Using diagnostics in supporting sustainable worm control in horses

Anthelmintic resistance in horses is an ongoing problem. Changes in methods of worming – from group worming to testing individual horses and worming appropriately – are important to help prevent any further resistance developing. There are various methods to test for worm burdens in horses, including faecal egg counts, blood tests and saliva tests. This article discusses these methods in relation to specific species of gastrointestinal parasites, in addition to how pasture management can be used to help maintain low levels of resistance.

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Gastrointestinal helminths present a serious threat to equine health and welfare. Horses can be infected by a range of worms, most commonly the cyathostomins (small strongyles, small redworm). Other helminths of importance include the tapeworm (*Anoplocephala perfoliata*), the pinworm (*Oxyuris equi*), and, in foals and yearlings, *Parascaris* spp. The large strongyle (*Strongylus vulgaris*), a parasite that can cause severe colic, is rarely found in herds subjected to years of broad spectrum anthelmintics, but has been observed to 're-emerge' in situations where use of these medicines has been reduced (Tydén et al, 2019). This parasite should still be considered in control programmes where there is low or negligible treatment frequency. Where horses are grazed with, or are on pastures recently grazed by, sheep or cattle, the liver fluke (*Fasciola hepatica*), should be taken into consideration, especially when there has been no opportunity to test or treat the ruminants.

Helminth control is hindered by anthelmintic resistance (Matthews, 2014; Nielsen, 2022), and the situation is most severe in cyathostomins. In some regions (for example, the UK and US), it is a serious threat because multi-drug resistance has been reported on numerous occasions. Anthelmintic resistance is common in *Parascaris* spp. on breeding farms, where there is pathogenic potential because of this parasite's possible clinical effect on susceptible animals. Currently available information indicates that no new equine anthelmintic classes will be licensed in the foreseeable future (Neilsen, 2022). For this reason, the equine sector needs to take action to preserve the remaining efficacy of the products available. This involves avoiding practices that have been previously demonstrated to increase selection pressure for drug resistance such as regular all-group treatments, treating animals then moving them to clean pasture and under-dosing. Instead, the decision to treat with anthelmintics needs to be based on assessing the risk of infection, implementing management approaches to reduce parasite transmission and, where appropriate, employing diagnostics to inform which horses require treatment. The key components of such approaches are shown in *Figure 1*.

Good pasture management can considerably moderate the risk of helminth infection. Strongyle egg hatching and speed of larval development is dependent on temperature and moisture (Mfitilodze and Hutchinson, 1987), meaning in the UK, the optimal time for larval development is summer. Although the microclimate at farm level will ultimately affect the speed of larval development and rate of survival, a general increase in ambient temperature, as has been observed in the UK's changing climate, is likely to accelerate egg hatching and larval development. Once third-stage larvae are available, mortality may increase because of more rapid use of the larvae's limited energy reserves. Ramsey et al (2004) demonstrated that, under summer conditions in Scotland, it takes 1–2 weeks for infective larvae to develop from cyathostomin eggs. Therefore, it is recommended that, to break the worm transmission cycle, faeces should be completely removed from paddocks at least once a week. Faecal removal should be applied year-round, especially on premises where horses have substantial grazing in all seasons. Herd (1986) demonstrated that dung removal significantly decreases pasture larval counts. Corbett et al found that faecal egg counts of equids grazing paddocks from which dung is removed are significantly lower than faecal egg counts of matched cohorts grazing paddocks not subjected to pasture hygiene measures. Low stocking densities will also limit helminth transmission; for example, Joó et al (2022) showed that strongyle egg excretion was significantly higher when horses were kept at high density (>30 animals/hectare) compared to those

