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Myostatin mutation causing double muscling could affect increased psoroptic mange sensitivity in dual purpose Belgian Blue cattle

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ABSTRACT

Belgian Blue cattle are known for their high degree of muscling and good carcass qualities. This high degree of muscling is mainly caused by a mutation in the myostatin gene (***MSTN***). Although the *MSTN* mutation is considered as fixed in the Belgian Blue breed, segregation is occurring in a sub-population bred for dual purpose. In the latter population, we observed an association between the mutation in *MSTN* and susceptibility to psoroptic mange, a skin disease caused by *Psoroptes ovis* mites that heavily plagues Belgian Blue cattle. In total, 291 animals were sampled and screened for their susceptibility for mange lesions and their *MSTN* genotype. Via linear mixed modelling, we observed that homozygous mutant animals had a significant increase in the size of mange lesions (+2.51% lesion extent) compared to homozygous wild type. These findings were confirmed with zero-inflated modelling, an animal model and odds analysis. Risk ratios for developing severe mange lesions were 5.9 times as high for homozygous mutant animals. All analyses confirmed an association between the *MSTN* genotype and psoroptic mange lesion size.

Implications

In the present study, we uncover an association between the mutation in the myostatin gene that causes double muscling in Belgian blue cattle, and the susceptibility to psoroptic mange. This skin disease, for which Belgian Blue cattle are very susceptible, is caused by mites and causes severe wounds, pain and animal suffering. Cattle with the double muscling mutation were more prone to developing psoroptic mange and had larger lesions. The association is of importance for other breeds and other livestock species, as it may point towards a negative role of myostatin in ectoparasitic susceptibility.

Introduction

The Belgian Blue cattle breed is known for its extreme degree of muscling, mainly caused by a loss-of-function mutation in the myostatin (***MSTN***) gene, which is also known as *GDF8* (Grobet et al., 1997). This gene is a member of the transforming growth factor beta superfamily (**TGF-β**) and encodes a chalone that inhibits muscular growth. The mutation in *MSTN* in Belgian Blue cattle is an 11 bp deletion, called *nt821(del11)*, causing a premature stop-codon and a dysfunctional protein (Grobet et al., 1997). Therefore, *nt821(del11)* causes muscular hypertrophy, also called "double muscling". Wild-type animals are denoted as *+/+*, heterozygous as *mh/+* and homozygous mutated as *mh/mh*. In Belgian Blue cattle, the mh/mh mutation is almost completely fixed and therefore, most animals exhibit the double-muscled phenotype (Druet et al., 2014; Dunner et al., 2003). However, in the 1970s, the breed, at that time called Race de Moyenne et Haute Belgique, was split into the current Belgian Blue cattle (all double-muscled) and a smaller sub-population called the dual purpose Belgian Blue (or in French: Bleue Mixte) (Colinet, 2010). This population comprises today about 3 500 animals, is not focused on extreme muscling and is used for dairy and meat production. In this sub-population, the *nt821(del11) MSTN* mutation is segregating. It is estimated that approximately 80% of all animals are carriers of the *mh* allele (Colinet, 2010).

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Belgian Blue cattle show excellent carcass qualities, but also have a high sensitivity for psoroptic mange. This highly contagious disease is caused by the *Psoroptes ovis* (*P. ovis*) mite evoking severe exudative dermatitis and pruritus. Moreover, the infestation can result in severe economic losses due to a loss in average daily gain (as much as 680 grams per day), treatment costs and decreased leather quality (Fisher and Wright, 1981; Lonneux et al., 1998; Rehbein et al., 2003). Severe infestations can lead to death of the animal. This genetic predisposition for developing psoroptic mange of Belgian Blue cattle, compared to other breeds, has been thoroughly described by Pouplard et al. (1990), Losson et al. (1999), Sarre et al. (2012) and involvement of genetic effects of the host have been suggested for a long time (Losson et al., 1999). Sarre et al. (2012) showed that 74% of all Belgian Blue farms in Northern Belgium (Flanders) had mange problems and almost half of the farmers regarded psoroptic mange as difficult to control or uncontrollable. Moreover, in non-endemic countries, outbreaks are often associated with imported Belgian Blue animals (Jones et al., 2008; Millar et al., 2011; Mitchell et al., 2012). Besides, differences in immune response against *P. ovis* between Belgian Blue cattle and Holstein-Friesian cattle were reported (Losson et al., 1999; Sarre et al., 2015; Chen et al., 2021).

