parasitologia

MDPI

Article

The Influence of Flock Variation, Sample Size, Flock Size and Mean Egg Count on the Accuracy and Precision of the Estimated Mean Egg Count

Michael Stear [1,*], Sarah Preston [1], David Piedrafita [1], George Cullimore [2] and Katarzyna Donskow-Łysoniewska [3]

[1] School of Science, Psychology and Sport, Federation University Australia, Ballarat, VIC 3350, Australia
[2] Performance Recorded Lleyn Breeders, Coombe Barn, Kelston, Bath BA1 9AJ, UK
[3] Department of Experimental Immunotherapy, Faculty of Medicine, Lazarski University, 02-662 Warsaw, Poland
* Correspondence: m.stear@latrobe.edu.au

Abstract: The control of parasitic nematode infection in sheep and other animals is threatened by the evolution of drug resistance in parasite populations. One recommendation to delay the onset of drug resistance is to estimate the flock mean faecal egg counts by sampling a subpopulation and to treat sheep only when egg counts are high. However, there is little research on the accuracy and precision of estimates of the flock mean obtained from samples. In silico sampling was used to quantify the influence of flock variation, sample size, flock size and mean egg count on the accuracy and precision of the estimated mean egg count. Commonly used and recommended sampling schemes gave alarmingly imprecise estimates of the true flock means. Simply providing a point estimate of the flock egg count can be seriously misleading. Therefore, quantiles were provided for the proportion of estimates in a plausible scenario that is likely to require treatment. It may be more informative to use these quantiles to predict the probability that the true flock mean is sufficiently high to consider treatment.

Keywords: faecal egg count; sampling; anthelmintic treatment; negative binomial distribution; sample size; modelling; *Teladorsagia circumcincta*

Citation: Stear, M.; Preston, S.; Piedrafita, D.; Cullimore, G.; Donskow-Łysoniewska, K. The Influence of Flock Variation, Sample Size, Flock Size and Mean Egg Count on the Accuracy and Precision of the Estimated Mean Egg Count. *Parasitologia* **2024**, *4*, 137–149. https://doi.org/10.3390/parasitologia4020012

Academic Editors: Theo de Waal and Geoff Hide

Received: 29 February 2024
Revised: 19 April 2024
Accepted: 25 April 2024
Published: 30 April 2024

1. Introduction

Nematodes threaten the very survival of sheep farming. Heavy infections cause disease and death, while even moderate infections can drastically reduce lamb growth and productivity [1]. Nematodes need to be controlled for humane and profitable livestock farming. Control is mainly achieved with anthelmintics, but the widespread evolution of drug resistance in parasite populations [2] hinders effective parasite control.

Two distinct recommendations to delay the development of drug resistance are to test for anthelmintic resistance and to treat animals only when necessary [3]. The development of statistically appropriate methods to detect drug resistance has received considerable attention [4–6]. In contrast, there has been little published research on when treatment is needed. The usual procedure is to sample faeces from some of the animals in a flock, count the concentration of eggs in the faeces and use the mean egg count as a diagnostic tool to help decide if treatment is required. However, there is little guidance available on the number of individuals required to provide a reliable estimate of the mean faecal egg count in eggs per gram (EPG) in the flock [7].

The statistical basis of sampling to estimate the population mean is well-developed [8]. One suggestion is to sample 10% of the population, but to sample at least 100 but no more than 1000 individuals https://survicate.com/blog/survey-sample-size/#:~:text=Many%20statisticians%20concur%20that%20a,it%20should%20not%20exceed%201000 (accessed on 28 February 2024). However, this recommendation is impractical for many sheep farmers

and is seldom followed. An industry-led group (SCOPS) suggests sampling at least 10 sheep https://www.scops.org.uk/internal-parasites/worms/using-worm-egg-counts/ (accessed on 28 February 2024). However, the reliability of an estimate obtained from ten sheep is not discussed.

In short, estimating the mean EPG of a flock is important information when deciding whether anthelmintic treatment is necessary, but there are few guidelines to help flock managers do this reliably. The purpose of this paper is to explore the influence of flock variation, sample size, flock size and proportion sampled on the accuracy and the precision of faecal counts with sampling regimes typical of sheep farms in the UK and elsewhere.

2. Results

2.1. Variation in the Flock

The influence of variation in the flock is presented in Figure 1, which presents the results of sampling 50 individuals from flocks of 100 animals. Each flock had different values of k (0.5, 1, 2, 3, and 4). The means and standard deviations of 1000 subsamples of 50 animals from each flock were 197.7 ± 29.1, 200.4 ± 23.4, 200.8 ± 16.7, 200.4 ± 13.6 and 200.3 ± 13.0, respectively. There appeared to be no systematic bias in the estimation of the mean. Figure 1 shows that the precision of the estimated mean egg count was negatively influenced by the flock variation. This visual observation was supported by the standard deviations, which declined as k increased. As the variation within the flock increased and k decreased, the spread of the estimated means increased, and consequently, the precision fell. For example, when k = 0.5, the estimated flock mean in 1000 samples of 50 sheep ranged from 103 to 277 epg. In contrast, when k = 4, the range of the estimated flock means was smaller, varying from 155 to 247 epg. Shapiro–Wilk tests showed that the distribution of estimated flock means was not significantly different from a normal distribution except when k = 1 ($p = 0.005$). However, this was not observed in replicate samples ($p > 0.05$) and was assumed to be a consequence of the fact that 1000 samples provide very sensitive tests to detect deviations from a normal distribution.

