GENETIC VARIATION IN RESISTANCE TO *HAEMONCHUS CONTORTUS* IN THE WAULDBY AND GROOTFONTEIN DOHNE MERINO FLOCKS

M.A. Snyman[1#], N.M. Dlamini[2,3], C. Visser[2], F.C. Muchadeyi[3] & P. Soma[4]

[1]Grootfontein Agricultural Development Institute, Private Bag X529, Middelburg (EC), 5900

[2]Department of Animal and Wildlife Sciences, University of Pretoria, Private Bag X20, Hatfield, 0028

[3]Biotechnology Platform, Agricultural Research Council, Private Bag X05, Onderstepoort, 0110

[4]Agricultural Research Council, Animal Production, Private Bag X2, Irene, 0062

[#]E-mail: GrethaSn@daff.gov.za

INTRODUCTION

Haemonchus contortus is one of the most economically important gastrointestinal nematodes infecting hundreds of millions of small ruminants worldwide. Anthelmintic resistance (McManus et al., 2014), the high cost of the drugs (Mpetile et al., 2015) and consumer concern about possible residual drug effects (Vijayasarathi et al., 2016), lead to the need for more sustainable, realistic and cost-effective helminth management strategies (Bath, 2014). Breeding programs for improved host genetic resistance to parasites could provide a long term solution to the problem (Bishop, 2012; Alba-Hurtado & Muñoz-Guzmán, 2013).

There is a significant variation in resistance to gastrointestinal nematodes within and between sheep breeds (Khusro et al., 2004; Cloete et al., 2007; Morris et al., 2010; Snyman & Fisher, 2019) which can be exploited in selection programmes. Selection for nematode resistance has been based mainly on indicator traits such as faecal egg count (Cloete et al., 2007; Morris et al., 2010; Bishop, 2012; Alba-Hurtado & Muñoz-Guzmán, 2013; Riggio et al., 2013; Snyman & Fisher, 2019), Famacha© score (Van Wyk & Bath, 2002; Snyman & Fisher, 2019) and body condition score (Cornelius et al., 2014; Snyman & Fisher, 2019).

The Ovine SNP50 BeadChip which became commercially available in 2008 provides 54241 equally spaced single nucleotide polymorphisms (SNPs) across the sheep genome for association analysis (Kijas et al., 2009). This technology 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 gastrointestinal nematodes. 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 gastrointestinal nematodes.

The aim of this study was to investigate genetic differences between *H. contortus* resistant or susceptible Dohne Merino sheep.

MATERIAL AND METHODS

The study was carried out 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).

Study sites 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 temperatures drop to 4 °C on average during the night (https://www.worldweatheronline.com/stutterheim-weather-history/eastern-cape/za.aspx).

The selection practices and data collection procedures in the flock have already been documented (Fisher & Van Sittert, 2013; Fisher et al., 2015; Snyman & Fisher, 2018; 2019). Data on faecal egg counts (FEC), Famacha© score (FAM) and body condition score (BCS) recorded on 940 animals from 2011 until 2014 were available. Between 10 and 12 two-weekly recordings of FEC were done over the years. From the available animals, 196 were selected on the basis of breeding values (EBV) for FEC (Table 1) for inclusion in this study. Animals were selected within years to account for any possible genetic trends.

Table 1. Selection of Wauldby animals for genotyping based on EBV for FEC

Year of birth	Number of 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
Total (196)	**48**	**48**	**52**	**48**

The Grootfontein Dohne Merino flock is kept under veld conditions at the Grootfontein Agricultural Development Institute (GADI), Middelburg (31° 28' South, 25° 1' East) in the Eastern Cape Province of South Africa. Mean annual rainfall is 373 mm, with 75% of that falling from October to March

inclusive (Du Toit & O'Connor, 2014). 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 FEC recordings per year were made in 2014 and 2016 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 2).

Table 2. Selection of Grootfontein Dohne Merino animals for genotyping based on FEC

Year of birth	**Number of animals**			
	Low FEC		**High FEC**	
	Ram lambs	**Ewe lambs**	**Ram lambs**	**Ewe lambs**
2014	6	6	6	6
2016	6	6	6	6
Total (48)	12	12	12	12

Genotyping

Blood samples of 196 Wauldby and 48 Grootfontein Dohne Merino animals were obtained from the GADI Bio-bank. DNA isolated from the blood samples were genotyped at the Agricultural Research Council, Biotechnology Platform (ARC-BTP) with the Illumina® Ovine SNP50 BeadChip (Illumina Inc., San Diego, CA).

