

SHORT COMMUNICATIONS

Counting nematode eggs in equine faecal samples

S. L. PRESLAND, E. R. MORGAN, G. C. COLES

THE control of gastrointestinal nematodes in horses often relies on routine anthelmintic prophylaxis, which aims to reduce parasite burden in individual animals and to limit contamination of the pasture with nematode eggs. However, treating all the horses in a group is likely to involve the unnecessary treatment of uninfected or lightly infected animals (Comer and others 2001), and to accelerate the development of anthelmintic resistance (Coles 2002). Routine chemoprophylaxis at fixed intervals is also likely to lead to unnecessary drug administration, and is insensitive to climate-driven fluctuations in the risk of infection (Reinemeyer 1999). Individual horses that are egesting large numbers of eggs should be identified and treated, protecting others in the group from high levels of infection, while leaving some parasites in refugia (Coles 2003). A major obstacle to such targeted anthelmintic prophylaxis in horses is the limited availability of

suitable faecal egg count tests. The widely used McMaster technique (MAFF 1986) was developed mainly for sheep and is poorly sensitive at low egg densities (Mes and others 2001) that might indicate that treatment is required in horses. It also requires specialist equipment, and the expense of laboratory diagnosis is likely to be a significant disincentive to many horse owners. More sensitive concentration techniques for egg detection generally require good laboratory facilities (Mes 2003), and are often less reliable for the estimation of high egg counts (Levine and others 1960, Rossanigo and Gruner 1991).

A new faecal egg count test devised by FECPAK NZ is used with cattle and sheep and has the potential to surmount these difficulties. A larger amount of faeces is examined than by the McMaster technique, so sensitivity should be greater (Hunter and Quenouille 1952). Centrifugation is not required, and the test is available as a complete kit which could be used by a veterinarian or horse owner on the premises. This short communication compares the FECPAK test and McMaster technique to examine the predicted improvements with the FECPAK system.

The FECPAK test, like the McMaster technique, is based on the flotation-dilution principle described by Stoll (1930). In

Veterinary Record (2005)
156, 208-210

S. L. Presland, BSc,
Department of Anatomy,
University of Bristol,
Southwell Street,
Bristol BS2 8EJ
E. R. Morgan, MA, PhD,
Med, MRCVS,
Department of Biological
Sciences, University of
Bristol, Woodland Road,
Bristol BS8 1US
G. C. Coles, MA, PhD,
CBiol, FIBiol,
Department of Clinical
Veterinary Science,
University of Bristol,
Langford House,
Bristol BS40 5DU

Correspondence to
Dr Coles

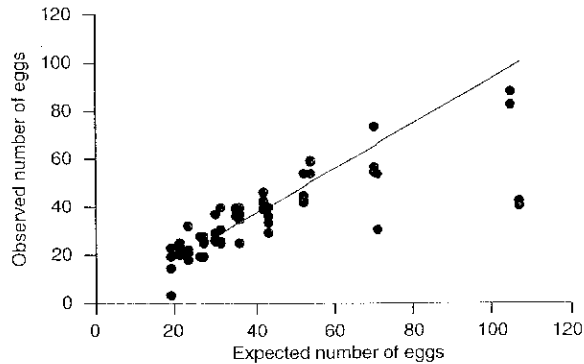


FIG 1: Effect of sample dilution on estimated strongyle egg density in well mixed equine faeces, using the FECPAK method. The line represents a 1:1 ratio between the expected and observed numbers of eggs

the standard FECPAK test for cattle, a minimum of 10 g faeces is mixed thoroughly in a plastic bag with three times the volume of water. A sample of the mixed suspension (45 ml) is then removed, made up to 230 ml with saturated salt solution and poured through a filter of 1 mm aperture to remove coarse debris. An aliquot is removed from the well mixed filtrate and placed in a custom-made acrylic counting chamber with a volume of 1 ml under the grids (compared with 0.3 ml on the McMaster slide). After 30 seconds, nematode eggs that have floated to the top of the chamber are counted under a microscope. The multiplication factor to transform the number of eggs counted to estimated faecal egg density depends on the dilution used: in this case, each egg counted represents 20 eggs per gram of faeces (epg).

