Recommended number of sheep sampled in programs for internal parasite control can lead to incorrect decisions

W. Bell[1], A.L. Sánchez[1], D. Castells[2], J.F. Ramos[1], E. Lorenzelli[3], I. Macchi[3] & R.W. Ponzoni[1]

[1] Universidad de la República, Facultad de Agronomía, Departamento de Producción Animal y Pasturas, Av. Eugenio Garzón 780, 12900, Montevideo, Uruguay
wbell@fagro.edu.uy (Corresponding Author)
[2]Secretariado Uruguayo de la Lana, Servando Gómez 2408, 12100, Montevideo, Uruguay
[3] Laboratorio Veterinario Dondo, Blanes 278, 50000, Salto, Uruguay

Summary

Worm egg count per gram (WEC) is used as a management tool to decide when to drench sheep to reduce the worm burden. It is also used in sheep genetic evaluations. In order to determine the appropriate time of drenching, or of recording a whole group in genetic evaluation, WEC is monitored in a random sample of animals from the group. The question that emerges is: what is the adequate sample size to determine if the average WEC has reached 500 to 1000? In this paper we examine two data sets with average WEC of 629 and 1499. They correspond to WECs frequently encountered in Uruguay. Thresholds beyond which drenching is recommended, or are considered appropriate for genetic evaluation, vary depending upon pasture availability and quality, and sheep condition. The former value is relevant for poor pastures and poor sheep condition, whereas the latter value is relevant for good pastures and sheep in good condition. When thresholds were set a 500 and 1200 in each data set, we found that with the recommeded sample size of 10 to 20 individuals, incorrect decisions would be frequently made. The minimum sample size should be 20 and preferably it should be greater. Given the insufficient sample size currently used, we recommend a revision of the topic and an experimentally and statistically based re-formulation of sampling guidelines.

Keywords: worm egg count, faeces, sheep, sample size

Introduction

Worm egg count in sheep faeces (WEC) is used as a management tool to decide when to drench to reduce the parasite burden (Court *et al*., 2010). It is also used in sheep genetic evaluations, where the aim is to select for lower WEC (Stear, 2010). In the latter case the records in a contemporary group are taken after WEC exceeds an arbitrary minimum of, say, 500 to 1000, the expectation being that the worm burden will be sufficient to enable the expression of between animal variation in the trait. In order to make decisions about when to drench, or about recording in a contemporary group for genetic evaluation purposes, WEC is monitored in a random sample of the sheep in question. The question arising is: what is the desired sample size to estimate if WEC has reached the threshold of 500 or 1000?

Recording WEC takes time and the cost is not trivial. Practical guides on parasite control make recommendations about the number of animals to be sampled. In Australia, WormBoss (2017) recommends sampling no less than 20 animals, whereas Fiel *et al*. (2011) in Argentina recommend a minimum of 10 animals and ‘ideally’ 20. In Uruguay, Pereira

(2002) recommends a minimum of 15 animals and a WEC of at least 600 to 800. Recently, Bell *et al*. (2017) examined this issue from a theoretical and numerical viewpoint and concluded that to have 'reasonable' confidence sample sizes should be considerably greater than those currently recommended. In this paper we study two data sets where all sheep had been recorded for WEC. We simulate and examine the consequences of sampling different number of sheep and we judge the decisions that would have been made using information from samples of various sizes.

Material and methods

Sheep and environment

WEC was recorded in eight to ten month old progeny from two flocks located in two different farms (La Magdalena, M, and Talitas, T) in 2012 and 2011 for M and T, respectively. Both farms are in the north of Uruguay (latitude and longitude 31°22'59" S and 57°58'00" W, respectively). Average rainfall is 1300 mm, distributed throughout the year. Average summer and winter temperatures are 24 and 13 °C, respectively. Ewes and lambs grazed native pastures. Lambs were born in September and weaned in January. Thereafter they also grazed on native pastures. Average age at recording WEC was nine months. Faeces samples were taken from the rectum, put in plastic bags, maintained at 3 to 5 °C and sent to a qualified laboratory.