The dual purpose Belgian Blue population is in general considered less susceptible to psoroptic mange but this disease still poses a serious threat. Moreover, dual purpose farmers often indicate that double-muscled animals (*mh/mh* genotype) are more susceptible for psoroptic mange, and these animals are often the first in their herds to show clinical signs of psoroptic mange. While this claim has been circulating amongst breeders and policymakers for years, we initiated a study on the association between psoroptic mange susceptibility and *MSTN* genotypes in dual purpose Belgian Blue cattle. The hypothesis of this study was that the *MSTN* genotype is associated with psoroptic mange susceptibility in Belgian Blue cattle. As *MSTN* plays a crucial role in the breeding programme of many beef cattle breeds, a potential causal relationship between *MSTN* and ectoparasitic resistance would be of major importance.

Material and methods

Animals

Farm visits were conducted between 2013 and 2019 during the winter housing period, in which a total of 291 dual purpose Belgian Blue cattle were sampled. These farm visits were initiated by farmers who were confronted with a psoroptic mange outbreak. Only herds where clinical signs of psoroptic mange were observed and without acaricide treatment at least 6 weeks before sampling were selected. Ten farms were visited and animals were assigned to 13 Farmer_Groups based on within-farm management groups (e.g. different age classes, originating from different summer pastures) (Supplementary Table S1). All data were collected in the same population during two consecutive projects, namely PSOROVIS (2013–2015, Project 1) and BOMANGE (2018–2019, Project 2).

The severity of mange lesions was expressed as the percentage of infested body surface, i.e. summed over all lesion spots (lesion extent), following the method proposed by Guillot (1981). The severity of every lesion was scored from 1 (almost completely healed) to 4 (active, thick crusted, wet lesions) (example figures added as Supplementary Fig. S1). In addition, three skin scrapings (each 4 cm^2) were taken at the predilection places for *P. ovis*, i.e. the neck, back and tail and were pooled per animal. These were used to confirm the presence of *P. ovis* mites and to exclude other ectoparasites (e.g. *Chorioptes bovis*, *Bovicola* (*Damalinia*) *bovis*, *Haematopinus eurysternus*). Skin colour was recorded during the farm visit, and checked with the colour registered by the herdbook.

Genotyping

DNA was extracted from EDTA blood samples, and animals were genotyped on either the EuroGenomics LD BeadChip genotyping array (Illumina, San Diego, CA, USA) (for 2013–2015, Project 1) or the EuroGenomics MD BeadChip array (Illumina, San Diego, CA, USA) (for 2018–2019, Project 2). Both arrays include single nucleotide polymorphism (**SNP**) markers to detect the *nt821(del11) MSTN* mutation. Using this array, *MSTN* genotypes were derived for all animals.

Stratification and variance inflation analysis

The data structure was somewhat unbalanced (*MSTN* genotypes vs. Farmer_Group, see Supplementary Table S1) and we verified if this would affect the results. First, a SNP-based principal component analysis was conducted to verify whether all studied animals were originating from the same population and whether population stratification was present. This analysis was performed using PLINK 1.9 (*--pca* command) (Chang et al., 2015) and visualised using *ggplot2* (Wickham, 2016). Next, a variance inflation analysis was performed to check the non-orthogonal distribution between Farmer_Group and *MSTN* genotypes (unbalanced distribution). This analysis was performed using the *car* package (Fox and Weisberg, 2019) in which a generalised variance inflation factor (**GVIF**) is computed (Fox and Monette, 1992). GVIF values above 10 indicate that (fixed) effects in a model are confounded which may affect estimates of the coefficients (Kutner et al., 2005).

