## **1** Interpretive Summary

2 The British national cattle register was used to analyse 21.2 million births and 21.6 million deaths

3 registered between 2011-2018. A significant proportion of on-farm mortality occurred before 3

4 months of age, and both dairy and male calves had higher mortality rates than beef and female calves

5 respectively. Month of birth and environmental temperature had a strong influence on mortality rate,

6 and it appears that providing optimal environmental conditions would greatly reduce mortality rate.

7 National cattle registers have great potential in monitoring mortality rates and further research is

8 needed to explore environmental factors likely to reduce calf mortality rates.

9	Calf mortality rates in Great Britain
10	Quantitative analysis of calf mortality in Great Britain
11	Robert M. Hyde <sup>*1</sup> , Martin J. Green <sup>*</sup> , Virginia E. Sherwin <sup>*</sup> , Chris Hudson <sup>*</sup> , Jenny Gibbons <sup>†</sup> ,
12	Tom Forshaw†, Mary Vickers†, Peter M. Down*
13	
14	*School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus,
15	Leicestershire, United Kingdom LE12 5RD
16	<sup>†</sup> Agriculture and Horticulture Development Board, Stoneleigh, Kenilworth, United Kingdom, CV8
17	2TL
18	
19	<sup>1</sup> Corresponding author: Robert M. Hyde
20	Email: Robert.hyde1@nottingham.ac.uk

## 21 ABSTRACT

22 National bodies in Great Britain (GB) have expressed concern over young stock health and welfare and identified calf survival as a priority, however no national data have been available to quantify 23 24 mortality rates. The aim of this study was to quantify the temporal incidence rate, distributional features and factors affecting variation in mortality rates in calves in GB since 2011. The purpose was 25 to provide information to national stakeholder groups to inform resource allocation both for 26 knowledge exchange and future research. Cattle birth and death registrations from the national British 27 Cattle Movement Service were analysed to determine rates of both slaughter and on-farm mortality. 28 The number of births and deaths registered between 2011-2018 within GB were 21.2 and 21.6 million 29 respectively. Of the 3.3 million on-farm deaths, 1.8 million occurred before 24 months of age (54%), 30 31 and 818,845 (25%) happened within the first 3 months of age. The on-farm mortality rate was 3.87% by 3 months of age, has remained relatively stable over time, and is higher for male calves (4.32%)32 33 than female calves (3.45%). Dairy calves experience higher on farm mortality rates than non-dairy 34 (beef) calves in the first 3 months of life, with 6.00% and 2.86% mortality rates respectively. The 0-3 35 month death rate at slaughterhouse for male dairy calves has increased from 17.40% in 2011 to 36 26.16% in 2018, and has remained low (<0.5%) for both female dairy calves, and beef calves of both 37 sexes. Multivariate adaptive regression spline (MARS) models were able to explain a large degree of the variation in mortality rates ( $R^2 = 96\%$ ). Mean monthly environmental temperature and month of 38 39 birth appeared to play an important role in neonatal on-farm mortality rates, with increased 40 temperatures significantly reducing mortality rates. Taking the optimal month of birth and 41 environmental temperature as indicators of the best possible environmental conditions, maintaining 42 these conditions throughout the year would be expected to result in a reduction in annual 0-3 month mortality of 37,571 deaths per year, with an estimated economic saving of around £11.6 million per 43 annum. National cattle registers have great potential for monitoring trends in calf mortality and can 44 provide valuable insights to the cattle industry. Environmental conditions play a significant role in 45 46 calf mortality rates and further research is needed to explore how to optimize conditions to reduce calf 47 mortality rates in GB.

# 48 Key words:

49 Calf mortality; monitoring; national data

## 50 INTRODUCTION

Neonatal mortality (defined as 1d of age – weaning, Compton et al., 2017) represents a significant 51 52 loss to the British cattle industry. Calf management is critical in rearing productive dairy cows 53 (Hultgren and Svensson, 2009), and represents a significant economic outlay, accounting for around 54 20% of total dairy farm expenditure (Gabler et al., 2000) with costs in Great Britain estimated at around £1819 per animal (Boulton et al., 2017). A study following 1097 calves from 19 farms in 55 England suggest 15% of liveborn dairy heifers die before reaching their first lactation (Brickell et al., 56 57 2009), with the cost of heifer mortality representing around £139 per animal when spread across surviving animals (Boulton et al., 2017). Effective calf management is also crucial for efficient beef 58 production (Mõtus et al., 2017), a significant industry for the UK, being the third largest producer of 59 60 beef in Europe (DEFRA, 2018).

