

# Genomic evaluation of resistance to *Haemonchus contortus* in a South African Dohne Merino flock

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## Highlights

- Investigated phenotypic and genetic differences in resistance to *H. contortus*.
- PCA indicated four distinct genetic clusters, not based on dosed classification.
- One superior cluster in terms of FEC and BCS was identified.
- Breeding for resistance against nematodes is feasible.

## Abstract

The aim of this study was to use genome-wide SNP data to investigate phenotypic and genetic differences in resistance to *Haemonchus contortus* between resistant and susceptible South African Dohne Merino sheep. The participating farm (Wauldby) has a history of heavy *H. contortus* challenge and implemented a selection strategy for resistance to *H. contortus* in 2011. Faecal egg count (FEC), body condition scores (BCS) and Famacha<sup>®</sup> scores (FAM) were recorded annually on lambs from weaning in January until the end of June from 2011 to 2014. Animals with FAM scores of  $\geq 2.5$  or BCS scores  $< 1.5$  were subjected to anthelmintic treatment and recorded as “Dosed” animals. Animals (196) were selected for genotyping based on EBV for FEC and classified into Case and Control groups on the basis of whether they were dosed or not. The Grootfontein Dohne Merino flock has never been subjected to selection for resistance to gastrointestinal parasites and 48 animals were selected on FEC for genotyping. DNA obtained from blood samples were genotyped using the Illumina<sup>®</sup> Ovine SNP50 BeadChip. Principal component analysis (PCA) resulted in four distinct genetic clusters, with the Grootfontein sheep population clustering separately. The Wauldby animals in Cluster 3 had significantly lower FEC and higher BCS than the animals in Clusters 2 and 4. FEC breeding values of  $114 \pm 97$ ,  $-629 \pm 84$  and  $-2 \pm 45$  were recorded for PCA-based Genetic cluster 2, 3 and 4 animals, respectively. The majority (88%) of animals in Cluster 3 were the progeny of sires from the resistant line. The average number of runs of homozygosity (ROH) was 44, 46, 54 and 47 per animal for Clusters 1, 2, 3 and 4 respectively. The highest number of ROHs was found on chromosomes 1, 2, 3, 4, 6, 9 and 10, on which QTL for resistance traits have been identified previously. Sires in Genetic cluster 3 were highly resistant and can be used in a breeding program to develop *H. contortus* resistant sheep. The results indicated genetic variation in host resistance against *H. contortus* in the Wauldby flock and breeding for resistance against nematodes is feasible.

**Key words:** body condition score, gastrointestinal nematodes, genomic, Famacha<sup>®</sup> score, faecal egg count

## 1. Introduction

Parasitic nematodes are a major constraint for sheep production throughout the world (Vijayasarithi et al., 2016), and is one of the most important restrictions affecting sheep production in South Africa (Kunene, 2010). Alba-Hurtado and Muñoz-Guzmán (2013) reported that losses due to gastrointestinal nematodes (GIN) have been estimated at approximately US\$ 400 million per annum in Australia and up to US\$ 26 million, US\$ 46 million and US\$ 103 million in Kenya, South Africa and India respectively. *Haemonchus contortus* is one of the most economically important GIN infecting hundreds of millions of small ruminants worldwide (Gasser et al., 2016).

The control of GIN in sheep has largely been based on the use of drugs (Bakunzi, 2003). The presence of anthelmintic resistance (AR) has however, made the use of anthelmintic drugs to control GIN unsustainable (McManus et al., 2014). Factors that further contribute to the unsustainability of anthelmintics use are the high cost of the drugs (Mpetile et al., 2015), consumer concern about possible residual effects (Vijayasarithi et al., 2016) and the fact that development of new, effective anthelmintics is too expensive (De Souza Chagas et al., 2016). Thus there is a need for more sustainable, realistic and cost-effective helminth management strategies (Bath, 2014). Breeding programs for enhanced host genetic resistance to parasites could provide a long term solution to the problems associated with the use of anthelmintic drugs (Bishop, 2012; Alba-Hurtado and Muñoz-Guzmán, 2013; Greer and Hamie, 2016).

There is a significant variation in resistance to GIN within and between sheep breeds, which is presumably due to underlying genetic diversity (McRae et al., 2014). Within-breed

genetic variation in resistance to nematode infection in sheep has been reported for various breeds (Khusro et al., 2004; Cloete et al., 2007; Morris et al., 2010; McRae et al., 2014).

Selection for nematode resistance has mainly been based on the use of indicator traits such as faecal egg count (Riggio et al., 2013), Famacha<sup>®</sup> score (Burke and Miller, 2008) and body condition score (Cornelius et al., 2014). In most within-breed studies of genetic resistance, FEC was used as the indicator trait for resistance (Cloete et al., 2007; Marshall et al., 2009; Morris et al., 2010; Bishop, 2012; Alba-Hurtado and Muñoz-Guzmán, 2013).

The Ovine SNP50 BeadChip which became commercially available in 2008 provides 54241 equally spaced SNPs across the sheep genome for association analysis (Kijas et al., 2009). Studies using the Ovine SNP50 BeadChip have yielded consistent results with an improved ability to detect contributions of QTL with small and moderate effects to resistance to GIN (Kemper et al., 2011; Riggio et al., 2013; McRae et al., 2014; Riggio et al., 2014). This technology also provides a fast way to detect regions under selection and could be used in the identification of genes under selection in sheep resistant or susceptible to GIN. An investigation of the phenotypic and genetic differences between resistant and susceptible lines can provide information for the development of breeding plans that could be used to control GIN.

Due to the difficulty of routinely collecting phenotypic data associated with resistance to internal parasites, suitable data sets to study resistance against *H. contortus* is scarce in South Africa. The history of and recent selection practices followed in the Dohne Merino sheep flock of the farm Wauldby, located in the Stutterheim district in the Eastern Cape Province of South Africa, makes it an ideal resource for research into resistance to *H. contortus* (Fisher et al., 2015; Snyman and Fisher, 2018). The aim of this study was to use Ovine SNP50 BeadChip technology to investigate phenotypic and genetic differences between GIN resistant or susceptible animals of the Wauldby Dohne Merino flock.

## 2. Material and Methods

### 2.1 Study site and experimental animals

The study was conducted at the farm Wauldby in the Stutterheim district in the Eastern Cape Province (27° 37' East, 32° 35' South) of South Africa. Rainfall and temperature averages are high from January to April and from October to December. The mean annual rainfall is 800 mm, with most rainfall occurring during summer. The average midday temperatures for Stutterheim range from 17.9 °C in June to 25.7 °C in February. The region is the coldest during July when the mercury drops to 4 °C on average during the night (<https://www.worldweatheronline.com/stutterheim-weather-history/eastern-cape/za.aspx>).

