PRACTICAL BREEDING FOR SHEEP RESISTANCE AND RESILIENCE TO HAEMONCHUS CONTORTUS

Trial to select Haemonchuscontortus resistant sheep under summer rainfall field conditions.

The "Parasol project" of 2006, looked at novel ways of combating the scourge of anthelmintic resistance under South African conditions. Prof Gareth Bath and Dr Jan van Wyk, selected one of my clients in the Stutterheim district as one of their experimental farms based on the major anthelmintic resistance problem we had previously identified on the farm.

At the time, it was predicted that within 10 years the farmer in question would no longer be farming with sheep due to the extent of the problem. This was exactly what was necessary to spur the farmer on and as the saying goes, "necessity is the mother of invention", and 9 years later the flock is thriving and producing sheep that can survive and flourish under extremely high Haemonchuscontortus field challenge.

Our challenge was to develop a system that could be both practical and cost effective that we could use or adapt for other South African stud and also commercial sheep farmers. We needed to produce "super sheep" and not "super worms" for his clients and for himself – he is his own biggest ram client!!

There are many success stories around the world regarding the breeding of sheep for nematode resistance, notably the NEMESIS PROGRAM, run by the CSIRO in Australia since 1994, that have proved beyond doubt that breeding worm resistant sheep is an effective and sustainable means to control internal parasites. This begs the question, why are SA farmers, veterinarians and animal scientists not routinely using genetic resistance to worms as at least one of the major selection criteria? After all, we are the undisputed world champions in Haemonchus anthelmintic resistance!

There is an enormous amount of research being conducted on selecting sheep for nematode resistance in all sheep producing areas of the world.
In a Mexican article published in 2012 (*Immune Responses Associated with Resistance to Haemonchosis in sheep, F Alba-Hurtado and MA Munoz-Guzman)* the authors list 104 references to related research papers.
Numerous trials worldwide aimed at identifying the genetic markers associated with nematode resistance and resilience are far advanced and in the near future DNA testing for the traits will become another useful and affordable tool in coping with the scourge of anthelmintic resistance.

This trial has been conducted on the farm Wauldby in the Stutterheim district, Eastern Cape, RSA.This is ideal Haemonchuscontortus country: high **SUMMER**rainfall (>750mm pa), hot, high humidity index, virtually frost-free, well grassed semi-sourveld. In addition, the present owner's predecessors had been very diligent in following a regular 3 to 4 week dosing program, which inadvertently selected the so-called "Stutterheim strain" of anthelmintic resistant wireworm. Worm eggs are regularly harvested and are routinely used as a reference strain for testing new drenches for anthelmintic resistance – the ultimate test for a new product.
Chemicals have virtuallybeen a placebo on the farm for many years. The extremely poor efficacy of anthelmintics was proven initiallyby Faecal egg count reduction tests in 2002,and confirmed many times since. Haemonchuscontortus showed major resistance to all registered chemicals at the time, (The fairly recently released Derquantel and Monopantel are effective and are used extremely conservatively to preserve their useful life-span). The result of the high anthelmintic resistance was

that sheep on the farm hadactually been unwittingly selected for Haemonchus resistance and resilience, resulting in sheep that were thriving despite the challenge.

Half the farm is endemic for Heartwater, and is rarely used for sheep. These camps are used for the Beef Shorthorn herd. The result is that the sheep veld camps (and Kikuyu lambing paddocks), have virtually no rotational grazing (either species rotation or significant rest periods) – and as a result sheep are exposed to huge natural field challenges of Haemonchus, virtually from birth, (up to 54,000 eggs per gram of faeces), and weaned lambs are not necessarily treatedeven at these levels unless they show severe anaemia.

The farm is also fairly unique in that larval cultures over the years have shown that Haemonchuscontortus is >99% of the nematode population (balance is Strongyloidespapillosus) – only summer worms to contend with. There is no liver fluke on the farm either.

AIMS AND STRUCTURE OF THE TRIAL

The aim is to select the most resistant sheep from the stud lamb crop each year, to be mated to selected rams at 18 months of age. The resultant lambs produced are compared to their peers after weaning.