Neonatal mortality not only represents an economic loss, but also delays genetic progress by
providing fewer replacements for voluntary culling (Raboisson *et al.*, 2013). Mortality has also been
explored as a marker for farm welfare surveillance, and has been suggested as an indicator of overall
health on cattle farms (Ortiz-Pelaez *et al.*, 2008; von Keyserlingk *et al.*, 2009). The effective
management of neonatal calves is essential for survival, welfare and productivity (Renaud *et al.*,
2018), and whilst mortality in calves is unlikely to be entirely eradicated, it should be a goal to reduce
it by as much as possible (Santman-Berends *et al.*, 2014).

In order to prevent disease and reduce mortality, it is essential to understand the incidence, 68 69 prevalence, distribution and key factors that influence disease variability; this is the basis of 70 epidemiology and is recognized as a first step in disease control (Dohoo, Martin and Stryhn, 2009). 71 National bodies in Great Britain (GB) have expressed concern over youngstock health and welfare, identifying calf survival as a priority (CHAWG, 2017, 2018b). However, there are currently no 72 national data being published and therefore the extent of the problem remains largely unknown. A 73 clear understanding of patterns of calf mortality on a national scale would inform stakeholders of 74 whether and where to allocate resources both for knowledge exchange and further research. 75

Furthermore, quantification of risk between groups (for example, beef or dairy calves, male or female
calves and specific times of year) would allow targeting of resources towards high risk populations
and time periods.

79 The use of national level data for epidemiological studies has been called for to help develop methods 80 of reducing morbidity and mortality (Santman-Berends et al., 2014; Veldhuis et al., 2016) but rates of 81 calf mortality in GB have not been evaluated nationally since 2007 (Gates, 2013). Whilst keepers of 82 bovine animals in GB must register births, deaths and movements of their animals through the Cattle 83 Tracing System, the data are not used to routinely report national incidence rates of calf mortality. 84 The aim of this study was to quantify the temporal incidence rate, distributional features and factors affecting variation in mortality rates in calves in Great Britain since 2011. The purpose was to provide 85 information to national stakeholder groups to inform resource allocation for both for knowledge 86 87 exchange and future research.

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## 90 MATERIALS AND METHODS

91 Birth and death data from 2010-2019 were requested from British Cattle Movement Services

92 (BCMS). Data were only available at county level, and included the number of births for each county, 93 country, breed, sex, month and year as an aggregated figure. Death data were provided in a similar 94 format, however excluded county level information, and contained the number of deaths for each age 95 at death (months), premises of death (i.e. "on farm" or "slaughterhouse"), country, sex, breed, month 96 and year. "On farm" deaths included animals that died on farm, and "slaughterhouse" deaths included 97 any animals that were transported to a slaughterhouse for slaughter. These datasets were combined to 98 allow calculation of estimated mortality rates for defined groups.

99 Births and deaths (of any age) were filtered to be after 2010 and before 2019. The premises of death comprised five categories; slaughterhouse, farm, market, hunt/knackers (colloquial terms for fallen 100 101 stock removal enterprises) and other. The vast majority of deaths (99.97%) were reported on-farm or at a slaughterhouse. Given that less than 0.03% of deaths were categorized as market, hunt/knackers 102 or other it was decided to exclude these from the dataset. Breeds were categorized as either "Dairy" or 103 "Non-dairy" (i.e. beef, including beef cross dairy) according to BCMS categories. A small proportion 104 105 (<0.05%) of animals were non-cattle (i.e. Bison, Yak and Water buffalo) and were removed from the dataset. 106

107 Cumulative mortality rates were calculated as a percentage of animals born in a specific month 108 subsequently dying within a defined time period. For example, of 100 calves born in January, two 109 calves dying in January at 0-1 months of age, two dying in February at 1-2 months of age, and one 110 dying in March at 2-3 months of age would result in a 0-3 month mortality rate of 5%. Country and 111 county were excluded from analysis to avoid the potentially erroneous assumption of zero cross 112 border transport between birth and death (e.g. the assumption that calves that die in Scotland were 113 also born in Scotland).

