

# **Final report**

# Comparison of FECPAK<sup>G2</sup> and Mini-FLOTAC methods for diagnosis of naturally acquired mixed infections of gastrointestinal nematodes in individual and pooled dung samples from goats

Project code:
Prepared by:

B.GOA.0130 Matthew C. Playford Dawbuts Pty Ltd

Date published:

30 June 2020

PUBLISHED BY Meat and Livestock Australia Limited PO Box 1961 NORTH SYDNEY NSW 2059

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

This publication is published by Meat & Livestock Australia Limited ABN 39 081 678 364 (MLA). Care is taken to ensure the accuracy of

the information contained in this publication. However MLA cannot accept responsibility of information or opinions contained in the publication. You should make your own enquiries before making decisions concerning your interests. Reproduction in whole or in part of this publication is prohibited without prior written consent of MLA.

#### **Executive summary**

- 1. The Australian goat industry is comprised of a diverse mixture of breeds and management methods, with goats kept for meat, dairy and fibre. A common theme across the sectors is the difficulty of worm control, partly due to the goat's differing behaviour and metabolism from other grazing herbivores and the lack of registered anthelmintic products for this species. This results in poor goat welfare and productivity as well as violation of residue limits of anthelmintics in goat meat samples that poses a risk to the ongoing export trade.
- Worm control decisions may be based on physical examination or objective testing. Diagnostic tools based on microscopic methods for counting worm eggs are available both as commercial laboratory-based providers and do-it-yourself options, but all services have a low uptake among goat producers, possibly due to cost and lack of convenience.
- 3. FECPAK<sup>G2</sup> is a novel device for worm egg counting using a microscopic camera and a webbased platform for connection to trained technicians, analysis and reporting. Dawbuts is the Australian distributor of FECPAK<sup>G2</sup>.
- 4. Validation studies for FECPAK<sup>G2</sup> have been conducted on sheep, cattle, alpacas and horses but none previously on goats. This study compared results of worm egg count testing in goats using FECPAK<sup>G2</sup> with those using Mini-FLOTAC on individual and pooled counts over 5 weeks across 9 mobs (each of 5 goats) on 6 farms in New South Wales.
- 5. Worm egg excretion by all mobs of goats was high during the 5-week trial. Mean worm egg counts across all mobs ranged from 623epg (Week 4) to 1408epg (Week 1). Individual counts ranged from 0epg to 9940epg (using Mini-FLOTAC).
- 6. A range of worm genera was observed, with *Haemonchus, Teladorsagia, Trichostrongylus* and *Chabertia* observed in larval cultures and *Nematodirus* eggs observed in worm egg counts.
- 7. Worm egg counts using the two methods were assessed on a week by week basis. Correlation of worm egg counts between the 2 methods was moderate to high (R<sup>2</sup> = 0.350.89) across the 5-week trial. Significance was higher (R<sup>2</sup> = 0.81-0.89) in the 4<sup>th</sup> and 5<sup>th</sup> weeks of the trial, indicating improved accuracy after familiarisation of the investigator with the new technology.
- 8. Results of the pooled samples across the 9 mobs were very similar to the mean of the individual counts, for both methods. There is a trend towards higher correlation for the FECPAK<sup>G2</sup> method. This means that goat producers can use pooled counts to save time and money on worm egg count testing, unless individual counts are required for anthelmintic resistance testing or for management reasons.
- 9. Sensitivity of the FECPAK<sup>G2</sup> unit is 35 eggs per gram, which is appropriate for Australian goat herds, that typically have worm egg counts over 300 eggs per gram. The level of zero counts for FECPAK<sup>G2</sup> was higher than Mini-FLOTAC, due to Mini-FLOTAC's sensitivity of 10 epg in this trial. The counts using Mini-FLOTAC were slightly higher than those using FECPAKG2 in each of the 5 weeks, due to the higher sensitivity. However the difference was not significant in Week 4.
- 10. Repeatability of the two methods was assessed by comparing repeat tests of the same sample. This was higher for FECPAK<sup>G2</sup> than Mini-FLOTAC.
- 11. Adoption activities should focus on educating goat producers, veterinarians and rural store staff on the risks posed by worms to Australian goats and the benefits of diagnosing worm burdens prior to serious morbidity and production losses occurring.