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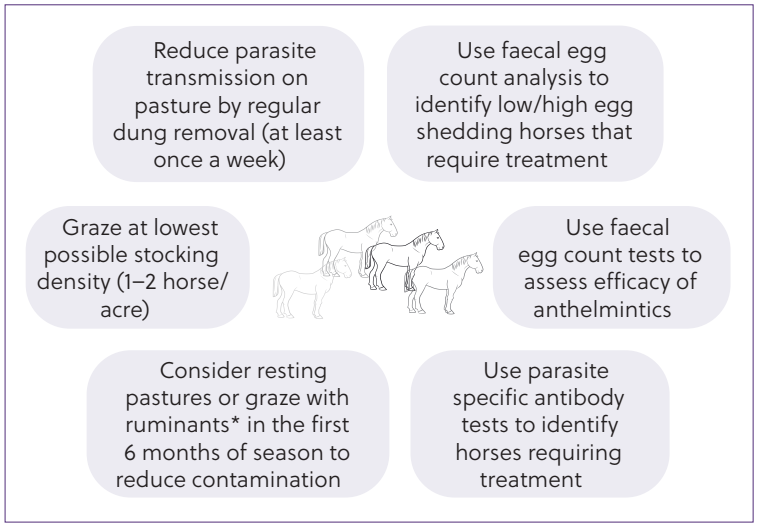

*Figure 1. Key components of sustainable equine helminth control programmes. *Note: this practice is not suitable or effective to reduce* F. hepatica.

grazed at lower densities (3–10 animals/hectare and 1–2 animals/hectare). Resting pastures for sufficient periods will reduce helminth contamination; infective strongyle larvae and *Parascaris* spp. eggs can survive over winter (Reinemeyer, 1986; Ihler, 1995).It is therefore recommended that pastures be rested until at least mid-way through the following grazing season. In the first half of the season, ruminants can be used as 'biological vacuum cleaners' as different helminth species infect horses and ruminants. A notable exception is *F. hepatica*. For this reason, a risk assessment should be undertaken before ruminants are moved to equine pastures and if required, they should be tested for liver fluke and treated if positive. Harrowing is often proposed as a method of helminth control. However, for it to be effective, worm eggs and larvae need to be exposed to sufficient sunny and dry conditions to kill them; these are rarely encountered for adequate periods in the UK for harrowing to be effective.

Applying diagnostics in sustainable control programmes

Faecal egg count tests

Faecal egg count methods such as the modified McMaster method can be used to reduce treatment frequency. This is because, in managed adult populations, helminths are not evenly distributed. Generally, less than 20% of a given population contribute over 80% of contamination onto pasture (Relf et al, 2013; Nielsen et al, 2018). Faecal egg count analysis will indicate which individuals shed eggs at a level (for example, 200–500 eggs per gram) that requires treatment to kill adult worms to mitigate egg excretion, whilst leaving a proportion of the parasite population unexposed to anthelmintics in untreated horses. Horses should be assessed by faecal egg count testing at least 2–3 times per year; this is because the probability that an individual will remain in a particular egg shedding category can be affected by age, disease, time since treatment, climate and changes of grazing (Lester et al, 2018). Testing should focus on higher transmission periods (mid-spring to late summer). In groups that graze for significant periods in autumn and winter, testing should be considered as faecal egg counts with >200 eggs per gram have been identified during this period in outwintered groups (Relf et al, 2013), especially in milder years or if dung removal is absent or infrequent. In foals and yearlings, faecal egg counts are useful for identifying which nematode parasite(s) are being excreted by foals, as *Parascaris* spp. eggs can be easily discriminated from strongyle eggs and anthelmintic sensitivity profiles are likely to be different between cyathostomins (commonly resistant to benzimidazole and pyrantel, less commonly resistant to macrocyclic lactones) and *Parascaris* spp. (commonly resistant to macrocyclic lactones, less commonly resistant to benzimidazole and pyrantel). To monitor anthelmintic efficacy, faecal egg count tests should be used annually. Here, samples are collected before treatment and 10–14 days after treatment. The mean percentage reduction in faecal egg count is calculated by comparing eggs counted in the post treatment sample to those counted pre-treatment. *Table 1* summarises the efficacy thresholds for reporting susceptibility or resistance.

Antibody testing for small strongyle infections

The Small Redworm Blood Test (Austin Davis Biologics Ltd) measures serum immunoglobulin G(T) to three cyathostomin recombinant antigens (Tzelos et al, 2020) and can be used as follows:

- To inform anti-cyathostomin anthelmintic treatments in certain situations
- To support differential diagnosis investigations in gastrointestinal disease cases

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Table 1. A guide to faecal egg count reduction thresholds when assessing anthelmintic efficacy

	Benzimidazole (eg fenbendazole)	Tetrahydropyrimidine (eg pyrantel embonate)	Macrocyclic lactone (eg ivermectin, moxidectin)
Expected efficacy if no resistance	99%	94–99%	99.9%
No evidence of resistance	>95%	>90%	>98%
Suspect resistance	90–95%	85–90%	95–98%
Resistance	<90%	<85%	<95%*

**Resistance has been rarely reported by faecal egg count reduction testing, so tests that demonstrate <95% mean reduction in faecal egg count after ivermectin or moxidectin administration should be repeated before confirming resistance. Efficacy below the expected threshold should be evident in most or all horses tested (adapted from the American Association of Equine Practitioners Guidelines, 2019).*

- To help monitor the effectiveness of recommended worm control programmes.