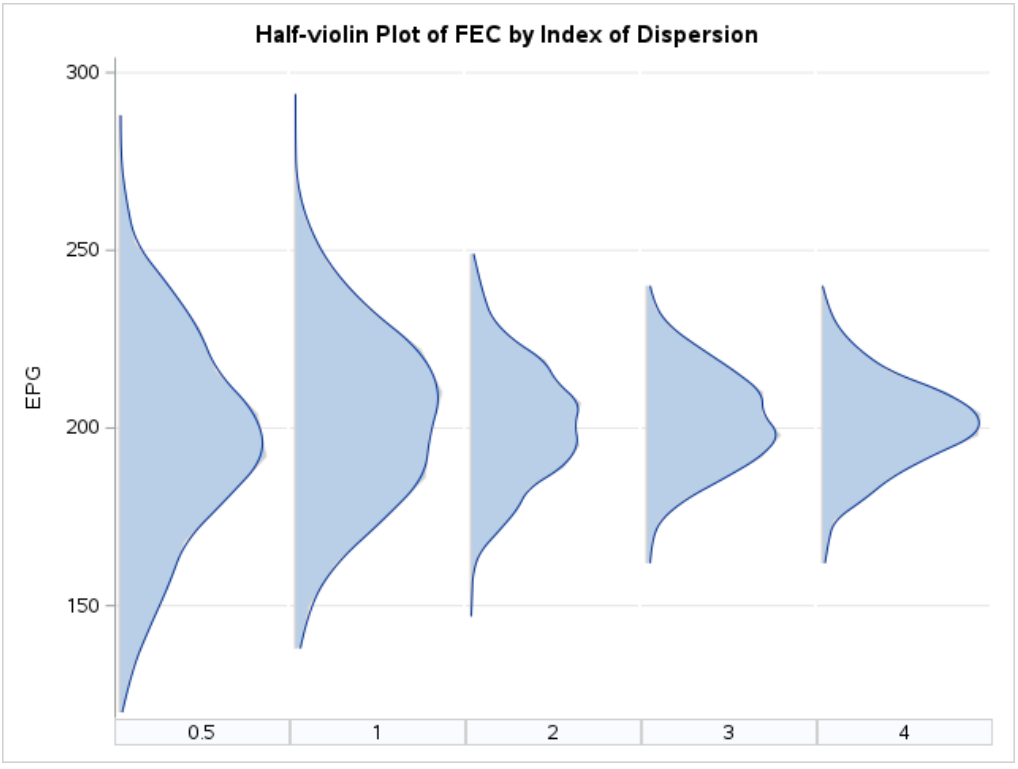


Figure 1. The influence of flock variation on the precision of the estimated mean faecal egg count. The *x*-axis lists the parameters of dispersion used for each set of samples.

2.2. Sample Size

Figure 2 shows the influence of sample size on the precision of the estimated mean EPG. For sample sizes of 10, 20, 30, 40 and 50, the means and standard deviations were 447.2 ± 136.4, 445.6 ± 90.0, 447.1 ± 75.5, 447.7 ± 50.2 and 453.3 ± 46.0, respectively. Shapiro–Wilk tests showed that the distribution of means obtained from sample sizes of 10 and 20 were inconsistent with a normal distribution ($p < 0.01$). In contrast, means obtained from sample sizes of 30, 40 and 50 were not significantly different from a normal distribution ($p > 0.05$). The was no apparent bias in the mean egg counts. As the number of animals in the sample increased, the estimated mean became more precise. There was considerable variation when only 10 animals were sampled; the estimated means in the 1000 replicates ranged from a low of 120 to a high of 965 epg. The 5% quantile was 235 epg, while the 95% quantile was 680 epg, i.e., 90% of the estimated means fell between 235 and 680 epg. When 50 animals were sampled, the range of the mean estimates was smaller. Ninety percent of the observations fell between 294 and 589 epg.

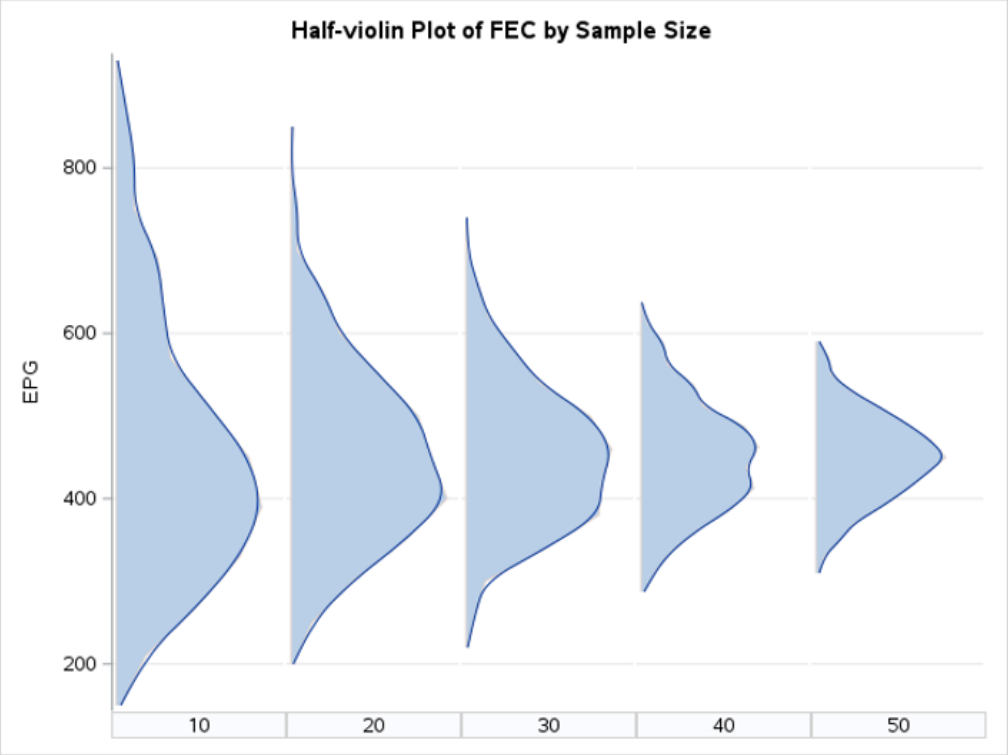


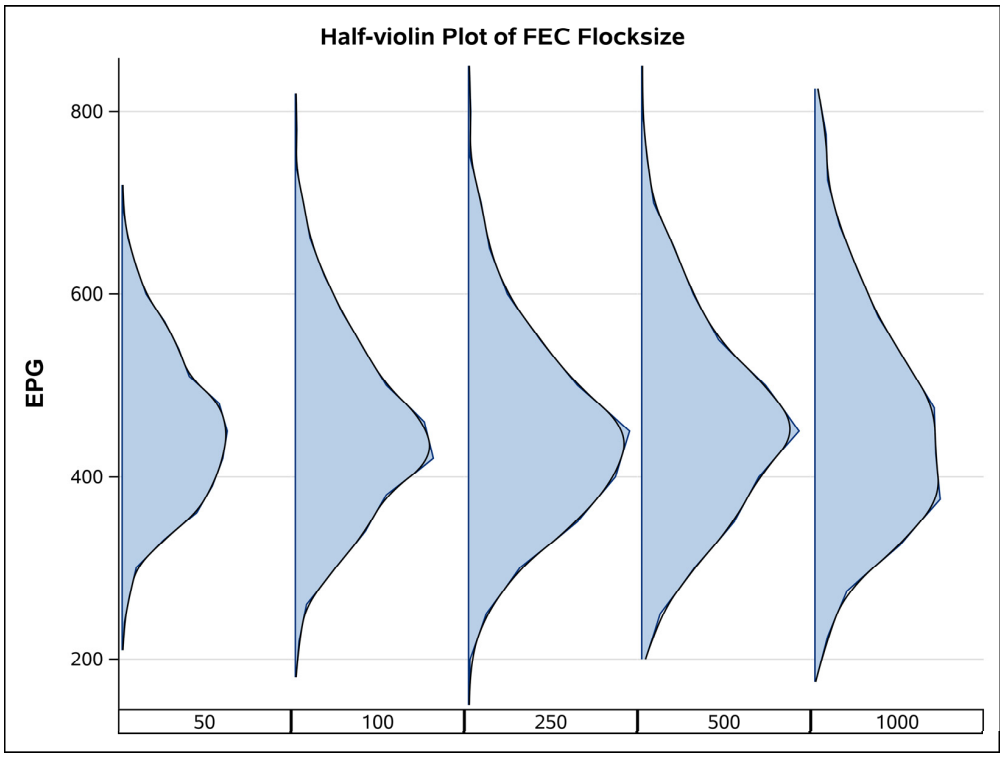
Figure 2. The influence of sample size on the estimated mean egg count. The *x*-axis lists the number of individuals in each sample.