Statistical analyses

To study the possible association between *MSTN* and psoroptic mange lesions, we used three types of analysis, to account for the limitations of the observational data. First, we fitted a linear mixed model to test for (significant) effects. Next, these results were used to fit an animal model that takes the pedigree into account. However, these models rely heavily on a Gaussian distribution of the data, and therefore might not be suited as there were a large number of zero-values (animals showing no lesions). Second, we used a zero-inflated model that takes the structure of the data into account. Zero-inflated models assume that zero-values are originating from a separate process and therefore can be modelled independently. The model fits both the odds of an animal to have a lesion (thus: lesion extent >0) and the lesion size of the animal, given that the animal has a lesion. Third, a different approach was followed to cancel out any potential effect of the underlying distribution of lesion extent: a risk and odds analysis was performed.

All statistical analyses were executed using R packages (R Core Team, 2020). Mixed linear models were estimated using the *nlme* package (Pinheiro et al., 2021) where random effect variances were estimated using REML (Patterson & Thompson, 1971). Potential fixed effects, besides the *MSTN* genotype, were coat colour, number of mites counted in skin scraping, sex, age at the moment of sampling, birth year, project and the two first principal components. Contrasts were tested with the *multcomp* package (*glht* function) (Hothorn et al., 2008). The animal model was fitted including a pedigree of 1 690 individuals (up to 13 generations deep), and SNP genotypes were included for relationship matrix calculations (ssGBLUP). For estimation of this animal model, gibbs3f90 with 1 000 000 iterations, 10 000 burnin and a thinning of 1 000 was used (Misztal et al., 2014). The zero-inflated model was fitted using the glmmTMB package (Brooks et al., 2017). The fitted model had a

random Farmer_Group effect and *MSTN* genotype, the first two principal components and Project as fixed effects, with a Gaussian error distribution. To determine the relative risk and odds ratios, both the chance of having a lesion, and having a large lesion (>average lesion size), were examined using the epiR package (Stevenson et al., 2021).

Results

Mange lesion description

Table 1 gives an overview of the observed lesion extent for all sampled animals (n = 291) and for the extreme lesions (scores 3 and 4, only recorded for animals sampled in project 2, n = 160). At the moment of sampling, 102 animals did not show any lesion. Skin scrapings did not always reveal *P. ovis*, but animals were kept in the dataset if in the concerned Farmer_Group at least one animal was found with *P. ovis*, as it confirmed the presence of an infestation at the group level. One hundred and fifty-five animals had a white coat colour, 39 were (spotted) black and 106 were (spotted) blue. The average age at sampling was 5 years (SD: 2.3 years), with a minimum of 1.3 years and maximum of 11.8 years.

Myostatin genotyping

All 291 sampled animals were genotyped for *MSTN*. A total of 59 animals were *+/+* (20%), 60 animals *mh/+* (21%) and 172 animals were *mh/mh* (59%). These proportions approximate the distribution of the *MSTN* genotypes known by the herdbook (G. Glorieux, Personal Communication) and by Colinet (2010). An overview of the distribution of *MSTN* genotypes per Farmer_Group is added in Supplementary Table S1.

Stratification and variance inflation analysis

Principal components were computed on the SNP genotype data, and individuals were labelled according to their *MSTN* genotype (Supplementary Fig. S2). The first principal component (**PC1**) explained 16.4% of the total variance, PC2 8.6% and PC3 5.4%. Moderate population stratification could be observed, somewhat separating *+/+* animals from *mh/mh* animals. Therefore, it was decided to include the first two principal components in all models to comply with possible population stratification effects, although they were not considered significant (*P*-value < 0.05) in any of the used models. Besides, a principal component analysis was performed excluding BTA 2, where *MSTN* is located (results not shown). This produced similar results and therefore we conclude that the observed stratification was independent from the *MSTN* genotype. In the variance inflation analysis, the calculated $\left(GVIF^{\frac{1}{2\cdot DF}}\right)^2$ values were below three for Farmer_Group, *MSTN* genotype and the first two principal components.