We have attempted to find practical and cost-effective selection parameters that can easily be applied to other stud or commercial flocks by farmers in conjunction with their veterinarian.

Group of approx. 250 weaned stud lambs (rams and ewesare run as separate flocks after weaning for obvious reasons) are deliberately exposed to continuous high field challenge of Haemonchus – they are left in the same camps as long as possible to maintain thehigh challenge. Standard management practices are maintained (vaccinations and minimal nutritional supplementation).

Expected Breeding Values (EBV) for worm resistance have been calculated and on the 17th March 2015 the first crop of rams with EBV values for FEC (Faecal egg counts) were offered and a considerable premium was paid for the rams with negative EBVs (low FEC) – buyers are becoming far more aware of the value of introducing genetically worm resistant rams into their flocks.

1000 EDTA blood samples have been collected and frozen to date from sheep identified with high, intermediate and low resistance rankings for eventual DNA genomic sequencing to identify genetic markers associated with resistance and compare the findings with research elsewhere.

Lambs are born in August each year (21 day lambing period), the entire lamb flock is dosed for worms when indicated by low Famacha and high FEC and again at weaning at the end of November)
NB Lambs are very susceptible to internal parasites and many lambs have very high FEC before developing an "acquired" or "adaptive" immunity and ridding themselves of parasites after exposure. There are also lambs born with an innate resistance that show very low FEC even at a young age, but it is advisable that selection processes should only be commenced after weaning once immunity has developed to prevent potential losses from parasites.

NB This trial is conducted under summer rainfall conditions with Haemonchuscontortusas the only significant nematode.
The protocol would need to be adapted considerably in winter rainfall areas or other areas where parasites such as Teladorsagia (Brown stomach worm) or Trichostrongylus(bankrupt worm) or other blood-sucking parasites such as Fasciola(liver fluke) are a significant factor.

Protocols in such areas should be developed and adapted in conjunction with parasitologists / veterinarians with a sound knowledge of local conditions.
Lambs are monitored after weaning and from mid-January to the onset of winter dormancy /hypobiosis of Haemonchus (early July on this farm).**FAMACHA scores** (read by an experienced shepherd), **Body Condition Scores (BCS)**(read by veterinarian, but canalso be read by the farmer or staff member) and **individual FEC**are measured and recorded every 14 days. The shepherds and the owner monitor and record Famacha scores in the intervening weeks.
There has consistently been a high correlation between both Body condition scores (repeatability of 0.68 +/-0.04) and FEC (repeatability 0.65 +/-0.04) as measured at the beginning of the trial in January when compared to values for the two traits throughout the rest of the summer.
Data collected over first three years of the trial, has indicated that **it is possible to reduce the number of individual faecal egg counts required to three samples per sheep**:
Individual faecal sample at the beginning of the season (January on this farm) from all lambs and two more individual FEC samples at peak season (March and April on this farm) from the untreated lambs only (dosed sheep have eliminated themselves and do not require further samples) without materially affecting the average FEC compared to two weekly faecal sampling. This reduces the laboratory costs associated with Faecal Egg Counts considerably.

(TST =Targeted Selected Treatment) The decision to dose a sheep is based mainly **ona Famacha score of 2.5or more**(scored by the experienced shepherd), evaluated in conjunction with BCS (<1.5) and also if FECare very high.

Any sheep that require dosing remain in the trial group, but are not regarded as resistant.Only sheep that were not drenched after weaning are considered for selection into the resistant group.

Individual sheep that do require dosing are treatedwith Moxidectini/m / Trichlorphon p/o. This is **strictly off-label usage**, but the combination has continued to be approx. 85% effective for the past 5 years, despite the actives being totally ineffective individually. This is ascribed to the fact that **Target Selected Treatment** is used routinely on all flocks – only avery small percentage of sheep are treated (on Famacha scores) and sheep areonly dosed in summer when the vast majority of the Haemonchus population on the farm are in **Refugia** (on the pasture, not in the sheep). Sheep are never dosed in winter when most of the Haemonchus population are in the sheep

The data collected is also used to evaluate the sires used based on the performance data of their lambs.