When only 10 or 20 sheep were sampled, the distribution of estimated means was right skewed and not symmetrical. A majority of estimates fell below the flock mean, while a relatively small proportion had estimated means in excess of the flock mean. Consequently, the modes of the distribution of estimated means were below the flock mean. For example, the mode occurred at 415 epg when only 10 sheep were sampled (Figure 2).

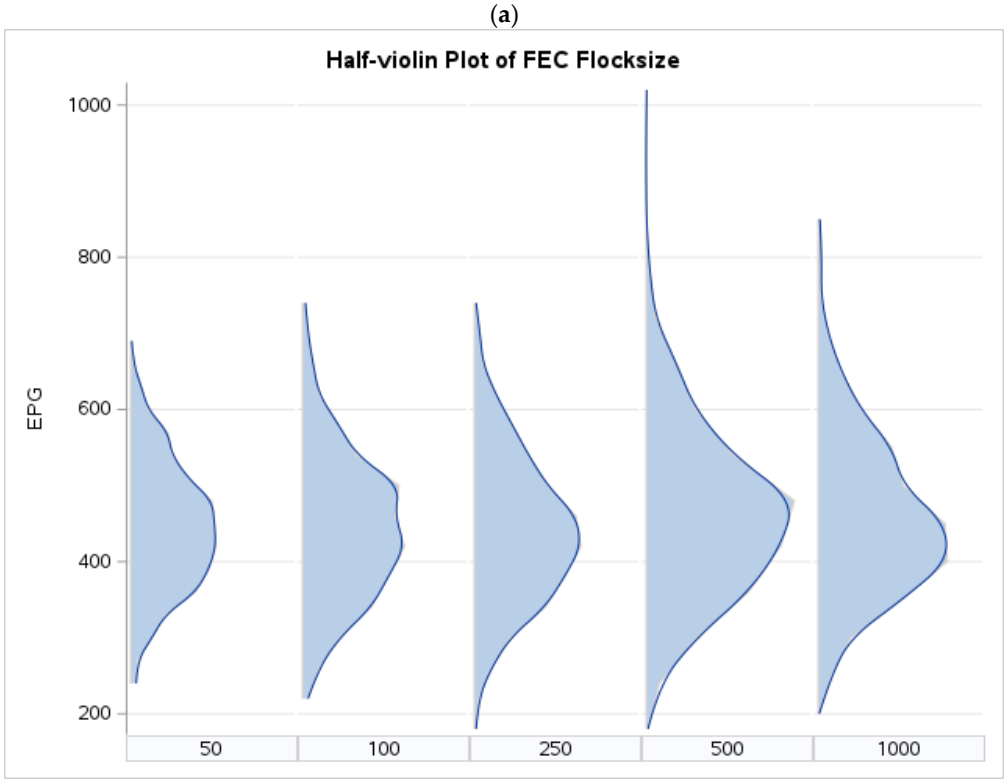
2.3. Flock Size

Figure 3a shows the influence of flock size when the same number (20) of sheep were sampled from each flock. As flock size increased and the proportion of sheep sampled fell from 40% (20 out of 50) to 2% (20 out of 1000), the dispersion increased slightly. The mean and standard deviation of the egg counts were 449.8 ± 82.1, 450.7 ± 95.7, 444.5 ± 99.3, 456.2 ± 104.3 and 459.5 ± 116.6 for flock sizes of 50, 100, 250, 500 and 1000, respectively. Sampling a small number of sheep from a large flock provides an unbiased but relatively

imprecise estimate of the mean flock faecal egg count. Shapiro–Wilk tests showed that none of the distributions of the sample means were normally distributed ($p < 0.01$).



(**a**)



(**b**)

Figure 3. (**a**) The influence of flock size when 20 sheep were sampled from each flock. The *x*-axis lists the number of individuals in each flock. (**b**) The influence of flock size on the precision of the estimated flock mean egg count when 10% of the flock was sampled. The *x*-axis lists the number of individuals in the sampled flock.

Figure 3b illustrates the effect of flock and sample size when sampling 10% of a flock. The flocks ranged in size from 50 to 1000 animals and the corresponding sample sizes ranged from 5 to 100 individuals. For sample sizes of 5, 10, 25, 50 and 100, the means and standard deviations were 451.9 ± 192.4, 458.6 ± 141.1, 436.2 ± 83.2, 453.8 ± 65.4, and 453.5 ± 46.2, respectively. There was no systematic bias in the estimation of the flock mean. All distributions were significantly different from a normal distribution ($p < 0.05$) except when 100 individuals were sampled from a flock of 1000 ($p = 0.758$). However, as the sample size increased from 5 to 100 individuals, the precision of the estimated mean increased, and the distribution of the estimated means appeared closer to normality. Clearly, sampling 10% of a flock does not give precise mean estimates unless the flock is large. A comparison of Figure 3a with Figure 3b suggests that the influence of sample size is more important than the influence of flock size in determining the precision of the estimated mean.

2.4. Flock Mean

Figure 4 demonstrates the influence of the flock mean epg on the precision of the estimated mean. Samples of 100 individuals were taken from flocks of 1000 sheep created by sampling from negative binomial distributions with means of 200, 450 and 750 eggs per gram. The values of k were set to 1 in each case. The means and standard deviations of the sample means were 198.8 ± 19.7, 456.6 ± 46.2 and 750.9 ± 79.5 for specified flock means of 200, 450 and 750, respectively. As the flock mean increased, the precision of the estimated means decreased. There was clearly more variation among the estimated means when the flock mean was higher.