Historically, to target potentially pathogenic encysted cyathostomin larvae that can emerge en masse to cause larval cyathostominosis, it was recommended to annually administer a larvicidal treatment in autumn or winter or the end of the grazing season to reduce disease risk (American Association of Equine Practitioners, 2019; Rendle et al, 2019). Such blanket applications may be unnecessary and are likely to exert a strong selection pressure for drug resistance. To reduce treatment frequency, experts have suggested that larvicidal treatments be focused on young or adolescent horses and other animals that display repeatedly high faecal egg counts, since these are most likely to harbour larger larval burdens (Nielsen et al, 2007). As an alternative to a blanket treatment approach, the Small Redworm Blood Test can be used to assess cyathostomin burdens in horses that do not fall under these categories to provide information for treatment decisions. The enzyme-linked immunosorbent assay detects levels of immunoglobulin G(T) specific to antigens expressed by mucosal and luminal cyathostomins (Tzelos et al, 2020), generating a 'serum score' from an immunoglobulin G-based calibration curve. The test exhibits excellent diagnostic performance (Lightbody et al, 2023), with selected serum scores shown to diagnose burdens above 1000 (serum score 14.37) and 10 000 (serum score 30.46) cyathostomins with a sensitivity of 97.65% and 91.55%, and a specificity of 85.19% and 75.61% respectively. Development studies that applied the test in equine populations managed under different conditions demonstrated that the proportion of horses that reported positive at these serum scores was associated with the level of strongyle transmission risk (Lightbody et al, 2023). These findings were used to develop test inclusion criteria based on assessment of infection risk *(Table 2)*.

Serum score results should be interpreted by veterinarians alongside their risk assessment to select a serum score cut-off for treatment. Using the test following the above guidance can result in considerable treatment reductions compared to an all-group treatment approach, as the test identifies low-burden individuals that would otherwise have received anthelmintics. For example, analysis of Austin Davis Biologics' commercial dataset containing a sport horse cohort demonstrates that, of 981 horses assessed, 62% demonstrated serum scores below the 1000 cyathostomin threshold, with 19% between the 1000–10 000 cyathostomin threshold, and 19% above the 10 000 cyathostomin threshold. Another example is demonstrated in *Figure 2,* which shows test results for a small herd of leisure horses grazing paddocks from which dung is removed daily.

Because the Small Redworm Blood Test can identify horses infected up to 10 000 cyathostomins with high sensitivity, it can be used in the differential diagnosis of intestinal disease (for example, colic) by ruling out cyathostomins in the aetiology of the symptoms. The test has been deployed in outbreaks of acute larval cyathostominosis to support diagnosis; a case report from a wel-

Table 2. A risk assessment must be performed before applying the Small Redworm Test for informing anthelmintic treatment decisions

Risk categories (combine individual factors to derive an overall level of risk)		
	Low	High
Management factors	Closed herd Adult horses (>4 years) Group grazed at low stocking density (<1–2 horses/acre) Dung removed more than once per week Or minimal grazing time (race horses, sport horses)	Not closed herd with no or poor quarantine procedures High proportions of young horses (<4 years) Stocking density (>2 horses/acre) Sporadic or no dung removal Anthelmintic resistance previously identified
Recent or previouss faecal egg count results in tested and co-grazing horses	Always <50 eggs per gram	Often or most recent test >200 eggs per gram
Action	Small Redworm testing should be considered to inform a treatment decision	Do not use the Small Redworm test for this purpose; most horses will return a positive serum score Consider applying larvicidal treatment late autumn or winter

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fare establishment in Ireland showed that all tested clinically affected horses (n=6) generated serum scores >50.0, designated as 'off scale' in the assay (Walshe et al, 2021). In cases where there is severe hypoproteinaemia, antigen-specific immunoglobulin G(T) may be low; this should be taken into account when interpreting results of the test alongside other clinico-pathological parameters. It may be worthwhile determining total plasma protein concentration in horses with clinical signs of larval cyathostominosis to rule out false negatives occurring because of low concentrations of immunoglobulin G(T) present in the sample.