Correlations between parasite burdens and body weights, growth rates, fleece weight and fibre diameter were investigated. Many sheep breeders believe that the “poor-doers” (due to worms on this farm) have lower body weights, (and are less fertile as a result), have lighter and finer fleeces and they question the wisdom of too much emphasis being placed on selecting sheep for finer wool. This has proved to be the case in the trial so far and further investigation is indicated.

RESULTS

- 43% of lambs did not require dosing the entire summer in the trial in 2012
- 56% were not dosed in 2013,
- And 46% were not dosed in 2014

Selection index

Only sheep that were not dosed were included in the data analysis.

Various indices for and combinations of FAM, BCS and FEC were evaluated as possible selection criteria.

The selection index that eventually provided the most suitable selection criteria was average FAM, average BCS and average FEC over the experimental period =

(FAM +BCS+FEC on a natural log transformation)

Rams and ewes are ranked on this selection index, as well as on average FEC over the summer test.

The trial is aimed at selecting sheep that thrive despite the adverse conditions.
In our trial, it is felt that Body condition score (BCS) is a better indicator than body mass (weight) of how well the sheep is doing, as larger framed sheep in poor condition will often weigh more than smaller framed lambs in a better condition.

By using the average FEC over the summer in the selection index, the resilient sheep that have good Famacha and BCS, but higher FEC are ranked far lower than resilient sheep with a low average FEC. This generally eliminates resilient sheep from selection as potential sires.
There is a good correlation between rankings on average FEC and rankings on the selection index too.

All ram and ewe lambs that did not require treatment for parasites are ranked on both the selection index and on average FEC.
An example of the first page of the data produced.

Selection Index: FAM + BCS + FEC (natural log transformation)
Equal weights awarded to each trait as heritability of traits is similar
(all 0.2 – 0.25) Average for each parameter over trial period.
Rams ranked as per selection index (**first page of report**)

Parasite resistance, body weight and fleece weight fine
Parasite Résistance fine, body weight average
Problems - not suitable

LAMNR	SIRE	Birth status	RANK Average selection index	SIR191	SIR152	SIR143	SIR254	SIR47	Ranking Sum FEC	Weaning weight index	Index 152	Index 213	Index 25	Index 206	Index 178	Fleece weight index 178
49	EN070010	1	1	11	25	1	38	6	1	117	127	125	129	126	129	90
119	B090121	2	2	22	35	6	33	2	5	85	94	92	90	89	88	85
84	B090121	1	3	4	28	5	29	8	7	109	111	111	108	100	102	112
93	B090238	2	4	26	20	4	34	26	4	85	80	84	82	88	90	103
240	EN080035	2	5	7	26	7	18	22	12	112	110	105	100	97	96	99
23	EN070010	2	6	10	21	2	23	12	8	102	101	105	102	96	93	103
124	EN080035	2	7	19	33	10	28	11	20	113	115	111	117	118	124	99
220	EN080035	2	8	16	22	24	26	7	16	125	130	126	125	130	126	112
29	EN070010	2	9	25	8	20	35	10	2	101	96	96	98	101	106	99
91	B090121	1	10	13	36	17	27	31	3	100	99	100	95	96	90	117
232	B090121	2	11	1	30	30	20	15	14	120	116	115	105	112	111	112

It was interesting the sheep selected as being highly resistant to Haemonchus on our selection index, also perform exceptionally well on the breed society indices – in the example below, the ram lamb with the highest ranking of the 2011 crop had a selection index of 140% on the breed society indices, a relative breeding value of R40.37 per lamb and a body EBV of +8.6kgbetter than his peers.