Statistical analysis

We calculated descriptive statistics for both data sets. Further to this initial examination of the data, we simulated taking 50 replicate samples of 5, 10, 15, 20, 30, 40, 50 and 100 animals from each data set. We used the SurveySelect Procedure in SAS (SAS Institute Inc., 2015) for this purpose.

We compared the means obtained from the different samples sizes by analysis of variance. The model fitted included sample size and replicate within sample size. We also estimated the least squares means for each sample within sample size to determine cases in which the 'correct' or 'incorrect' decision would have been made from each sample size.

Results

Table 1 shows descriptive statistics for data sets M and T.

Table 1.Descriptive statistics for WEC in lambs from La Magdalena (M) and Talitas (T).

Farm	Number of observations	Mean	Minimum	Maximum	Standard deviation	Fraction of zero counts
M	486	629	0	4500	599	0.10
T	503	1499	0	7500	1097	0.01

There were no significant differences in WEC among sample sizes. Table 2 shows the corresponding least squares means. The overall values for WEC were 632 and 1478 for M and T, respectively.

Table 2. Least squares means (LSM) and standard errors (SE), range and confidence interval (CI) for WEC in La Magdalena (M) and Talitas (T).

Sample size	M			T		
	LSM (SE)	Range	CI	LSM (SE)	Range	CI
5	705 (375)	632 to 779	147	1376 (69)	1239 to 1512	273
10	621 (265)	569 to 673	104	1462 (49)	1365 to 1558	193
15	606 (217)	563 to 648	85	1538 (40)	1460 to 1617	157
20	644 (188)	607 to 681	74	1443 (35)	1375 to 1511	136
30	613 (153)	583 to 643	60	1505 (28)	1449 to 1560	111
40	615 (133)	589 to 641	52	1505 (24)	1456 to 1553	96
50	613 (119)	589 to 636	47	1498 (22)	1455 to 1541	86
100	638 (84)	621 to 654	33	1496 (15)	1465 to 1526	61

Figures 1 and 2 in the Appendix show the distribution of WEC for different sample sizes in M and T, respectively. In both farms the range of values was smaller for larger sample sizes. The dotted line in each histogram indicates an arbitrary limit below which a sample mean could have resulted in an incorrect decision (e.g. decide not to drench when actual WEC was beyond the threshold).

Discussion and conclusions

There were no significant differences in WEC among sample sizes in neither of the two data sets analysed. However, the standard error and the confidence interval were affected (smaller standard errors and narrower confidence intervals were associated with larger sample sizes, Table 2). For monitoring and decision making purposes current protocols indicate sampling for WEC in 10 to 20 animals. Thresholds to decide drenching or conducting a whole mob recording for genetic evaluation may vary typically between 500 and 1200 depending on circumstances and the environment (WormBoss, 2017). In Figure 1 and 2 (see Appendix) we plotted the frequency of WEC for different sample sizes for both data sets. We indicate with a dotted line an assumed threshold beyond which a decision would be made to treat the animals (500 and 1200 for data sets M and T, respectively). Counts to the left of the dotted line indicate that an incorrect decision would have been made (i.e. decide not to drench when actual WEC was above the threshold). For example, for data set M (Figure 1), with sample sizes 10, 20 and 100, incorrect decisions would have been made in 30, 18 and 2 % of cases, respectively.

Jointly considered, results from both data sets indicate that adherence to current sampling protocols may lead to incorrect decisions about the need for drenching. The frequency of incorrect decisions would be greater for smaller samples sizes.

The problem of internal parasites in sheep has been addressed mainly via the administration of chemicals. The approach has not been entirely successful, and among other, the issue of parasite resistance to chemicals has emerged. Decisions on what to administer and when, are at best based on WEC recorded in a sample of 10 to 20 individuals. This sample size is below what Bell *et al*. (2017) recommend in a theoretical and numerical study. Consistent with their findings, in this paper we show that such samples size would often lead to incorrect decisions. Avoiding the conclusion that small sample size may be one of the reasons for the failure to control internal parasites in sheep, is difficult. Given the importance of internal parasites in many sheep producing regions and the reliance on chemical

treatments, we recommend that the issue of sample size on which to base decisions should be revised.