Wauldby has a well-documented history of heavy *H. contortus* challenge and AR of the *H. contortus* strains present on the farm. In the past, the farm was used for several trials relating to resistance of *H. contortus* to anthelmintics (Fisher et al., 2015). The severe AR problem on the farm has inadvertently resulted in selection of sheep over many years with a high degree of resistance to internal parasites as drenching with anthelmintics has been largely ineffective (Fisher et al., 2015; Snyman and Fisher, 2018). In 2011, a breeding plan aimed at improved resistance against *H. contortus* was implemented in the Wauldby Dohne Merino flock. Selection was based on faecal egg counts (FEC), Famacha<sup>®</sup> score (FAM) and body condition score (BCS). The selection procedures are described in detail by Snyman and Fisher (2018) and Snyman and Fisher (2019).

FEC, BCS and 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. FEC were recorded with the McMaster procedure (Van Schalkwyk et al., 1994), while FAM was performed according to the method described by Malan et al. (2000). Body condition score was assessed on a scale of one to five, with one being an emaciated sheep, three being a sheep in average condition and five being an obese sheep (Thompson and Meyer, 1994). Only one

assessor was allocated to conduct the BCS and FAM scoring in order to eliminate operator variance. FAM was recorded weekly, while FEC and BCS scores were recorded at two-weekly intervals. Between 10 and 12 two-weekly recordings of FEC were performed per year. Animals with FAM scores of  $\geq 2.5$  or BCS scores  $< 1.5$  were subjected to anthelmintic treatment and recorded as “Dosed” animals. Animals that did not receive any anthelmintic treatment were recorded as “Not dosed”. Replacement lambs for the resistant line were selected from those lambs that did not receive any anthelmintic treatment. The available data recorded from 2012 (2011-born lambs) until 2015 (2014-born lambs) were used for this study.

Phenotypes for FEC, BCS and FAM from 940 individuals were recorded over the four year period. From these animals, 196 were selected on the basis of the estimated breeding values (EBV) for FEC (Table 1) for inclusion in this study. Among the Dosed and Not dosed groups, animals with the highest and lowest EBV for FEC were selected within years to account for any possible genetic trends.

**Table 1** Number of Wauldby and Grootfontein animals selected for genotyping

Year of birth	Number of Wauldby animals			
	Dosed (Case)		Not dosed (Control)	
	Low EBV FEC	High EBV FEC	Low EBV FEC	High EBV FEC
2011	7	7	7	7
2012	6	6	13	10
2013	18	18	17	16
2014	17	17	15	15
<b>Total (196)</b>	<b>48</b>	<b>48</b>	<b>52</b>	<b>48</b>

Year of birth	Number of Grootfontein animals			
	Low FEC		High FEC	
	Ram lambs	Ewe lambs	Ram lambs	Ewe lambs
2014	6	6	6	6
2016	6	6	6	6
<b>Total (48)</b>	<b>12</b>	<b>12</b>	<b>12</b>	<b>12</b>

The Grootfontein Dohne Merino flock is kept under veld conditions at the Grootfontein Agricultural Development Institute (GADI) near Middelburg (31° 28' South, 25° 1' East) in the Eastern Cape Province of South Africa. The mean annual rainfall at Grootfontein is 373 mm,

with 22% occurring in spring, 39% in summer, 30% in autumn and 9% in winter. The average minimum temperature (July) is -0.4 °C and the average maximum temperature (January) is 30.3 °C. The Grootfontein Dohne Merino animals were included as a reference population in the study, as they have not been subjected to any selection for resistance against *H. contortus*. However, since 2005, animals in the flock only received anthelmintic treatment when pooled sample faecal egg counts were high. Data recorded on 619 animals born in 2014 and 469 animals born in 2016 were available. Three recordings per year of FEC in 2014 and 2016, as well as 2 recordings of FAM in 2016 were done on the Grootfontein animals. As data from only two years were available, no EBVs were estimated, but animals with the highest and lowest FEC within each year were selected for genotyping (Table 1).

## 2.2 Statistical Analysis

Data on FEC were transformed to logarithms ( $\text{Log}_{10}(\text{FEC}+10)$ ) to improve the distribution of the data. Both the untransformed data (FEC) and the log-transformed data (LFEC) were used in all the analyses.

The minimum, maximum, mean, standard deviation (SD) and coefficient of variation (CV) for FAM, BCS, FEC and LFEC for all animals alive at the end of the trials from 2011 to 2014 for the different recordings were obtained with PROC MEANS of SAS (SAS, 2016). For these analyses, the average of recording 1 for all available FEC records in 2011, 2012, 2013 and 2014 was calculated to obtain FEC1. The average of recording 2 for all available FEC records in 2011, 2012, 2013 and 2014 was calculated to obtain FEC2, and so forth to obtain averages for all traits, for all 12 recordings. Recording 5 was excluded from the analyses for the Wauldby animals due to incomplete data. An Average FEC record was calculated for each animal for each year as the average FEC over all 12 recordings for that specific animal for that

specific year (FECA). Average FAM (FAMA), Average BCS (BCSA) and Average LFEC (LFECA) were calculated in the same way.

The influence of non-genetic effects on these phenotypic traits was estimated. The non-genetic effects tested for significance were year of birth, sex, birth status (1 = lambs born as singles, 2 = lambs born as twins, 3 = lambs born as triplets), how many times (0 to 4) an animal was dosed, genotyping group (Case or Control – Wauldby; High or Low FEC – Grootfontein) and the respective two way interactions. Age of the animal at recording was included as a covariate. The PROC GLM procedure of the SAS statistical package was used to determine which of these fixed effects had a significant influence on the average of the resistance traits recorded over the experimental period (SAS, 2016). None of the two way interactions, or age at recording had a significant influence on any of the resistance traits.

Analyses of the resistance data recorded on the Wauldby animals indicated that a combination of information recorded at the first, sixth and ninth recordings (FEC169, LFEC169, BCS169, FAM169 etc.) could be used as basis for selection of resistance against *H. contortus* (Snyman et al., 2018). Least-square means for these combination traits, as well as FECA, LFECA, FAMA and BCSA were compared amongst the three genetic clusters of the Wauldby animals obtained with principle component analysis (PCA). Furthermore, estimated breeding values for FECA, LFECA, FAMA and BCSA of the Wauldby animals, as well as their sires, were also compared between the three genetic clusters (SAS, 2016).