Dohne breed society indices on 2 rams selected on our selection index as sires for mating to ewes selected for resistance in April 2013

RAM INFORMATION

RAM NUMBER	SIRE	BIRTH STATUS	BODY EBV	WOOL EBV	FIBRE DIAMETER EBV	REV	SELECTION INDEX %	RANK
B11.49	EN7.10	SINGLE	+8.6	-0.084	-1.03	40.37	140	1
B11.124	EN8.35	TWIN	+5.06	-0.059	-0.45	22.31	118	9

EBV	Expected Breeding Value
BODY WEIGHT EBV	Is in actual KG's
WOOL WEIGHT EBV	Is in actual kilograms
FIBRE DIAMETER EBV	Is in actual microns
REV	Relative Economic value in Rands

SELECTION INDEX TAKES INTO ACCOUNT ALL OF THE ABOVE 3 TRAITS IN ONE INDEX AS COMPARED TO THE GROUP OF 109 OTHER CONTEMPORIES.

RANKING IS OUT OF 109 RAMS.

Our top ram, no 49, came out top on the Dohne society selection index as well by a large margin (**selection index140%)** and a body **EBV (+8.6kg),** best growth rate , **REV of R40.27** and fine wool (-1.03 micron) as a bonus too , **AA stud ram**

PROTOCOLS FOR SELECTING COMMERCIAL SHEEP FLOCKS FOR RESILIENCE /RESISTANCE

The laboratory costs associated with Faecal egg counts (FEC) remain the biggest cost factor in selection for resistance and protocols have been developed for use in commercial sheep flocks (as opposed to stud flocks), where sheep are selected for **Resistance and/or Resilience without costly individual faecal egg counts.**

If one does not monitor individual faecal egg counts it is not possible to distinguishbetween **resistant sheep (low egg counts) and resilient sheep (higher egg counts, but able to tolerate the presence of fairly high worm burdens** without severe negative effects being observed). Resilient sheep are likely to produce lambs with higher worm burdens that shed far more eggs onto the pastures and are more likely to succumb in drought situations or in times of nutritional stress than sheep with very low parasite burdens.

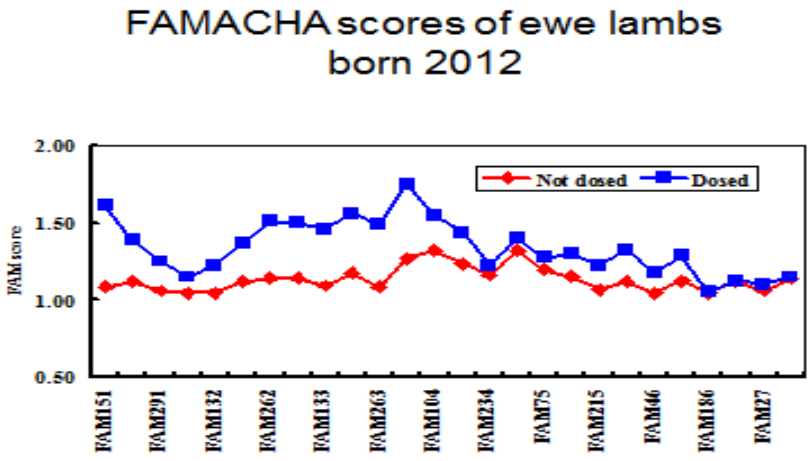
The protocols we have applied to the **commercial flocks** on this farm and on other commercial sheep flocks in the region are in many ways similar to the protocols for resistance selection, but with considerably less faecal samples required:-