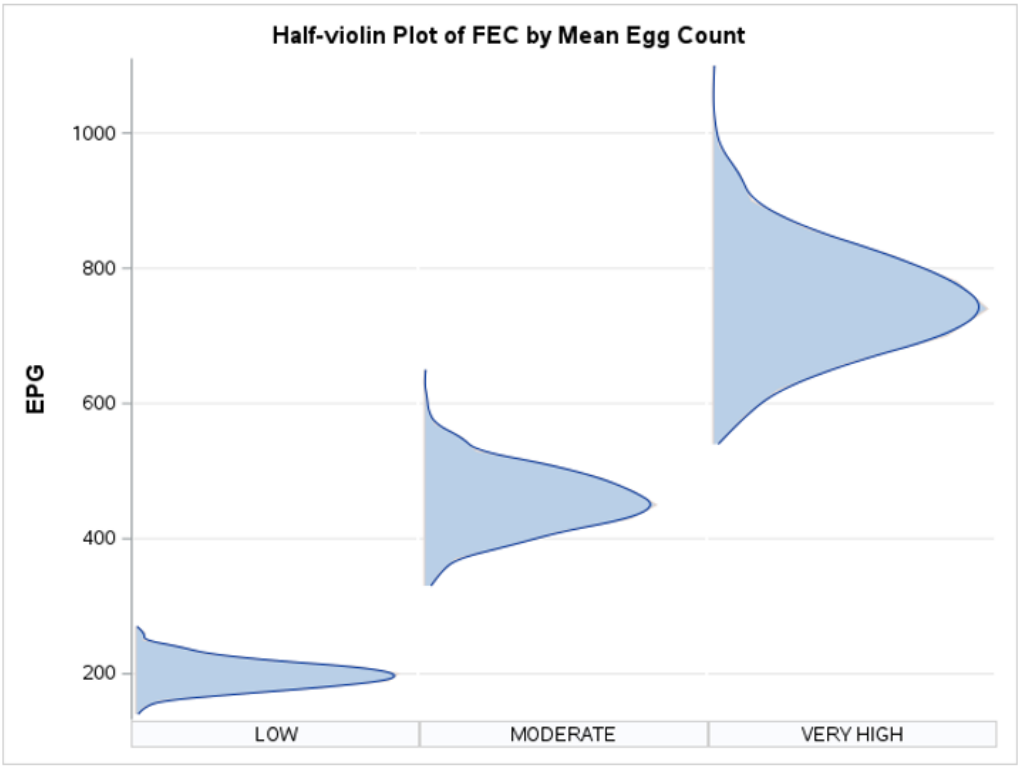


Figure 4. The influence of the flock mean on the precision of the estimated mean faecal egg count. The x-axis provides the mean of the flock EPG. Low = 200, moderate = 450 and high = 750 epg.

2.5. A Plausible Scenario That Estimates the Flock Mean with Low Precision

Figure 5 presents a half-violin plot of sampling in a plausible scenario using options that produce low precision in the estimated mean. The options were k = 0.5, a sample size of 10, 20, 30, 40, 50 or 100 individuals from a simulated flock of 1000 individuals and a mean egg count of 750 eggs per gram. The means and standard deviations for the distribution of

mean egg counts from samples of 10, 20, 30, 40, 50 or 100 individuals were 736.1 ± 388.2, 756.0 ± 276.5, 742.8 ± 212.2, 745.8 ± 188.4, 749.2 ± 163.5 and 749.2 ± 114.4. The sample means were clustered around 750, indicating that the means were not systematically biased. The standard deviations were quite large but fell from 388 to 114 as the sample size increased from 10 to 100 individuals per sample. The Shapiro–Wilk test showed that the observed distribution of means was significantly different from a normal distribution for all sample sizes from 10 to 100 ($p < 0.01$). The distribution was markedly right skewed mean for a sample size of 10, but the skewness decreased as the sample size increased. One consequence of the pronounced right skew with sample sizes of 10 individuals is that most estimates of the mean will fall below the flock mean. The mode of the distribution was only 525 eggs per gram, while a small number of means overestimated the flock mean. The estimated flock mean egg counts ranged from 80 to 3010 eggs per gram.

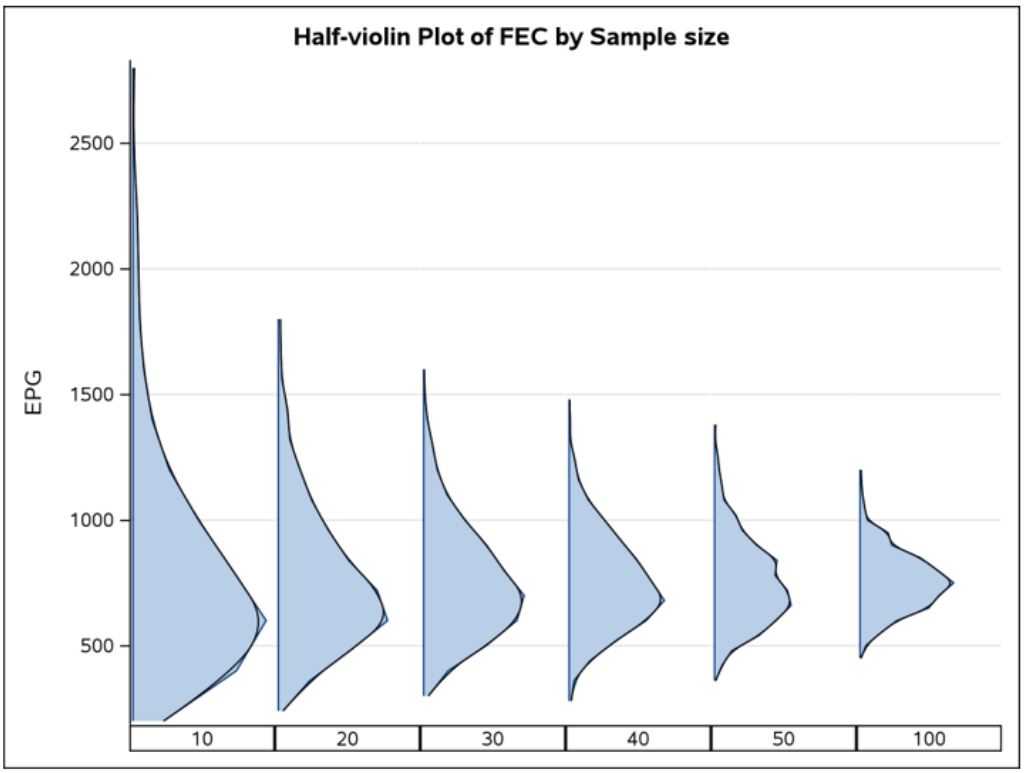


Figure 5. The influence of sample size on the flock mean egg count using options that produce low precision in the estimated mean. The options were k = 0.5, sample size of 10, 20, 30, 40, 50 or 100 individuals from a simulated flock of 1000 individuals and a mean egg count of 750 eggs per gram. The *x*-axis indicates the number of individuals in each of the subsets sampled from the flock.