### **2.3 Genotyping**

Blood samples from 244 animals were received from GADI Bio-bank with approval from the Ethical Committee of GADI (GVE/AP2/21/1) and the Animal Ethics Committee of the Faculty of Natural and Agricultural Sciences at the University of Pretoria (EC161205-088). DNA was isolated using the DNA isolation NucleoMag<sup>®</sup> VET kit (NucleoMag -



MACHEREY-NAGEL GmbH and Co KG, Düren, Germany). Animals were genotyped at the Agricultural Research Council, Biotechnology Platform (ARC-BTP) with the Illumina® Ovine SNP50 BeadChip (Illumina Inc., San Diego, CA). In total 196 Dohne Merino sheep from Wauldby and 48 Dohne Merino sheep from Grootfontein were genotyped. SNP-calling was done using the Illumina® Genome Studio software v2.0 (Illumina, San Diego, California 92122 U.S.A).

## **2.4 Quality control**

The SNP genotype data were subjected to quality control measures using PLINK v1.07 software (Purcell et al., 2007) for missingness per individual (MIND), Hardy-Weinberg equilibrium test (HWE), missingness per marker (GENO) and minor allele frequency (MAF). After data quality control, 2 individuals were removed for low genotyping (MIND >0.1) and were excluded from further downstream analysis. Four hundred and eighty markers were excluded based on the HWE test ( $P \leq 0.001$ ), 3260 SNPs failed the missingness test (GENO >0.1) and 6166 SNPs failed the frequency test (MAF <0.02). After pruning, there were 47518 SNPs with an average call rate of 0.94 available for further analyses.

## **2.5 Population genetic clustering**

A principal component analysis (PCA) was performed to determine the relationship between individuals of the two Dohne Merino sheep populations using SNP and Variation Suite (SVS) software (GoldenHelix Inc., Bozeman, MT, USA). Animals in the data set were allocated to a specific genetic cluster based on PCA clustering. Genetic clusters were described in terms of the year of birth, sex, birth status, dosing status, genotyping groups and parentage (in the case of the Wauldby animals).

## **2.6 Runs of Homozygosity (ROH)**

Runs of homozygosity (ROH) were identified for each PCA-based genetic cluster using PLINK v1.07 software (Purcell et al., 2007). The PLINK algorithm defines ROH as regions of homozygous genotypes that were greater than 1000 kb in length identified in the genome with a sliding window of 50 SNPs. The algorithm allowed one heterozygous SNP per run and no more than 3 missing genotypes within a window.

A search for QTLs associated with parasite resistance on chromosomes that had a high number of runs of homozygosity was undertaken. The Sheep Quantitative Trait Loci Database (QTLdb) was used (<http://www.animalgenome.org/QTLdb/sheep>) for this purpose.

## **3. Results**

### **3.1 Descriptive statistics of phenotypic traits**

Descriptive statistics for the phenotypic traits recorded over the experimental period for all the Wauldby and Grootfontein animals are presented in Table 2. Mean FEC peaked at the third recording and remained high until the seventh recording, after which it declined steadily. From the maximum FEC values recorded, it is clear that the lambs were subjected to high *Haemonchus* challenge, even at the last two recordings during June. Despite this high challenge, mean FAM was still low, implying no anaemia. A FAM score of 4.0 was only awarded during the first three recordings.

CV for FEC was very high, emphasizing the large phenotypic variation in FEC in this flock. It is evident that log transformation of FEC resulted in coefficients of variation that decreased from between 114% and 163% to below 30%. CV for FAM were also higher than for most production traits, while the CV for BCS were in the range of that generally recorded for body weight in sheep.

**Table 2** Descriptive statistics of the phenotypic data recorded on all the Wauldby animals from 2011 until 2014 and on all the Grootfontein animals during 2014 and 2016

Trait	Recording <sup>a</sup>	n <sup>c</sup>	Minimum	Maximum	Mean	SD	CV	
<b>Wauldby animals</b>								
FAM	1	940	1.0	4.0	1.31	0.58	44.35	
	2	940	1.0	4.0	1.29	0.50	38.82	
	3	940	1.0	4.0	1.40	0.59	42.02	
	4	940	1.0	3.0	1.29	0.51	39.80	
	6	940	1.0	3.0	1.41	0.58	41.17	
	7	940	1.0	3.0	1.38	0.55	39.59	
	8	940	1.0	3.0	1.30	0.49	38.01	
	9	940	1.0	3.0	1.21	0.42	34.67	
	10	940	1.0	2.0	1.11	0.31	27.79	
	11	940	1.0	3.0	1.08	0.30	27.87	
	12	940	1.0	3.0	1.13	0.34	30.30	
	FAMAvg <sup>b</sup>			1.0	3.18	1.26	0.47	36.76
	BCS	1	940	1.2	3.5	2.31	0.39	17.02
2		940	1.0	3.0	2.14	0.35	16.28	
3		940	1.0	3.0	2.09	0.35	16.80	
4		940	1.4	3.0	2.09	0.33	15.61	
6		940	1.3	3.0	2.13	0.31	14.75	
7		940	1.0	3.5	2.14	0.31	14.26	
8		940	1.4	3.0	2.15	0.29	13.39	
9		940	1.4	3.0	2.12	0.30	14.29	
10		940	1.3	3.0	2.13	0.27	12.47	
11		940	1.0	3.0	2.10	0.27	13.07	
12		940	1.4	3.0	2.06	0.27	13.13	
BCSAvg				1.85	3.09	2.13	0.35	13.73
FEC		1	940	0	28600	3672	4260	116
	2	940	0	36900	3376	4335	128	
	3	940	0	38100	5150	5450	106	
	4	940	0	45800	3727	4890	131	
	6	940	0	35900	4022	4924	122	
	7	940	0	37900	3996	4657	117	
	8	940	0	29000	3212	3992	124	
	9	940	0	37600	2853	3934	138	
	10	940	0	40100	2500	3941	158	
	11	940	0	52500	1991	3027	152	
	12	940	0	26700	1596	2303	144	
	FECAvg			0	37191	3281	4156	131
	LFEC	1	940	1.00	4.46	3.17	0.77	24.41
2		940	1.00	4.57	3.12	0.76	24.34	
3		940	1.00	4.58	3.37	0.74	21.93	
4		940	1.00	4.66	3.09	0.87	28.30	
6		940	1.00	4.56	3.13	0.87	27.66	
7		940	1.00	4.58	3.22	0.76	23.67	
8		940	1.00	4.46	3.10	0.76	24.66	
9		940	1.00	4.58	3.03	0.77	25.42	
10		940	1.00	4.60	3.00	0.71	23.60	
11		940	1.00	4.72	2.91	0.73	25.04	
12		940	1.00	4.43	2.77	0.76	27.48	
LFECAvg				1.00	4.58	3.08	0.77	25.14
<b>Grootfontein animals</b>								
FAM	2	469	1	5	2.3	0.83	36.0	
	3	450	1	3	1.4	0.54	37.8	
FEC	1	1075	0	5600	106	341	321	
	2	1557	0	147000	9937	8388	84	
	3	1194	0	30200	2176	3888	179	
LFEC	1	1075	1	3.45	1.39	0.65	46.9	
	2	1557	1	2.18	3.86	0.40	10.4	
	3	1194	1	4.48	2.70	0.91	33.5	

<sup>a</sup> Recording: FAM1 = The average of recording 1 of FAM in 2011, 2012, 2013 and 2014 (Wauldby) or 2014 and 2016 (Grootfontein) was calculated to obtain FAM1, etc. <sup>b</sup> FAMAvg: The average FAM over all 12 recordings per animal for each year was calculated. These average FAM values obtained for 2011 to 2014 were averaged to obtain the FAMAvg presented in the table for the Wauldby animals. BCSAvg, FECAvg and LFECavg were calculated the same way.

