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Abstract

We analyzed juvenile mortality rates per sire family for four different periods in a population of Holstein heifers born between 2017 and 2020 in France and Wallony. The global mortality rate from birth to one year of life was 11.8% in the population of 1,001 sires, while it reached 30.6% and 19.9% in the progeny of two bulls, Mo and Pa, respectively. Various investigations were carried out on these two bulls and their progeny, including clinical examination, karyotyping, genetic mapping, and whole genome sequencing. Mo was found to carry a partial translocation from BTA29 to BTA26 while Pa was mosaic for a dominant nonsense mutation in the *GATA6* gene causing cardiac outflow tract defect in its progeny.

Introduction

Reducing juvenile mortality in cattle is important for both economic and animal welfare reasons. Previous studies have revealed a large variability between breeds and sire progeny groups (e.g. Leclerc et al. 2016), with some extreme cases due to dominant mutations causing various syndromes among the descendants of mosaic bulls (Bourneuf et al. 2017). The purpose of this study was twofold: to fine monitor calf mortality at the level of sire families within the French and Walloon Holstein populations, and to use this information to detect genetic defects that might have been overlooked by lack of specific symptoms.

Material & Methods

Analysis of mortality rates at different ages in the progeny of individual sires.

Information on the pedigree, sex, date of birth, date of death, and cause of death (either natural or slaughter) of Holstein animals were recovered from the French and Walloon databases. To focus on the most reliable data, only female calves born between 2017 and 2020, which remained on their farm of birth during their first year of life, were selected. The final data set comprised 2.25 million daughters from 1001 sires. The size of the half-sib families ranged from 100 to 37,839 individuals, with a mean of 2,246. Natural mortality rate was computed during the first year of life and for four sub-periods (perinatal, postnatal, pre-weaning and post-weaning) corresponding to distinct causes of death (Table 1; Santman-Berends et al, 2019).

Clinical examination.

Nine calves (both sexes) sired by two half-brother bulls (Mo: n=3 and Pa: n=6) that showed very high mortality rates were necropsied. Gross phenotypic description was also available for eight and seven additional presumably affected calves of each sire, respectively.

Molecular characterization of the genetic defects of sires Mo and Pa.

Mo was karyotyped to screen for chromosomal abnormality while Pa was dead at time of the study and thus not available for blood sampling. Illumina Bovine SNP50 phased and imputed genotypes from Pa and 203 of its progeny were extracted from the French genomic evaluation database. Assuming a dominant inheritance with somatic mosaicism in Pa, we performed transmission disequilibrium tests for 16,487 informative markers, 14 progeny that died before 50 days of age (incl. four necropsied), and 189 half-sib controls still alive at 2 years. We then sequenced the genome of one case with Illumina technology (150 bp paired-end reads), processed the data in accordance with the guidelines of the 1000 Bull Genomes Project (Hayes and Daetwyler 2019), and we used 5,116 genomes from run 9 of the latter project as controls.

Results and discussion

The natural mortality rate of heifers during their first year of life was of 11.8% on average, with 4.2% for perinatal, 2.9% for postnatal, 3.1% for pre-weaning and 3.2% for post-weaning mortalities (Table 1, Figure 1). These rates were lower than most of those reported in the literature (e.g. Leclerc et al. 2016), probably because we considered only females and sex is known to have a significant effect on juvenile mortality. Interestingly, natural mortality rates per period and per half-sib family showed approximately normal distribution suggesting quantitative inheritance. Yet we observed outlier families with possible mono or oligogenic inheritance of excess mortality and focused on the worst sire per category.

Table 1 Mortality rates in % for the worst bull per category (bolded) vs population means

Bull	Bull sire	NB of Calves	Perinatal 0-2 days	Postnatal 3-14 days	Pre-weaning 15-55 days	Post-weaning 55-365 days	Global mortality 1-365 days
Mean of 1001 bulls		2,250	4.2	2.9	3.1	3.2	11.8
Mo	Mogul	108	**16.7**	**6.7**	2.4	**7.3**	**30.6**
Pa	Mogul	2,297	5.3	6.1	**6.3**	4.8	19.9