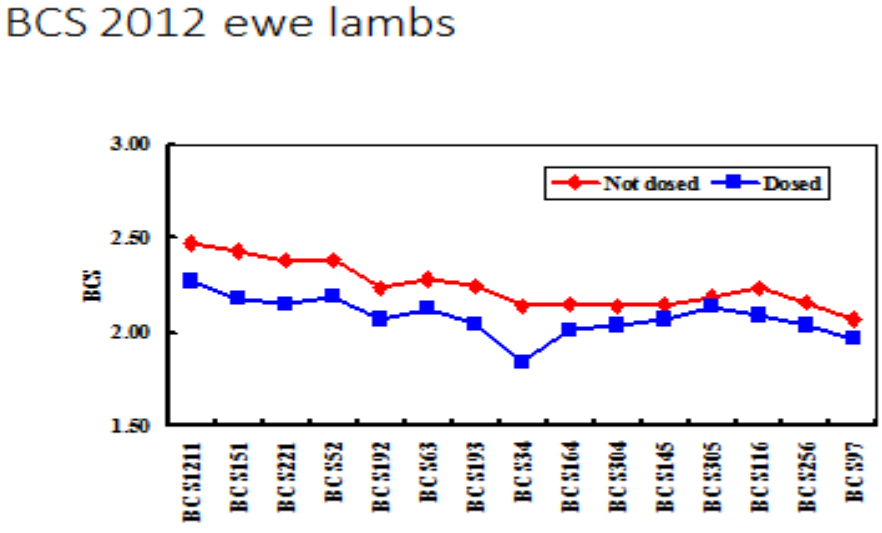
- Normal parasite management up to weaning
- Only ewe lambs are assessed after weaning, as ram lambs are castrated and do not contribute to the genetics of the flock.
- As with the resistance selection protocol lambs are monitored after weaning until parasite levels start to increase - routine grouped flock dung samples (from the same 10 sheep) help to anticipate increases in parasite burdens timeously, and Famacha and Body condition scores are monitored every 1-2 weeks (depending on level of parasite challenge at the time).
- Individual sheep are only treated (TST) if theyshow a Famacha score of >2.5 and are treated with an active proven to be effective on that farm. Mark any sheep requiring treatment with a coloured ear tag. Add a second or third tag (or different colour) with each subsequent treatment required.
- Cull out all ewes at the end of the summer that required dosing (or required dosing more than once, depending on percentage of sheep treated). Remove susceptible sheep before breeding age is reached (heritable)
- **Sires**: Leave adult ram flock without dosing for as long a period as possible and then perform individual FEC on all rams and cull out rams with unacceptably high faecal egg counts before the breeding season

- If at all possible, only buy in rams that have been selected for parasite resistance.
- Practice Targeted Selected Treatment (TST) on all adult ewe flocks if possible – if a low percentage of flock show anaemia (Famacha 3 or more) or have “bottle-jaw”, tag and dose only the individual sheep requiring treatment and cull them at the end of the season.

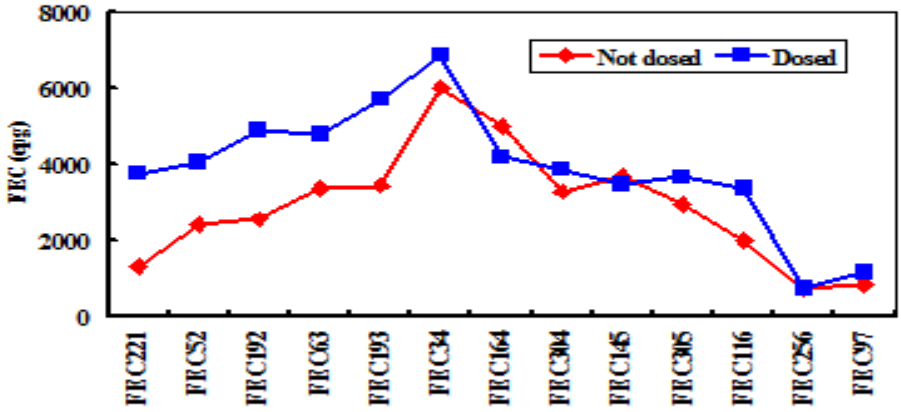
The end result of this protocol is similar to selecting for resistance only, but will take longer to achieve due to the resilient sheep not being identified.