In the UK, veterinarians are using the test as a 'rule out' option in the differential diagnosis of gastrointestinal diseases, in addition to informing treatment decisions and as a herd level-tool to evaluate the effectiveness of their clients' parasite control programmes.

Antibody testing for tapeworm infection

Standard faecal egg count methods are insensitive for *A. perfoliata,* especially as the eggs are not evenly distributed in faeces (Nilsson et al, 1995) and it is not known whether eggs are released continuously from the adult parasites, or whether there is an impact of season or diurnal variation. Instead, tests can be applied to measure immunoglobulin G(T) to parasite excretory/secretory antigens. Antibody-based saliva and serum tests have been available for several years in Europe. The serum test measures antigen-specific immunoglobulin G(T), levels of which show strong positive correlations (Spearman's correlation 0.78) with *A. perfoliata* infection intensity (Proudman and Trees, 1996). The commercial version of this enzyme-linked immunosorbent assay (Tapeworm Blood Test, Austin Davis Biologics Ltd) incorporates a calibration curve that generates a 'serum score' for each horse and acts as an internal quality control (Lightbody et al, 2016). Serum score results are categorised as 'low', 'borderline' or 'moderate/high' and anti-tapeworm treatment should be considered for horses that report in the latter two categories. A saliva test, EquiSal® Tapeworm (Austin Davis Biologics Ltd), is also available that uses the same antigens and like the blood test, has been thoroughly validated by comparing antigen-specific immunoglobulin G(T) with *A. perfoliata* counts in horses (Lightbody et al, 2016). This test also categorises results as 'low', 'borderline' and 'moderate/high'. The saliva test has been demonstrated to accurately identify all horses (Lightbody et al, 2016) with what has been described as a clinically relevant burden of over 20 tapeworms (Proudman and Trees, 1999). When used to inform treatment decisions, the saliva test can considerably reduce anthelmintic use compared to blanket treatment protocols; in a study of 237 horses from an equine charity, 85% of tests reported below the treatment threshold (Lightbody et al, 2018). Despite a sizeable reduction in anti-cestode treatments, no increase in tapeworm prevalence was reported at these premises over the two years of the study. Analysis of the commercial dataset (UK samples, 2015–2022 inclusive) demonstrated the levels of reduction in anthelmintic use compared to a blanket treatment approach; of 164 002 samples assessed in this time frame, 68.2% (111 927 samples) reported as under the recommended treatment threshold.

The timing and frequency of tapeworm testing should be based on an assessment of infection risk. Horses grazing pasture where previous testing has identified higher burdens that require treatment, or where there has been tapeworm-related disease or where there are high proportions of individuals <5 years old should be tested in spring and autumn. Horses considered at lower risk (for example, groups where previous tests indicate no or low levels of infection, no previous history of tapeworm-related disease or comprise animals >5 years) can be tested in spring or autumn.

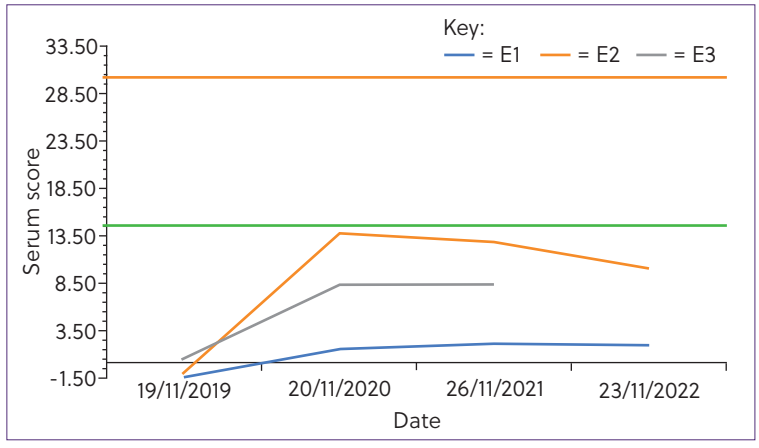

Figure 2. Small Redworm Blood Test serum scores of three horses (E1, E2, E3) that graze paddocks where dung is removed daily. E3 moved from the premises before November 2022. The green horizontal line depicts the 14.37 serum score cut off for 1000 cyathostomins, and the orange horizontal line depicts the 30.46 serum score cut off for 10 000 cyathostomins.