British cattle must be dual tagged within 20d of birth, and all births and deaths must legally beregistered through BCMS. There is a requirement that deaths that occur prior to tagging also be

recorded within the holding register, and inspectors visit a proportion of UK farms (at least 3% of
holdings annually) to validate identification and record keeping protocols (UK Government, 2014).
Whilst it is unlikely that many stillborn (0-24hour mortality) calves will be included within registered
deaths, there remains uncertainty as to the current stillborn rate in GB.

120 Descriptive and statistical analyses were conducted in R (R Core Team, 2017). On farm mortality was treated separately to slaughterhouse deaths, and descriptive graphical analysis was conducted by 121 breed, sex, age and month. Linear regression methods were employed to provide insight into 122 potentially influential factors associated with calf mortality at 0-3 months of age. These factors 123 124 included the breed, sex, month of birth and Met Office meteorological data (mean, minimum and maximum monthly temperatures across the UK). To explore interactions between variables and non-125 linearities within the data, multivariate adaptive regression spline models (Friedman, 1991) were 126 employed using the earth (Milborrow, 2019) and caret (Kuhn. et al., 2018) R packages. Interactions 127 128 up to order 3 were tested and the maximum number of terms available to the model was explored and 129 optimized using a dense grid of values between 2 and 25 in increments of 1. Ten -fold crossvalidation repeated 10 times was used to identify the optimal value of these tuning parameters; 130 131 optimization was based on minimizing model mean absolute error (MAE). Final model covariates 132 were therefore selected based on minimizing the cross-validation model MAE; covariate selection is an integral part of the MARS procedure (Friedman, 1991). An evaluation of model fit and the extent 133 of over-fitting was assessed by a comparison of MAE and R<sup>2</sup> computed from the final model based on 134 135 the full dataset ('internal fit') and computed from 10-fold cross validation ('cv-fit'). Residuals were 136 examined to ensure model fit, by examining fitted values against residuals to ensure the model was 137 not over-, or under-predicting mortality rates, particularly at the extremities of fitted values.

To estimate the effect of optimizing environmental conditions on calf mortality, covariates in the final
model were used to predict how mortality might change with different environmental temperatures or
different months of birth.

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#### RESULTS 143

144 The cattle population in the UK is around 9.6 million (UK Government, 2018). The number of births and deaths registered between 2011-2018 across GB were 21.2 and 21.6 million respectively, of 145 146 which 18.3 million and 3.3 million deaths were at slaughterhouse and farm respectively. Of the total 147 on-farm deaths 1.8 million occurred before 24 months of age (54%, Figure 1), and 819,703 (25%) happened within the first 3 months of age. Of the 818,845 dairy cattle that died on farm before 24 148 149 months of age, 409,612 (50%) died within the first 3 months of age, and of the 956,293 non-dairy 150 cattle that died on farm before 24 months of age, 410,091 (43%) died within 3 months of age. Of the 151 18.3 million deaths that were recorded at slaughterhouses, 644,848 (3.5%) were before 3 months of 152 age. The 0-3 month death rate at slaughterhouses (and excluding any on farm mortality) was 0.49%, 153 0.21% and 0.34% for dairy females, non-dairy females and non-dairy males respectively, and was 154 19.94% for dairy males.

155 The on-farm (excluding any slaughterhouse deaths) mortality rate was 3.87% by 3 months of age, and 156 was higher for male than female calves, with male calves experiencing a mortality rate of 4.32%, 157 compared with 3.45% for female calves. Dairy calves experienced higher mortality rates than nondairy calves within 3 months of age, with 6.00% and 2.86% mortality rates respectively. Male dairy 158 159 calves had the highest on farm mortality rate within 3 months of 7.37% compared with a mortality 160 rate of 4.96% for female dairy calves. Female non-dairy calves had the lowest mortality rate within 3 161 months of 2.61% compared with a mortality rate of 3.10% for male non-dairy calves.

