Short communication: Lethal mutations in Vorderwald cattle through Montbéliarde incrossings

S. Reinartz and O. Distl*

Institute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover, 30559 Hannover, Germany

ABSTRACT

Vorderwald cattle are a dual-purpose cattle breed with high migrant contributions from Montbéliarde bulls in the recent past. Through the wide use of Montbéliarde bulls, undesirable alleles were also disseminated into the Vorderwald population. Haplotypes on bovine chromosome 19 (MH1) and 29 (MH2), supposed to harbor lethal mutations, were identified in Montbéliarde cattle. A study in French Montbéliarde cattle identified the *PFAS*:g.28511199C>T (rs455876205) variant as the most likely MH1 embryonic lethal mutation. The objective of the present study was to determine whether the *PFAS*:g.28511199C>T variant was introduced into Vorderwald cattle through Montbéliarde bulls and disseminated in this population. The present study expands on previous work on the deleterious *SLC37A2* variant (ss2019324563) of the MH2 locus. Herein, we traced the ss2019324563 variant back to the Montbéliarde bull, which was the most likely source for this deleterious mutation in Vorderwald cattle. We genotyped 354 Vorderwald cattle for the *PFAS* variant, resulting in 41 heterozygous individuals and a T allele frequency of 0.058. An aborted fetus homozygous mutant for *SLC37A2* from our previous study on the MH2 locus in Vorderwald cattle was wild type for the *PFAS* variant. Both lethal mutations were segregating independently of each other, and we found no indications of joint occurrence in a larger number of animals. Neither *SLC37A2* nor *PFAS* double heterozygous mutants were lethal. The earliest animal with a heterozygous *PFAS* genotype was 1 of 5 migrant Montbéliarde bulls, and this bull was the most likely origin of the deleterious *PFAS* allele in Vorderwald cattle. All Vorderwald cattle under study born before introgression of this Montbéliarde bull were homozygous wild type. In addition, all 41 heterozygous Vorderwald cattle had genetic contributions

from this Montbéliarde bull, whereas in 74 Vorderwald cattle without genes from Montbéliarde bulls, the *PFAS* T allele was not observed. In a sample of actual German Fleckvieh the *PFAS* T allele could be found at a very low frequency. Our study demonstrated the introgression of lethal variants through Montbéliarde bulls into a traditional cattle breed highly adapted to harsh local conditions. These findings underline the need to screen bulls for lethal mutations before their wide use in breeding, particularly in breeds with a focus on preservation of their genetic uniqueness.

Key words: lethal mutant, Vorderwald, incrossing, phosphoribosylformylglycinamidine synthase

Short Communication

Vorderwald cattle are an important dual-purpose breed adapted to the mountainous region of the Black Forest in Baden-Württemberg, in the southwest of Germany. Their small population size makes it difficult to achieve high genetic progress in performance traits similar to that of German Fleckvieh, German Brown, or Holsteins. To compensate for the lower genetic improvement and to reduce rates of inbreeding, Holstein, Ayrshire, and Montbéliarde bulls were introduced for crossbreeding with Vorderwald cows for limited periods (Sambraus, 1994; Hartwig et al., 2014). Contributions from these sires had significant positive effects on milk performance traits in Vorderwald cattle (Hartwig et al., 2015). The largest migrant contributions came from 5 Montbéliarde bulls in the 1990s (Hartwig et al., 2014; Reinartz and Distl, 2016). In Montbéliarde cattle, 2 recessive lethal haplotypes, MH1 and MH2, have been detected (Fritz et al., 2013). Variants in sex hormone binding globulin (*SHBG*) and solute carrier family 37 A2 (*SLC37A2*) were considered to be the responsible lethal mutations in MH1 and MH2, respectively. Migrant Montbéliarde bulls introduced the deleterious *SLC37A2* mutation (MH2) into Vorderwald cattle (Reinartz and Distl, 2016). An investigation of an aborted Vorderwald fetus demonstrated the embryonic lethal effect of the *SLC37A2*:g.28879810C>T mutation and its wide

Received July 5, 2019.

Accepted September 9, 2019.

