Genetic diversity and flock clustering of a South African Dohne Merino flock selected for resistance to *Haemonchus contortus*

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Summary

Gastrointestinal parasitism is a major problem for livestock productivity worldwide and small ruminant production is affected the most. Resistance of gastrointestinal nematodes (GIN) to anthelmintics has become a widespread problem, with resistance of *Haemonchus contortus* in South Africa being one of the most severe. Farming with animals resistant to nematode infestation has been proposed as a sustainable alternative. The farm Wauldby in the Stutterheim district of South Africa has a well-documented history of heavy *H. contortus* challenge. In 2011, a project aimed at genetic selection for resistance to *H. contortus* was implemented at Wauldby. Annually, faecal egg counts (FEC), Famacha© score (FAM) and body condition score (BCS) were recorded on all lambs from weaning in January at 4 months of age, until the end of June when the *H. contortus* challenge decreased. Lambs were only drenched with an anthelmintic when they had a FAM of 2.5 or higher. Breeding values for FEC were estimated for Wauldby animals born from 2011 to 2014. In this study, genome-wide SNP data generated using the Illumina® Ovine SNP50 BeadChip was used to investigate flock clustering of the Wauldby Dohne Merino flock and its association with resistance to *H. contortus*. A total of 192 animals were selected for inclusion in the study. Within years, animals with the highest and lowest breeding values for FEC were selected among the animals that needed drenching (cases), and those that did not need any drenching (controls). Animals from the Grootfontein Dohne merino flock, which had not been subjected to any selection for resistance, were used as a reference population. The principal component analysis (PCA) plot was performed using SNP & Variation Suite (SVS) from Golden Helix to illustrate the population genetic structure of animals within the Wauldby Dohne Merino and GADI Dohne Merino sheep. Four distinct genetic clusters were observed, with the GADI Dohne Merino sheep population clustering separately. The Wauldby Dohne Merino population differentiated into 3 distinct clusters. Average FEC, LFEC (log-transformed FEC), BCS and FAM recorded over the study period were compared between the different clusters for the Wauldby animals. These results indicated that it should be possible to select for resistance to *H. contortus* on the basis of the phenotypic traits included in the study.

Key words: body condition score, gastrointestinal nematodes, Famacha©, faecal egg count

Introduction

Gastrointestinal parasitism is a major problem for livestock productivity worldwide and has a significant, long-term effect on small ruminant health as well as cause suffering and financial losses annually (Alba-Hurtado & Muñoz-Guzmán, 2012; Geurden *et al.,* 2014). Continuous use of chemical drenches to control gastrointestinal nematodes (GIN) in sheep has led to the development of anthelmintic resistance (AR) of GIN (Riggio *et al.,* 2013). AR has been reported in most sheep producing regions such as Australia (Falzon *et al.,* 2014), New Zealand (Hooda *et al.,* 1999), North, Central and South America (De Graef *et al.,* 2013), Africa (Vatta *et al.,* 2002) and Europe (Alba-Hurtado & Muñoz-Guzmán, 2012). Breeding for host genetic resistance is seen as a long-term strategy for controlling GIN in a sustainable way (Greer & Hamie, 2016). *Haemonchus contortus* is one of the most economically important gastrointestinal nematodes infecting hundreds of millions of small ruminants worldwide (Riggio *et al.,* 2013). Genetic resistance to this worm both between and within breeds has been documented in previous studies (Hooda *et al.,* 1999; De Souza Chagas *et al.,* 2016). The aim of this study was to use high-throughput genome-wide SNP data generated using the Illumina® Ovine SNP50 BeadChip to investigate genetic diversity and flock clustering of a Dohne Merino flock and its association with resistance to *H. contortus*.

Materials and Methods

The farm Wauldby in the Stutterheim district in the Eastern Cape province of South Africa has a well-documented history of heavy *H. contortus* challenge and *Haemonchus* resistance to all available anthelmintics. In 2011, a *Haemonchus* resistant line was established in the Dohne Merino stud at Wauldby. Since 2011, faecal egg counts (FEC), body condition score (BCS) and Famacha© score (FAM) were collected and recorded annually on all the lambs from January at 4 months of age, until the end of June when the *H. contortus* challenge decreased. FAM was recorded weekly, while FEC and BCS scores were recorded at two-weekly intervals. Animals with FAM scores of ≥ 2.5 or BCS scores < 1.5 were subjected to anthelmintic treatment and recorded as dosed animals. Replacement lambs for the resistant line were selected from those lambs that did not receive any anthelminthic treatment.