Association analysis

Fig. 1 shows an overlay of a boxplot (blue) and individual observations (red) of lesion extent, per *MSTN* genotype. Animals with *mh/mh* genotype clearly exhibit on average larger lesions, with a higher variance and thus more extreme lesions.

A linear mixed model for lesion extent with Farmer_Group as random effect and the first two principal components, project (1 or 2) and *MSTN* genotype as fixed effect, was fitted. Effect estimates are shown in Table 2. Animals with white coats had larger lesions (+0.85% lesion extent compared to black colour and +0.60% lesion extent compared to blue coat colour) but the overall effect of coat colour was not significant at 0.05. Therefore, it was not withheld in the final model (shown in Table 2). Other fixed effects - number of mites counted, sex, age at the moment of sampling and birthyear - were considered insignificant as well (*P*-value > 0.05). For extreme lesions (sum of lesions with scores 3 and 4), a linear mixed model was fitted with *MSTN* genotype and the first two principal components as fixed effect and Farmer_Group as random effect. The project effect was not included as all animals in this analysis were sampled in project 2. Results are also shown in Table 2. Results of the posthoc contrast analysis between the different *MSTN* genotypes are shown in Table 3. Both contrasts between *mh/+* and *mh/mh*, and [*+/+* and *mh/+*] versus *mh/mh* were considered significantly different ($P < 0.05$, or $P < 0.01$ with Bonferroni correction for multiple testing).

The same linear mixed model for lesion extent was also fitted as an animal model to take the pedigree and genomic relationship between animals into account. The model estimated the relative effect of *mh/+* compared to *+/+* at −0.09% lesion extent (SE = 0.75%), and the relative effect of *mh/mh* compared to *+/+* at 2.64% lesion extent (SE = 0.66%). Additive genetic standard deviance was estimated at 1.55% lesion extent, Farmer_Group SD at 1.28% lesion extent and residual SD at 3.81% lesion extent. These estimations result in a heritability of 12.7% for susceptibility to psoroptic mange in the sampled population.

Subsequently, the zero-inflation model was fitted. The risk of being among those who have no lesions was 2.38, expressed as odds ratio (=ratio of the probability of having no lesion and the probability of having a lesion). The odds were decreased for animals with *mh/+* with −0.82 (*P*-value = 0.28) and for animals with *mh/mh* with −2.45 (*P*-value = <0.01); thus, these animals have a higher chance of developing a lesion. The odds were decreased for animals sampled in Project 1 with −0.63 (*P*-value = 0.22). The effect of the first two principal components was considered insignificant (*P*-value > 0.20). The baseline lesion extent was 3.63% of body surface for animals who had a chance of having lesions. *Mh/+* decreased it with −3.25% lesion extent (*P*-value = 0.36), whereas having *mh/mh* decreased it with −1.86% lesion extent (*P*-value = 0.63), both estimates were considered insignificant. Sampling in Project 1 increased with 4.95% lesion extent among those who have a chance of having lesions (*P*-value < 0.001). The effect of the first two principal components

Table 1
Overview of lesion characteristics at the moment of visit for all dual purpose Belgian Blue cattle sampled in both projects (n = 291), for projects 1 and 2 separately (n = 131 and n = 160, respectively) and for severe lesion (score 3 + score 4) for animals sampled in project 2 (n = 160).

