk factors

275   highlight the strong role of prior infection in the ewe and flock for current IMM.

276   Underfeeding energy in lactation was a significant risk for IMM (> 6-fold odds), again

277   highlighting the role of poor nutrition in mastitis in these suckler ewes. Teat lesions and

278   udder conformation was also associated with IMM.

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281   *3.2.3. Factors associated with intramammary masses in pregnant ewes*

282   Data from 1427 ewes were included in the model investigating intramammary masses in

283   pregnant ewes in year 2 (Table 6). As with IMM in lactation, previous infection and poor

284   nutrition were the key risks: ewes with IMM at previous examinations in year 1 were

285   significantly more likely to have IMM when pregnant in year 2 than those without IMM at

286   previous examinations and underfeeding energy in lactation in year 1 was associated with an

287   increased risk of IMM while underfeeding protein in lactation in year 1 was associated with a

288   decreased risk of IMM when pregnant in year 2. No other variables were significantly

289   associated with an IMM in pregnancy in year 2.

290   *3.2.4. Factors associated with lamb daily live weight gain*

291   Data from 6453 lambs were included in the model investigating lamb DLWG (Table 7). Key

292   results were that lambs reared by ewes that had AM, an IMM, a traumatic teat lesion or a

293   non-traumatic teat lesion, had lower DLWG than lambs reared by ewes without these issues.

294   *3.2.5. Factors associated with udder conformation and teat lesions in lactating ewes*

295   Results from the mixed effect multivariable models of the udder conformation variables,

296   traumatic and non-traumatic teat lesions are included in the supplementary material (Tables

297   S4 – S11). Key results were that generally across the models, udder and teat conformation

298   were associated with increasing age (i.e. increasing parities) and the mastitis disease

299   variables, suggesting that conformation is poorer with increasing age and disease. Latent

300   class analysis indicated that there were four classes of ewe; young ewes with good teat and

301   udder conformation; young-middle aged ewes with poorer conformation, young-middle aged

302   ewes with good conformation and older ewes with poorer conformation (data not shown).

303   IMM when lactating and rearing $\geq$ 2 lambs were associated with an increased risk for both

304   types of teat lesions.

305   The model fits were good (Figures S2.1 – S2.14).

306   **4. Discussion**

307   This is the first prospective, longitudinal study of suckler ewes on the risks and

308   interrelationships between AM, IMM, udder and teat conformation and lamb growth rate.

309   The associations between AM, IMM and udder conformation are complex with, for example,

310   dependent variables in one model (e.g. AM) being explanatory variables in another. The aim

311   of investigating each aspect of udder health and conformation in multivariable models was to

312   elucidate associations and develop hypotheses for development of AM and IMM. Udder

313   conformation was investigated because in previous papers (Casu et al., 2010; Huntley et al.,

314   2012) some udder and teat conformations have been linked to intramammary infections.

315   One key result was that AM was strongly associated with IMM in lactation. This 12-fold

316   odds is indicative of causality with IMM a result of an episode of AM. Not all IMM were

317   associated with AM, this could indicate that farmers are not observing all cases of AM

318   because some are mild and others are missed, as suggested in dairy ewes (Lafi et al., 1998)

319   and as observed by researchers in the current study when AM had not been recorded by

320   farmers in some instances. The risk of IMM in lactation was also associated with previous

321   IMM and flock percentage of IMM. This suggests that IMM can be a source of infection to

322   other ewes in the flock, as is commonly thought and given as a reason for culling ewes with

323   IMM (Gelasakis et al., 2015).

324   There was a tendency for IMM in pregnancy the same year or lactation the previous year to

325   be associated with a higher risk of AM in the subsequent lactation. However, ewes were

326   culled, sold or died throughout the study for many reasons, including some ewes with IMM

327   removed from some flocks between years 1 and 2, and this might have weakened the

328   association detected between IMM and subsequent IMM and AM. We can hypothesise that

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329   IMM are a result of an acute disease event and are themselves chronic, persistent infection

330   that may increase a ewe's risk of subsequent AM.

331   The percentage of IMM was very high in the smaller flocks in the current study. These were

332   pedigree flocks where the farmers were less likely to cull ewes with IMM and so the flock

333   prevalence of IMM would have increased each year. Consequently a high percentage of IMM

334   could be due to spread of disease being more rapid in these flocks where affected and

335   unaffected ewes and lambs were kept together, or because of management decisions to retain

336   affected ewes.

337   In the current study, IMM were not detected at all subsequent examinations once first

338   detected, however, ewes with previously detected IMM were approximately 3 - 5 times more

339   likely to have IMM at a later date (Tables 5 & 6). IMM in suckler ewes are typically

340   abscesses (Smith et al., 2015). Abscesses are thought to be polymicrobial (Brook, 2002); they

341   develop and rupture as part of their maturation cycle. Rupture facilitates the spread of

342   bacteria which can subsequently reform abscesses elsewhere within their environment

343   (Cheng et al., 2011). This cycle of growth and rupture might explain why IMM were present

344   at one examination and not at a second but then present again at the third or fourth

345   examination.

