MCMASTER METHOD OF WORM EGG COUNT FROM FAECAL SAMPLES OF GOATS: A COMPARISON OF SINGLE AND DOUBLE CHAMBER ENUMERATION OF WORM EGGS

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ABSTRACT. Many parasitology laboratories practiced the McMaster technique as a method in obtaining the quantitative diagnosis of *Strongyle* eggs burden in farm animals especially ruminants. The McMaster technique also play a crucial role in faecal egg count reduction test (FECRT) for anthelmintic resistance identification. Some laboratories recommend two-chamber counting method while some recommend single chamber counting method. This study focuses on the comparison between single and double counting in McMaster technique for detection of *Strongyle* egg count. In this study, it is shown that there is no significant difference between both methods based on the *p*-value obtained which is $p>0.05$ from 127 fresh goat faecal samples. The techniques practised during the study follow the standard established technique. Single chamber counting is suitable for a large number of faecal samples from big herds because it is faster, less laborious and produces sensitive and reliable results in *Strongyle* egg count. As more commercial farms are set up, there is a need to conduct a fast and efficient test to help farmers evaluate their livestock worm burden.

Keywords: McMaster technique, EPG, single chamber, double chamber

INTRODUCTION

The McMaster technique is a quantitative method to demonstrate and count *Strongyle* eggs in faeces of herbivores. The eggs are floated in a known volume of faecal suspension and counted microscopically on a McMaster slide. McMaster technique utilizes faecal sample to determine faecal egg count enabling the detection of parasitic element (e.g. helminths egg and larvae, protozoa oocysts and cysts) which is a widely used method to count parasite eggs (Cringoli *et al*., 2010). Faecal egg count is done for monitoring helminthiasis burdens in herd and flocks, determining the degree of pasture contamination and in epidemiological studies or anthelmintic resistance identification (Coles *et al*., 1992).

RE# MJVR -0036-2014

The McMaster technique is developed and improved at the McMaster laboratory of the University of Sydney (Gordon and Whitlock, 1939) which is the most universally used technique for estimating the number of helminth eggs in faeces. In literature, many variations of the McMaster technique can be found and a lot of modifications is done to this method (Cringoli *et al*., 2004). Different McMaster method modifications use various weight of faeces examined, volumes and types of floatation solutions, sample dilutions, floatation times, applications of additional centrifugation, duration and speeds of centrifugation, numbers of sections of McMaster slide counted and different coefficients for interpretation (Cringoli *et al*., 2004). These variation factors can limit the accuracy and significance of diagnostic faecal egg count in the McMaster technique. The choice of single and double chamber counting in McMaster can also affect the reliability of the McMaster technique in estimating real egg per gram (EPG) from faeces.

Thus, the aim of this study is to compare the sensitivity and relationship between single chamber and double chamber counting in the McMaster technique by assessing the number of strongyle eggs per gram (EPG) in fresh faecal sample of goats.

METHODOLOGY

A total of 127 faecal samples were collected from goats of various ages (three months to three years) from nine smallholder goat farms around the vicinity of Ipoh. About five grams of faeces was collected using plastic gloves, per recta of each goat, selected randomly from the flock. The faecal sample was processed immediately within two hours of collection. An amount of three grams of faeces from each goat was weighed, placed in a jar, and saturated salt (sodium chloride) solution was added. The saturated salt solution was prepared by diluting common salt in distilled water in an electric mixer until it cannot be dissolved anymore. The ratio of faeces to the sodium chloride solution is 1 g:15 ml. The faeces and sodium chloride mixture was then poured into a mortar through a tea-sieve and ground to a paste using a pestle. The waste produced in the tea-sieve is discarded while the liquid in the mortar is poured back into a clean jar. The jar is gently spun to stir the sediment in it. Using a clean pipette, the sediment is pipetted by placing the pipette in the centre of the jar to the centre of the overall depth of the sediments. Next, this sediment is pipetted into the chambers of the McMaster slides in which one chamber is for single reading and the other two-chamber is for double chamber reading where the eggs from both chamber is counted and an average faecal egg count (FEC) is estimated. For example, number of eggs found in chamber A of a McMaster slide is 300 and for chamber B is 200, so the total number of eggs for that faecal samples is 500 EPG. From this value, only the average FEC is calculated and used in the data, which is 250 EPG.

The McMaster slide is then immediately observed under the microscope to prevent crystallization of the salt solution. This study focused only on strongyle eggs (MAFF, 1986).