On-farm mortality rates remained relatively stable over time (Figure 2). Male dairy calves appeared to 162 be the only category of animal being routinely sent for slaughter at 0-3 months of age, and this

appeared to show an upward trend over time, from 17.40% in 2011 to 26.16% in 2018 (Figure 3). 164

165 There appeared to be a strong seasonal component to 0-3 month on farm mortality rates across both

breed types and sexes (Figure 4), with dairy calves having a lower 0-3 month mortality rate during 166

167 summer, with a rate of 6.61%, 6.11%, 4.79% and 6.74% for dairy calves born during winter, spring, summer and autumn respectively. Similarly, non-dairy calves had a lower mortality rate when born
during summer, and also had a lower rate during spring, with the highest rate being autumn (winter:
2.88%, spring; 2.56%, summer: 2.56% and autumn: 3.98%).

171 Results of the final MARS model are provided in Table 1. Both sex and breed type were associated 172 with differences in mortality rate, with an increased rate for male calves, and decreased rate for beef 173 calves respectively. Month of birth had an effect on mortality rate, and the mortality rate in the first 3 months of life increased for calves born in December by +1.1%. Whilst mortality rate was reduced for 174 calves born during February (-1.4%), there was an interaction between breed type and month of birth 175 176 (Feb\*Non-dairy) of +1.0%, resulting in a predicted mortality rate change of -0.4% for Non-dairy calves born in February (as opposed to dairy calves born in February which had a predicted rate of -177 1.4%). Similarly, dairy calves born in November had a mortality rate change of +0.3%, compared to 178 +0.9% for Non-dairy calves (the interaction effect of Nov\*Non-dairy = +0.6%). There were 179 180 interactions between month and breed type for February, March, October and November, and interactions between month and sex for January and August indicating that these subsets of animals at 181 182 these specific times had different predicted risks of mortality.

183 MARS models identified a hinge point within the mean monthly temperature variable at 4.8°C, and at 184 9.6°C where an interaction effect with Non-dairy (beef) calves was included. This indicated that reduced mean monthly temperatures were associated with increased calf mortality regardless of 185 186 month, and this association was slightly stronger below 4.8°C. An interaction term for non-dairy breed 187 type and temperature was present; Non-dairy\*h(Mean temperature -9.6). This interaction term coefficient indicated a change in mortality rate for non-dairy calves (as denoted by non-dairy\*h) for 188 each 1°C above 9.6°C (as denoted by (mean temperature -9.6)) of -0.2%, effectively neutralizing the 189 190 0.2% decrease in mortality above 4.8% suggested by the term h(4.8 – Mean temperature). In short, 191 this indicated that the mortality of non-dairy calves increased by 0.2% for every 1°C decrease in 192 temperature, but that this effect was limited to below 9.6°C only, and there was minimal effect of 193 temperature on the mortality rate of non-dairy calves above this point.

Analysis of model fit showed an R<sup>2</sup> value of 95.77%, RMSE of 0.44 and mean absolute error (MAE) 194 of 0.34 when using 10-fold cross validation, and an R<sup>2</sup> value of 96.24%, RMSE of 0.40 and (MAE) of 195 0.31 when using internal fit, indicating there were no signs of model overfitting. Optimal model 196 197 parameters were a degree of 2 interactions, and the number of terms for inclusion (nprune) set at 16. 198 To examine the effect of environmental conditions on calf mortality the final model was used to 199 predict mortality given optimal month and environmental temperature. Calves born in February were 200 shown to have the lowest mortality rate independent of temperature, and increased mean monthly 201 environmental temperature was shown to decrease mortality rates at 0-3months of age independent of 202 month (the maximum monthly mean temperature recorded was 17.3°C). 203 Predictions of mortality were made by assuming all calves were born in February and with the environmental temperature set constantly at 17.3°C. This resulted in an estimated total reduction of 204

300,570 deaths at 0-3 months of age over the period 2011-2018, equating to a mean reduction of
37,571 fewer deaths per year.