# Table of contents

Ex	Executive summary2					
1.	Backgroun	nd	5			
	1.1 The	Australian goat industry	5			
	1.1.1 Imp	oortance and value of the goat industry	5			
	1.1.2 Dia	gnosing and treating internal parasites in goats	5			
2.	Project ob	jectives	7			
	2.1 Diag	nostic methods for worm egg counts	7			
	2.1.1 Vali	dation of FECPAK <sup>G2</sup> against microscope-based methods	7			
	2.1.2 Seri	al observation of small goat flocks	7			
	2.1.3 Poo	led versus individual worm egg counts	7			
3.	Methodol	ogy	7			
	3.1 Farn	n and laboratory details	7			
	3.1.1 Farr	m and animal selection	7			
	3.1.2 Sam	nple collection and testing schedule	8			
	3.1.3 Laboratory methods and personnel					
	3.1.4 Mini-FLOTAC					
	3.1.5 FEC	PAK <sup>G2</sup>				
	3.1.6 Stat	tistical analysis				
4.	Results		11			
	4.1 Wor	m egg counts	11			
	4.1.1 Sam	nples collected and counted				
	4.1.1.1	Week 1				
	4.1.1.2	Week 2				
	4.1.1.3	Week 3				
	4.1.1.4	Week 4				
	4.1.1.5	Week 5				
	4.1.1.6	Weeks 1-5 summary				
	4.2 Poo	led and individual worm egg counts	16			

	4.3 Pattern of worm egg counts	17
	4.4 Zero counts	17
	4.5 Larval culture	17
	4.6 Repeatability of worm egg counts using Mini-FLOTAC and FECPAK <sup>G2</sup>	18
5. D	Discussion	19
	5.1 Concordance between Mini-FLOTAC and FECPAK <sup>G2</sup>	19
	5.1.1 Correlation over time	19
	5.1.2 Number of zero counts	19
	5.2 Pooled and individual worm egg counts	19
	5.3 Pattern of excretion of worm eggs in goats in small mobs	20
	5.4 Repeatability of worm egg counts	20
	5.5 Worm genera detected	20
6. C	onclusions/recommendations	20
	6.1 FECPAK <sup>G2</sup> as a method for monitoring worm burdens and diagnosing	20
	anthelmintic resistance in goats	20
7. K	ev messages	21
,,,,,	7.1 Routine use of worm egg counts is valuable for goat management	· . 21
	7 1 1 Monitoring	21
	7.1.2 Drench resistance testing.	21
	7.1.3 FECPAK <sup>G2</sup> is a reliable method of conducting worm egg counts	21
8. A	ppendix	23
	8.1 Appendix 1- Description of methods for worm egg counts	23
	8.1.1 Mini-FLOTAC Instructions for use	23
	8.1.2 FECPAK <sup>G2</sup> Instructions for use	24
	8.1.4 Statistical analysis summary	24
9. R	eferences	27

# 1. Background

#### **1.1 The Australian goat industry**

#### **1.1.1 Importance and value of the goat industry**

The Australian goat industry is comprised of dairy, fibre and meat flocks. The value of goatmeat is A\$182m (2018 estimate), down from the 2017 peak of A\$255m. Australia is the world's leading exporter of goatmeat and the majority goes to the United States of America, Taiwan, South Korea Canada and The West Indies. About 90,000 head/year go to live export.

#### 1.1.2 Diagnosing and treating internal parasites in goats

Due to their natural browsing habit<sup>i</sup>, goats have anatomical and physiological differences in their gastrointestinal tract to cattle and sheep and tend to pick up larger burdens of gastrointestinal nematodes (GIN) on pasture than sheep. Goats kept under farming conditions have particularly high worm burdens and suffer production losses and mortality as a result<sup>2</sup>. Diagnosing worm infections relies on clinical inspection for diarrhoea (scours), anaemia (indicated by pale mucous membranes), body condition score, hypoproteinaemia (indicated by dependent oedema including bottle jaw) and weight loss.

By the time clinical signs are detectable by goat managers, the flock has usually suffered production loss, mainly in the form of low weight gain in growing animals, loss of body condition score in mature animals and low fibre and milk production.

Diagnosis of parasite infection to prevent production losses via treatment, change of paddock, supplementary feeding or other management intervention is critical to keeping goats on farms. Conventional methods such as microscopic observation of dung samples to count worm eggs are useful for proactively monitoring worm burdens, but have low uptake among goat producers due to either inconvenience and cost (if sending samples to a laboratory) or the need for specialist training and accreditation (if doing testing themselves).

WormBoss, the Meat & Livestock Australia (MLA) and Australian Wool Innovation-funded website, is the premier authority on goat worm control in Australia. The Drench Decision Guide published on WormBoss Goats determines that worm egg counts should be taken both to make a decision to treat goats and also to diagnose drench resistance and monitor efficacy of treatments<sup>ii</sup>.

FECPAK<sup>G2</sup> is an internet-connected, image-based diagnostic platform used to conduct faecal egg counts (FEC) tests on animals<sup>iii</sup>. This technology will make FEC testing easier and faster with the ability to do it on site by any operator without specialist training. The system is image based, making it auditable with permanent records. This also removes the need to transport samples to a laboratory and fast with most results delivered within the hour.

FECPAK<sup>G2</sup> has been in use in Australia for two years and there are currently about 60 units in veterinary clinics, on farms and in rural retail stores. The dynamics of worm egg output and use of FECPAK<sup>G2</sup> in camelids, sheep, horses and cattle are relatively well-researched, but for goats there is a gap in research knowledge.