346   Another key finding of this study was that dietary levels of energy and protein in pregnancy

347   and lactation impacted significantly on AM and IMM. One Flock (E) underfed protein to all

348   ewes in pregnancy in year 1 and many flocks underfed energy and protein in lactation by

349   current guidelines. Whilst underfeeding protein in pregnancy was not common on study

350   farms, it warrants discussion because of the risk should this occur on any farm; insufficient

351   protein in pregnancy will lead to reduced mammary development and inadequate milk supply

352   throughout lactation (Fthenakis et al., 2012). Underfeeding energy in pregnancy and

353   lactation was also a risk for IMM and the latter was common on our study farms (Table S3),

354   but underfeeding protein was apparently protective. One recent study reported that if energy

355   in the diet is adequate increasing protein beyond requirements has no benefit for lamb growth

356   rate (Van Emon et al., 2014). Another study (Barbagianni et al., 2015) reported that

357   experimental ewes fed insufficient energy during pregnancy (to bring on pregnancy

358   toxaemia) had more mastitis after lambing, these authors suggested that this was due to an

359   impaired immune response caused by the increased concentrations of β-hydroxybutyrate, but

360   it could have been a direct effect of low energy in the diet. It is possible that current

361   guidelines on the absolute amount of energy and protein required, or the ratio between the

362   two, are wrong.

363   AM might occur from poor diet because an inadequate milk supply leads to hungry lambs

364   that traumatise the teats and udder (Cooper et al., 2013). Although we did not find an

365   association between traumatic teat lesions and AM (in univariable or multivariable analysis)

366   this was probably because we examined ewes at approximately 9 weeks after lambing and so

367   lesions would have healed: Cooper et al. (2013) found that the incidence of traumatic teat

368   lesions was greatest 3 - 4 weeks after lambing and healed within two weeks.

369   We studied a small number of convenience-selected farms, but if inadequate diets are

370   common on GB sheep farms, nutrition could be a large attributable risk to udder health and

371   farmers could improve udder health considerably using current nutritional guidelines

372   (Agricultural and Food Research Council, 1993). We did not detect associations between ewe

373   body condition and AM or IMM as in other studies (Arsenault et al., 2008; Huntley et al.,

374   2012; Onnasch et al., 2002). This may be because we measured BCS at two time points, once

375   in pregnancy and once lactation, when most ewes had adequate body condition. Huntley et al.

376 (2012) reported an association between low BC and high SCC when BCS was measured

377 weekly.

378 The majority of ewes in our study had udders with good conformation (teat angle 5, teat

379 position 3, udder drop 7; Figure S1) to suckle lambs (Huntley et al., 2012). Udder

380 conformation that differed from this optimum was associated with a small but significant

381 increased risk of IMM and AM (Tables 4 & 5), as reported previously (Casu et al., 2010;

382 Huntley et al., 2012). This association is likely to be causal in some instances, with increased

383 exposure of the teat end to contamination or poor teat positioning making suckling difficult

384 for lambs and so causing teat lesions. Udder conformation also changed in some ewes with

385 IMM and after a severe case of AM (CG personal observation). These results, and the latent

386 class analysis, suggest udder conformation is generally good but that age and udder disease

387 can impact on udder and teat conformation. Breeders should be aware of avoiding selecting

388 away from good udder conformation in any selection programme that inadvertently selects

389 for altered phenotype. That said, in general, not selecting replacement ewes from ewes with

390 poor udder conformation would be sensible.

391 Several other factors were associated with AM or IMM, including increasing ewe age and

392 larger litter size. These risks are important and have been reported previously (Arsenault et

393 al., 2008; Larsgard and Vaabenoe, 1993; Pereira et al., 2014; Waage and Vatn, 2008). We

394 conclude that age needs to be managed through planned culling of older ewes. Where ewes

395 are not able to feed their lambs adequately (due to age or large litter sizes / weights) then

396 supplementing lambs' feed would reduce the risk of over-demand for milk from ewes.

397 Importantly for farmers, the presence of AM, IMM in lactation, traumatic teat lesions and

398 non-traumatic teat lesions all impacted negatively on lamb daily live weight gain (Table 7).

399 This is in line with previous findings (Arsenault et al., 2008; Huntley et al., 2012) and could

400   be due to decreased milk production in the diseased gland or to ewes preventing lambs from

401   suckling. Whatever the reason, this association adds to the economic cost of AM and IMM.