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Variable	Coefficient
Intercept	6.0

Breed type (Non-Dairy)	-2.7
Sex (Male)	2.4
h(Mean temperature -4.8)	-0.2
h(4.8- Mean temperature)	0.2
Sex (Male) * Breed type (Non Dairy)	-1.9
Month (Mar) * Breed type (Non Dairy)	-1.2
Month (Feb)	-1.4
Breed type (Non Dairy) * h(Mean temperature-9.6)	0.2
Month (Dec)	1.1
Month (Nov)	0.3
Month (Feb) * Breed type (Non Dairy)	1.0
Month (Oct) * Breed type (Non Dairy)	0.7
Month (Jan) * Sex (Male)	0.6
Month (Nov) * Breed type (Non Dairy)	
Month (Aug) * Sex (Male)	

210 Table 1. Results from multivariate adaptive regression splines (MARS) model; "h" denotes hinge

211 point.

### 213 **DISCUSSION**

This research represents one of the largest calf mortality datasets ever reported and suggests minimal 214 change in GB calf mortality rates between 2011 and 2018. The results indicate that environmental 215 216 temperature, time of year, sex, and breed type are strong predictors of mortality rate, and account for around 96% of the total variation in on-farm mortality. Importantly, it appears that relatively low 217 218 mortality rates are achievable at specific times of year, in certain groups of animals and in relatively 219 warm temperatures. For example, in the lowest risk groups for both dairy and non-dairy breed types (female calves born in February) a mortality rate of <2% would be expected at temperatures of  $17.3^{\circ}C$ 220 (the maximum monthly temperature recorded). If the environmental conditions provided for these 221 222 groups of animals could be identified and replicated through improvements in housing management, a 223 reasonable national target for the British cattle industry could be to reduce overall 0-3 month calf 224 mortality to <2%. To achieve this, further research into and understanding of these specific 225 environmental conditions is required; these areas are discussed below.

The effect of environmental temperature and month of birth appear to play a significant role in 226 227 neonatal mortality rates. Reductions in mortality during summer has been previously reported in both 228 dairy (Lombard et al., 2007; Raboisson et al., 2013) and beef calves (Mõtus et al., 2017), and colder 229 temperatures have been suggested as an important factor in mortality rates, with temperatures of  $\geq 10$ 230 °C being associated with lower mortality (Pannwitz, 2015). Colder weather may also have a negative 231 effect on calf vigour, which may subsequently affect the transfer of passive immunity (Olson et al., 232 1980; Robison et al., 1988); an essential component in reducing morbidity and mortality (Godden, 233 2008; Cuttance et al., 2018). There may also be an effect of increased infection pressure during 234 housing through winter, which might subsequently increase the risk of morbidity and mortality 235 (Raboisson et al., 2013). The large effects of month and temperature suggest a substantial 236 environmental component to the risk of mortality; if calf housing was able to replicate optimal 237 environmental conditions all year round, major reductions in mortality could result. The potential 238 mean annual reduction in mortality of 37,571 fewer deaths identified in this study as attributable to sub-optimal environmental conditions, represents a potential economic saving of around £11.6 million 239

per year when using an estimate of £310 per calf death (Kossaibati and Esslemont, 1997) as well as
having obvious welfare implications. The impact of environmental conditions on calf mortality rates
in GB should certainly be the subject of future research.

243 Higher neonatal mortality rates in males compared with females has been previously reported 244 (Raboisson et al., 2013; Pannwitz, 2015; Cuttance et al., 2017), potentially related to the higher mortality associated with concomitant dystocia (Johanson et al., 2011), particularly for breeds with 245 heavier birth weights (Gundelach et al., 2009; Linden et al., 2009). Male dairy calves are also often 246 regarded as less economically valuable as heifer calves, and thus may not receive the same standard of 247 248 care (Renaud et al., 2017), potentially resulting in higher morbidity and mortality rates. Variations in neonatal mortality rates between breed types has previously been reported and is possibly due to 249 250 management of the breeds rather than the breed themselves (Raboisson et al., 2013). Whilst mortality risk might be hypothesised to be higher in beef crossed calves due to increased dystocia (Raboisson et 251 252 al., 2013), this study suggests that dairy animals are at increased risk of mortality. This has been previously reported in a study of 1.3 million Slovenian calves reporting a 2-30d mortality rate of 253 2.68%, and indicating calves from Holstein Friesian dams having higher mortality rates than calves 254 255 from other breeds, with herd size and calving season also being important factors influencing 256 mortality rate (Voljč et al., 2017). Results from the current study suggest there is an urgent need for 257 additional research to identify strategies to reduce mortality rates in male dairy calves in particular. The increasing trend of calves being sent for slaughter by 3 months of age has not been previously 258 reported in GB, and whilst it is not possible to identify reasons for this increase in this study, it is 259 260 likely that there are a range of social and economic factors involved. An increase in the number of 261 male dairy calves entering the beef chain rose by 59% from 2006 to 2015, and recent estimates indicate 81% of all male dairy calves in 2015 were reared for beef in GB (CHAWG, 2018a). The fate 262 263 of male dairy calves is an important issue for the industry (Renaud *et al.*, 2018), and the increase in 264 male dairy calves entering the beef chain may, in part, be due to this increased rate of early slaughter. 265 There is a slight reduction in on farm mortality of male dairy calves at 0-3 months of age, however 266 this may also be due to the increased rate of early slaughter of male dairy calves.