*Corresponding author: ottmar.distl@tiho-hannover.de

dissemination in Vorderwald cattle through admixture with Montbéliarde bulls (Reinartz and Distl, 2016). The *SHBG* locus (*SHBG*:g.27956790C>T, rs38377500) was ruled out as a candidate harboring a deleterious MH1 variant in Vorderwald (Reinartz and Distl, 2016) and later in Montbéliarde cattle (Michot et al., 2017). The *SHBG* T allele frequency was 0.12 in Vorderwald cattle and was common in other cattle breeds (Reinartz and Distl, 2016). Further study of the MH1 haplotype identified a variant within phosphoribosylformylglycinamide synthase (*PFAS*; *PFAS*:g.28511199C>T, rs455876205) as the most likely candidate for embryonic lethality among French Montbéliarde (Michot et al., 2017). In Montbéliarde cattle, the MH1 haplotype was spread through the bull Boislevin and his sons. Recombination events occurring in his grandson Redon and great-grandson Rapallo allowed fine mapping of MH1 and identification of the *PFAS*:g.28511199C>T variant (Michot et al., 2017). This variant is located in a class I glutamine amidotransferase-like domain, causing a substitution of an arginine by a cysteine (p.R1205C). This amino acid is highly conserved within eukaryotes, displaying an important role of the *PFAS* gene in purine synthesis. Synthesis of DNA and RNA as well as energy supply for cells are important tasks of this gene (Henikoff, 1987; Clark, 1994; Bønsdorff et al., 2004).

The objectives of the present study were to prove whether the *PFAS*:g.28511199C>T variant was introgressed into Vorderwald cattle and whether migrant Montbéliarde bulls may be identified as having contributed to dissemination of this deleterious mutation. Through analysis of pedigrees with MH1 and MH2 carriers, we searched for the most likely sources of these deleterious alleles among migrant Montbéliarde bulls. In addition, we screened our data for animals carrying both deleterious MH1 and MH2 mutations. This study expands on a previous analysis among Vorderwald cattle by Reinartz and Distl (2016), on the candidate mutations *SHBG*:g.27956790C>T within MH1 and *SLC37A2*:g.28879810C>T within MH2 (Fritz et al., 2013).

Sampling included 354 Vorderwald cattle, 106 German Fleckvieh, 99 German Brown, and 68 animals of the breeds Angus, Blonde d'Aquitaine, Charolais, Holstein, Limousin, and Salers. Genomic DNA was isolated from EDTA blood using standard protocols. Genotyping of the *PFAS*:g.28511199C>T (rs455876205) variant was performed using a mismatch PCR-RFLP and the restriction enzyme *BmgBI* (Supplemental Table S1; <https://doi.org/10.3168/jds.2019-17213>).

Polymorphism information content, heterozygosity, allele and genotype frequencies, and χ^2 tests for

Hardy–Weinberg equilibrium were calculated using the ALLELE procedure of SAS/Genetics, version 9.4 (SAS Institute, Cary, NC). The software OPTI-MATE, version 3.88 (Schmidt and Wrede, 2006) was used to calculate the proportions of Montbéliarde and Ayrshire blood.

Allele frequency of the *PFAS* T allele in 354 Vorderwald cattle was 0.058. In French Montbéliarde cattle, the allele frequency was slightly higher, with an estimate of 0.067 (Michot et al., 2017). We found 41 heterozygous mutant and 313 homozygous wild type but no homozygous mutant individuals (Table 1). All 74 Vorderwald cattle without genetic contributions from Montbéliarde bulls were genotyped as homozygous wild type for the *PFAS* variant. All carriers of the *PFAS* variant had Montbéliarde blood proportions at 18.8 to 53.1%, whereas Ayrshire blood proportions were at 0.2 to 2.2%. Homozygous wild-type Vorderwald cattle had Ayrshire blood proportions up to 13%. The earliest heterozygous animal was a bull born in 1986 and used in AI for Vorderwald cattle (Figure 1). This was 1 of the 5 Montbéliarde bulls (bull E, Figure 1) migrating into Vorderwald cattle in the 1990s. A French study traced the MH1 haplotype to the Montbéliarde bull Boislevin (Michot et al., 2017). Our pedigree analysis showed the maternal grandsire of Boislevin, Debout, as the most recent common ancestor to the earliest Montbéliarde bull genotyped as a *PFAS* carrier in the present study. The Montbéliarde bull Debout is very likely the origin for the *PFAS* variant transmitted via the maternal path to Montbéliarde bull E. All *PFAS* heterozygous Vorderwald cattle had bull E as an ancestor in their pedigrees, with relationship coefficients from 0.2 to 50%. It was also obvious that the deleterious *PFAS* mutation was maintained up to the actual population.