Results have been very consistent over the three years of completed data:
A few graphs are included to show the trends

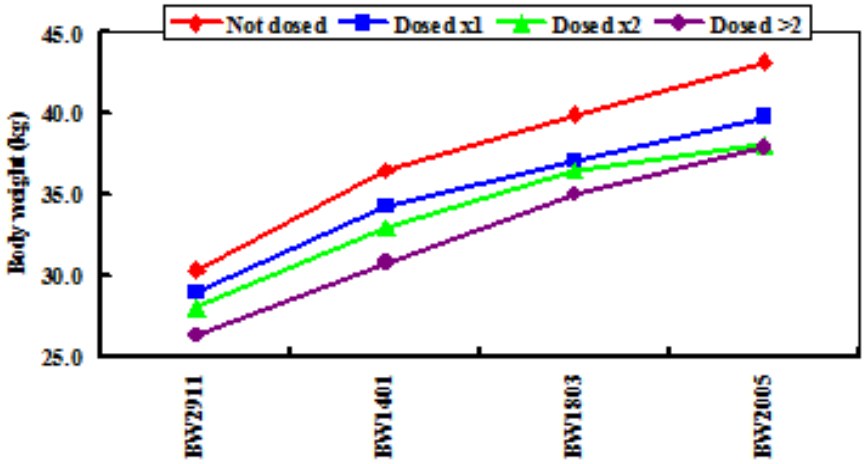


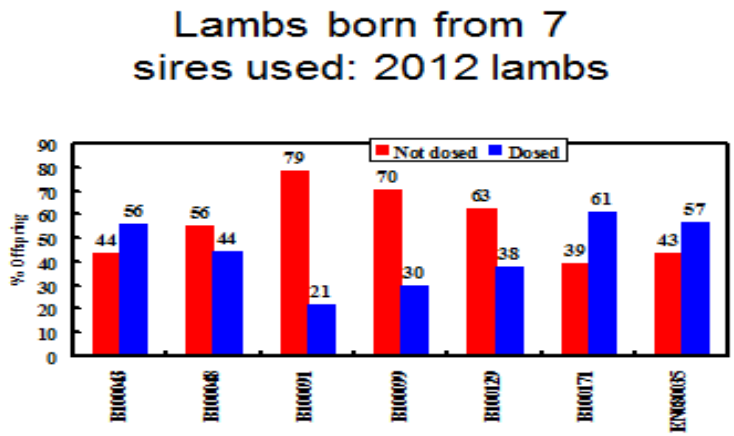


FEC of Not-dosed and Dosed ewe lambs over the experimental period summer 2013



Growth curve of 2013 ram lambs over the experimental period





Comparison of protocols to select Resistant or Resistant / Resilient sheep

Resistant vs Resistant & Resilient

NB not the same genes / Summer rainfall areas

Target: Stud breeders and group breeding schemes

- Normal worm management pre-weaning. Individual FEC on rams before first treatment – select innate resistant rams
- Selection group: **Ram and Ewe lambs** post-weaning
- FAMACHA and BCS every 1-2 weeks after weaning in summer
- Individual animal FEC 3x @beginning of summer and 2 samples at peak worm season
- Only treat FAMACHA >2.5 (TST)
- Evaluate rams on their lambs' performance (FAM, BCS, FEC)
- Buy in only resistant sires
- Mark (and cull) all lambs needing dosing
- Select lambs on ave FAM:BCS:FEC index (High ave FEC will eliminate resilient lambs) after first summer season.
- Calculate EBV values for FEC
- (Adult ewes - only treat on TST and mark and cull out high FAMACHA)

Target: Commercial sheep flocks

- Normal worm management pre-weaning. Only routine bulk FEC
- Selection group : **Ewe lambs** post weaning
- FAMACHA and BCS every 1-2 weeks in summer after weaning. No individual samples
- Group dung samples monthly to monitor parasite levels (from the same 10 sheep)
- Only treat FAMACHA >2.5 (TST)
- Ram evaluation: Dung sample **SIRES** at peak of season (untreated) FEC and cull out high counts
- Buy in only resistant sires
- Mark and cull all lambs needing dosing (more than once?)
- Select lambs on FAM: BCS only
- (Adult ewes - only treat TST and mark and cull out high FAMACHA)

DISCUSSION

It is estimated that even in flocks previously unselected for resistance and / or resilience, one will see a difference within three years and a marked improvement within 5 years

There were significant differences in the number of lambs that required dosing. from the various sires used.