267 Previous on farm mortality estimates for GB calves are relatively sparse, with previous estimates from national CTS data suggesting an on-farm 0-6 month mortality rate of 2.47% for beef and 7.42% for 268 dairy calves born in 2007 (Gates, 2013), compared with 4.15% and 8.31% 0-6 month mortality rates 269 found in the current study for beef and dairy respectively. Research from 11 farms in the south-east of 270 271 England in 2011-2012 estimated 24hr-2mo dairy calf mortality at 4.5% (Johnson et al., 2017), similar to the current studies' finding of a 4.87% mortality rate between 0-2 months. Mortality rates have 272 273 been reported to be highly variable between herds (Brickell *et al.*, 2009), with a recent study reporting 274 a 2-56d mortality rate of 12.7% for one dairy herd (Mahendran et al., 2017). Mortality rates in GB do not appear to have altered dramatically over time, with historic mortality rates up to six months, 275 276 estimated at around 5.2% in 1952 (Withers, 1952), extremely similar to the current study reporting 277 5.5%.

Previous research suggests a significant portion of on farm mortality occurs within early life, with the majority of calves dying within the first month (Gates, 2013; Santman-Berends *et al.*, 2014), and around two thirds of cattle mortality being within the first 4 months (Struchen *et al.*, 2015). There are a number of factors that affect calf mortality rates up to 3 months of age (Windeyer *et al.*, 2014), which are largely beyond the scope of this article, however neonatal diarrhea and pneumonia are likely to play a significant role (Compton *et al.*, 2017).

Whilst calf mortality is a commonly reported metric in many countries, there are a wide variety of
metrics being reported, and recent research has evaluated and assessed 10 definitions for calf
mortality (Santman-Berends *et al.*, 2019). Age classes for mortality studies also differ considerably
between studies (Raboisson *et al.*, 2013), making comparisons challenging. A systematic review and
meta-analysis, however, found the rate of perinatal (defined as 0-2d of age) mortality ranged from 39%, and was increasing over time, with neonatal (defined as 1d-weaning) mortality ranging from 511%, which was not found to have changed over time (Compton *et al.*, 2017).

291 As previously reported, there are several limitations to using BCMS databases for research purposes

292 (Gates, 2013), particularly the potential exclusion of stillborn calves and early mortality prior to

tagging. Previous estimates suggest around 7.9% of dairy calves die before 24 hours of age (Brickell

294 et al., 2009), and therefore the current studies estimates of mortality are likely to exclude perinatal 295 mortality. Despite the legal requirement to register all deaths, including those before the registration 296 and tagging of calves, there is a small risk that not all deaths/euthanasias that occur prior to tagging 297 and registration are recorded. The absence of individual farm and calf level information available for 298 this study meant some assumptions had to be made in order to calculate mortality figures, particularly 299 that births and deaths were evenly distributed throughout months. Whilst this is important to 300 recognize, the effects of these potential errors are likely to be small due to the large scale of the data and are unlikely to have significant bearing on the interpretation of the data. Data quality of CTS data 301 has been reported to have improved over time (Green and Kao, 2007) and whilst it is unlikely to be 302 completely free of error (Gates, 2014), the large scale of data being collected means extremely useful 303 304 insights can be made.