The counted strongyle eggs will be calculated using the following formula to obtain the EPG value:

EPG = Total number of eggs counted × 100

For example, if 2 eggs were counted, the EPG is 200.

RESULTS

Statistical analysis was conducted using a 20.0 version SPSS system where direct calculation of mean, median, variance, and standard variation for 127 sample sizes including a histogram were presented in Table 1, Table 2, Table 3, Table 4, and Figure 1, Figure 2 and Figure 3. Kolmogorov-Smirnov and Shapiro-Wilk tests were used for normality test of data. A nonparametric Mann-Whitney U test was chosen by the system as suitable method for this experiment base on data provided and sample size. It evaluates

Table 1. Descriptive statistic of single chamber data.

Worm Egg Count (WEC)		Statistic	Std. Error
Mean		1086.61	218.834
95% Confidence Interval for Mean	Lower Bound	653.55	
	Upper Bound	1519.68	
5% Trimmed Mean		669.73	
Median		100.00	
Variance		6081803.525	
Std. Deviation		2466.131	
Minimum		0	
Maximum		16500	
Range		16500	
Interquartile Range		1100	
Skewness		4.123	.215
Kurtosis		19.941	.427

Table 2. Test of Normality.

	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
WEC	.330	127	.000	.483	127	.000

a. Lilliefors Significance Correction

Table 3. Descriptive statistic of double chamber data.

WEC		Statistic	Std. Error
Mean		932.68	195.628
95% Confidence Interval for Mean	Lower Bound	545.54	
	Upper Bound	1319.82	
5% Trimmed Mean		554.13	
Median		100.00	
Variance		4860332.458	
Std. Deviation		2204.616	
Minimum		0	
Maximum		15400	
Range		15400	
Interquartile Range		800	
Skewness		4.473	0.215
Kurtosis		23.970	0.427

WEC: Worm Egg Count

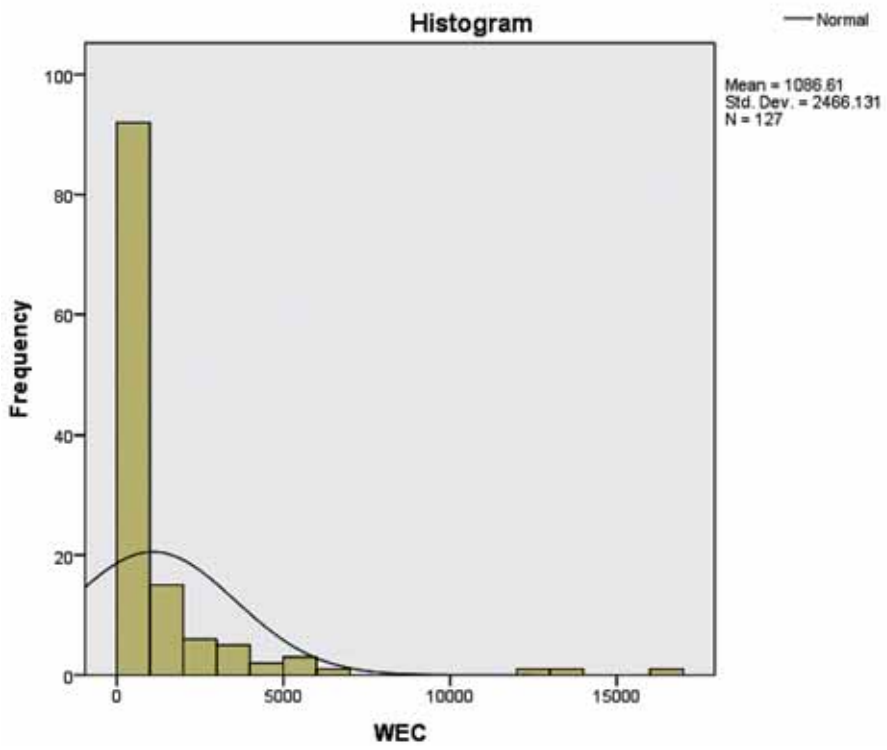

Figure 1. Data for single chamber method presented in histogram and the bell shape graph showing the normality of the data skewed to the right.