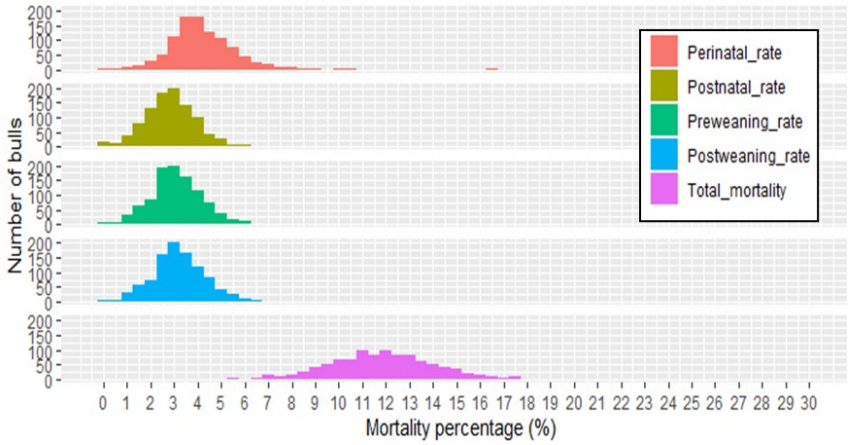


Figure 1: Histogram of distribution of mortality rates for each period of death

Among them, two bulls with high mortality rates (Mo and Pa), sired by the most popular bull sire of the cohort (Mogul) particularly retained our attention and were selected for further analysis. Karyotyping of Mo revealed a translocation of approximately the first half of bovine chromosome 29 (BTA29) onto BTA26 (Figure 2, A). A survey by French and Belgian veterinarians enabled us to gather phenotypic information for six affected calves. The main clinical features included symptoms reminiscent of Jacobsen syndrome in human (i.e. cranial dimorphism, seizure and heart defects), a rare genetic defect caused by haploinsufficiency for more than 100 genes (Rodríguez-López et al. 2021) located in a region of HSA11q orthologous to the translocated segment of BTA29 (Figure 2, B). In the near future, the genotyping of the affected calves with SNP arrays should allow us to confirm the diagnostic of partial monosomy of BTA29 and to precise the breakpoints of the translocation.

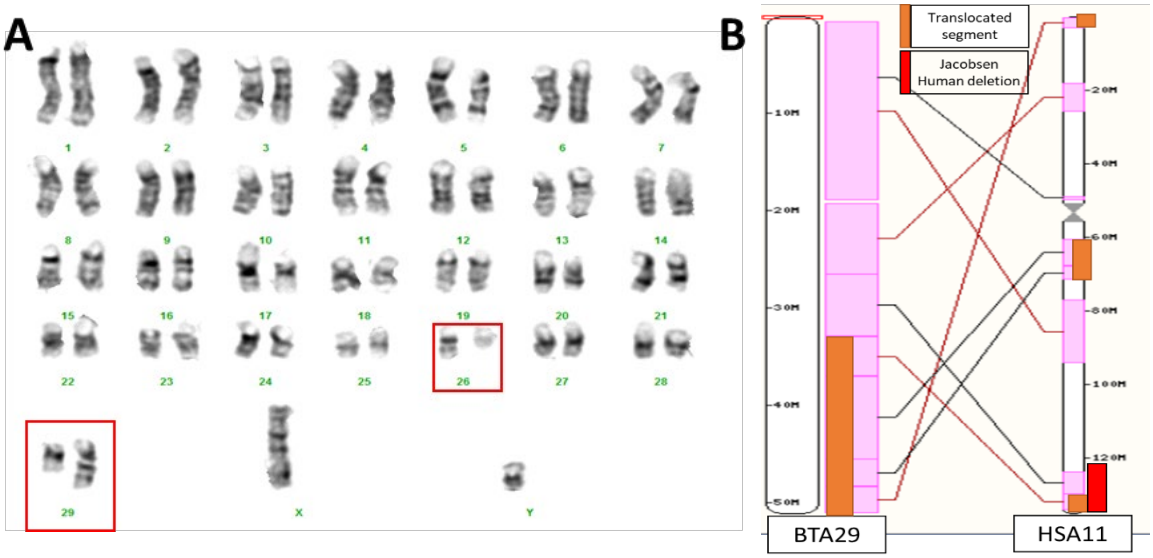
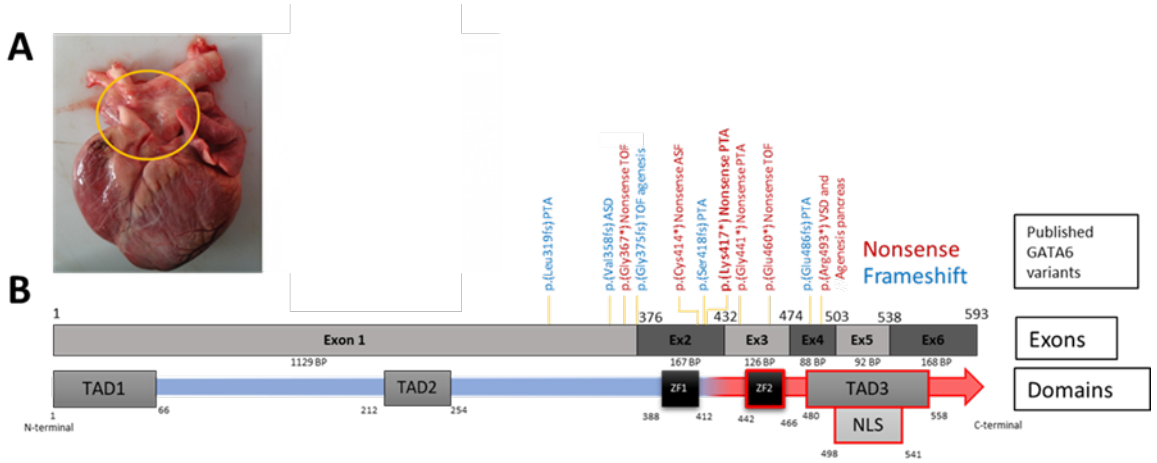