In common with other tests of this type, excessive dilution reduces sensitivity, whereas insufficient dilution inhibits egg flotation and visibility and also leads to an underestimation of egg density (Dunn and Keymer 1986). The optimum dilution should therefore take into account the consistency of the faecal material and the sensitivity required from the test. To ascertain the best dilution for use in horses, faeces from two horses with a naturally acquired mixed strongyle infection were analysed using the FECPAK test. Initial dilutions of 1 g faeces in 1 ml water (for a high number of expected eggs) to 1 g in 10 ml were used. The expected number of eggs was calculated from the mean number of epg in the three most dilute samples \times the mass of faeces examined in the counting chamber. In less diluted suspensions, fewer eggs were counted than expected (Fig 1). A 4 ml sample of water added initially to each gram of faeces represented the most concentrated workable dilution. At this dilution, each egg counted represents 25 epg.

The relative sensitivities of each test were compared using faeces from an uninfected horse to which a known number of strongyle eggs had been added to final densities of 50, 100 and 200 epg. Each test was repeated five times at each egg density. Using the FECPAK test, eggs were detected in each case, whereas using the McMaster technique, six false negative results were obtained (three of five at 50 epg and three of five at 100 epg). The greater sensitivity of the FECPAK test is to be expected, because more faeces are examined than by the McMaster technique, and the chances of missing eggs present at low density are consequently reduced.

Assuming that the faecal sample is well mixed and that eggs are randomly distributed in suspension, the expected proportion of false negatives at each faecal egg density can be predicted from the Poisson distribution, with the mean being equal to the expected number of eggs per slide (Peters and Leiper 1940). The assumption of Poisson distribution was tested by taking serial counts from well mixed samples of naturally infected horse faeces using both the McMaster and the FECPAK methods (three series with each). Deviation from Poisson was assessed using the Kolmogorov-Smirnov test

TABLE 1. Results of serial egg counts performed on two unmixed samples of horse faeces using the McMaster technique and FECPAK test

	Horse 1		Horse 2	
	McMaster	FECPAK	McMaster	FECPAK
n	20	20	20	20
Mean	323	301	148	183
Range	0-600	175-450	0-400	125-350
Variance	29,862	7465	8020	4480
sd	172	86	90	67
Predicted variance*	16,125	7531	7375	4563

* Calculated as the mean number of eggs counted \times the square of the multiplication factor for transformation to eggs per gram of faeces (epg), and assumes that the eggs are Poisson distributed and therefore that the variance of the raw egg counts equals their mean. Estimated mean epg for each sample was not significantly different using either test (198 for horse 1 and 147 for horses 1 and 2, Mann-Whitney U test)

(SPSS software; SPSS), and in each case found to be non-significant ($n=25, 25, 21, 21, 24, 24$; $Z=0.35$ to 0.95 , $P=0.33$ to 1.0). Probabilities of zero counts at each egg density were then computed using the formula for the Poisson distribution given in Hilborn and Mangel (1997). At 50 epg, it was predicted that 37 per cent of tests using the McMaster technique and 14 per cent of FECPAK tests would give false negative results. At 100 epg the corresponding values were 14 per cent and 2 per cent, respectively, and at 200 epg 2 per cent and below 0.01 per cent, respectively. The FECPAK test can therefore be said to be more sensitive than the McMaster technique at low egg densities.