Table 4. Tests of Normality

	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
WEC	.336	127	.000	.460	127	.000
a. Lilliefors Significance Correction						

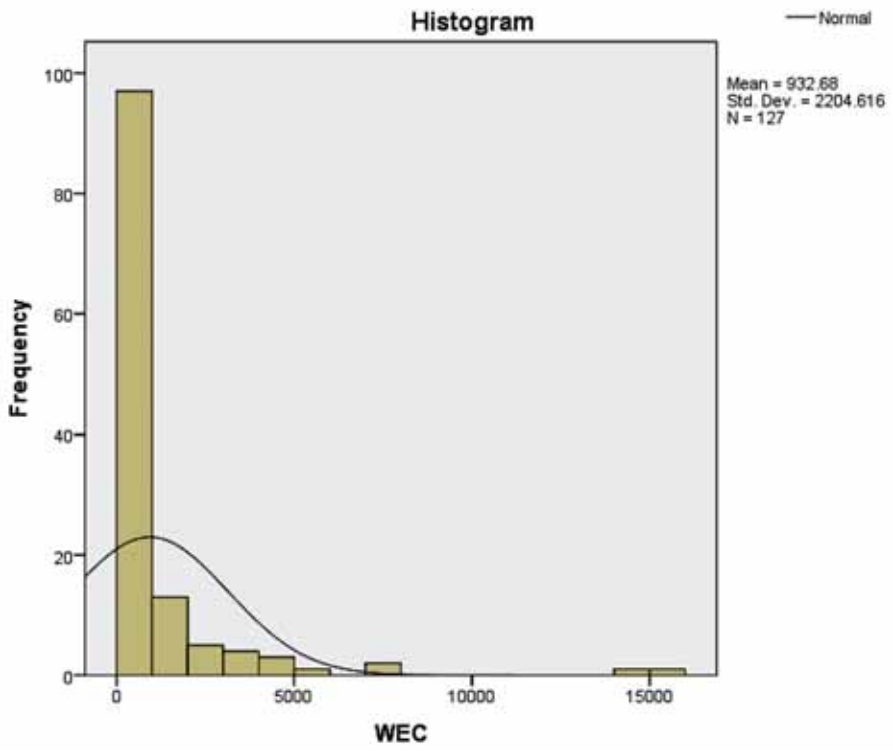

Figure 2. Data for double chamber method presented in histogram and the bell shape graph showing the normality of the data skewed to the right.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of WEC is the same across categories of Chamber.	Independent-Samples Mann-Whitney U Test	.950	Retain the null hypothesis.

Figure 3. Nonparametric Tests. Asymptotic significances are displayed. The significance level is 0.05

the statistical differences among the two conducted McMaster methods. Based on Mann-Whitney U test, *p*-value = 0.950 > x=0.05, hence we accept H_0. Meaning that there is no significant difference in egg per gram (EPG) value when single or double chamber McMaster counting is used. In this case, there is no significant reduction in EPG value when either one of the McMaster method is being used.

DISCUSSION

There are many factors in the methodology of the McMaster faecal egg count technique, that can affect the result of both counting techniques. One of the most important factors that affects the result is the weight of faeces examined. Variation in amount of faecal sample will affect the amount of strongyle eggs seen and counted. Precise measurement of faecal egg count can be determined using electronic balance to weigh the faeces. The next factor in methodology that can affect the accuracy of both counting methods in McMaster technique is sampling error arising from the fact that the eggs are not evenly distributed through the faeces, especially if the consistency of faeces is variable; where faeces may be soft, diarrhoeic, hard, normal or constipated.

Other factors that can contribute to variation of results during experiments are bias in pipetting the mixture; which can be avoided by pipetting the sample from the centre of the jar after stirring its content to allow even distribution of helminth eggs in the jar. Moreover, pipetting at the centre of the jar instead of at the base of the jar will prevent pipetting up of heavier eggs sedimented at the base of the jar and almost no strongyle eggs as it is lighter and might have floated.

Apart from that, other factors that can influence strongyle egg count in both counting methods include parasite biology (fecundity of the species, parasite number, prepatent period, arrested development), host physiological status (history of prior exposure to parasite, nutrition), host management factors (treatments, anthelmintic resistance, herd or flock density) or technician's skill level (Vadlejch *et al.,* 2011).

Both single and double chamber counting methods in the McMaster technique can be used in determination of helminth eggs. The mean egg count of double chamber counting gives the same reading of strongyle eggs when compared with single chamber counting. Single chamber counting method is faster, less laborious and produces sensitive and reliable results of strongyle egg count with repeated measurement over time.

CONCLUSION

In conclusion, the findings of the present study proved that there is no difference on results obtained when single chamber counting or double chamber counting is being used. Hence, this experiment has aided the laboratory practitioner in choosing a suitable method for their EPG

counting which is time-saving especially when it involves many samples.

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