	Lesion extent (in % body coverage)			Severe lesion extent (in % body coverage)
	Project 1 + 2	Project 1	Project 2	Project 2
Mean	2.45	4.26	0.90	0.67
Median	0.57	1.71	0.29	0
SD	4.55	5.67	1.56	1.81
Max	32.0	32.0	10.0	10.0

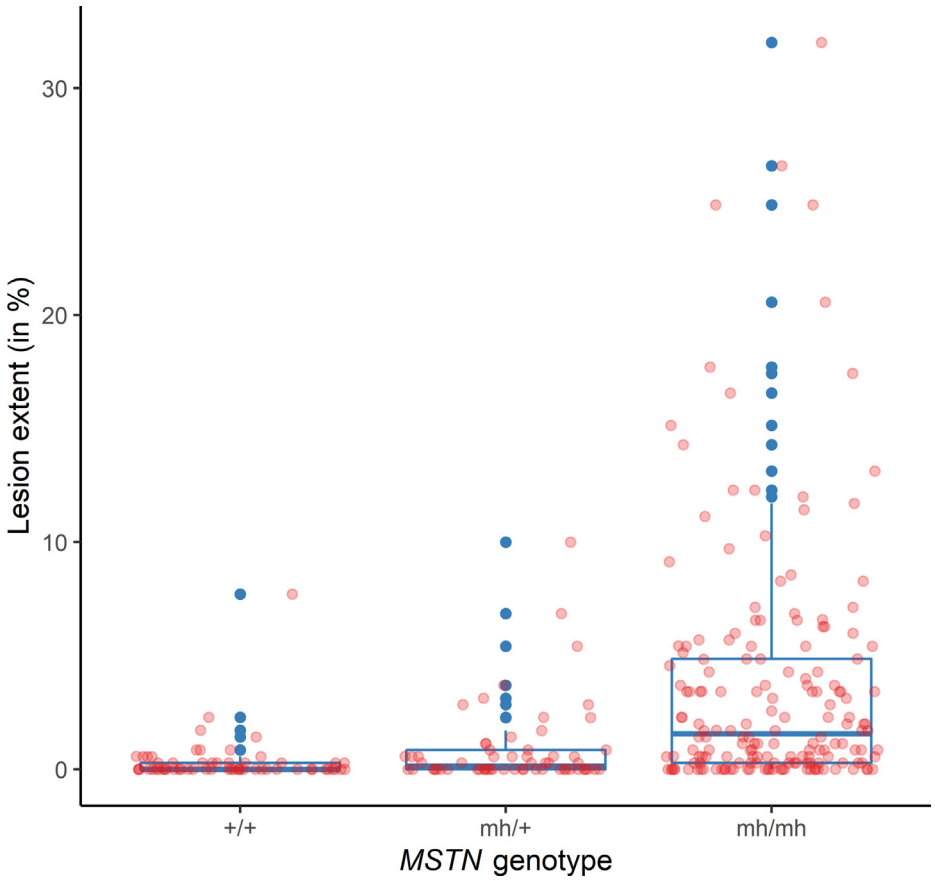

Fig. 1. Boxplot (blue) of the observed lesion extent in dual purpose Belgian Blue cattle in the three groups of *MSTN* genotypes and individual (red) observations of all psoroptic mange lesions grouped by *MSTN* genotype. Blue dots in the boxplot can be considered as the extreme values per group. SD in the *+/+* group is 1.07%, for the *mh/+* group 1.80% and for the *mh/mh* group 5.45%. Abbreviations: *MSTN* = Myostatin gene.

Table 2
Overview of the fitted effect estimates in the linear mixed model for lesion extent (n = 291) and severe lesion extent (score 3 + 4, n = 160, only Project 2) in dual purpose Belgian Blue cattle.