The 5%, 10% and 25% quantiles for a sample size of 10 were 252.5, 320 and 462.5 eggs per gram. In other words, if the estimated mean is 252.5 epg or less, then only 5% of the time is this consistent with a true flock mean of 750 epg or greater. Similarly, only 25% of estimated flock means will fall below 465 epg if the true flock mean is 750 epg or greater. These empirical confidence limits could form, with appropriate qualifications, the basis of advice to farmers and breeders. Table 1 provides estimates of the percentage of mean estimates that would be expected to fall below certain egg counts. These percentages were obtained from a separate sampling and provided the observed proportion of estimates falling below these values when 1000 samples of the specified sizes were taken to estimate the flock mean.

Table 1. Percentage of individuals from a flock with a specific negative binomial distribution falling below specified egg count values.

Number Sampled/Egg Count	10	20	30	40	50	100
≤100	0.1					
≤200	1.5	0.3				
≤300	8.1	1.1	0.2			
≤400	16.9	6.0	3.1	0.8	0.4	
≤500	28.8	17.0	11.6	6.4	5.2	1.4
≤600	40.6	30.6	27.5	21.7	18.6	16.1
≤700	51.6	49	45.4	45.0	41.6	36.1

For example, if the true flock mean is 750 eggs per gram, then about 52% of samples of the ten animals will have means below 700 eggs per gram when the dispersion parameter k is 0.5 and the flock size is 1000. Similarly, for a flock of the same size and with the same dispersion parameter k, if 20 sheep are sampled, about 49% of the time, the mean will fall below 700 eggs per gram. If 30 sheep are sampled under the same conditions, then about 45% of the sample means will fall below 700 eggs per gram.

If only 10 sheep are randomly sampled from a flock of 1000 sheep with a dispersion parameter of 0.5, then, as already stated, a slight majority of these subsets of 10 sheep (52%) will have means below 700 eggs per gram. About 41% of the samples of size 10 will have a mean of 600 eggs per gram or below, and about 29% of the subsets of size 10 will have a mean of 500 eggs per gram or below. These values illustrate that there is a real risk of falsely assuming that the egg count is not high enough to consider treatment even when the egg count is alarmingly high. This risk is high when the sample size is only 10 animals, but the risk decreases as the number of sheep sampled increases.

3. Discussion

This in silico study has shown that flock variation, number of individuals sampled, flock size and true mean egg count all influence the precision of the estimated flock mean. The estimates are alarmingly imprecise for typical sampling schemes. The imprecision of the estimated means suggests that simply stating the estimated flock mean egg count could be seriously misleading. A plausible scenario was simulated and quantiles were estimated for samples of different sizes when true egg counts were high (750 epg). This scenario was chosen to provide high levels of variation in the mean estimates and reduce the chance of not providing treatment when needed.

Rather than only reporting an egg count of (say) 400 epg, it may be more useful to state that the estimated flock mean of 400 epg has less than a 17% chance of coming from a flock with a true mean of 750 epg or above. This applies to a sample of 10 individuals from a flock of 1000 sheep, and Table 1 provides estimates appropriate for different sample sizes. A more detailed description of the meaning of the estimated mean egg count may make it easier for farmers and breeders to integrate this information with other information, such as the condition of the animals, their susceptibility to nematodes, the quality of feed and other management variables before making a decision on whether or not to treat.

An alternative procedure would be to indicate the confidence limits of the flock mean egg count. However, classical confidence limits assume that samples come from a normal distribution, and this assumption is not always valid for flock mean egg counts estimated from samples, particularly when flock variation is high, the number of individuals sampled is low, the true flock size is large and the true flock mean is high.

Caution is required in the interpretation of these results. The analysis has assumed that egg counts follow a negative binomial distribution. The negative binomial has a long history of describing parasite distributions [9], but other distributions have been

suggested [10–12]. In addition, the negative binomial distribution does not always provide a good fit for mixed natural infections in sheep [13]. The choice of the negative binomial is largely empirical. However, if variation among animals follows a gamma distribution and variation in the counting process follows a Poisson distribution, then the number of eggs counted would be expected to follow a negative binomial distribution [14]. The gamma distribution is quite similar to the log-normal distribution [15], especially at some parameter values. A log-normal distribution of susceptibility among animals coupled with Poisson variation in the counting process could produce distributions similar to the negative binomial [16]. A log-normal distribution could arise if the genes underlying susceptibility were interacting in a multiplicative rather than an additive fashion.

This analysis supports the conclusions of Kahn, who used a resampling procedure from a flock of Australian sheep predominantly infected with *H. contortus* [7] and concluded that sampling ten animals may not provide an accurate and precise estimate of the flock mean egg count. The results are inconsistent with the conclusions from a previous modelling study [17]. Although Morgan et al. did not examine the influence of the number of samples on the flock mean obtained by counting individual samples but examined the effect of sample size on the composite mean created by pooling aliquots of faeces from different sheep. Pooling samples before counting is widely used [18,19] and can reduce the cost. Morgan et al. modelled variation in the composite mean by assuming that variation among animals followed a negative binomial distribution and variation in the counting process for the pooled sample followed a poisson distribution. This double sampling should create even more variation than sampling individual sheep. However, Morgan et al. concluded that a composite mean obtained by pooling aliquots from ten individuals could provide an adequate estimate in the majority of situations. Their modelling showed that if the flock mean was 500 eggs per gram (epg), then 42% of composite means created from ten animals would fall below 300 epg if the negative binomial dispersion factor (k) was 0.1, 19% of composite means would fall below 300 epg if k = 0.5 and 9% of samples would fall below 300 epg if k = 1. The acceptable proportion of tests with false results is partly a decision for each flock manager, but failure to treat animals when necessary is unethical and even illegal in many countries.

Modelling allows variables to be isolated and analysed while minimizing the effect of other variables. However, in natural infections, there are often interactions between variables. For example, the inverse index of dispersion (k) was strongly and positively correlated with the mean egg count [20].

In addition, these results are only directly applicable to predominantly *T. circumcincta* infections. Unfortunately, there is insufficient publicly available information to model sampling sheep with nematode infections that are dominated by *H. contortus* or *Trichostongylus colubriformis*. Further, egg counting procedures other than the McMaster cannot be easily and reliably modelled because there is insufficient information on the variation among animals with these procedures. However, once such information becomes available, the programs (Files S1 and S2) will require only minor modifications to be applied to these species and techniques. Indeed, similar programs could be applied to other parasites in other species.

Two potential sources of bias in sampling are selection bias and measurement bias [21]. Selection bias occurs when the selected animals are not representative of the flock. In principle, this can be minimised by selecting animals at random. In practice, this can be difficult. For example, if the lambs are gathered and the first animals into the race are sampled, these animals might have lower levels of nematode infection because they will be more active and nematode infection has been associated with reduced activity [22]. Increasing the proportion of a flock that is sampled could reduce selection bias.

