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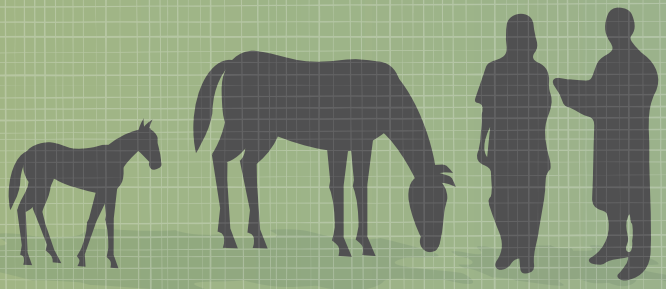

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How to avoid the pitfalls of faecal worm egg counts and the best way to interpret the results

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Techniques for determining faecal egg counts are numerous and they exist in countless modifications. Main principles are flotation-based platforms, where a given quantity of faeces is suspended in a set volume of flotation medium, wherein eggs are allowed to float to separate them from faecal debris. The eggs are counted under the microscope and multiplied by a factor to estimate the number of eggs per gram of faeces.

Detection limit vs. diagnostic sensitivity

It is important that veterinary practitioners know the diagnostic performance of the test they are using. Unfortunately, information about parameters for evaluating the diagnostic performance of parasite faecal egg counting techniques is often confusing and erroneous as many misconceptions exist. This presentation will attempt to address some of the most common misconceptions.

First of all, each egg counting technique has a detection limit, which is synonymous with the multiplication factor applied to calculate the amount of eggs per gram (EPG) of faeces. The detection limit is a theoretically derived number based on the amount of faeces used in the analysis, the volume of flotation medium in which the faeces are suspended, and the volume of suspension examined under the microscope. This is theoretical, because it ignores any degree of egg loss during the homogenisation and filtration steps and it assumes a perfectly even suspension of eggs within the suspension. Thus, detection limit does not inform about the diagnostic sensitivity of the technique. The diagnostic sensitivity, defined as the proportion of true positive samples detected positive with the test is not a function of detection limit. In other words a technique with a lower detection limit is not necessarily more sensitive than a technique with a higher detection limit, and it is therefore misleading to advertise a given technique as 'more sensitive' based on a theoretical detection limit without appropriate documentation.

Quantitative parameters for diagnostic performance

Despite the discussion above, diagnostic sensitivity is not a very important parameter with faecal egg counts. The reason is that sensitivity really only comes into play at very low egg count levels, and it often has little or no practical implication whether a given sample comes back as 0 or 12 EPG, for example. In both cases, the horse is a low shedder.

The most important parameter for evaluating the performance of faecal egg count techniques is precision. Precision is also referred to as repeatability and is a measure of variation between repeated measures with the same technique on the same sample. Precision affects the classification of horses into low, moderate and high strongyle egg shedding categories, and it influences resistance testing with the faecal egg count reduction procedure. A common measure for precision is coefficient of variation (CV). Any diagnostic service offering faecal egg counts should provide validation data demonstrating precision of their technique upon request.

Precision should not be confused with accuracy, which is a measure of how close a given technique measures to the true count of a sample. The true egg count is never truly known and accuracy can only be estimated by spiking known numbers of eggs to egg-free samples and then determining the egg counts with the technique in question. However, this approach will never fully mimic the distribution of eggs in a sample from a naturally infected horse, so will only be an approximation. Accuracy is not important as long as it is constant across egg count levels. In other words, a technique may underestimate the egg count by 30%, but that can be accounted for by adjusting the thresholds for low, moderate and high shedding. The faecal egg count reduction test will not be affected by accuracy, as long as the same technique is used for pre- and post-treatment egg counts, because it measures a per cent reduction between the two time points.

Application of faecal egg counts

Egg counts remain useful for at least three different purposes: evaluating anthelmintic treatment efficacy by use of the faecal egg count reduction test; identification of low, moderate and high strongyle egg shedders in mature horses; and identification of *Parascaris* spp. infection in foals, weanlings and yearlings. The latter is important as *Parascaris* spp. parasites often require treatment with different anthelmintic drug classes than the omnipresent cyathostomins.

Egg counts, however, are not reliable as a clinical diagnostic tool in individual horses. In other words, an egg count determined from a horse showing clinical signs suggesting parasitic disease is not going to yield useful information. There are several reasons for this. First of all, there is no direct linear correlation between egg counts and worm counts, and negative predictive values are low. More eggs do not mean more worms. Secondly, adult (egg shedding) worms rarely, if ever, cause parasitic disease, whereas migrating or encysted larvae have a higher pathogenic potential. Finally, there are more than 50 species of strongyle parasites infecting horses, but their eggs are indistinguishable from each other. Thus, more pathogenic parasites such as *Strongylus vulgaris* cannot be detected by faecal egg counts.

In recent years, an automated image analysis-based faecal egg count technology has been developed and launched. This system uses fluorescence staining and an image analysis algorithm to determine ascarid and strongyle faecal egg counts without the use of a microscope. Validation studies have documented higher precision and accuracy compared with the classical McMaster technique. Counts can be completed in three minutes and a permanent data record is created by capturing an image of the sample analysed with all counted eggs circled.