We estimated a nonrecombinant MH1 haplotype in the *PFAS* heterozygous Montbéliarde bull E with both mutated alleles of the MH1 variants, *SHBG* and *PFAS*. All progeny of Montbéliarde bull E investigated here showed haplotypes containing both mutant alleles of *SHBG* and *PFAS* on one chromosome and wild-type alleles on the other. We did not verify recombinant hap-

Table 1. Number and origin of Vorderwald cattle genotyped for the *PFAS*:g.28511199C>T (rs455876205) variant

Origin of sample	N	<i>PFAS</i> genotype	
		C/T	C/C
Experimental herd	30	2	28
Aborted fetus	1	0	1
Farms	323	39	284
Total	354	41	313

Figure 1. Pedigree shows the introgression of the *PFAS-SHBG* haplotype with mutant T alleles due to crossbreeding of Montbéliarde bulls with Vorderwald cattle. The *PFAS*:g.28511199C>T variant with the deleterious T allele located on bovine chromosome 19 is the most likely locus of MH1. Bulls A through E are French Montbéliarde bulls. Ancestor A may be the most likely source of the *PFAS*:g.28511199C>T mutation, which was transmitted through bulls B through D to bull E, and from this bull into the Vorderwald cattle population.

Figure 2. Pedigree shows the distribution of the *SLC37A2*:g.28879810C>T alleles in the Vorderwald cattle population. Bull F is the likely source, and his son (bull G) and grandson (bull H) spread the lethal allele into the Vorderwald cattle population.

lotypes as reported by Michot et al. (2017). Vorderwald cattle with the *PFAS* T allele were, in all cases, also carriers of the *SHBG* T allele. Thus, estimated haplotype frequency with both mutated alleles of *SHBG* and *PFAS* was identical with the allele frequency for the *PFAS* T allele (0.058). The haplotype with the mutated *SHBG* T allele and wild-type *PFAS* C allele had a frequency of 0.052. All other haplotypes were wild type (0.89) for both alleles.

Among the 126 Vorderwald males sampled, we found 16 to be heterozygous, and among the 228 Vorderwald females, 29 were heterozygous. Allele frequencies in males and females were 0.064 and 0.037, respectively. No significant deviations in genotype and allele frequencies were found between the sexes.

To validate the origin of the *PFAS* variant in Montbéliarde cattle, we genotyped 68 animals from 7 different cattle breeds and 99 German Brown. We did not find the *PFAS* T allele, indicative of an origin in Montbéliarde cattle, in any animals from these breeds. In 106 German Fleckvieh samples from the birth years 2008 to 2016, 2 bulls were heterozygous for the *PFAS* T allele. The *PFAS* heterozygous German Fleckvieh bulls were paternal half-siblings born in 2012 and 2015. These 2 bulls had the same paternal grandsire (Malefiz, DE09 15079575), which sired the known *PFAS* carrier Mangfall (DE09 35463615), as reported by Michot et al. (2017). Possibly segregation of the *PFAS* T allele happened via the Fleckvieh bull Malefiz.

A previous study showed a homozygous mutant genotype of the lethal *SLC37A2*:g.28879810C>T mutation in an aborted Vorderwald fetus and dissemination of the mutant *SLC37A2* allele through admixture with Montbéliarde (Reinartz and Distl, 2016). We searched the available pedigrees of *SLC37A2* T allele carriers to locate the source of this *SLC37A2* variant. The *SLC37A2* T allele was introgressed in the Vorderwald population through another of the 5 Montbéliarde bulls (Figure 2). Here, we identified Montbéliarde bull F as the likely origin of the deleterious *SLC37A2* T allele. A son (bull G) and a grandson (bull H) of Montbéliarde bull F were also heterozygous and contributed to dissemination of the *SLC37A2* T allele. Montbéliarde bull E was homozygous wild type for the *SLC37A2* variant. Only 3 out of 344 animals were double heterozygous for both variants within *SLC37A2* and *PFAS* (Table 2). The aborted Vorderwald fetus was homozygous mutant for *SLC37A2* and homozygous wild type for *PFAS*, as were his parents and grandparents. With an estimate of 0.0424, the *SLC37A2* T allele frequency was lower than that of the *PFAS* T allele frequency.