Rashid et al. (2018) used FECPAK<sup>G2</sup> in a comparison with McMaster method to count GIN eggs from alpacas and found an overall 'moderate to good agreement' (Lin's concordance correlation coefficient 0.78 for salt and 0.84 for sugar) between the methods<sup>iv</sup>. Previous experience with research teams in our own and other laboratories suggest that although most are familiar with the McMaster technique, it takes a few weeks of use before operators are confidently and competently using the FECPAK<sup>G2</sup> method.

WormBoss published 'Worm Control in goats- advice for Australian veterinarians' in 2016 to enable veterinarians to prescribe 'off-label' anthelmintics to goat owners<sup>v</sup>. The list of anthelmintic products that are registered for use in goats in Australia is limited and only includes 3 active ingredients; abamectin, fenbendazole and morantel.

Since these single active products have low efficacy against worms in goats and extension advice to goat producers directs them to use combination products, many use sheep or cattle products 'offlabel', with or without veterinary advice. This creates a high risk of residue violation in products from goats. The National Residue Survey annual summary of goat meat residues indicates that 3% of 100 samples of goatmeat tested for moxidectin in 2017-18 and 2% of the 100 samples in 2015-16 returned a level higher than the Maximum Residue levels (MRL)<sup>vi</sup>. Note that moxidectin is not registered for goats.

This finding puts the entire goatmeat export industry at risk and provides a strong warning for the industry to provide better worm control methods and extension to goat producers to allow for worm control without resorting to high-risk practices.

FECPAK<sup>G2</sup> has already been assessed as a rapid and simple means for conducting worm egg counts in herbivore faecal samples. If the accuracy and sensitivity of this method is appropriate to monitoring worm burdens and diagnosing anthelmintic resistance in goats, then it would provide an extremely valuable tool to overcome risks both to goat welfare and residue violations.



Figure 1: Collecting fresh goat dung samples from Rangeland goat farm on Razorback, NSW.

# 2. Project objectives

#### 2.1 Diagnostic methods for worm egg counts

#### 2.1.1 Validation of FECPAK<sup>G2</sup> against microscope-based methods

The primary objective of this study was to validate the use of FECPAK<sup>G2</sup> in goats by comparing worm egg counts conducted using both FECPAK<sup>G2</sup> and Mini-FLOTAC, the current standard for international research worm egg counts for livestock. This was done by statistical analysis of repeat (within method) and comparison (between method) results.

#### 2.1.2 Serial observation of small goat flocks

This allows investigators to describe patterns of worm egg excretion in small goat flocks

#### 2.1.3 Pooled versus individual worm egg counts

The major merit of conducting individual worm egg counts is that the manager gains knowledge of the range, including lows and highs, of worm egg counts within a flock. However, due to expense, many livestock producers compromise by taking insufficient individual samples. This study helps describe the relationship between individual and pooled worm egg counts in small goat flocks.

# 3. Methodology

#### 3.1 Farm and laboratory details

#### 3.1.1 Farm and animal selection

Five small goat flocks in the Camden, NSW region and one in Burragate, NSW were purposivelyselected and recruited. A selection of breeds was included to allow for variation between breeds and purpose.

Flock parasitological history and/or pre-screening indicated the source flocks as harbouring burdens of naturally-occurring mixed infections of gastrointestinal nematodes (data not shown).

Inclusion criteria:

- 1. under 20 head of goats/mob with one to three trial mobs per farm of healthy goats.
- 2. able to cooperate with investigator to collect individual dung samples for weekly worm egg counts for 6 weeks
- 3. willing to keep a log of treatments and other management inputs, including metabolic status (kidding, lactating), nutritional inputs, anthelmintic or external parasite treatments.

Farm details are included in Appendix 8.3. Only one of the farms used worm egg counts to routinely monitor goat worm burden (Mobs 6 and 7). Another farm (Mob 4) used Bioworma (*Duddingtonia flagrans*- International Animal Health) as an in-feed supplement to inhibit the development of and restrict contamination by worm larvae on pasture.



Figure 2: Boer goats on co-operator farm (Mob 5)

The total number of mobs monitored was 9. Samples were collected from 5 head per week from each mob. One pooled sample was made up from samples from the individual goats, making a total of 6 samples per week per mob= 54 samples per week in total.

#### 3.1.2 Sample collection and testing schedule

Samples were collected from goats for 5 consecutive weeks, from 25 May to 23 June 2020. Samples were then analysed at the Razorback laboratory from 25 May to 26 June 2020. On all farms, dung samples were collected from the ground using a gloved hand after goats were observed to defecate. Samples were immediately placed into a pre-labelled, sealable plastic sandwich bag and goat identity and date recorded.