402   The study design was selected so that longitudinal data could be collected. This could only be

403   done on a small number of farms and so the intention was not to identify representative farms

404   but rather strengths of associations between factors to improve our understanding of the

405   biology of AM, IMM and teat and udder conformation. Because of the need to visit farms on

406   four occasions and to have extra data on AM, lamb weights and litter sizes between visits, we

407   convenience-selected farms that were already collecting such data and where farmers were

408   compliant with the study demands. Overall this was successful, although we aimed to follow

409   4000 ewes over 2 years but due to one large commercial farm dropping out of the study in

410   year 2 and a high rate of attrition among the ewes, surprisingly only 1307 ewes were seen at

411   all 4 examinations. Therefore we may not have had sufficient power to detect all risks,

412   particularly when ewes were culled or sold at the end of year 1 due to AM and IMM.

413   Another potential weakness of this study is that 7 of the 10 farms were pedigree sheep

414   breeding farms where management practices are different from large commercial farms, e.g.

415   pedigree sheep breeders may be less likely to cull ewes for IMM as ewes are more valuable

416   and replacement costs are higher, however, these flocks were selected because we could

417   study the impact of retaining ewes with IMM.

418   Despite these weaknesses the results from this study are likely to be qualitatively

419   generalisable to other flocks. Due consideration of diet, management of older ewes, ewes

420   with large litters and ewes with IMM is necessary to reduce the risk of AM and IMM and to

421   maintain growth rates of lambs.

422

423   **5. Conclusions**

424   We conclude that this study of 10 flocks indicates that IMM, diet and udder conformation all

425   contribute to cases of acute and chronic mastitis in suckler ewes. The relationships are

426   complex, but the pattern of events appears to be that ewes fed inadequate protein in

427   pregnancy and inadequate energy in pregnancy and lactation are at greater risk of AM and

428   IMM. AM leads to development of IMM, IMM persist and may cause more IMM and AM

429   within a flock. We conclude that feeding appropriate levels of energy and protein both in

430   pregnancy and lactation and managing IMM would increase udder health and consequently

431   increase flock productivity and profitability.

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433   **Conflict of interest**

434   The authors have no conflicts of interest to declare.

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513   Table 1. Summary of the ten study farms.

Farm	Main Breed	Lambing		No. ewes Yr. 1	No. ewes Yr. 2	No. ewes present Yr. 1 & 2
		Month	Indoor / Outdoor			
A	Lleyn	Apr/May	O*	321	322	225
B	Charollais	Dec	I	145	155	75
C	Charollais	Dec/Jan	I	60	56	37
D	Charollais	Dec	I	74	93	44
E	Texel	Feb/Mar	I	116	89	72
F	Lleyn	Apr/May	O*	1522	1509	1151
G	Texel	Mar/Apr	I	165	**NV**	*NA*
H	Texel	Feb	I	87	**NV**	*NA*
I	Crossbreeds / Lleyn	Mar/Apr	I	1160	**NV** (1113)†	(689)
J	Texel	Feb/Mar	I	**NV**	34	*NA*
Total number of ewes				3650	3371	2293 (1307 **PFE**)

514   I: Indoor lambing; O*: Outdoor lambing /small number lambed indoors; NV: Not visited; NA: Data not
515   available for this farm; PFE: Present at all four exams; †: Not visited year 2 but provided data.

516

517 Table 2. Number and percentage of acute mastitis and intramammary masses in lactation and
518 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**	

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

520

521

522 Table 3. Number and percentage of intramammary masses (IMM) in pregnancy and lactation

523 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
			Yes: 1 (100%)

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526

527 Table 4. Three-level binary logistic model of factors associated with acute mastitis in 3847
528 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					

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

532

533

534 Table 5. Three-level binary logistic model of factors associated with intramammary masses in
535 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		

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

539

540

541

542   Table 6. Two-level binary logistic model of factors associated with intramammary masses in

543   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					

544   *N*: Number; OR: Odds ratio; CI: Confidence interval; IMM: Intramammary mass; SD: Standard deviation;

545   Reference: baseline category for comparison.

546   Where categories are in **bold** they are statistically different from the reference category at $P < 0.05$.

547

548

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**

		N			
Lamb BW (kg)		8667	**-0.003**	**-0.005**	**-0.001**
Lamb age when when weighed (days)	^ 1	8681	**0.032**	**0.01**	**0.05**
	^ 2		**-0.0009**	**-0.0015**	**-0.0004**
	^ 3		**0.00001**	**0.000006**	**0.000018**
	^ 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		

551 *N:* Number; CI: Confidence interval; BW: Birth weight; BCS: Body condition score; SD: Standard deviation;

552 Reference: baseline category for comparison. (Lamb age when weighed (days) was entered as a quadratic term).

553 Where categories are in **bold** they are statistically different from the reference category at $P < 0.05$.

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