<sup>c</sup> Number of data records used for calculations

### 3.2 Influence of non-genetic effects on phenotypic traits

The effect of year of birth, sex, birth status, dosing status and group on the phenotypic traits recorded over the experimental period are summarised in Table 3 for all the animals. For the Wauldby animals, year of birth had a significant effect at  $P < 0.01$  on all traits. Sex had no effect on BCS and birth status had no effect on FAM. Male lambs had a higher average FEC and FAM than female lambs, while single-born lambs had a higher average FEC than twins and triplets. The number of times a lamb was dosed had a significant effect on all the phenotypic traits. Lambs that were not dosed had lower FEC, LFEC and FAM and a higher BCS than lambs that were dosed once or more. There were no significant differences between the genotyped Case and Control Wauldby animals in any of the traits.

**Table 3** Effect of year of birth, sex, birth status and dosing status on the phenotypic traits ( $\pm$  s.e.) recorded over the experimental period for all the Wauldby and Grootfontein animals

Effect	FAM	BCS	FEC	LFEC
<b>Wauldby animals</b>				
<b>Year of birth</b>				
2011	1.41 <sup>a</sup> $\pm$ 0.10	2.55 <sup>a</sup> $\pm$ 0.06	6543 <sup>a</sup> $\pm$ 771	3.67 <sup>a</sup> $\pm$ 0.14
2012	1.66 <sup>b</sup> $\pm$ 0.10	2.06 <sup>b</sup> $\pm$ 0.05	4297 <sup>b</sup> $\pm$ 766	2.96 <sup>b</sup> $\pm$ 0.14
2013	1.80 <sup>c</sup> $\pm$ 0.10	1.92 <sup>c</sup> $\pm$ 0.05	5330 <sup>c</sup> $\pm$ 760	3.37 <sup>c</sup> $\pm$ 0.14
2014	1.79 <sup>c</sup> $\pm$ 0.10	1.77 <sup>d</sup> $\pm$ 0.06	5604 <sup>c</sup> $\pm$ 793	3.58 <sup>a</sup> $\pm$ 0.14
<b>Sex</b>				
Male	1.82 <sup>a</sup> $\pm$ 0.10	2.08 <sup>a</sup> $\pm$ 0.05	6359 <sup>a</sup> $\pm$ 749	3.54 <sup>a</sup> $\pm$ 0.13
Female	1.51 <sup>b</sup> $\pm$ 0.10	2.07 <sup>a</sup> $\pm$ 0.05	4528 <sup>b</sup> $\pm$ 755	3.25 <sup>b</sup> $\pm$ 0.13
<b>Birth status</b>				
1	1.80 <sup>a</sup> $\pm$ 0.06	2.11 <sup>a</sup> $\pm$ 0.04	6609 <sup>a</sup> $\pm$ 506	3.54 <sup>a</sup> $\pm$ 0.09
2	1.78 <sup>a</sup> $\pm$ 0.06	2.04 <sup>b</sup> $\pm$ 0.04	5889 <sup>b</sup> $\pm$ 500	3.41 <sup>b</sup> $\pm$ 0.09
3	1.42 <sup>a</sup> $\pm$ 0.23	2.08 <sup>ab</sup> $\pm$ 0.13	3831 <sup>b</sup> $\pm$ 1763	3.23 <sup>ab</sup> $\pm$ 0.31
<b>Number of times dosed</b>				
0	1.01 <sup>a</sup> $\pm$ 0.08	2.39 <sup>a</sup> $\pm$ 0.04	1862 <sup>a</sup> $\pm$ 584	2.92 <sup>a</sup> $\pm$ 0.10
1	1.24 <sup>b</sup> $\pm$ 0.08	2.28 <sup>b</sup> $\pm$ 0.04	3291 <sup>b</sup> $\pm$ 600	3.20 <sup>b</sup> $\pm$ 0.11
2	1.68 <sup>c</sup> $\pm$ 0.09	2.16 <sup>c</sup> $\pm$ 0.05	5469 <sup>c</sup> $\pm$ 682	3.47 <sup>c</sup> $\pm$ 0.12
3	1.79 <sup>c</sup> $\pm$ 0.14	1.83 <sup>d</sup> $\pm$ 0.08	7392 <sup>c</sup> $\pm$ 1085	3.48 <sup>bc</sup> $\pm$ 0.19
4	2.60 <sup>d</sup> $\pm$ 0.29	1.72 <sup>d</sup> $\pm$ 0.16	9201 <sup>c</sup> $\pm$ 2261	3.90 <sup>bc</sup> $\pm$ 0.40
<b>Group*</b>				
Case (Dosed)	1.61 <sup>a</sup> $\pm$ 0.14	2.17 <sup>a</sup> $\pm$ 0.07	5599 <sup>a</sup> $\pm$ 1038	3.27 <sup>a</sup> $\pm$ 0.22
Control (Not dosed)	1.42 <sup>a</sup> $\pm$ 0.35	2.29 <sup>a</sup> $\pm$ 0.19	5919 <sup>a</sup> $\pm$ 2609	3.63 <sup>a</sup> $\pm$ 0.55
<b>Grootfontein animals</b>				
<b>Year of birth</b>				
2014	-		9103 <sup>a</sup> $\pm$ 323	3.79 <sup>a</sup> $\pm$ 0.02
2016	1.94 $\pm$ 0.04		6402 <sup>b</sup> $\pm$ 364	3.65 <sup>a</sup> $\pm$ 0.02
<b>Sex</b>				
Male	1.93 <sup>a</sup> $\pm$ 0.05		5677 <sup>a</sup> $\pm$ 335	3.60 <sup>a</sup> $\pm$ 0.02
Female	1.96 <sup>a</sup> $\pm$ 0.05		9828 <sup>b</sup> $\pm$ 345	3.84 <sup>b</sup> $\pm$ 0.02
<b>Birth status</b>				
1	1.85 <sup>a</sup> $\pm$ 0.05		7665 <sup>a</sup> $\pm$ 324	3.73 <sup>a</sup> $\pm$ 0.02
2	1.88 <sup>ab</sup> $\pm$ 0.03		7416 <sup>a</sup> $\pm$ 234	3.73 <sup>a</sup> $\pm$ 0.01
3	2.10 <sup>b</sup> $\pm$ 0.11		8177 <sup>a</sup> $\pm$ 763	3.71 <sup>a</sup> $\pm$ 0.05
<b>Group*</b>				
High	2.47 <sup>a</sup> $\pm$ 0.18		20181 <sup>a</sup> $\pm$ 1501	4.24 <sup>a</sup> $\pm$ 0.05
Low	1.73 <sup>b</sup> $\pm$ 0.18		1125 <sup>b</sup> $\pm$ 1573	2.93 <sup>b</sup> $\pm$ 0.05