Statistical Analysis

Data on FEC were transformed to logarithms (Log_{10} (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 and mean for FEC were obtained with PROC MEANS of SAS (SAS, 2016). For these analyses, the average of recording 1 of FEC in 2011, 2012, 2013 and 2014 was calculated to obtain FEC1. The average of recording 2 of FEC in 2011, 2012, 2013 and 2014 was calculated to obtain FEC2, and so forth to obtain averages for all traits for all 12 recordings for the Wauldby animals. For the Grootfontein animals, the average of recording 1 of FEC in 2014 and 2016 was calculated to obtain FEC1, etc.

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

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. 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).

RESULTS AND DISCUSSION

Faecal egg counts for the Wauldby and Grootfontein animals recorded over the experimental period are summarised in Table 3. Mean FEC peaked at the third recording and remained high until the seventh recording in the Wauldby animals, after which it declined steadily. From the maximum FEC values recorded, it is clear that the lambs were subjected to a high *Haemonchus* challenge, even at the last two recordings during June. The untransformed FEC in the Wauldby animals ranged from 0 to 52500 eggs per gram, compared to the range of 0 to 147000 eggs per gram of the Grootfontein animals. Large individual variation is expected in untransformed FEC data and is commonly reported in literature. Pollott & Greeff (2004) and Cloete et al. (2007) 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. The descriptive statistics of the other traits is discussed in detail by Snyman & Fisher (2019).

Table 3. Faecal egg counts for the Wauldby animals from 2011 until 2014 and the Grootfontein animals in 2014 and 2016

Recording	Minimum	Maximum	Mean
Wauldby [a]			
FEC1	0	28600	3672
FEC2	0	36900	3376
FEC3	0	38100	5150
FEC4	0	45800	3727
FEC6	0	35900	4022
FEC7	0	37900	3996
FEC8	0	29000	3212
FEC9	0	37600	2853
FEC10	0	40100	2500
FEC11	0	52500	1991
FEC12	0	26700	1596
Grootfontein [b]			
FEC1	0	5600	106
FEC2	0	147000	9937
FEC3	0	30200	2176

[a] FEC1 = The average of recording 1 of FEC in 2011, 2012, 2013 and 2014 was calculated to obtain FEC1, etc. for the Wauldby animals

[b] FEC1 = The average of recording 1 of FEC in 2014 and 2016 was calculated to obtain FEC1, etc. for the Grootfontein animals

The PCA plot including genotypes of all the Wauldby and Grootfontein Dohne Merino animals (without pre-defining any possible groups) is depicted in Figure 1. Four distinct genetic clusters were observed from the PCA. The Grootfontein Dohne Merino sheep population had its own separate genetic cluster (Genetic 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 (Dosed) and Controls (Not dosed) in all clusters.

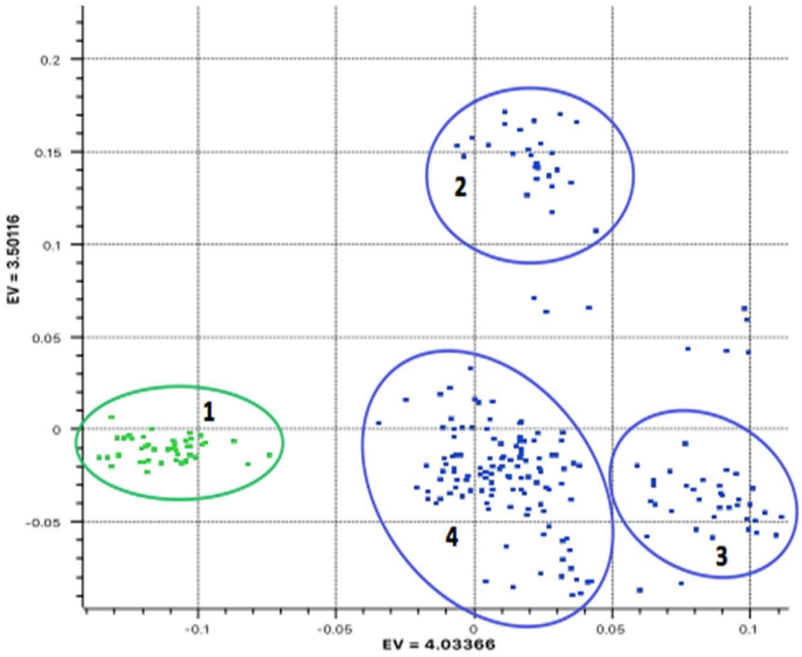

Figure 1. PCA based clustering of the Wauldby and Grootfontein Dohne Merino sheep populations. Genetic cluster 1 = Grootfontein Dohne Merino animals; Genetic cluster 2, 3 and 4 = Wauldby Dohne Merino animals; Outliers = Wauldby Dohne Merino animals

The decision whether a lamb should be dosed or not was based on its FAM, in combination with its BCS. FEC was not taken into consideration, in order to allow resilient lambs to be available for the selection line as well (Snyman & 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.