Measurement bias occurs when the estimate differs consistently from the true value [21]. When only a small number of animals are sampled, right-skewed distributions can give biased results because most animals have values below the mean and heavily infected animals may be underrepresented in some samples [23]. Faecal egg counts are usually

estimated by the McMaster procedure [24] or a variant. This procedure consistently underestimates the concentration of eggs in the faeces [25]. This is not a serious problem because guidelines for treatment are usually based on results from the McMaster procedure or a modification. Newer automated procedures [26] can give higher counts and these could lead to unnecessary applications of anthelmintics if measurement bias is ignored.

These results are also relevant to research experiments. The substantial variation in EPG is partly due to variation among animals, which includes genetic and non-genetic variation in sheep [27–31] as well as variation in the counting process. Animal variation can be minimised by careful experimental design, but even using the same animals in before and after treatment designs will not completely eliminate variation. Experiments in inbred mice often use five or so animals in a group. This can be appropriate for inbred strains of mice that are genetically identical, but care is needed when the animals are genetically heterogeneous.

In summary, this in silico trial has quantified variation in EPG among samples of sheep. The results show that sampling a small number of animals can give misleading results and wrongly indicate that treatment is or is not necessary. The conclusion is that larger sample sizes than 10 sheep are needed to provide reliable estimates of the mean flock EPG. The exact size will depend upon the amount of risk that the flock manager is willing to take. These models used parameter values obtained from flocks with predominantly *T. circumcincta* infections. However, the procedure can be easily applied to other infections if the distribution of parasites has been quantified.

4. Materials and Methods

All modelling was done in SAS on demand for Academics from November 2022 to November 2023; SAS release: 9.04.01M7P08062020. Modelling proceeded in two stages. Firstly, flocks of various sizes were created. Then, these flocks were sampled under different scenarios. Half-violin plots were used to plot the range of mean egg counts in 1000 replicate samples for each scenario. Half-violin plots were chosen to facilitate the comparison of distributions obtained from different scenarios.

Simulation of flocks. The amount of variation in a flock will depend in part upon the nematode species present. For the gastrointestinal nematodes that infect sheep, the world can be divided into four zones. In zone 1, there is little nematode infection because conditions are unsuitable; for example, the climate is too dry. In zone 2, the egg count is dominated by *Haemonchus contortus*; this includes hot areas such as Kenya [32] and some of Australia [33] but also countries like Sweden [34]. In zone 3, the egg count is mainly due to *Trichostrongylus* species with a contribution from *Teladorsagia circumcincta*; this includes countries with a warm climate, such as New Zealand [35] but also south western and south eastern Australia [33]. In the fourth zone, the faecal egg count is largely due to *T. circumcincta*; this is the predominant nematode in temperate areas with cool climates, such as Scotland [13] and northern England [36]. These delineations can be quite flexible; for example, even in Scotland a small number of *H. contortus* can be found in infected sheep during exceptionally hot years [37]. As a rough guide, lambs with high egg counts will have values in the tens of thousands in areas where *H. contortus* is dominant, thousands in areas dominated by *Trichostrongylus* species, but values in the hundreds in areas dominated by *T. circumcincta.* Even in areas dominated by *T. circumcincta*, egg counts in the thousands can occur at the end of the grazing season, possibly due to the contribution of other species of nematodes [38].

The McMaster procedure [24] is a widely used procedure for counting nematode eggs in faeces. Typically, about 3 g of faeces is sampled from each sheep, but only the equivalent of 0.02 g faeces are counted and eggs per gram (epg) is obtained from the number of eggs counted multiplied by 50. The eggs counted were assumed to follow a negative binomial distribution [13]. The negative binomial is a two-parameter distribution which has a variety of parameterisations. In parasitology, the two parameters are the mean and k, which is an index of dispersion and is also known as a dispersion parameter. The statistical packages

SAS and R model k differently; R models k as an inverse index of dispersion while SAS models k as a positive index of dispersion. An alternative parameterisation for the negative binomial models is the number of failures before a specified number of successes. Here the two parameters are *p* the probability of success and r the number of successes. The mean is r(1 − p)/(p). The inverse index of dispersion (k) is equivalent to the number of successes (r) in the alternative parameterisation. The RAND ('NEGBINOMIAL', p,r) function was used to sample from a negative binomial distribution with the required mean and k values. The negative binomial distribution has been comprehensively explored [23,39,40]. This study does not aim to replicate this work but simply aims to explore the validity of sampling schemes in widespread use in veterinary parasitology.

The mean egg counts and the estimated values of k in the simulated flocks varied from the values specified in the RAND function, especially when the number of individuals in the simulated flock was small. To minimise the complications caused by these deviations, each flock was created at least 20 times, and the flocks with parameter values closest to the RAND function were used. Table 2 lists the values in the simulated flocks; these flocks provided the samples that were used to create the half-violin plots. All flocks used in the modelling had mean egg counts within five eggs of the values specified in the RAND function. All values of k fell within the 95% WALD confidence limits of the specified value.

Table 2. Realised parameters of the negative binomial distribution in simulated flocks. S.d = standard deviation, k = inverse index of dispersion, CL = confidence limits, N = number of individuals sampled, T= total flock size. Mean = mean egg count (eggs per gram).

Figure	Identity	Mean	S.d.	1/k	Wald 95% CL
1	K = 0.5	196.5	288.9	2.25	1.58–3.21
1	K = 1	201.5	230.6	1.16	0.79–1.68
1	K = 2	200.5	171.7	0.51	0.33–0.80
1	K = 3	201.0	138.3	0.27	0.15–0.49
1	K = 4	200.5	130.0	0.17	0.08–0.34
2	N = 10–50	450.5	467.0	0.998	0.73–1.36
3a	N = 5/50	448	452.3	0.92	0.60–1.40
3a	N = 10/100	450.5	467.0	0.998	0.73–1.36
3a	N = 25/250	446.6	451.0	1.00	0.82–1.23
3a	N = 50/500	454.8	481.7	0.97	0.84–1.11
3a	N = 100/1000	454.4	488.7	1.02	0.93–1.13
3b	T = 50	449.0	489.5	1.01	0.65–1.56
3b	T = 100	450.5	467.0	0.998	0.73–1.36
3b	T = 250	446.6	451.0	1.00	0.82–1.23
3b	T = 500	454.8	481.7	0.97	0.84–1.11
3b	T = 1000	454.4	488.7	1.02	0.93–1.13
4	Mean = 200	199.6	210.8	0.92	0.82–1.04
4	Mean = 450	454.3	488.7	1.02	0.93–1.13
4	Mean = 750	753.0	829.2	1.04	0.95–1.14
5	T = 1000	753.4	1232.8	2.01	1.84–2.19

The deviations of the mean egg counts and the estimated values of k in the simulated flocks from the original distribution have important consequences. They indicate that sampling directly from a specified distribution could give misleading results. Sampling directly from a theoretical distribution ignores the role of flock size and could create a sample that differs considerably from the specified distribution.