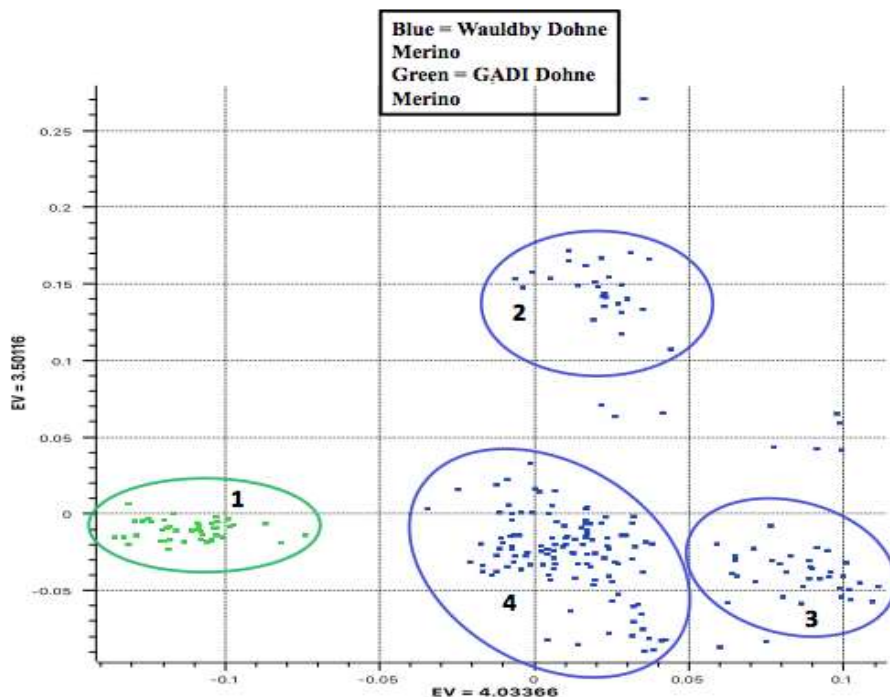
<sup>a,b,c,d</sup> Values with different superscripts within effects and traits differed significantly ( $P < 0.01$ )

\* Case/Control and High/Low = Only for the genotyped animals

The 2014-born Grootfontein lambs had a higher FEC than the 2016-born lambs. In contrast to the Wauldby animals, ewe lambs had higher FEC than male lambs in the Grootfontein flock. Lambs born as triplets had a higher FAM than single lambs. Group (High/Low FEC) had a significant effect on all traits and lambs in the high FEC category also had a higher FAM than the lambs in the lower FEC category.

### 3.3 Population genetic clustering

The PCA plot including only the Wauldby Dohne Merino animals revealed that the animals did not cluster according to Cases and Controls. The same trend was observed regarding the High and Low FEC animals of the Grootfontein flock. Subsequently, PCA was performed on the genotypes of all animals (without pre-defining any possible groups) to investigate the genetic differentiation between all animals. The resulting PCA plot can be seen in Figure 1.



**Figure 1** PCA based clustering of the Wauldby and Grootfontein Dohne Merino sheep populations. Genetic cluster 1 = Grootfontein Dohne Merino animals; Genetic cluster 2, 3 and 4 = Wauldby Dohne Merino animals; Outliers = Wauldby Dohne Merino animals

Four distinct genetic clusters were observed from the PCA. The Grootfontein Dohne Merino sheep population had its own separate genetic cluster (Cluster 1). The Wauldby Dohne Merino population differentiated into three distinct genetic clusters, consisting of a mixture of lambs born between 2011 and 2014, including both Cases and Controls in all clusters.

**Table 4** Composition of Clusters in terms of fixed effect classes, group and parentage of animals in each genetic cluster

Effect	No of animals per genetic cluster				Outliers
	1	2	3	4	
No of animals	48	25	34	116	12
<b>Year of birth</b>					
2011	-	5	0	22	0
2012	-	3	9	21	2
2013	-	6	0	55	4
2014	24	11	25	18	6
2016	24	-	-	-	-
<b>Sex</b>					
Male	24	11	23	50	5
Female	24	14	11	66	7
<b>Birth status</b>					
1	12	15	20	70	12
2	30	10	14	46	0
3	6	-	-	-	-
<b>Dosing status</b>					
10	-	13	19	62	4
21	-	8	11	43	7
22	-	2	4	11	1
23	-	2	0	0	0
<b>Case/Control</b>					
Case	-	12	15	55	8
Control	-	13	19	61	4
<b>FEC</b>					
High	25	-	-	-	-
Low	23	-	-	-	-
<b>Parentage (Animals having selected/other sires/dams as parents)</b>					
Selected sire / selected dam	-	1	27	9	6
Selected sire / other dam	-	0	3	0	0
Other sire / selected dam	-	9	2	35	3
Other sire / other dam	-	15	2	72	3

Genetic cluster 1 = Grootfontein Dohne Merino animals

Genetic cluster 2, 3 and 4 = Wauldby Dohne Merino animals

Outliers = Wauldby Dohne Merino animals

The description of the genetic clusters in terms of year of birth, sex, birth status, dosing status, Case/Control groups, High/Low FEC and parentage is presented in Table 4. Genetic

cluster 4 was the largest and comprised 116 individuals. One of the animals in Genetic cluster 2, 30 in Genetic cluster 3, and nine in Genetic cluster 4 were progeny of sires that were selected for the resistant line.

### 3.4 Least-square means of the traits by cluster for the genotyped Wauldby animals

Least square means for the various phenotypic traits associated with resistance for the different clusters of the Wauldby animals are presented in Table 5. Animals in Cluster 3 had significantly lower FEC169, LFEC169 and higher BCS169 than the animals in Clusters 2 and 4. Superior EBVs for all traits except FAM were also observed for Genetic cluster 3.