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.

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

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

Least square means for the resistance traits, trait combinations, as well as EBVs of the traits for the different genetic clusters of the Wauldby animals are presented in Table 5. Animals in Genetic cluster 3 had lower FEC than the animals in Genetic clusters 2 and 4. Animals in Genetic cluster 3 also had significantly lower FEC169, LFEC169 and higher BCS169 than the animals in Genetic clusters 2 and 4. Superior EBVs for all traits except FAM were also observed for Genetic cluster 3.

Table 5. Least square means for the resistance traits, trait combinations, as well as EBVs for the traits (± s.e) for the different genetic clusters

Trait	Genetic cluster 2	Genetic cluster 3	Genetic cluster 4
FECA	$7269^{a} \pm 788$	$3936^{b} \pm 862$	$5338^{b} \pm 714$
FEC169	$4661^{a} \pm 678$	$1362^{b} \pm 742$	$3821^{a} \pm 615$
LFECA	$3.602^{a} \pm 0.170$	$3.111^{b} \pm 0.186$	$3.278^{ab} \pm 0.154$
LFEC169	$3.15^{a} \pm 0.14$	$2.62^{b} \pm 0.15$	$3.10^{a} \pm 0.13$
FAMA	$1.53^{a} \pm 0.11$	$1.59^{a} \pm 0.12$	$1.60^{a} \pm 0.10$
FAM169	$1.38^{a} \pm 0.07$	$1.36^{a} \pm 0.07$	$1.41^{a} \pm 0.06$
BCSA	$2.17^{ab} \pm 0.08$	$2.29^{a} \pm 0.09$	$2.16^{b} \pm 0.08$
BCS169	$2.07^{a} \pm 0.04$	$2.20^{b} \pm 0.04$	$2.09^{a} \pm 0.04$
EBV-FEC	$114^{a} \pm 97$	$-629^{b} \pm 84$	$-2^{a} \pm 45$
EBV-LFEC	$0.037^{a} \pm 0.025$	$-0.209^{b} \pm 0.022$	$0.005^{a} \pm 0.012$
EBV-FAM	$-0.029^{a} \pm 0.011$	$-0.025^{a} \pm 0.010$	$0.015^{b} \pm 0.005$
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; Values with the same superscripts did not differ significantly ($P \geq 0.05$) between clusters within rows; FECA = Faecal egg count averaged over all recordings per year; FEC169 = Average faecal egg count for the 1st, 6th and 9th 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 summarised in Table 6. 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 Genetic cluster 2 had intermediate EBVs for all the traits. The majority (88%) of animals in Genetic cluster 3 were the progeny of sires selected for the resistant line, while only 4.0% and 7.8% of the animals in Genetic 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 (Genetic cluster 3) of animals.

Table 6. Averages for estimated breeding values (± s.e.) for the traits of the sires in the different genetic clusters of the Wauldby Dohne Merino sheep

Trait	Genetic cluster 2	Genetic cluster 3	Genetic cluster 4
EBV-FEC	$-328^{ab} \pm 300$	$-790^{a} \pm 300$	$-51^{b} \pm 146$
EBV-LFEC	$-0.060^{ab} \pm 0.077$	$-0.227^{a} \pm 0.077$	$-0.002^{b} \pm 0.038$
EBV-FAM	$-0.036^{a} \pm 0.025$	$-0.040^{a} \pm 0.025$	$0.024^{b} \pm 0.012$
EBV-BCS	$-0.002^{ab} \pm 0.035$	$0.072^{a} \pm 0.035$	$-0.009^{b} \pm 0.017$

[a,b] 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; EBV-FEC = Estimated breeding value for faecal egg count, etc.

Genetic resistance to parasites is arguably the most sustainable way of gastrointestinal nematode control, and can be achieved through selection of sires with favourable 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 Genetic 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.

CONCLUSION

In this study, genetic and phenotypic differences in FEC, BCS and FAM within the resistant Wauldby Dohne Merino sheep population are evident. Sires in Genetic cluster 3 are highly resistant and can be used in a breeding program to develop sheep that are resistant to *H. contortus* 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. The separate genetic cluster observed for the Grootfontein Dohne Merinos indicated that this flock is genetically distinct from the Wauldby population. More information on resistance traits in the Grootfontein flock is needed before accurate conclusions regarding its genetic resistance to *H. contortus* could be made.

ACKNOWLEDGMENTS

The Agricultural Research Council and Red Meat Research and Development South Africa are hereby acknowledged for funding the genotyping of the animals.