In order to investigate accuracy and repeatability at higher egg densities, faeces were taken from the rectums of two naturally infected horses, and each test was repeated 20 times on the unmixed bulk sample. For the McMaster technique, 3 g faeces were taken and suspended in 45 ml of flotation fluid, and for the FECPAK test 20 g of faeces were taken and suspended initially in 80 ml of water, before being processed as described above. For each sample, the transformed counts, that is, estimated epg, were not significantly different between tests, but the variance of the counts was lower for the FECPAK test and the range was narrower (Table 1). The variance of the estimated epg was higher than that predicted assuming random (Poisson) distribution within the faecal mass for the McMaster technique, but not for the FECPAK test. This suggests that error due to the clumping of eggs within the faecal mass was effectively negated by the relatively large samples taken for the FECPAK test. The range of the McMaster counts included 0 for both horses, but the FECPAK test returned no false negative results. The FECPAK method is therefore more sensitive than the McMaster method at standard dilutions, and is less likely to underestimate mean epg over a wide range of faecal egg densities. It is able to estimate egg density more accurately by virtue of the larger amount of faeces examined. It is also simpler to use and requires less specialised equipment than the McMaster technique.

Attempts to achieve sustainable control of gastrointestinal nematodes in horses through targeted anthelmintic prophylaxis will be successful only if horse owners are able to administer treatments appropriately. With a little training, the FECPAK test system enables on-farm monitoring of faecal egg output from individual animals cheaply, quickly and reliably, allowing treatment of the correct horses at the correct time. The test could also potentially be extended to other situations where accurate detection of low egg densities is important, for example, faecal monitoring of trematode infections (with denser flotation fluid), and faecal egg count reduction tests for anthelmintic resistance in a range of host-parasite combinations.

ACKNOWLEDGEMENTS

This study was a third-year student's honours project in Equine Science (S. L. P.) supported by a gift from FECPAK.

References

- COLES, G. C. (2002) Sustainable use of anthelmintics in grazing animals. *Veterinary Record* **151**, 165-169
- COLES, G. C. (2003) Strategies to minimise anthelmintic resistance in large animal practice. *In Practice* **25**, 494-499
- COMER, K., COLES, G. C. & HILLYER, M. H. (2001) A national survey for anthelmintic resistant nematodes in thoroughbreds. Eighteenth International Conference of the World Association for the Advancement of Veterinary Parasitology, Stresa, Italy, August 26 to 30, 2001. p 166
- DUNN, A. & KEYMER, A. (1986) Factors affecting the reliability of the McMaster technique. *Journal of Helminthology* **60**, 260-262
- HILBORN, R. & MANGEL, M. (1997) Probability and probability models: know your data. In *The Ecological Detective: Confronting Models with Data*. Princeton, Princeton University Press. p 70
- HUNTER, G. C. & QUENOUILLE, M. H. (1952) A statistical examination of the worm egg count sampling technique for sheep. *Journal of Helminthology* **4**, 157-170
- LEVINE, N. D., KRISHNA, N. M., CLARK, D. T. & AVES, I. J. (1960) A comparison of nematode egg counting techniques for cattle and sheep feces. *American Journal of Veterinary Research* **21**, 511-515
- MAFF (1986) *Manual of Veterinary Parasitological Laboratory Techniques*. London, Her Majesty's Stationery Office
- MES, T. H. M. (2003) Technical variability and required sample size of helminth egg isolation procedures. *Veterinary Parasitology* **115**, 311-320
- MES, T. H. M., PLOEGER, H. W., TERLOU, M., KOOYMAN, F. N. J., VAN DER PLOEG, M. P. J. & EYSKER, M. (2001) A novel method for the isolation of gastro-intestinal nematode eggs that allows automated analysis of digital images of egg preparations and high throughput screening. *Parasitology* **123**, 309-314
- PETERS, B. G. & LEIPER, J. W. G. (1940) Variation in dilution-counts of helminth eggs. *Journal of Helminthology* **18**, 117-142
- REINEMEYER, C. R. (1999) Current concerns about control programs in temperate climates. *Veterinary Parasitology* **85**, 163-172
- ROSSANIGO, C. E. & GRUNER, L. (1991) Accuracy of two methods for counting eggs of sheep nematode parasites. *Veterinary Parasitology* **39**, 115-121
- STOLL, N. R. (1930) On methods of counting nematode ova in sheep dung. *Parasitology* **22**, 116-136