**Table 5** Averages for resistance traits, phenotypic trait combinations, as well as estimated breeding values (EBV) ( $\pm$  s.e) for the genotyped offspring, as well as their sires, in the different genetic clusters

Trait	Genetic cluster 2	Genetic cluster 3	Genetic cluster 4
<b>Genotyped offspring</b>			
FECA	7269 <sup>a</sup> $\pm$ 788	3936 <sup>b</sup> $\pm$ 862	5338 <sup>b</sup> $\pm$ 714
FEC169	4661 <sup>a</sup> $\pm$ 678	1362 <sup>b</sup> $\pm$ 742	3821 <sup>a</sup> $\pm$ 615
LFECA	3.60 <sup>a</sup> $\pm$ 0.17	3.11 <sup>b</sup> $\pm$ 0.19	3.28 <sup>ab</sup> $\pm$ 0.15
LFEC169	3.15 <sup>a</sup> $\pm$ 0.14	2.62 <sup>b</sup> $\pm$ 0.15	3.10 <sup>a</sup> $\pm$ 0.13
FAMA	1.53 <sup>a</sup> $\pm$ 0.11	1.59 <sup>a</sup> $\pm$ 0.12	1.60 <sup>a</sup> $\pm$ 0.10
FAM169	1.38 <sup>a</sup> $\pm$ 0.07	1.36 <sup>a</sup> $\pm$ 0.07	1.41 <sup>a</sup> $\pm$ 0.06
BCSA	2.17 <sup>ab</sup> $\pm$ 0.08	2.29 <sup>a</sup> $\pm$ 0.09	2.16 <sup>b</sup> $\pm$ 0.08
BCS169	2.07 <sup>a</sup> $\pm$ 0.04	2.20 <sup>b</sup> $\pm$ 0.04	2.09 <sup>a</sup> $\pm$ 0.04
EBV-FEC	114 <sup>a</sup> $\pm$ 97	-629 <sup>b</sup> $\pm$ 84	-2 <sup>a</sup> $\pm$ 45
EBV-LFEC	0.037 <sup>a</sup> $\pm$ 0.025	-0.209 <sup>b</sup> $\pm$ 0.022	0.005 <sup>a</sup> $\pm$ 0.012
EBV-FAM	-0.029 <sup>a</sup> $\pm$ 0.011	-0.025 <sup>a</sup> $\pm$ 0.010	0.015 <sup>b</sup> $\pm$ 0.005
EBV-BCS	-0.024 <sup>a</sup> $\pm$ 0.009	0.058 <sup>b</sup> $\pm$ 0.008	0.005 <sup>c</sup> $\pm$ 0.004
<b>Sires of genotyped offspring</b>			
EBV-FEC	-328 <sup>ab</sup> $\pm$ 300	-790 <sup>a</sup> $\pm$ 300	-51 <sup>b</sup> $\pm$ 146
EBV-LFEC	-0.060 <sup>ab</sup> $\pm$ 0.077	-0.227 <sup>a</sup> $\pm$ 0.077	-0.002 <sup>b</sup> $\pm$ 0.038
EBV-FAM	-0.036 <sup>a</sup> $\pm$ 0.025	-0.040 <sup>a</sup> $\pm$ 0.025	0.024 <sup>b</sup> $\pm$ 0.012
EBV-BCS	-0.002 <sup>ab</sup> $\pm$ 0.035	0.072 <sup>a</sup> $\pm$ 0.035	-0.009 <sup>b</sup> $\pm$ 0.017

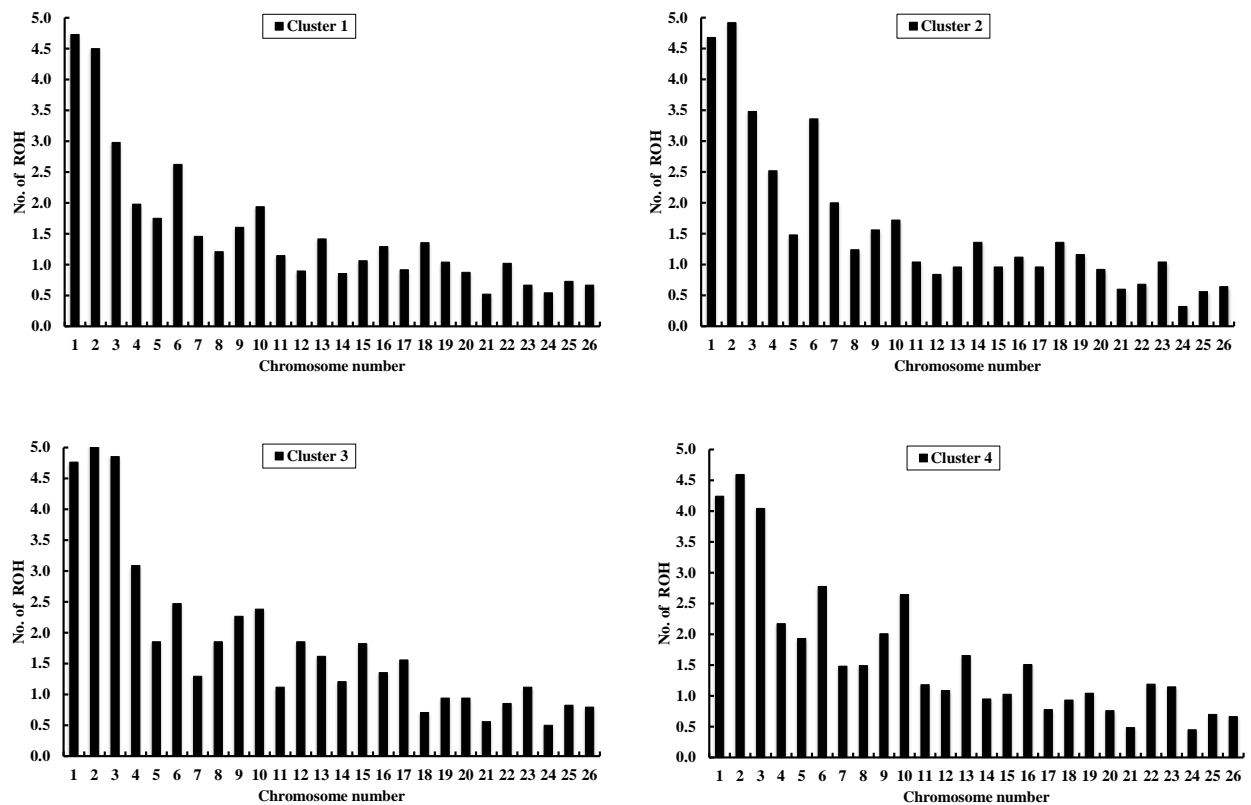
<sup>a,b,c</sup> Values with different superscripts differ significantly ( $P < 0.05$ ) between clusters within rows; Values with the same superscripts did not differ significantly ( $P > 0.05$ ) between clusters within rows; FECA = Faecal egg count averaged over all recordings per year; FEC169 = Average faecal egg count for the 1<sup>st</sup>, 6<sup>th</sup> and 9<sup>th</sup> recordings; EBV-FEC = Estimated breeding value for faecal egg count, etc.