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Appendix

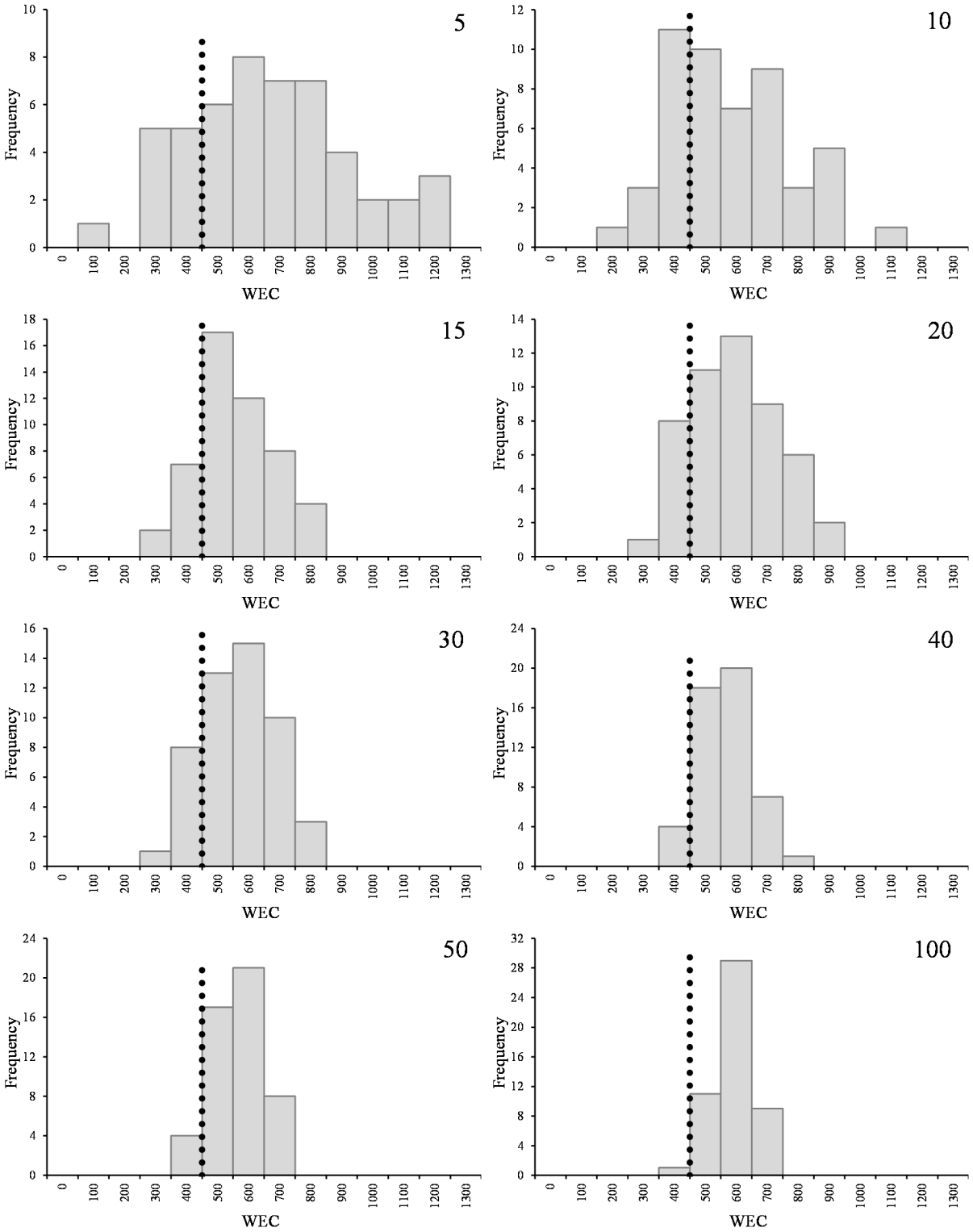

Figure 1. Distribution of WEC by sample size (5, 10, 15, 20, 30, 40, 50 and 100) in La Magdalena.