Anthelmintics licensed for treatment of tapeworm have a short half-life, reaching negligible levels in around 24 hours. After this, horses can become reinfected if grazing contaminated pasture. Therefore, it is important to reduce reinfection risk by good pasture management such as dung removal and maintaining low stocking densities. The saliva test can be used to investigate reductions in immunoglobulin G(T) in follow-up samples taken 10–12 weeks after treatment as antibody levels fall more rapidly in saliva compared to in serum. Lightbody et al (2016) demonstrated that in over 70% of praziquantel-treated horses, *A. perfoliata*-specific salivary immunoglobulin G(T) reduced to below treatment threshold levels within 5 weeks. If anti-tapeworm salivary immunoglobulin G(T) is high beyond 10–12 weeks, Lightbody et al (2016) suggested ongoing transmission where the tested individual is grazed, and the individual should be moved to clean pastures or paddock hygiene improved. A tapeworm treatment may be considered at this time to mitigate further contamination of pasture, especially where the animal cannot be moved or it is not possible to improve pasture management practices. The test should not be repeated within 10–12 weeks of treatment to inform on additional dosing as antibodies detected at this point may be indicative of previous infection. All horses on the same paddock should be tested as they may be acting as a source of contamination.

Testing for *Oxyuris equi*

Pinworm can be a persistent problem on some premises. There is no validated method for detection of *O. equi* infection and standard faecal egg count tests are of no value in diagnosing this parasite. The current best option is to perform a 'tape test' which involves taking an 8–10 cm strip of clear sticky tape and applying it firmly to the skin around the anus (this is where female worms lay their eggs). Fold the tape so that the sticky sides are together and submit to a diagnostic laboratory or view under the microscope to identify the characteris-

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KEY POINTS

- Anthelmintic resistance is an increasing problem in equines.
- There are various ways to test for worm excretion or worm burdens, including faecal egg counts, blood tests and saliva tests.
- Tests should be used to inform anthelmintic treatment decisions to reduce selection pressure for anthelmintic resistance.
- Excellent pasture management can help moderate levels of worm challenge from the environment; this will help reduce selection pressure for anthelmintic resistance.

tic eggs. Because the sensitivity of this method is low, at least three samples per test should be taken.

Testing for *Strongylus vulgaris*

An enzyme-linked immunosorbent assay has been developed to detect *S. vulgaris* infection (Andersen et al, 2013). This has not been commercialised and is not available in the UK, so testing for this parasite relies on coprological assessment. Large strongyles eggs cannot be differentiated from cyathostomin eggs. Faecal samples require culture in a parasitology laboratory for 2 weeks, after which third-stage larvae are obtained by Baermannisation and then can be discriminated from small strongyle larvae on the basis of the intestinal cell morphology based on published keys (Russell, 1948).

Testing for liver fluke

Fasciola hepatica can occasionally be found in horses, especially if they co-graze with ruminants on wet or marshy land. Eggs can be detected in equine faeces using flotation methods that use a solution with a high specific gravity. However, fluke infections in horses often do not reach maturity and in those that do, the prepatent period may be longer or eggs may not be excreted in faeces (Nansen et al, 1975). This leads to difficulty in diagnosis using faecal egg detection methods. A serum enzyme-linked immunosorbent assay has been developed, which can help identify if a horse has been exposed to *F. hepatica* infection (Salimi-Bejestani et al, 2005; Howell et al, 2020). Ideally, ruminants that co-graze with horses should be tested for infection.

Conclusions

Sustainable approaches to helminth control need to balance reducing treatment frequency whilst minimising the risk of parasite-associated disease in susceptible individuals. Improvements in pasture management should reduce the overall burden in grazing horses, and, if combined with the appropriate diagnostic tests, will lead to reductions in anthelmintic use, which will lessen selection pressure for resistance. EQ

Conflicts of interest

The authors are employed by Austin Davis Biologics Ltd, the company that markets EquiSal, the Tapeworm Blood Test and the Small Redworm Blood Test.

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