		Lesion extent				Severe Lesion extent		
		Estimate	SE	*P*-value		Estimate	SE	*P*-value
Intercept		−0.32	0.96	0.74		0.51	1.00	0.61
Fixed effects								
MSTN genotype								
mh/+		−0.11	0.96	0.90		0.06	0.96	0.95
mh/mh		2.51	1.20	0.03		3.01	1.20	0.01
Principal components								
PC1		−4.84	7.32	0.51		−8.00	8.22	0.33
PC2		9.67	6.95	0.17		4.68	7.29	0.52
Project								
Project 2		2.53	0.80	0.01				
Random effects								
Var(Farmer_Group)	(n = 13)	0.71			(n = 8)	1.98		
Residual variances		16.14				16.10		

Abbreviations: *MSTN* = Myostatin gene. All units in % lesion extent. In the lesion extent model, intercept includes the *+/+ MSTN* genotype and the Project 1 effect, with PC1 (principal component) and PC2 scores equal to 0. For the severe lesion extent, the intercept includes the *+/+ MSTN* genotype and with PC1 and PC2 scores equal to 0. The given *P*-values are indicatory for whether the estimate is different from zero. PC1 scores: [−0.11; 0.08], PC2 scores: [−0.16; 0.10].

was considered insignificant (*P*-value > 0.40). In conclusion, there was a significant effect of the *MSTN* genotype on the occurrence of lesions in this zero-inflated model, but once an animal shows lesions, the effect of *MSTN* was insignificant for the lesion size.

Finally, relative risks and odds ratios were estimated by grouping the animals for the presence of the wild-type *MSTN* allele (*+*), thus either *+/+* or *mh/+* versus *mh/mh*, for two situations: (1) the risk or chance of having a lesion at the moment of sampling, thus lesion extent > 0% and (2) the risk or chance of having a lesion larger than the mean lesion size (>2.45% lesion extent). Table 4 shows the contingency tables for both scenarios. For scenario (1): animals with the *mh/mh* genotype were 1.82 times more likely to develop a lesion compared to animals with the *+/+* or *mh/+* genotype, and the odds of animals with *mh/mh* genotype having a lesion were five times the odds of animals with *+/+* or *mh/+* genotype (95% confidence interval: 3.03–8.33). For scenario (2): animals with the *mh/*

Table 3
Contrast analysis in dual purpose Belgian Blue cattle between the different *MSTN* genotypes in a linear mixed model with *MSTN* genotype, project and the first two principal components as fixed effects, and the Farmer_Group as random effect.

Tested contrast	Estimate	SE	*P*-value
mh/mh vs *mh/+*	+2.62	0.77	<0.001
mh/+ vs *+/+*	+0.21	1.78	0.91
mh/mh vs *+/+*	+2.83	2.07	0.17
[*mh/+* and *mh/mh*] vs *+/+*	+1.51	1.89	0.42
mh/mh vs [*+/+* and *mh/+*]	+2.72	1.28	<0.01

Abbreviations: *MSTN* = Myostatin gene.

mh genotype were 5.88 times more likely to develop a lesion larger than the average measured lesion size compared to animals with the *+/+* or *mh/+* genotype, and the odds of animals with *mh/mh* genotype having a lesion larger than the average measured lesion size were nine times the odds of animals with *+/+* or *mh/+* genotype (95% confidence interval: 4.35–20).

Discussion

The aim of the study was to investigate whether the Belgian Blue's high susceptibility for psoroptic mange is linked to muscular hypertrophy caused by the *nt821(del11) MSTN* mutation. As the main population of Belgian Blue is fixed for this mutation, we could not study the potential association between *MSTN* and mange lesion development in this population. Therefore, we studied this potential association in the dual purpose Belgian Blue population that still segregates this mutation. Accordingly, we studied the psoroptic mange lesions of 291 dual purpose Belgian Blue cattle and related this to their *MSTN* genotype. A significant association was found between the *nt821(del11) MSTN* mutation and the psoroptic mange lesion size.