## 306 CONCLUSIONS

- 307 The GB national cattle register provides an important resource in identifying neonatal mortality trends
- in the GB herd and will provide invaluable insights to the cattle industry if reported on a regular basis.
- 309 Environmental conditions appear to play a significant role in calf mortality rates, and further research
- 310 is needed to explore precise environmental factors likely to reduce calf mortality rates in GB.

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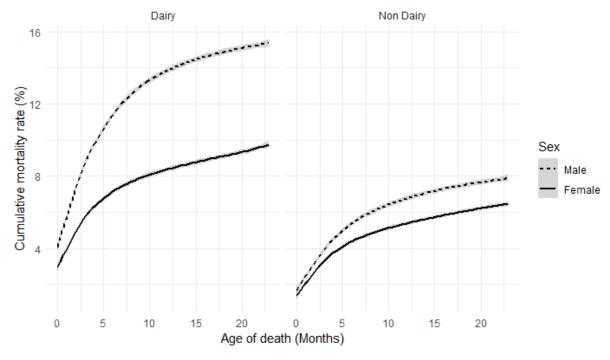
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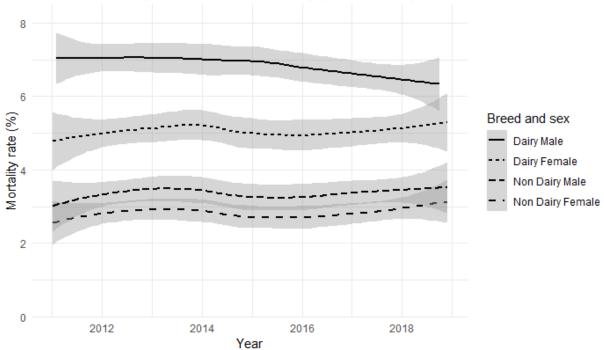
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Cumulative on farm mortality rate of calves by sex and breed type

437 Figure 1: Cumulative on-farm mortality rates by age, sex and breed type. 95% confidence interval as

<sup>438</sup> grey shading.



On farm mortality rate 0-3 months (%) by year, breed type and sex

441 Figure 2: On-farm mortality rate 0-3months of age (%) by year breed type and sex from 2011-2018.

442 95% confidence interval as grey shading.

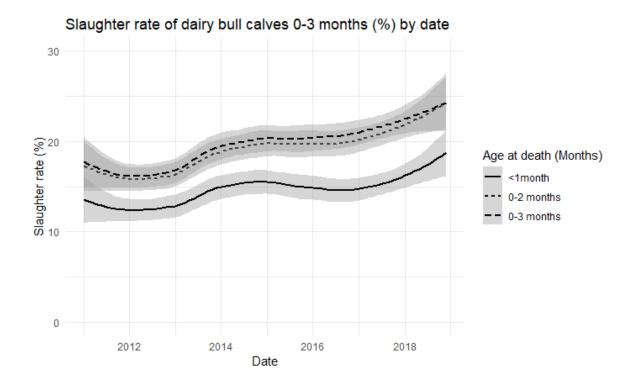
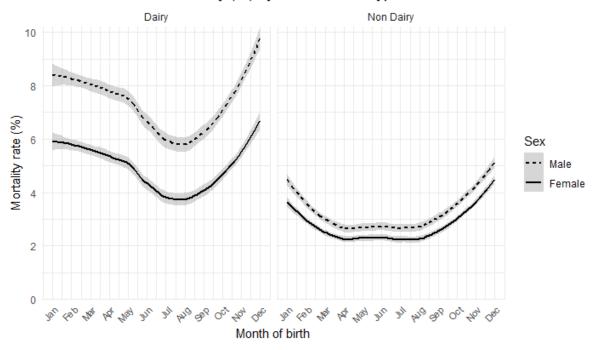


Figure 3: Slaughter rate over time within 1 month, 0-2 months and 0-3 months of age. 95%

<sup>446</sup> confidence interval as grey shading.



On farm 0-3month mortality (%) by month, breed type and sex

450 Figure 4: Seasonal patterns in on-farm 0-3 month mortality rates by breed type and sex. 95%

452

<sup>451</sup> confidence interval as grey shading.