There are surprisingly few published estimates for the parameters of the negative binomial distribution applied to faecal egg counts in sheep [5,13,17], and some of these are unsuitable because they are based on very few animals or the nematode species infecting the sheep are not provided [5,17]. We followed the sampling principles described above and used estimates obtained by sampling more than 100 individuals. These estimates came from a single flock of straight-bred Scottish Blackface sheep kept on an upland farm in Strathclyde [13]. Necropsy of 530 lambs from this flock indicated that the infection was predominantly *T. circumcincta* [41]. Mean egg counts varied from 52 to 572 eggs per gram (epg). The two samples taken in May, when the lambs were one month old and suckling had k values of 0.09 and 0.17. Many animals had zero egg counts and were probably not grazing and, therefore, not exposed to infection. The remaining values of k varied from 0.50 to 2.59.

Sampling from flocks Sample sizes varied from 10 to 100 individuals in different comparisons. Flock means vary over the course of a grazing season. The decision to treat lambs with an anthelmintic is not determined solely by the value of the mean EPG, but in general, a value of 200 would not trigger treatment, an EPG of 450 would be considered for treatment, while a value of 750 EPG would suggest treatment is required (https://www.scops.org.uk/internal-parasites/worms/using-worm-egg-counts/ accessed on 28 February 2024).

The univariate procedure was used to estimate means and standard deviations and to determine quantiles as well as the extreme values in each sample. The univariate procedure also provided Shapiro–Wilk tests to test the fit of the 1000 means to the normal distribution and drew histograms of the distributions of faecal egg counts. Proc genmod was used to estimate the value of k and provided 95% Wald confidence limits. The surveyselect procedure was used to sample the required number of individuals at random from each flock. Sampling was without replacement. The sgpanel procedure was used to create half violin plots using the counts generated by the univariate procedure to produce histograms. The Spline statement was used to create smooth curves with a quadratic Bèzier interpolation. A program used to create a simulated flock is shown in File S1. A program used to sample sheep from a simulated flock and create a half-violin plot is provided in File S2.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/parasitologia4020012/s1. File S1: A SAS program to create a simulated flock. File S2: A SAS program to sample sheep from a simulated flock.

Author Contributions: Conception G.C.; modelling and first draft M.S.; review and rewriting S.P., D.P., G.C. and K.D.-Ł. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data can be generated from Files S1 and S2.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Coop, R.L.; Sykes, A.R.; Angus, K.W. The effect of three levels of *Ostertagia circumcincta* larvae on growth rate, food intake and body composition of growing lambs. *J. Agric. Sci.* **1982**, *98*, 247–255. [CrossRef]
2. Burgess, C.G.; Bartley, Y.; Redman, E.; Skuce, P.J.; Nath, M.; Whitelaw, F.; Tait, A.; Gilleard, J.S.; Jackson, F. A survey of the trichostrongylid nematode species present on UK sheep farms and associated anthelmintic control practices. *Vet. Parasitol.* **2012**, *189*, 299–307. [CrossRef] [PubMed]
3. Stubbings, L.; Bartley, D.; Busin, V.; Lovatt, F.; Page, P.; Rose Vineer, H.; Skuce, P. *SCOPS Technical Manual*; OSF: Charlottesville, VA, USA, 2020. [CrossRef]
4. Kaplan, R.M.; Denwood, M.J.; Nielsen, M.K.; Thamsborg, S.M.; Torgerson, P.R.; Gilleard, J.S.; Dobson, R.J.; Vercruysse, J.; Levecke, B. World Association for the Advancement of Veterinary Parasitology (WAAVP) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in ruminants, horses and swine. *Vet. Parasitol.* **2023**, *318*, 109936. [CrossRef]