REFERENCES

Alba-Hurtado, F. & Muñoz-Guzmán, M.A., 2013. Immune responses associated with resistance to haemonchosis in sheep. BioMed. Res. Int. 2013, 1-11. Available Online: http://dx.doi.org/10.1155/2013/162158.

Bath, G.F., 2014. The "BIG FIVE" - A South African perspective on sustainable holistic internal parasite management in sheep and goats. Small Rumin. Res. 118, 48-55.

Bishop, S., 2012. Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. Animal 6, 741-747.

Cloete, S., Olivier, J., Du Toit, E. & Dreyer F., 2007. Genetic analysis of faecal worm egg count in South African Merinos under natural challenge. S. Afr. J. Anim. Sci. 37, 237-247.

Cloete, S., Mpetile, Z. & Dzama, K., 2016. Genetic parameters involving subjective FAMACHA© scores and faecal worm egg counts on two farms in the Mediterranean region of South Africa. Small Rumin. Res. 145, 33-43.

Cornelius, M., Jacobson, C. & Besier, R., 2014. Body condition score as a selection tool for targeted selective treatment-based nematode control strategies in Merino ewes. Vet. Parasitol. 206, 173-181.

Du Toit, J.C.O. & O'Connor, T.G., 2014. Changes in rainfall pattern in the eastern Karoo, South Africa, over the past 123 years. Water SA. 40, 453-460.

Fisher, A., Snyman, M.A. & Blaine, R., 2015. Practical breeding for resistance and resilience to *Haemonchus contortus* in sheep. VetNews, July 2015, 2-9.

Fisher, A.D. & Van Sittert, S.J., 2013. The efficacy of a slow-release albendazole capsule against *Haemonchus contortus* with known resistance to albendazole. J. S. Afr. Vet. Ass. 84, Art. #1000, 5 pages. http://dx.doi.org/10.4102/jsava.v84i1.1000.

Khusro, M., Van der Werf, J.H.J., Brown, D.J. & Ball, A., 2004. Across flock (co)variance components for faecal worm egg count, live weight, and fleece traits for Australian Merinos. Livest. Prod. Sci. 91, 35-43.

Kijas, J.W., Townley, D., Dalrymple, B.P., Heaton, M.P., Maddox, J.F., McGrath, A., Wilson, P., Ingersoll, R.G., McCulloch, R., McWilliam, S., Tang, D., McEwan, J., Cockett, N., Oddy, V.H., Nicholas, F.W. & Raadsma, H., International Sheep Genomics Consortium, 2009. A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. PLoS ONE 4, e4668.

McManus, C., Do Prado Paim, T., De Melo, C.B., Brasil, B.S. & Paiva, S.R., 2014. Selection methods for resistance to and tolerance of helminths in livestock. Parasit. 21, 56-70.

Morris, C., Bisset, S., Vlassoff, A., Wheeler, M., West, C., Devantier, B. & Mackay A., 2010. Selecting for resilience in Romney sheep under nematode parasite challenge, 1994-2007. New Zeal. J. Agr. Res. 53, 245-261.

Mpetile, Z., Cloete, S., Kruger, A. & Dzama, K., 2015. Environmental and genetic factors affecting faecal worm egg counts in Merinos divergently selected for reproduction. S. Afr. J. Anim. Sci. 45, 510-520.

Pollott, G. & Greeff, J., 2004. Genetic relationships between faecal egg count and production traits in commercial Merino sheep flocks. Anim. Sci. 79, 21-32.

Riggio, V., Matika, O., Pong-Wong, R., Stear, M. J. & Bishop, S. C., 2013. Genome-wide association and regional heritability mapping to identify loci underlying variation in nematode resistance and body weight in Scottish Blackface lambs. Heredity 110, 420-429.

SAS Institute Inc. 2016. SAS OnlineDoc® 9.4 Procedures Guide, Sixth Edition. Cary, NC: SAS Institute Inc.

Snyman, M.A. & Fisher, A.D., 2018. Progress in selection for resistance to *Haemonchus contortus* in a South African Dohne Merino flock. Grootfontein Agric 18, 1-19.

Snyman, M.A. & Fisher, A.D., 2019. Genetic parameters for traits associated with resistance to *Haemonchus contortus* in a South African Dohne Merino sheep flock. Small Rumin. Res. https://doi.org/10.1016/j.smallrumres.2019.01.004.

Van Wyk, J.A. & Bath, G.F., 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet. Res. 33, 509-529.

Vijayasarathi, M., Sreekumar, C., Venkataramanan, R. & Raman, M., 2016. Influence of sustained deworming pressure on the AR status in strongyles of sheep under field conditions. Trop. Anim. Health Prod. 48, 55-62. www.animalgenome.org/cgi-bin/QTLdb/OA/browse (accessed 15.06.17).