In agreement with Michot et al. (2017), we found no homozygous *PFAS* mutant individuals, and, thus,

Table 2. Joint distribution of the *PFAS*:g.28511199C>T and *SLC37A2*:g.28879810C>T genotypes in 344 Vorderwald cattle

Item	<i>PFAS</i> : C/C	<i>PFAS</i> : C/T	<i>PFAS</i> : T/T	Total
<i>SLC37A2</i> : C/C	278	38	0	316
<i>SLC37A2</i> : C/T	24	3	0	27
<i>SLC37A2</i> : T/T	1	0	0	1
Total	303	41	0	344

our data may support a deleterious effect of the *PFAS*:g.28511199C>T variant. Pedigree analysis and genotyping of a large number of Vorderwald cattle with migrant contributions from Montbéliarde bulls showed introgression of the deleterious mutations in *SLC37A2* and *PFAS* through 1 Montbéliarde bull each. Dissemination of the deleterious *PFAS* variant in Vorderwald cattle, with an actual population size of approximately 12,460 cows, reached a high *PFAS* T allele frequency similar to that of the French Montbéliarde population. Heterozygosity for both variants, *SLC37A2* and *PFAS*, was not lethal. In conclusion, genotyping of the deleterious *SLC37A2* and *PFAS* variants must be initiated among Vorderwald cattle to avoid further dissemination of these variants. A compulsory *PFAS* screening program also must be set up for Fleckvieh bulls. All bulls intended for breeding must be homozygous wild type for both deleterious variants, according to German animal welfare legislation.

ACKNOWLEDGMENTS

We thank all cattle breeders for the providing samples from their animals and for sharing pedigree data with us. For their support in collecting samples and providing pedigree data, we particularly thank F. Maus and Michaela Reinhardt, Landratsamt Schwarzwald-Baar-Kreis, Sachgebiet Tierzucht, Germany. We also thank AI centers in Bavaria and Baden-Württemberg for their readiness to donate semen samples for this study.

REFERENCES

- Bønsdorff, T., M. Gautier, W. Farstad, K. Rønningen, F. Lingaas, and I. Olsaker. 2004. Mapping of the bovine genes of the de novo AMP synthesis pathway 1. *Anim. Genet.* 35:438–444.
- Clark, D. V. 1994. Molecular and genetic analyses of *Drosophila* Prt, which encodes the first enzyme of de novo purine biosynthesis. *Genetics* 136:547–557.
- Fritz, S., A. Capitan, A. Djari, S. C. Rodriguez, A. Barbat, A. Baur, C. Grohs, B. Weiss, M. Boussaha, D. Esquerre, C. Klopp, D. Rocha, and D. Boichard. 2013. Detection of haplotypes associated with prenatal death in dairy cattle and identification of deleterious mutations in *GART*, *SHBG* and *SLC37A2*. *PLoS One* 8:e65550.
- Hartwig, S., R. Wellmann, R. Emmerling, H. Hamann, and J. Bennewitz. 2015. Importance of introgression for milk traits in the German Vorderwald and Hinterwald cattle. *J. Dairy Sci.* 98:2033–2038.

- Hartwig, S., R. Wellmann, H. Hamann, and J. Bennewitz. 2014. The contribution of migrant breeds to the genetic gain of beef traits of German Vorderwald and Hinterwald cattle. *J. Anim. Breed. Genet.* 131:496–503.
- Henikoff, S. 1987. Multifunctional polypeptides for purine de novo synthesis. *BioEssays* 6:8–13.
- Michot, P., S. Fritz, A. Barbat, M. Boussaha, M.-C. Deloche, C. Grohs, C. Hoze, L. Le Berre, D. Le Bourhis, O. Desnoes, P. Salvetti, L. Schibler, D. Boichard, and A. Capitan. 2017. A missense mutation in PFAS (phosphoribosylformylglycinamide synthase) is likely causal for embryonic lethality associated with the MH1 haplotype in Montbéliarde dairy cattle. *J. Dairy Sci.* 100:8176–8187.
- Reinartz, S., and O. Distl. 2016. Validation of deleterious mutations in Vorderwald cattle. *PLoS One* 11:e0160013.
- Samraus, H. 1994. Gefährdete Nutztierassen. Ihre Zuchtgeschichte, Nutzung und Bewahrung. Ulmer, Stuttgart, Germany.
- Schmidt, T., and J. Wrede. 2006. OPTI-MATE Version 3.88: Software for the management of endangered populations. Hannover, Institute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover, Germany.

ORCIDS

O. Distl <https://orcid.org/0000-0002-3516-836X>