Sens	sitivity	35epg	10epg	35epg	10epg	
Week	No. mobs	<b>Individual</b> FECPAK <sup>G2</sup>	Individual MiniFLOTAC	<b>Pooled</b> FECPAK <sup>G2</sup>	Pooled MiniFLOTAC	No. goats in mob
1	9	90	90	18	18	5
2	9	90	90	18	18	5
3	9	90	90	18	18	5
4	9	90	90	18	18	5
5	9	90	90	18	18	5
тс	DTAL	450	450	90	90	

#### Table 1: Details of samples collected and analysed from goat farms in the trial

#### 3.1.3 Laboratory methods and personnel

The Kamiya Laboratory at Dawbuts office in Camden NSW has offered parasitology diagnostic services for livestock producers since 2009. Laboratory Manager is Phil Stein. Due to full utilisation of the main laboratory, a field laboratory was established at Razorback, NSW using equipment and methods from the Dawbuts laboratory. Training of the project investigator, Sandra Playford, was done at Dawbuts Laboratory using standard techniques for Mini-FLOTAC and FECPAK<sup>G2</sup>.

#### 3.1.4 Mini-FLOTAC

The Mini-FLOTAC method was developed by Giuseppe Cringoli at the University of Naples Federico II. It is more sensitive than the standard McMaster technique due to a higher volume of faeces used per sample and a larger volume of floatation fluid introduced into the slide. This is possible due to a sliding mechanism that, after leaving eggs to float in the saline solution for 10 minutes, is turned to remove the floating eggs from the debris beneath<sup>vii</sup>.

The Mini-FLOTAC method has been used in a range of published research trials on livestock and humans<sup>viii</sup>. Comparison with microscope-based methods such as the original version of FECPAK (a modified McMaster method) have been conducted<sup>ix</sup>. Mini-FLOTAC was found to be more sensitive than the McMaster method for diagnosing GIN infections in sheep, with a subsequent advantage in diagnosing anthelmintic resistance using faecal egg count reductions tests (FECRT)<sup>x</sup>.

Instructions for use are included in Appendix One. The sensitivity used was 10 eggs per gram.



Figure 3: Investigator Sandra Playford preparing Mini-FLOTAC slides for worm egg counts using microscope at the Razorback Laboratory. Note use of Fill-FLOTAC for sample homogenisation and slide filling.

#### 3.1.5 FECPAK<sup>G2</sup>

Dung samples are weighed and mixed with water prior to a 'sedimentation phase' to remove lipids that can interfere with visualisation of eggs. After the supernatant is discarded, the precipitate is mixed with saline and filtered prior to a pipette being used to load the cassette. Worm eggs float to the top of the well in the cassette and are photographed at five levels of the meniscus. The images are melded into a single image that is sent via internet connection to a trained operator to 'mark up' the image by highlighting strongyle and *Nematodirus* eggs using different coloured electronic markers. The markers are then counted and the worm egg count is calculated. Results are sent to the submitter by email. Details of preparation are included in Appendix 1.



The sensitivity of this method for sheep and goats is 35 eggs per gram.

Figure 4: Photograph of eggs accumulated in well in cassette of FECPAK<sup>G2</sup>. Note strongyle eggs marked with red marker and *Nematodirus* eggs marked with green.

#### **3.1.6 Statistical analysis**

Data from the original laboratory data capture sheets was entered into an Excel (Microsoft Corporation) spreadsheet and forwarded for statistical analysis. Dr. Andrew Hodge of Zoetis Australia analysed the dataset and provided a highly detailed report of the variation in data between and within methods.

# 4. Results

#### 4.1 Worm egg counts

#### 4.1.1 Samples collected and counted

A total of 54 samples each week were tested twice each using Mini-FLOTAC, read at 10 eggs per gram (epg) sensitivity and FECPAK<sup>G2</sup> read at 35 epg sensitivity.

#### 4.1.1.1 Week 1

In Week 1, counts using Mini-FLOTAC ranged from 5 to 6960 epg with an average of 1406 epg. Counts using FECPAK<sup>G2</sup> ranged from 0 to 8050epg with an average of 967.7epg.



The correlation between the two methods was moderate,  $R^2 = 0.49$ .

Figure 5: Graphs showing correlation between calculated worm egg count values for goats using FECPAK<sup>G2</sup> and Mini-FLOTAC in Week 1.

#### 4.1.1.2 Week 2

The highest count noted in Week 2 was 5160 epg with an average of 798.9epg (Mini-FLOTAC) and 4340 epg with an average of 572.3epg (FECPAK<sup>G2</sup>). Correlation between the two methods was moderate,  $R^2 = 0.55$ .



# Figure 6: Graphs showing correlation between calculated worm egg count values for goats using FECPAK<sup>G2</sup> and Mini-FLOTAC in Week 2.

#### 4.1.1.3 Week 3

In Week 3, worm egg counts ranged from 0-9940 epg with an average of 928.6epg (Mini-FLOTAC) and 0-2590 epg with an average of 474.6epg (FECPAK<sup>G2</sup>). Correlation between the two methods was medium,  $R^2 = 0.35$ .