The possibility of population stratification in the sampled data was studied via principal component analysis. Based on this analysis, it was concluded that moderate population stratification was present, which was countered by including the first two principal components (together explaining 25% of the total variation) in all used models. This addition was considered non-significant in all tested models. Therefore, we deem the effect of population stratification in the data on the presented models as limited. Moreover, when using an animal model, any possible population stratification will be captured by the (SNP and pedigree based) relationship matrix in the animal model. Besides, animals sampled in both projects clustered together, indicating that they originate from the same base population (results not shown). We sampled about 10% of the whole population, which we considered representative for the whole population based on principal component analysis and herdbook records. The variance inflation factor analysis was performed to check whether the non-orthogonal design between *MSTN* genotypes and Farmer_Groups caused bias. This analysis indicated no significant problem due to the distribution of genotypes over Farmer_Groups, as the GVIF was estimated below three for all effects in the model, where 10 is frequently considered to be the threshold (Kutner et al., 2005).

When modelling lesion extent in dual purpose Belgian Blue cattle, the preliminary linear model and the animal model yielded similar results. Both models showed a significant association between *MSTN* genotype and mange lesion size: animals with the *mh/mh* genotype had significantly larger lesions. Also, when specifically estimating contrasts (posthoc analysis), lesions of the *mh/mh* genotype differed significantly from the group of animals with *+/+* and *mh/+* genotypes. On an allele basis, animals with at least one + allele had significantly smaller lesions at the moment of sampling. To our knowledge, we are the first to confirm the association between *MSTN* and psoroptic mange susceptibility. The heritability for mange susceptibility in the sampled population was estimated at 12.7%, which can be considered as low. Similar heritabilities were observed in other studies for ectoparasitic susceptibility such as tick resistance in cattle (Mapholi et al., 2016; Turner et al., 2010).

To take the high number of animals with no lesions (lesion extent = 0%) into account, we have fitted a zero-inflated model. This model first fits the probability of an individual to have a lesion, and next, it fits a model for the size of the lesion for those animals who have a probability to have a lesion in the first part of the model. The *mh/mh* genotype was significantly associated with the probability of development of a lesion, but it did not significantly influence the actual size of the lesion. Or simply put, animals with the + allele had a significantly lower chance of developing lesions, which was also the outcome of the posthoc contrast analysis.

Next, a non-parametric risk and odds analysis was performed. These analyses are irrespective of the underlying distribution of the data, but purely focus on the occurrence of an event (the development of a lesion). We showed that the relative risk of developing a psoroptic mange lesion is higher for animals with *mh/mh*, and this risk even increases when considering severe lesions. Similar results were found for the odds to develop a psoroptic mange lesion.

Irrespective of the statistical method that was used, all analyses pointed to a clear association between the *nt821(del11) MSTN* mutation and the psoroptic mange lesion size in the dual purpose Belgian Blue cattle breed.

However, this association does not immediately imply causality. Based on this research, we cannot exclude the possibility that a causative gene is co-inherited with *MSTN*. After all, *MSTN* mutations also appear in other cattle breeds, and only Belgian Blue cattle shows such a high degree of psoroptic mange susceptibility. Moreover, given that the Belgian Blue population is completely fixed for the *mh MSTN* allele, *MSTN* is not the sole source of variability in psoroptic mange susceptibility observed within the breed and between other breeds. More research is needed to elucidate a (causative) role of *MSTN* in the increased psoroptic mange sensitivity. The use of *MSTN*-null mutant animals could be a valuable

Table 4
Contingency table of the 291 analysed dual purpose Belgian Blue cattle in two scenarios: (1) whether an animal has developed a lesion at the moment of sampling, and (2) whether an animal has developed a lesion larger than the average lesion size (2.45%) at the moment of sampling.

	Scenario 1 (Lesion extent > 0%)		Scenario 2 (Lesion extent > mean lesion extent)	
	Lesion		Lesion	
	Yes	No	Yes	No
MSTN genotype				
+/+ or *mh/+*	52	67	8	111
mh/mh	137	35	70	102

Abbreviations: *MSTN* = Myostatin gene.

approach but such a study in cattle seems currently unfeasible because of the large number of animals needed and the complexity of the trial. A null mutant study in mice could be an option, but the different mite species in mice (*Myocoptes musculinus*, *Myobia musculi* or *Radfordia affinis*) could impact the representativeness of the study.