The best sheep selected on the combination (ave FAM+BCS+FEC) selection index were also the best performing sheep selected on the Dohne merino society selection indices.
Worm resistant / resilient sheep in this trial had superior performance on all parameters, compared to the sheep that required dosing.
Nutrition plays a very important role in the ability of sheep to cope with heavy worm challenge – if nutrition is improved (particularly protein intake) the FEC drop dramatically
Minerals also play an important role in the ability of the animal to withstand the deleterious effects of internal parasites and also boost the immune system – FEC consistently drop by up to 50% after administration of an oral mineral preparation.
Coccidiosis has a negative effect on the ability of the animal to contain worm burdens and FEC increase when coccidial burdens are high.
Individual FEC are of little value indeciding when a particular sheep requires treatment – resilient sheep may have high burdens (>50,000 epg on occasion), but not be anaemic and consequently not require treatment, but in contrast some sheep show severe anaemia at relatively low FEC (some as low as 2500 epg) in the same flock and require treatment.
Individual FEC values are valuable in selecting for resistant sheep and grouped faecal samples from flocks are certainly a valuable indicator of changes in parasite levels and an early warning of impending outbreaks of haemonchuscontortus.

Sheep that **required dosing** (sheep with high worm burdens and low resilience) –

- On average, remained more anaemic, despite being dosed
- Remained in a poorer body condition score than sheep that were never dosed
- Average FEC were similar despite dosing (skewed by resilient sheep not dosed)
- Had considerably poorer growth rates
- Produced lighter and finer (lower micron) fleeces
- Cost money (anthelmintic remedy), but did not perform

Lessons learnt from this trial so far:

Resistance to internal parasites is certainly heritable

- There was a very high repeatability of both FEC (65%) and BCS (68%) measurements recorded at the first peak of the wireworm season (19th January on this farm in 2012)
- It is feasible to select onregular BCS, FAM andwith just threeindividual faecal egg samples on untreated weaned lambs (or possibly just on ram lambs) taken before and at the peak of the worm season – to cut costs.
- Farmer and staff can monitor Famacha and BCS for the rest of the season and only treat on TST (Targeted Selected Treatment).
- In this trial, in 2012, 43% of the lambs did not require treatment from weaning to winter, 56% in 2013 and 46% in 2014.
- Mark sheep requiring treatment and cull “repeat offenders”
- Only select rams and (if numbers allow)replacement ewes from the untreated sheep
- Leave adult sires untreated for as long as feasible, perform individual FEC and cull out high FEC
- Should one be breeding from rams that are genetically susceptible to worm infestation? Particularly stud sires
- Consider selecting a resistant line within stud flocks to fast track selection

The question really should be: Can we afford not to select for resistance?

As the trial progresses over the next few years, it is anticipated that the selected (resistant) group will grow. Second and third generation selected sheep are already showing higher resistance characteristics.

This particular flock had a 10 year head start, thanks to anthelmintic resistance.

In phase 2 and 3 of the trial (lambs born Aug 2012 and 2013) a number of unweaned lambs had very lowfaecal egg counts of 0-500 eggs per gram (average FEC 11,000+). These may be naturally resistant lambs, or lambs with particularly effective immune systems and will be monitored.

The 2nd phase of the trial supports findings elsewhere in the world that genetically resistant lambs develop very high worm burdens initially at a young age and then mount an immune response to the challenge (cell and antibody) - response is less effective in susceptible sheep.

The study of the mechanisms that govern resistance to nematodes is a rapidly developing field and the role of $CD4^+$ T helper (Th2) cells in the immune response to internal parasites as well as major differences in genes (41 known genes in one NZ study - mostly stress response related functions) between sheep susceptible and resistant to gastrointestinal parasites.
Genotyping using the ovine SNP50 BeadChip is showing very positive results

Selection of sheep for either resistance or resilience/resistance should not be seen in isolation, but is an important advance in the fight against anthelmintic resistance by nematodes (particularly Haemonchus under South African conditions), and needs to be used in conjunction with other good management practices to reduce the worm challenge and boost the animal's ability to compensate for the deleterious effects of the parasites, as have been discussed in other papers at the WWW congress.

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