Figure 7: Graphs showing correlation between calculated worm egg count values for goats using FECPAK<sup>G2</sup> and Mini-FLOTAC in Week 3.

#### 4.1.1.4 Week 4

In Week 4, worm egg counts ranged from 20 to 3260epg with an average of 623 epg (Mini-FLOTAC) and from 0 to 5425 epg with an average of 653.5epg (FECPAK<sup>G2</sup>). Correlation between the two methods was high,  $R^2 = 0.81$ .



Figure 8: Graphs showing correlation between calculated worm egg count values for goats using FECPAK<sup>G2</sup> and Mini-FLOTAC in Week 4.

#### 4.1.1.5 Week 5

In Week 5, worm egg counts ranged from 5 to 5605 epg with an average of 828.3epg (Mini-FLOTAC) and 0 to 3325epg with an average of 647.4epg (FECPAKG2). Correlation between the methods was high at  $R^2 = 0.89$ .



Figure 9: Graphs showing correlation between calculated worm egg count values for goats using FECPAK<sup>G2</sup> and Mini-FLOTAC in Week 5.

#### 4.1.1.6 Weeks 1-5 summary



Figure 10: Correlation (expressed as R<sup>2</sup>) between Mini-FLOTAC and FECPAK<sup>G2</sup> over the course of the 5-week trial.

#### 4.2 Pooled and individual worm egg counts

Each mob consisted of 5 goats that had their individual worm egg counts conducted each week by each method. The samples were then pooled, homogenised and a sub-sample taken to conduct pooled worm egg counts.

Table 2 shows the difference (expressed as a percent of the pooled count) in Week 5 between the mean of the 5 individuals and the pooled count when the samples were mixed.

Table 2: Percentage difference between average of the 5 individual counts and the pooled count of
the 5 samples across 9 mobs of goats for samples collected and counted in Week 5.

% difference av. V pool					
Mini-					
Mob	Flotac	FECPAK <sup>G2</sup>			
1	-9%	20%			
2	44%	52%			
3	-91%	-14%			
4	34%	31%			
5	15%	29%			
6	28%	35%			
7	-1%	11%			
8	-95%	0%			
9	-221%	-198%			
Summary	-33%	-4%			

### 4.3 Pattern of worm egg counts

The pattern of worm egg excretion from each mob of goats over 5 weeks is shown in Figure 10.





#### 4.4 Zero counts

The number of zero counts (and total number of counts) for each method is shown in Table 3.

Table 3: Number of times a zero count (= no worm eggs) was recorded for each of the 2 worm egg count methods in each of the 5 weeks of the trial.

Week	Mini-FLOTAC	FECPAK <sup>G2</sup>
1	0 (54)	3(54)
2	3(54)	8(54)
3	1(54)	9(54)
4	0(54)	2(54)
5	0(54)	4(54)
Total	4 (270)	26 (270)
% zero counts	1.5%	9.6%

#### 4.5 Larval culture

Each farm had a pooled larval culture conducted once in the final week of the trial. Larval culture results are presented in Table 4.

#### Table 4: Larval culture and differentiation results for each of the farms in the trial.

Mobs Date Haemonchus Teladorsagia Trichostrongylus Cooperia Chabertia
---

1, 2 & 3 (pooled)	23JUNE2020	0	23	77	0	0
4	24JUNE2020	100	0	0	0	0
5	23JUNE2020	71	0	6	0	23
6, 7	26JUNE2020	0	22	78	0	0
(pooled)						
8	26JUNE2020	77	5	18	0	0
9	24JUNE2020	21	13	64	0	2

# 4.6 Repeatability of worm egg counts using Mini-FLOTAC and FECPAK<sup>G2</sup>

Table 5 summarises repeatability (difference in the two FEC values) for Mini-FLOTAC, vs. the overall mean. There were significant trends in all cases on either the original or log scales (though not necessarily both).

Datas		
et	Original scale	Log scale
Week 1	No significant trend in difference vs. overall mean	Significant trend in difference vs. overall mean (p=0.0002)
Week 2	No significant trend in difference vs. overall mean	Significant trend in difference vs. overall mean (p=0.0161)
Week 3	No significant trend in difference vs. overall mean	Significant trend in difference vs. overall mean (p=0.0132)
Week 4	Significant trend in difference vs. overall mean (p=0.004)	No significant trend in difference vs. overall mean
Week 5	Significant trend in difference vs. overall mean (p=0.0004)	No significant trend in difference vs. overall mean
Over all	No significant trend in difference vs. overall mean	Significant trend in difference vs. overall mean (p<0.0001)

Table 5: Repeatability – Mini-FLOTAC

Table 6 summarises repeatability (difference in the two FEC values) for the FECPAK<sup>G2</sup> method, vs. the overall mean. There were significant trends on the original scale for Week 1 and overall, but not on the log scale. This may indicate slightly better repeatability data compared to the current method (Table 5).