Although this study is the first to describe this association between *MSTN* and the susceptibility for an ectoparasitic disease, MSTN has been related to other phenotypes such as skin healing and immunity. Zhang et al. (2012) reported that MSTN is expressed in the skin of both mice and humans, where it plays a role in wound healing. They found that *MSTN*-null mice showed a delayed skin wound healing in both the epidermis, due to a reduced keratinocyte migration and protracted keratinocyte proliferation, and in the dermis, where both fibroblast-to-myoblast transformation and collagen disposition were reduced. This resulted in a delayed re-epithelialisation and wound contraction. Moreover, Wallner et al. (2016) also suggested that MSTN could play a role in future wound healing treatments in humans. Up to date, there are no indications that this would differ in cattle. If this would be the case, the abrasive feeding of *P. ovis* at the epidermis could be promoted by a delayed wound healing. Furthermore, Zhang et al. (2011) found that pharmacological inhibition of myostatin suppressed the expression of inflammatory cytokines in mice. Also, Lyons et al. (2010) found a similar relation between MSTN and proinflammatory cytokines (IL-17, IL1β and IFN-γ) in high-fat induced obese mice.

As there is no literature available on *MSTN*-null mutants in mammals in relation to the immune system, some effects of MSTN on the immune system in fish are described. However, caution must be taken when interpreting results from fish to mammals as they have two *MSTN* copies (Stinckens et al., 2011). Wang et al. (2018) found in null-*MSTN* transgenic zebrafish (*Danio rerio*) that individuals had an impaired NF-κB pathway. Similar results were also found by Wu et al. (2020) in Crucian carps (*Carassius auratus*). Also, in *MSTN* transgenic medaka (*Oryzias latipes*), the immune system appeared to be at least partially suppressed (Chiang et al., 2016).

Given these various roles MSTN plays in other species, it is not ruled out that MSTN also plays a role in the immune system in cattle, although this has never been studied before. The association between MSTN and the susceptibility for psoroptic mange in Belgian Blue cattle that we observed may support this idea.

This research confirms the common belief of Belgian Blue farmers that "double-muscled" (*mh/mh* genotype) dual purpose Belgian Blue cattle are more prone to psoroptic mange than others. This finding could also (partially) explain the high level of susceptibility for psoroptic mange in the Belgian Blue main population. Unfortunately, it was impossible to test for this association in the meat-type Belgian Blue population, and therefore, we performed the research in the related dual purpose sub-population. The association was confirmed using different statistical approaches, where it was shown that animals with *mh/mh* genotype developed significantly larger lesions (+2.51% lesion extent). Moreover, animals with the *mh/mh* genotype had a higher risk for developing psoroptic mange lesions and the odds of these animals developing a large lesion were nine times the odds of animals with *+/+* or *mh/+* genotype. Besides its effects on muscle development, the literature describes an effect of MSTN on both skin healing and immune suppression in other species, adding evidence for the possible link between MSTN and mange susceptibility in cattle. As MSTN plays an important role (in breeding) for many livestock breeds, this potential causal relation between MSTN and ectoparasitic resistance is of utmost importance. However, more research has to be carried out into the molecular mechanisms to better understand this putative association.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100460.

Ethics approval

The sampled animals lived on commercial Belgian Blue farms in Belgium. All farm visits and animal sampling were conducted following the procedures approved by the ethical committee of KU Leuven (P152/2011 & P225/2017).

Data and model availability statement

All data (genotypes, mange phenotypes and pedigree) can be found on the Figshare repository (10.6084/m9.figshare.15081798). The model was not deposited in an official repository.

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Declaration of interest

None.

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