The Grootfontein Dohne Merino stud is kept at Grootfontein Agricultural Development Institute (GADI) near Middelburg in the Eastern Cape Province under veld conditions. The GADI Dohne Merino sheep were used as a reference flock for the Wauldby animals, as no specific selection for helminth resistance was done in the GADI flock

Breeding values for FEC were estimated from the data available on the Wauldby animals from 2011 to 2014. Within years, animals with the highest and lowest EBV for FEC were selected among the dosed (n=48, Low EBV FEC; n= 48, High EBV FEC), as well as the not dosed (n=52, Low EBV FEC; n=48, High EBV FEC) groups. Animals were selected within years to account for any possible genetic trends. In the case of the Grootfontein Dohne Merino animals, FEC data for the 2014 and 2016 born lambs were available. Animals with the highest and lowest FEC within each year were selected for genotyping (n=25/year).

DNA was isolated using the DNA isolation NucleoMag® VET kit (NucleoMag - MACHEREY-NAGEL GmbH & Co KG, Düren, Germany) and samples with ≥25ng/ul of DNA were genotyped at the Agricultural Research Council, Biotechnology Platform using the Illumina® Ovine SNP50 BeadChip (Illumina Inc., San Diego, CA).

The SNP genotype data were subjected to quality control using PLINK v1.07 software (Purcell *et al.,* 2007) for missingness per individual (MIND)>0.1; Hardy-Weinberg

equilibrium test p<=0.001; missingness per marker (GENO)>0.1 and minor allele frequency (MAF)<0.02. After quality control, 238 individuals and 47518 SNPs with an avergae call rate of 0.94 were available for further analyses.

Principal component analysis (PCA) was performed using SNP & Variation Suite (SVS) from Golden Helix on all Dohne Merino sheep from Wauldby farm and GADI. Average FEC, LFEC (log-transformed FEC), BCS and FAM recorded over the study period were compared between the different genetic clusters for the Wauldby animals.

Results and discussion

The PCA plot is depicted in Figure 1. Four distinct clusters were observed, with the GADI Dohne Merino sheep population clustering on its own (Cluster 1). Three distinct clusters (Clusters 2 to 4) were observed for the Wauldby Dohne Merinos consisting of a mixture of animals from the case and control groups.

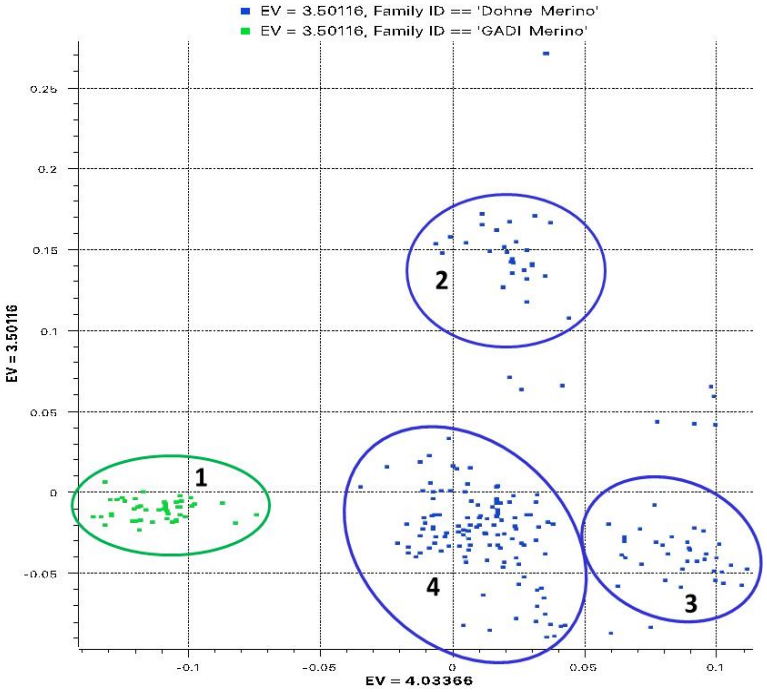

Figure 1. PCA based clustering of GADI Dohne Merino (Cluster1) and Wauldby farm Dohne Merino (Clusters 2-4) sheep.

The averages for the various individual resistance traits or combinations of traits are summarised in Table 1 and Appendix Table A1 for the three genetic clusters of the Wauldby Dohne Merino sheep. Animals in Cluster 3 had lower FEC, lower FAM, higher BCS and higher selection index values than the animals in Clusters 2 and 4. The majority of the sires (88%) of the animals in Cluster 3 was selected for the resistant line, while only 4.0% and 7.8% of the sires in Clusters 2 and 4 respectively, were selected sires.

Conclusion

The Wauldby Dohne Merino and GADI Dohne Merino populations are two genetically different populations. The results indicated that selection for resistance has resulted in genetic differentiation between animals, and the establishment of a more resistant line of animals. It should be possible to select for resistance to *H. contortus* on the basis of the phenotypic traits

included in the study.

Table 1. Averages for resistance traits for the different genetic clusters of the Wauldby Dohne Merino sheep.