Dataset	Original scale	Log scale
	Significant trend in difference vs. overall mean	No significant trend in difference vs.
Week 1	(p=0.0036)	overall mean
		No significant trend in difference vs.
Week 2	No significant trend in difference vs. overall mean	overall mean
		No significant trend in difference vs.
Week 3	No significant trend in difference vs. overall mean	overall mean

#### Table 6: Repeatability – FECPAK<sup>G2</sup> Method

Week 4	No significant trend in difference vs. overall mean	No significant trend in difference vs. overall mean
Week 5	No significant trend in difference vs. overall mean	No significant trend in difference vs. overall mean
Overall	Significant trend in difference vs. overall mean (p=0.0162)	No significant trend in difference vs. overall mean

# 5. Discussion

#### 5.1 Concordance between Mini-FLOTAC and FECPAK<sup>G2</sup>

#### 5.1.1 Correlation over time

The range and average values of the weekly sum of 54 worm egg counts were similar in the first 3 weeks but had clear discrepancies over some individual tests. In particular, Mini-FLOTAC counts were higher over this period, particularly in Week 3 when the average values for eggs per gram calculated was almost double those of FECPAK<sup>G2</sup>.

However, in the 4<sup>th</sup> and 5th weeks of the trial, the correlation between the two methods was very high (0.82 and 0.88 respectively). This is thought to be due to the operator gaining increasing familiarity with the details of the FECPAK<sup>G2</sup> method and carrying out the preparation in a more competent manner.

In summary, correlations between the two worm egg count methods were moderate in the first 3 weeks, but were high in the final 2 weeks (see Figure 10.)

#### 5.1.2 Number of zero counts

Mini-FLOTAC sensitivity is higher, with a limit of quantification of 10 epg, compared to FECPAK<sup>G2</sup> with 35 epg. This means a lower number of zero counts observed. Over the course of the trial, MiniFLOTAC recorded only 4 zero counts, while FECPAK<sup>G2</sup> recorded 26 zero counts. However, on each occasion that FECPAK<sup>G2</sup> gave a zero count, the count on Mini-FLOTAC was close to zero (data not shown).

#### 5.2 Pooled and individual worm egg counts

Overall, there was a reasonably high correlation between pooled and the average of the individual worm egg counts from goats in the trial. As shown in Table 3, when the correlation varied it was often seen in both methods. This could be due to the nature of mixing and homogenising samples or the distribution of worm eggs in different parts of the mixing chamber.

Given that repeat counts of aliquots from a single chamber of dung containing worm eggs suspended in saline solution follows a Poisson distribution, where the mean and the variance are

equal, the 'true mean' of a series of worm egg counts will be unlikely to match the pooled count, but the average of repeat measures will be expected to approach the true mean.

Table 2 shows that in Week 5 of this trial, FECPAK<sup>G2</sup> showed pooled counts that were closer to the average of individual counts. This is possibly due to the method of pooling for FECPAK<sup>G2</sup>, which involves making a slurry and homogenising by squeezing the samples inside a sealed plastic bag.

#### 5.3 Pattern of excretion of worm eggs in goats in small mobs

All mobs of goats had high worm egg counts across the trial. Figure 10 shows the variation, in particular illustrating the effect of drenching on worm egg counts. For example, Mobs A, B and C were treated for worms after the first week. Results of Weeks 2 and 3 show a decline in worm egg numbers. In contrast, Mob G used Bioworma in feed and had good worm control (shown by low worm egg numbers) for the first 3 weeks of the trial. Worm egg counts increased substantially in the last 2 weeks of the trial, for reasons unknown. All of the worm larvae identified on this farm were *Haemonchus*.

#### 5.4 Repeatability of worm egg counts

Analysis of repeated measures for the same sample showed that despite some variation in the first week, there were no significant differences between repeat samples using FECPAK<sup>G2</sup> in Weeks 2-5. Using Mini-FLOTAC there were significant differences at each time point, in either the original scale or when analysed by log scale.

These results indicate that the FECPAK<sup>G2</sup> worm egg counts are more repeatable than the MiniFLOTAC method.

#### 5.5 Worm genera detected

Differentiation of third stage larvae showed that a range of worm genera were present on trial farms. On one farm only *Haemonchus* (Barber's Pole Worm) was detected, while on other farms mixed burdens of worms were detected (see Table 4). The 5 genera detected are typically present on Australian goat farms.

# 6. Conclusions/recommendations

# 6.1 FECPAK<sup>G2</sup> as a method for monitoring worm burdens and diagnosing

#### anthelmintic resistance in goats

The results of repeated worm egg counting on dung samples from goats over 5 weeks show that, despite unfamiliarity with the novel method (FECPAK<sup>G2</sup>) in the early part of the trial, a very high correlation between the methods was observed after 3 weeks. The 4<sup>th</sup> and 5<sup>th</sup> week correlations were very high ( $r^2$ = 0.81 and 0.89 respectively).