Figure 2. Karyotype of sire Mo (A) and synteny between BTA 29 and HSA11 (B).

A different etiology was suspected for the excess of mortality observed among Pa's progeny, since the peak of mortality was later than for Mo's daughters and the symptoms were different. Indeed, necropsy of six affected calves revealed persistent *truncus arteriosus* (Figure 3, A) and symptoms compatible with heart defects in seven additional cases. To gain insights into the molecular basis of this defect we analyzed Illumina Bovine SNP50 genotypes from 14 calves that died prematurely and 189 half-sib controls that had been genotyped for genomic evaluation. In doing so, we mapped the locus on BTA24 between positions 19,770,957 and 37,706,580 on ARS-UCD1 assembly. Subsequent sequencing of the genome of a necropsied calf and filtering for variants observed in 5116 control genomes revealed a single heterozygous candidate variant within the interval: a thymine-to-adenine substitution in exon 2 of *GATA6* predicted to introduce a premature stop codon (chr24: g.34,187,181T>A; GATA6 p.417K>X). If translated the mutant protein would lack about 30% of amino acid sequence including important functional domains (ZF2, NLS and TAD3; Figure 3). Interestingly the loss of GATA6 heterozygosity has been associated with various defects of differentiation, development, and function of the heart in vertebrates, including abnormal septation of the aortopulmonary trunk (e.g. Kodo et al. 2009 and Figure 3, B).



TAD, transcriptional activation domain; ZF, zinc finger domain; NLS, nuclear localization signal domain (Polar residues); TOF, Tetralogy of Fallot; ASD, Atrial septal defect; VSD, ventricular septal defect

Figure 3: Bovine heart with persistent *truncus arteriosus* (A) and scheme of GATA6 with bovine mutation p.417K>X and deleterious variants reported in human (B).

PCR and Sanger sequencing of the sire Pa, three affected calves and three control carrying the same paternal haplotype but in the non mutated version, confirmed the de novo nature of the nonsense variant. Finally, analyzing segregation distorsion of adjacent markers within the control calves (57:132 ratio) we estimated a proportion of 28.4 % of mutants in the progeny of Pa.

In conclusion, by focusing on the progeny of extreme bulls for juvenile mortality, we have described bovine models for two syndromes also observed in humans. Our results demonstrate the suitability of our method to reveal genetic defects that are hardly detectable with traditional heredo-surveillance. Beyond this proof of concept, the calculation of mortality rates at different ages for the whole population of bulls pave the way for future detection of QTL associated with juvenile mortality.

Acknowledgments
F. Besnard is supported by a CIFRE PhD grant from IDELE, with the financial support of the Association Nationale de la Recherche et de la Technologie and APIS-GENE (Paris, France).

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