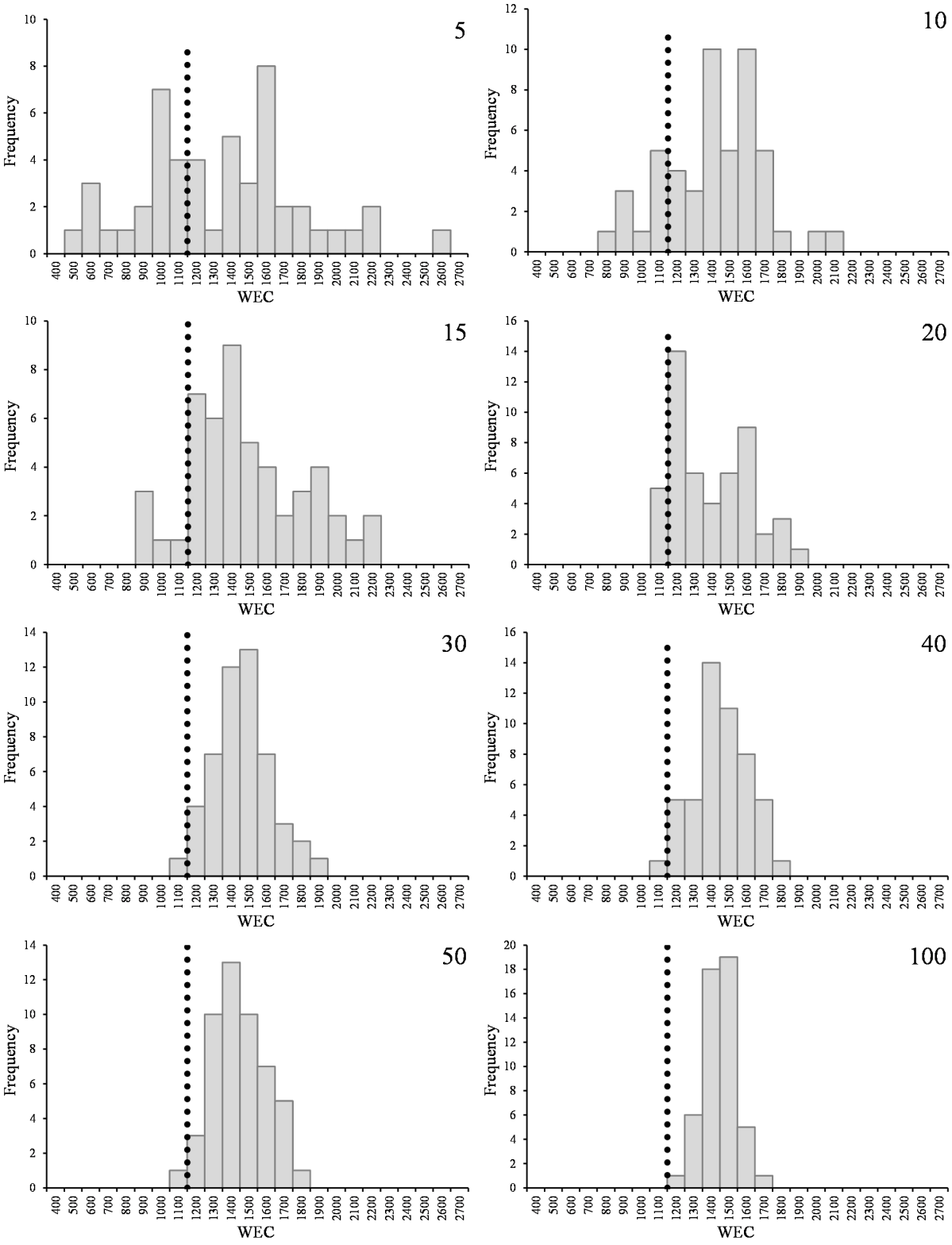

Figure 2. Distribution of WEC by sample size (5, 10, 15, 20, 30, 40, 50 and 100) in Talitas.