The FECPAK<sup>G2</sup> testing method is available in Australia for use by goat producers themselves, or alternatively in veterinary clinics or rural stores that service goat farms. One key advantage is that testing can be done at the point of care or at a nearby service provider without the need for sending dung samples through the mail to an accredited laboratory, or risking inaccurate results being done

by an unaccredited provider. Another is that feedback on preparation and image capture can be given immediately to the operator and images of each test are kept on record for auditing.

These features result in higher quality assurance, lower cost, greater convenience and lower biosecurity risk for both animals and humans compared to conventional methods.

Adoption activities should aim to educate producers and promote routine use of worm egg counts using FECPAK<sup>G2</sup> by farmers, rural stores and veterinarians, with a view to making the service commercially self-sufficient. This model has already been adopted by about 60 providers in Australia, but the rate of testing is still very low.

Adoption of FECPAK<sup>G2</sup> for both routine monitoring of goat worm burdens and for testing the efficacy of drenches will lead to improved worm control in goats. This will in turn improve productivity and reduce the risk of violations of residues in goat meat or unacceptable animal welfare outcomes that threaten the sustainability of the Australian goat industry.

# 7. Key messages

#### 7.1 Routine use of worm egg counts is valuable for goat management

#### 7.1.1 Monitoring

Using worm egg counts enables goat producers to test and treat goats before they are at risk of death or reduced production.

#### 7.1.2 Drench resistance testing

Producers who have access to convenient low-cost worm egg count testing are more likely to conduct drench resistance testing. This will lead to less abuse of drenches due to using anthelmintics with low efficacy.

#### 7.1.3 FECPAK<sup>G2</sup> is a reliable method of conducting worm egg counts

As well as microscopic methods such as Mini-FLOTAC and the McMaster method, FECPAKG2 can be used as a point-of-care testing platform for rapid and convenient worm egg counts.



Figure 12: Goat from co-operator farm displaying browsing behaviour.

# 8. Appendix

# 8.1 Appendix 1- Description of methods for worm egg counts

#### 8.1.1 Mini-FLOTAC Instructions for use



#### 8.1.2 FECPAK<sup>G2</sup> Instructions for use



#### 8.1.4 Statistical analysis summary

Dr. Andrew Hodge of Zoetis Australia analysed the dataset and provided a highly detailed report of the variation in data between and within methods.

Overview:

• Table 1 summarises comparison of mean FECs for the two methods on original and log scales. Means were higher with the current method in all cases except Week 4.

Datas		
et	Original scale	Log scale
Week 1	Mean higher with current method; mean diff. 439 (95% CI 75-802)	Mean higher with current method
Week 2	Mean higher with current method; mean diff. 227 (95% CI 16-437)	Mean higher with current method
Week 3	Mean higher with current method; mean diff. 454 (95% CI 67-841)	Mean higher with current method
Week 4	No. sig. diff. in means; mean diff31 (95% CI -154 to 93)	No sig. diff. in means

Week	Mean higher with current method; mean diff. 184 (95% CI	Mean higher with current
5	93 to 275)	method
Overa ll	Mean higher with current method; mean diff. 254 (95% CI 137-372)	Mean higher with current method

• Table 2 summarises correlation coefficients between the FECs for the two methods. All correlations were highly significant, with R-squared values from 0.35 to 0.89. Table 2: Correlation of FEC data from the two methods

Dataset	Original scale	Log scale
Week 1	Significant correlation; R <sup>2</sup> =0.49	Significant correlation; R <sup>2</sup> =0.61
Week 2	Significant correlation; R <sup>2</sup> =0.55	Significant correlation; R <sup>2</sup> =0.74
Week 3	Significant correlation; R <sup>2</sup> =0.35	Significant correlation; R <sup>2</sup> =0.62
Week 4	Significant correlation; R <sup>2</sup> =0.81	Significant correlation; R <sup>2</sup> =0.68
Week 5	Significant correlation; R <sup>2</sup> =0.89	Significant correlation; R <sup>2</sup> =0.76
Overall	Significant correlation; R <sup>2</sup> =0.50	Significant correlation; R <sup>2</sup> =0.66

• Table 3 summarises the method agreement (difference in results between methods compared vs. overall mean FEC). There were a lot of significant trends here. Table 3: Method Agreements

Datas		
et	Original scale	Log scale
Week	No significant trend in difference vs. overall	No significant trend in difference vs. overall
1	mean	mean
Week 2	Significant trend in difference vs. overall mean (p=0.04)	No significant trend in difference vs. overall mean
Week 3	Significant trend in difference vs. overall mean (p<0.0001)	Significant trend in difference vs. overall mean (p=0.0195)
Week 4	Significant trend in difference vs. overall mean (p=0.002)	No significant trend in difference vs. overall mean
Week 5	Significant trend in difference vs. overall mean (p<0.0001)	Significant trend in difference vs. overall mean (p=0.0002)
Over all	Significant trend in difference vs. overall mean (p<0.0001)	Significant trend in difference vs. overall mean (p<0.0001)

• Table 4 summarises repeatability (difference in the two FEC values) for the current method, vs. the overall mean. There were significant trends in all cases on either the original or log scales (though not necessarily both).