EBVs for the traits of the sires of the animals in the three genetic clusters of the Wauldby animals are also summarised in Table 5. Genetic clusters 3 and 4 differed significantly for all traits, with sires of animals in Genetic cluster 3 showing superior

performance regarding resistance. Sires of animals in Cluster 2 had intermediate EBVs for all the traits.

### 3.5 Runs of homozygosity

The total number of runs of homozygosity observed for all animals was 10546. Of these ROH, 2113 were found in Cluster 1 (48 animals), 1148 in Cluster 2 (25 animals), 1838 in Cluster 3 (34 animals) and 5447 in Cluster 4 (116 animals). The average number of ROH was 44, 46, 54 and 47 per animal for Clusters 1, 2, 3 and 4 respectively. The average number of ROH per animal for each chromosome for the four genetic clusters is depicted in Figure 2.



**Figure 2** The average number of runs of homozygosity (ROH) per animal for each chromosome for Genetic cluster 1 (Grootfontein) and Genetic clusters 2, 3 and 4 (Wauldby)

Chromosomes 1, 2, 3, 4, 6, 9 and 10 had more than two ROH per animal in Genetic clusters 3 and 4. Animals in Genetic cluster 2 had the highest number of ROH per chromosome



(more than two) on Ovine chromosome (OAR) 1, 2, 3, 4, 6, and 7. Grootfontein animals only had more than two ROH per chromosome on OAR 1, 2, 3 and 6. The Sheep Quantitative Trait Loci Database (QTLdb) (<http://www.animalgenome.org/QTLdb/sheep>) was used to identify QTLs associated with parasite resistance. The location of these QTLs in the sheep genome are summarised in Table 6. Parasite resistance traits have been reported throughout the sheep genome and are found almost on all chromosomes. Reported QTLs for faecal egg count and *Haemonchus contortus* FEC are also found on OAR 1, 2, 3, 4, 6, 9 and 10, which corresponds to the chromosomes with the highest number of ROH in the Wauldby and Grootfontein Dohne Merino populations.

**Table 6** The location of different parasite resistance traits in the sheep genome

<b>Parasite resistance traits</b>	<b>QTL location*</b>
Faecal egg count	Chr <b>1, 2, 3, 4, 5, 6, 7, 8, 9, 10</b> , 11, 13, 14, 15, 16, 17, 19, 21, 22, 23, 24, 25 and 26
Eggs per worm	Chr 26
<i>Haemonchus contortus</i> FEC	Chr <b>1, 2, 3, 4, 5, 6, 7, 8, 9, 10</b> , 11, 12, 13, 15, 16, 18, 20, 21, 22, 23, 24, 25 and 26
<i>Haemonchus contortus</i> FEC2	Chr 12, 13, 16, 20, 23
Worm count	Chr <b>2, 12, 16, 18, 22, 23, 24, 25</b> and 26
Nematodirus FEC	Chr <b>2, 3</b> , 11, 14, 15
<i>Trichostrongylus colubriformis</i> FEC	Chr <b>1, 3, 6</b> , 11, 12, 18, 22
Change in haematocrit	Chr <b>2, 3</b> , 11, 13 and 26
Haematocrit	Chr <b>1, 3, 5, 8, 14, 15, 18, 20, 22</b> and 25

\*Chromosomes in bold had a high number of ROH in the present study

#### 4. Discussion

Descriptive statistics for the phenotypic traits (FEC, FAM, BCS and LFEC) recorded over the experimental period were estimated for all the Wauldby and Grootfontein animals. Although high FAM scores of up to 4 were recorded at the beginning of the annual recording period for the Wauldby animals, the mean FAM value over recordings and years were 1.26 (CV = 36.76%). This was marginally lower but in line with mean FAM values (coefficients of variation in brackets) of 1.23 (33%), 1.40 (43%) and 1.90 (42%) which were reported under conditions of low, moderate and peak nematode challenge (Riley and Van Wyk, 2009).

However, the latter study was done on adult ewes, compared to lambs used in the present study. Riley and Van Wyk (2011) also reported a mean FAM of 1.92 (43%) in a South African Merino flock. The mean FAM of the Grootfontein animals varied substantially between recordings, although the CVs were similar to those of the Wauldby animals.

The overall average BCS recorded for the Wauldby animals for all the years was 2.13 (13.73%), but some individual BCS scores recorded from 2011 to 2014 were critically low (1.0). The average BCS of each recording and the overall BCS average were higher than 2.

The maximum FEC values recorded from 2011 to 2014 in the Wauldby flock were very high. This indicates a severe *Haemonchus* challenge at Wauldby. In this study, the observed CVs for FEC were very high (above 100%), suggestive of a very high variation and a non-normal distribution of FEC recordings over the experimental period. The untransformed FEC in the Wauldby animals ranged from 0 to 52500 epg, compared to the range of 0 to 147000 epg of the Grootfontein animals. Large individual variation is expected in untransformed FEC data and is commonly reported in literature. Cloete et al. (2007) and Pollott and Greeff (2004) also found similar distributions and variation in FEC. The results provided in this study are consistent with those reported in the literature (Khusro et al., 2004; Mpetile et al., 2015; Cloete et al., 2016). Differences in the range of FEC observed in different studies are due to breed, nematode species and environmental differences.

FEC is known to be exceedingly variable and skewed, needing transformation prior to analysis to normalize the data (Cloete et al., 2007). Data on FEC were transformed to logarithms to the base of 10 (FEC + 10) to normalise the skewed distribution. The application of transformations on FEC data improved the data as indicated by lower CVs in the log-transformed data. Cloete et al. (2007) and Matebesi-Ranthimo et al. (2014) also reported a CV in faecal worm egg count exceeding 100% before transformation, which reduced to below 20% after log transformation. A similar trend was found in this study, although the level of CV was

slightly higher (up to 28% after transformation). According to Mpetile et al. (2015) high CV values are indicative of significant phenotypic variation, which could lead to improved genetic gains and response to selection.

Year of birth had a significant effect on all phenotypic traits in the Wauldby ( $P < 0.01$ ) and Grootfontein ( $P < 0.05$ ) flocks. The effect of birth year was expected and corresponded to previous literature (Mpetile et al., 2015; Burke et al., 2016).