5. Denwood, M.J.; Kaplan, R.M.; McKendrick, I.J.; Thamsborg, S.M.; Nielsen, M.K.; Levecke, B. A statistical framework for calculating prospective sample sizes and classifying efficacy results for faecal egg count reduction tests in ruminants, horses and swine. *Vet. Parasitol.* **2023**, *314*, 109867. [CrossRef] [PubMed]
6. Torgerson, P.R.; Schnyder, M.; Hertzberg, H. Detection of anthelmintic resistance: A comparison of mathematical techniques. *Vet. Parasitol.* **2005**, *128*, 291–298. [CrossRef]
7. Kahn, L. Accuracy and Precision of Sampling for Worm Egg Count Estimates (Abstract). In Proceedings of the 23rd International Conference of the World Association of the Advancement of Veterinary Parasitology, Buenos Aires, Argentina, 21–25 August 2011. Available online: https://hdl.handle.net/1959.11/15773 (accessed on 28 February 2024).
8. Cochran, W.G. *Sampling Techniques*; John Wiley & Sons: Hoboken, NJ, USA, 1977.
9. Bliss, C.I.; Fisher, R.A. Fitting the Negative Binomial Distribution to Biological Data. *Biometrika* **1953**, *9*, 176–200. [CrossRef]
10. Gaba, S.; Ginot, V.; Cabaret, J. Modelling macroparasite aggregation using a nematode-sheep system: The Weibull distribution as an alternative to the negative binomial distribution? *Parasitology* **2005**, *131*, 393–401. [CrossRef]
11. Taylor, L.R. Aggregation, variance and the mean. *Nature* **1961**, *189*, 732–735. [CrossRef]
12. McVinish, R.; Lester, R.J.G. Measuring aggregation in parasite populations. *J. R. Soc. Interface/R. Soc.* **2020**, *17*, 20190886. [CrossRef]
13. Stear, M.J.; Bairden, K.; Duncan, J.L.; Gettinby, G.; McKellar, Q.A.; Murray, M.; Wallace, D.S. The distribution of faecal nematode egg counts in Scottish Blackface lambs following natural, predominantly *Ostertagia circumcincta* infection. *Parasitology* **1995**, *110*, 573–581. [CrossRef]
14. Evans, M.; Hastings, N.; Peacock, B. *Statistical Distributions*; Wiley: New York, NY, USA, 1993.
15. Vaz, M.F.; Fortes, M. Grain size distribution: The lognormal and the gamma distribution functions. *Scr. Metall.* **1988**, *22*, 35–40. [CrossRef]
16. Hunter, G.C.; Quenouille, M.H. A statistical examination of the worm egg count sampling technique for sheep. *J. Helminthol.* **1952**, *26*, 157–170. [CrossRef]
17. Morgan, E.R.; Cavill, L.; Curry, G.E.; Wood, R.M.; Mitchell, E.S. Effects of aggregation and sample size on composite faecal egg counts in sheep. *Vet. Parasitol.* **2005**, *131*, 79–87. [CrossRef] [PubMed]
18. Kenyon, F.; Rinaldi, L.; McBean, D.; Pepe, P.; Bosco, A.; Melville, L.; Devin, L.; Mitchell, G.; Ianniello, D.; Charlier, J.; et al. Pooling sheep faecal samples for the assessment of anthelmintic drug efficacy using McMaster and Mini-FLOTAC in gastrointestinal strongyle and Nematodirus infection. *Vet. Parasitol.* **2016**, *225*, 53–60. [CrossRef] [PubMed]
19. Rinaldi, L.; Levecke, B.; Bosco, A.; Ianniello, D.; Pepe, P.; Charlier, J.; Cringoli, G.; Vercruysse, J. 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. *Vet. Parasitol.* **2014**, *205*, 216–223. [CrossRef] [PubMed]
20. Grenfell, B.T.; Wilson, K.; Isham, V.S.; Boyd, H.E.G.; Dietz, K. Modelling patterns of parasite aggregation in natural populations: Trichostrongylid nematode–ruminant interactions as a case study. *Parasitology* **1995**, *111*, S135–S151. [CrossRef] [PubMed]
21. Lohr, S.L. *Sampling: Design and Analysis*; Brooks/Cole, Cengage Learning: Boston, MA, USA, 2010.
22. Ikurior, S.J.; Pomroy, W.E.; Scott, I.; Corner-Thomas, R.; Marquetoux, N.; Leu, S.T. Gastrointestinal nematode infection affects overall activity in young sheep monitored with tri-axial accelerometers. *Vet. Parasitol.* **2020**, *283*, 109188. [CrossRef]
23. Lloyd-Smith, J.O. Maximum likelihood estimation of the negative binomial dispersion parameter for highly overdispersed data, with applications to infectious diseases. *PLoS ONE* **2007**, *2*, e180. [CrossRef] [PubMed]
24. Gordon, H.M.; Whitlock, H.V. A new technique for counting nematode eggs in sheep faeces. *J. Counc. Sci. Ind. Res. Aust.* **1939**, *12*, 50–52.
25. Rossanigo, C.E.; Gruner, L. Accuracy of two methods for counting eggs of sheep nematode parasites. *Vet. Parasitol.* **1991**, *39*, 115–121. [CrossRef]
26. Cringoli, G.; Rinaldi, L.; Albonico, M.; Bergquist, R.; Utzinger, J. Geospatial (s)tools: Integration of advanced epidemiological sampling and novel diagnostics. *Geospat. Health* **2013**, *7*, 399–404. [CrossRef]
27. Stear, M.J.; Bairden, K.; Bishop, S.C.; Buitkamp, J.; Duncan, J.L.; Gettinby, G.; McKellar, Q.A.; Park, M.; Parkins, J.J.; Reid, S.W.J.; et al. The genetic basis of resistance to *Ostertagia circumcincta* in lambs. *Vet. J.* **1997**, *154*, 111–119. [CrossRef]
28. Raadsma, H.W.; Gray, G.D.; Woolaston, R.R. Breeding for disease resistance in Merino sheep in Australia. *Rev. Sci. Tech. L'office Int. Epizoot.* **2009**, *17*, 315–328. [CrossRef]
29. Bisset, S.A.; Morris, C.A.; McEwan, J.C.; Vlassoff, A. Breeding sheep in New Zealand that are less reliant on anthelmintics to maintain health and productivity. *New Zealand Vet. J.* **2001**, *49*, 236–246. [CrossRef]
30. Benavides, M.V.; Sonstegard, T.S.; Kemp, S.; Mugambi, J.M.; Gibson, J.P.; Baker, R.L.; Hanotte, O.; Marshall, K.; Van Tassell, C. Identification of Novel Loci Associated with Gastrointestinal Parasite Resistance in a Red Maasai x Dorper Backcross Population. *PLoS ONE* **2015**, *10*, e0122797. [CrossRef]
31. Bishop, S.; Bairden, K.; McKellar, Q.; Park, M.; Stear, M. Genetic parameters for faecal egg count following mixed, natural, predominantly Ostertagia circumcincta infection and relationships with live weight in young lambs. *Anim. Sci.* **1996**, *63*, 423–428. [CrossRef]
32. Mugambi, J.M.; Wanyangu, S.W.; Bain, R.K.; Owango, M.O.; Duncan, J.L.; Stear, M.J. Response of Dorper and Red Maasai lambs to trickle *Haemonchus contortus* infections. *Res. Vet. Sci.* **1996**, *61*, 218–221. [CrossRef]
33. Emery, D.L.; Hunt, P.W.; Le Jambre, L.F. *Haemonchus contortus*: The then and now, and where to from here? *Int. J. Parasitol.* **2016**, *46*, 755–769. [CrossRef]

34. Waller, P.J.; Bernes, G.; Rudby-Martin, L.; Ljungstrõm, B.; Rydzik, A. Evaluation of copper supplementation to control *Haemonchus contortus* infections of sheep in Sweden. *Acta Vet. Scand.* **2004**, *45*, 149–160. [CrossRef]
35. Douch, P.G.C.; Green, R.S.; Morris, C.A.; McEwan, J.C.; Windon, R.G. Phenotypic markers for selection of nematode-resistant sheep. *Int. J. Parasitol.* **1996**, *26*, 899–911. [CrossRef]
36. Thomas, R.J.; Boag, B. Epidemiological studies on gastro-intestinal nematode parasites of sheep. The control of infection in lambs on contaminated pasture. *Res. Vet. Sci.* **1973**, *15*, 238–249. [CrossRef] [PubMed]
37. Stear, M.J.; Bairden, K.; Bishop, S.C.; Gettinby, G.; McKellar, Q.A.; Park, M.; Strain, S.A.J.; Wallace, D.S. The processes influencing the distribution of parasitic nematodes among naturally infected lambs. *Parasitology* **1998**, *117*, 165–171. [CrossRef] [PubMed]
38. Stear, M.J.; Singleton, D.; Matthews, L. An evolutionary perspective on gastrointestinal nematodes of sheep. *J. Helminthol.* **2011**, *85*, 113–120. [CrossRef] [PubMed]
39. Grafen, A.; Woolhouse, M.E.J. Does the negative binomial distribution add up? *Parasitol. Today* **1993**, *9*, 475–477. [CrossRef]
40. Anscombe, F.J. Sampling theory of the negative binomial and logarithmic series distributions. *Biometrika* **1950**, *37*, 358–382.
41. Stear, M.J.; Abuagob, O.; Benothman, M.; Bishop, S.C.; Innocent, G.; Kerr, A.; Mitchell, S. Variation among faecal egg counts following natural nematode infection in Scottish Blackface lambs. *Parasitology* **2006**, *132*, 275–280. [CrossRef]