Trait	Genetic clusters		
	2	**3**	**4**
FECA	$7249^{a} \pm 770$	$3927^{b} \pm 840$	$5321^{b} \pm 697$
FEC179	$4853^{a} \pm 995$	$1554^{b} \pm 1030$	$4012^{a} \pm 975$
BCSA	2.19 ± 0.08	$2.31^{a} \pm 0.09$	$2.18^{b} \pm 0.08$
BCS179	$2.16^{a} \pm 0.06$	$2.30^{b} \pm 0.06$	$2.19^{a} \pm 0.06$
SI179	$7.68^{a} \pm 0.27$	$8.36^{b} \pm 0.28$	$7.74^{a} \pm 0.27$
EBV-FEC	$115^{a} \pm 98$	-629 ± 84^{b}	-2 ± 45^{a}
EBV-FAM	$-0.029^{a} \pm 0.012$	$-0.024^{a} \pm 0.010$	$0.01\,5^{b} \pm 0.005$
EBV-BCS	$-0.024^{a} \pm 0.009$	$0.058^{b} \pm 0.008$	$0.005^{c} \pm 0.004$

[a,b,c] Values with different superscripts differ significantly ($P < 0.05$) between clusters within rows; FECA/BCSA = Faecal egg count / Body condition score averaged over all recordings per year; FEC179/BCS179= Average faecal egg / body condition score count for the 1st, 7th and 9th recordings; Selection index = Body condition score – Log-transformed FEC – Famacha© score; EBV-FEC = Estimated breeding value for faecal egg count, etc.

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APPENDIX A

Table A1. Averages for resistance traits for the different genetic clusters of the Wauldby Dohne Merino sheep.

Trait	Genetic clusters		
	2	**3**	**4**
FEC1	$7249^{a} \pm 770$	$3927^{b} \pm 840$	$5320^{b} \pm 700$
FEC7	$3993^{a} \pm 1245$	$97^{b} \pm 1358$	$3513^{a} \pm 1126$
FEC8	$4238^{a} \pm 988$	$379^{b} \pm 1077$	$3404^{a} \pm 894$
FEC9	$2722^{a} \pm 778$	$53^{b} \pm 848$	$2581^{a} \pm 704$
FEC11	$2068^{a} \pm 464$	$771^{b} \pm 506$	$1983^{a} \pm 420$
FECA	$7249^{a} \pm 770$	$3927^{b} \pm 840$	$5321^{b} \pm 697$
FEC179	$4853^{a} \pm 995$	$1554^{b} \pm 1030$	$4012^{a} \pm 975$
FAM9	0.94 ± 0.13	$0.79^{a} \pm 0.13$	$0.97^{b} \pm 0.13$
FAM10	$1.06^{a} \pm 0.11$	$0.87^{b} \pm 0.11$	$1.02^{a} \pm 0.10$
FAM179	1.70 ± 0.19	1.91 ± 0.20	1.93 ± 0.19
BCS7	$2.12^{a} \pm 0.07$	$2.31^{b} \pm 0.08$	2.21 ± 0.07
BCS8	$2.14^{a} \pm 0.07$	$2.32^{b} \pm 0.07$	$2.24^{b} \pm 0.07$
BCS9	2.19 ± 0.06	2.26 ± 0.07	2.18 ± 0.06
BCS10	$2.23^{a} \pm 0.06$	$2.35^{b} \pm 0.07$	$2.32^{b} \pm 0.06$
BCSA	2.19 ± 0.08	$2.31^{a} \pm 0.09$	$2.18^{b} \pm 0.08$
BCS179	$2.16^{a} \pm 0.06$	$2.30^{b} \pm 0.06$	$2.19^{a} \pm 0.06$
SI179	$7.68^{a} \pm 0.27$	$8.36^{b} \pm 0.28$	$7.74^{a} \pm 0.27$
EBV-FEC	115 ± 98^{a}	-629 ± 84^{b}	-2 ± 45^{a}
EBV-LFEC	0.067 ± 0.026^{a}	-0.209 ± 0.022^{b}	0.005 ± 0.012^{a}
EBV-FAM	-0.029 ± 0.012^{a}	-0.024 ± 0.010^{a}	0.015 ± 0.005^{b}
EBV-BCS	-0.024 ± 0.009^{a}	0.058 ± 0.008^{b}	0.005 ± 0.004^{c}

[a,b,c] Values with different superscripts differ significantly ($P < 0.05$) between clusters within rows

FEC1 = Faecal egg count of 1st recording, etc.; FECA = Faecal egg count averaged over all recordings per year; FEC169 = Average faecal egg count for the 1st, 6th and 9th recordings, etc; FAM9 = Famacha© score for the 9th recording; FAM179 = Average Famacha© score for the 1st, 7th and 9th recordings; BCS7 = Body condition score for the 7th recording; BCSA = Body condition score averaged over all recordings per year; BCS179 = Average body condition score for the 1st, 7th and 9th recordings; Selection index = Body condition score – Log-transformed FEC – Famacha© score; EBV-FEC = Estimated breeding value for faecal egg count, etc.