In the Wauldby flock, male animals had a higher ( $P < 0.01$ ) average FEC than females. This is consistent with results reported by Cloete et al. (2007) and Matebesi-Ranthimo et al. (2014), who observed lower FEC in ewes than in rams. Mpetile et al. (2015) also found that the overall log-transformed FEC of ram lambs was almost double that of ewe lambs ( $P < 0.001$ ). In contrast, female lambs of the Grootfontein flock had higher ( $P < 0.05$ ) FEC than the male lambs. Khusro et al. (2004) also found varying results of sex effects on FEC, where yearling ewes had higher FEC than rams, while the opposite was true in hoggets. Variable results were reported by Abuargob et al. (2014) over a three year period, where rams had higher FEC than ewes in some months, while ewes had higher FEC in other months. Abuargob et al. (2014) furthermore suggested that differences between females and males could be due to differences in behavior, morphology or physiological status of sex and that differences in hormonal status of the sexes may affect the immunological responses of the animal to *H. contortus*. According to Haile et al. (2007), the reported sex differences in favor of females regarding response to GIN infections in many studies suggest that the male flock should be given more attention in order to maintain lower worm burdens.

In the current study, birth status had a significant ( $P < 0.01$ ) effect on FEC in the Wauldby flock, but not in the Grootfontein flock. Single lambs had a higher FECA than twins or triplets. Cloete et al. (2007) and Mpetile et al. (2015) also reported higher FECs in single than in multiple birth types. These results indicate that multiple birth types are able to tolerate

the pathogenic effect of parasites than singles.

Dosing status had a significant effect on all phenotypic traits. Lambs that were not dosed had a lower FEC, higher BCS and lower FAM compared to the lambs that were dosed. In the Wauldby lambs, there were no significant differences in FAM, BCS, FEC or LFEC between the Case and Control group lambs. The decision whether a lamb should be dosed or not was based on its FAM, in combination with its BCS. The FEC was not taken into consideration, in order to allow resilient lambs to be available for the selection line as well (Snyman and Fisher, 2018). Animals were classified into the Case and Control groups based on whether they were dosed or not. Therefore significant differences between the Case and Control groups in the phenotypic traits under investigation *per se* were not expected. This was supported by the fact that the Case and Control animals did not form separate clusters in the PCA.

The three genetic clusters that were formed during PCA for the Wauldby animals might be due to genetic divergence as a result of selection. A high level of genetic diversity was observed in the Wauldby Dohne Merino population. The separate genetic cluster observed for the Grootfontein Dohne Merinos indicated that this flock is genetically distinct from the Wauldby population.

Differences between the three Wauldby genetic clusters were investigated on a phenotypic level. Animals in Cluster 3 had lower FEC and higher BCS than the animals in Clusters 2 and 4. The majority (88%) of animals in Cluster 3 were the progeny of sires selected for the resistant line, while only 4.0% and 7.8% of the animals in Clusters 2 and 4 respectively, were progeny of resistant line sires. These results of both individual traits as well as the combination of recordings one, six and nine indicate that selection for resistance has resulted in genetic differentiation between animals, and the establishment of a more resistant line (Cluster 3) of animals.

Genetic resistance to parasites is arguably the most sustainable way of GIN control, and can be achieved through selection of sires with favorable EBVs. Superior animals will generate offspring with lower FEC, lower FAM and higher BCS. EBVs for the phenotypic traits of the sires differed significantly ( $P < 0.05$ ) between the different genetic clusters and Cluster 3's sires had the lowest EBV-FEC and EBV-FAM. These results show that sires of offspring in Genetic cluster 3 have genetic potential for establishing a *H. contortus* resistant line.

The prevalence and distribution of runs of homozygosity in the Wauldby and Grootfontein Dohne Merino sheep populations and their association with parasite resistance traits were investigated. Selection increases homozygosity around the target region (Peripolli et al., 2017) and therefore, long or high ROHs are expected in regions under selection. The Wauldby farm animals are under selection for parasite resistance and QTLs associated with parasite resistance were expected to be located in high ROH regions. Animals in Cluster 3 had the most ROH per animal (54), which confirms the results from the phenotypic trait differences between the genetic clusters, that animals in Cluster 3 are the most resistant. Grootfontein animals had the least ROH per animal (44), which corroborates that this flock have never been selected for parasite resistance.

Several parasite resistance traits have been investigated in sheep, including *Haemonchus contortus* FEC, worm count and Strongyle FEC. Studies on QTL identification for parasite resistance in sheep have been carried out in different sheep populations and breeds, resulting in little consensus among the results (Marshall et al., 2009; Gutiérrez-Gil et al., 2009; Kemper et al., 2011; Silva et al., 2012; Riggio et al., 2014). The QTL for resistance traits have been identified on a range of chromosomes, including OAR 1, 2, 3, 4, 6, 9 and 10, on which the highest number of ROHs was found in the present study.

Although the animals in the Grootfontein flock have not been subjected to goal-oriented selection for parasite resistance, the presence of ROH on the same chromosomes to those of

the Wauldby animals, which had been subjected to selection for resistance for several years, indicates that these two populations have been selected for the same traits. This could be explained by the fact that the South African Dohne Merino Breeders' Society has a selection index which is used by all breeders. Body weight forms an important part of this selection index, and QTL for body weight have been reported on OAR 1, 2, 3, 4 and 6 in several sheep breeds (Walling et al., 2004; McRae et al., 2005; Raadsma et al., 2009; Roldan et al., 2010; Matika et al., 2011). These chromosomes correspond to those having the highest number of ROHs in the present study.

From the published studies it is evident that a large number of QTL contribute to nematode resistance through various pathways (Periasamy et al., 2014). Those relating to host response and immunological mechanisms are not yet fully understood. More research is needed to validate the presence of nematode-associated QTL in the Wauldby and Grootfontein Dohne Merino flocks.

## **5. Conclusion**

In this study, genetic and phenotypic differences in FEC, BCS and FAM within the parasite/nematode resistant Wauldby Dohne Merino sheep population were evident. Sires in Genetic cluster 3 are highly resistant and can be used in a breeding program to develop sheep that are resistant to GIN infections. The use of resistant sires in a breeding program will provide a practical, sustainable and cost-effective helminth management strategy. The results obtained from this study indicate that there is genetic variation in host resistance against *H. contortus* in the Wauldby Dohne Merino flock and breeding for resistance against nematodes in this population is therefore feasible. However, before large scale commercial nematode resistance breeding programs are introduced, further research is required to assess host response mechanisms and also to understand the biological processes underlying host resistance to GIN

challenges. Investigations on candidate immune variants for genes involved in host response to GIN infections would help determine the immunological mechanisms responsible for resistance to GINs.

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