#### Table 4: Repeatability - Current Method

Datas		
et	Original scale	Log scale

Week 1	No significant trend in difference vs. overall mean	Significant trend in difference vs. overall mean (p=0.0002)
Week 2	No significant trend in difference vs. overall mean	Significant trend in difference vs. overall mean (p=0.0161)
Week 3	No significant trend in difference vs. overall mean	Significant trend in difference vs. overall mean (p=0.0132)
Week 4	Significant trend in difference vs. overall mean (p=0.004)	No significant trend in difference vs. overall mean
Week 5	Significant trend in difference vs. overall mean (p=0.0004)	No significant trend in difference vs. overall mean
Over all	No significant trend in difference vs. overall mean	Significant trend in difference vs. overall mean (p<0.0001)

• Table 5 summarises repeatability (difference in the two FEC values) for the FECPAK method, vs. the overall mean. There were significant trends on the original scale for Week 1 and overall, but not on the log scale. This may indicate slightly better repeatability data compared to the current method (Table 4).

Datas		
et	Original scale	Log scale
Week	Significant trend in difference vs. overall mean	No significant trend in difference vs.
1	(p=0.0036)	overall mean
Week	No significant trend in difference vs. overall	No significant trend in difference vs.
2	mean	overall mean
Week	No significant trend in difference vs. overall	No significant trend in difference vs.
3	mean	overall mean
Week	No significant trend in difference vs. overall	No significant trend in difference vs.
4	mean	overall mean
Week	No significant trend in difference vs. overall	No significant trend in difference vs.
5	mean	overall mean
Overa II	Significant trend in difference vs. overall mean (p=0.0162)	No significant trend in difference vs. overall mean

#### Table 5: Repeatability - FECPAK Method

 Table 6 summarises the diagnosis agreement for the original counts for the two methods, from the cross-tabulation of Low-Medium-High values (as per the definitions for sheep). Same category indicates any pairs of results that are L-L, M-M or H-H; within one category also includes pairs such as L-M or M-H, i.e. anything except L-H or H-L.

Dataset	Comment
Week 1	57% same category; 91% within +/- one category
Week 2	74% same category; 93% within +/- one category
Week 3	65% same category; 94% within +/- one category

Table 6:	Diagnosis	Agreement
----------	-----------	-----------

Week 4	78% same category; 100% within +/- one category
Week 5	69% same category; 98% within +/- one category
Overall	69% same category; 95% within +/- one category

# 9. References

- <sup>i</sup> Jolly S (2013) Goat nutrition in Australia Literature review B.GOA.0055 Meat and Livestock Australia, North Sydney, Australia.
- <sup>ii</sup> http://www.wormboss.com.au/sheep-goats/tests-tools/drench-decision-guide/goats/nsw-cssw.php accessed 29 June 2020
- iii https://www.techion.com/ accessed 29 June 2020
- <sup>iv</sup> Rashid MH, MA Stevenson, S Waenga, G Mirams, AJD Campbell, JL Vaughan, A Jabbar (2018) Comparison of McMaster and FECPAKG2 methods for counting nematode eggs in the faeces of alpacas Parasites & Vectors 11:278
- <sup>v</sup> Baxendall S, M Lyndal-Murphy, D Maxwell, L Kahn (2016) Worm control in goats- advice for Australian veterinarians- WormBoss.
- <sup>vi</sup> National Residue Survey (2018) Goat Residue Testing Annual Datasets 2017-2018, Department of Agriculture and Water Resources, Government of Australia
- <sup>vii</sup> Cringoli G, MP Maurelli, B Levecke, A Bosco, J Vercruysse, J Utzinger, L Rinaldi (2017) The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nature Protocols 12, 9:1723
- viii B Barda, P Cajal, E Villagran, M Juarez, A Krolewiecki, L Rinaldi, G Cringoli, R Burioni, M Albonico (2014) MiniFLOTAC, Kato-Katz and McMaster: three methods, one goal; highlights from north Argentina. Parasites & Vectors 7: 271
- <sup>ix</sup> Godber OF, CJ Pythian, A Bosco, D Ianiello, G Coles, L Rinaldi, G Cringoli (2015) A comparison of the FECPAK and Mini-FLOTAC faecal egg counting techniques. Veterinary Parasitology 207, 3–4: 342-345
- <sup>x</sup> Rinaldi L, B Levecke, A Bosco, D Ianniello, P Pepe, J Charlier, G Cringoli, J Vercruysse (2014) Comparison of individual and pooled faecal samples in sheep for the assessment of gastrointestinal strongyle infection intensity and anthelmintic drug efficacy using McMaster and Mini-FLOTAC. Veterinary Parasitology 205, 1–2: 216-223