austindavis biologics ltd

EQUISTATUS™ TAPEWORM SCIENTIFIC AND TECHNICAL INFORMATION

Background

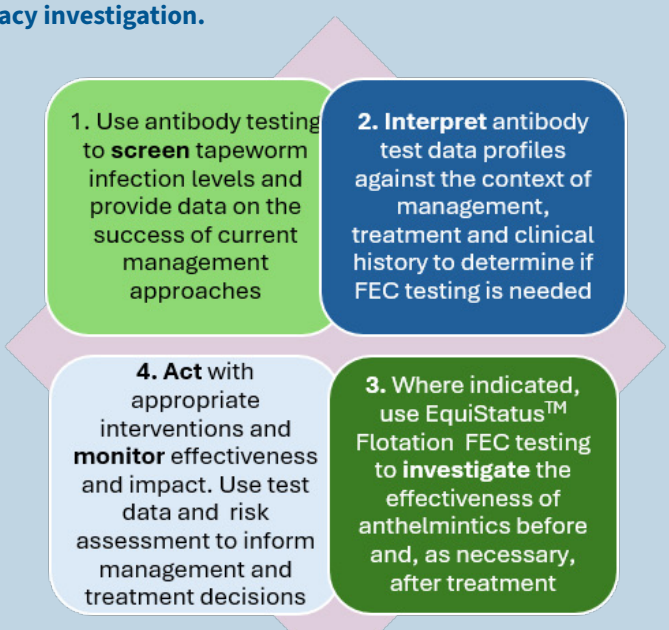
The changing landscape of tapeworm control

The common tapeworm, *Anoplocephala perfoliata*, is an important pathogen of horses. Adult tapeworms attach to the intestinal mucosa, especially around the ileocaecal junction, where they cause inflammation and pathology. Higher tapeworm burdens are associated with more severe mucosal thickening and ulceration (Pearson et al. 1993; Williamson et al. 1997; Pavone et al. 2011; Hreinsdóttir et al. 2019). The damage caused has been linked to an increased risk of colic (Back et al. 2013; Proudman & Holdstock 2000), including ileal impaction and spasmodic colic (Proudman & Edwards 1993; Proudman et al. 1998) with the risk of disease increasing at higher infection intensities (Proudman & Trees 1996). In severe cases, infection can be fatal (Ryu et al. 2001). For these reasons, *A. perfoliata* has been a key target of parasite control programmes, with routine treatment using praziquantel and pyrantel salts promoted for many years.

More recently, diagnostic-led approaches have been recommended in which test results are used to guide treatment decisions to reduce unnecessary anthelmintic use (Rendle et al. 2019; Matthews et al. 2023; Matthews & Peachey 2026). This is increasingly important given growing concerns about reduced anthelmintic efficacy against *A. perfoliata* (Nielsen 2023; Matthews & Peachey 2026). If treatments are ineffective, significant burdens will persist, increasing the risk of disease and allowing continued egg shedding and pasture contamination. Identifying groups in which reduced treatment efficacy is suspected is an important first step in determining when further investigation using faecal egg count (FEC) testing is warranted.

EquiStatus™ Tapeworm combines saliva or blood antibody testing with treatment and management history to identify groups that require further coprological investigation. Where indicated, EquiStatus™ Faecal Flotation FEC testing is then used before and after treatment to assess anthelmintic efficacy. This stepwise approach has been developed through detailed research and systematic evaluation of its individual test components and their interpretation, with each stage informing whether further investigation is warranted and how it should be undertaken (Figure 1).

Figure 1. Overview of EquiStatus™ approach to anthelmintic efficacy investigation.



Praziquantel and double-dose pyrantel salts are the only licensed anthelmintics for the treatment and control of *A. perfoliata.* Historically, both demonstrated high efficacy when assessed by comparing the recovery of tapeworms following treatment. For example, pyrantel pamoate (13.2 mg pyrantel base/kg) demonstrated 95.5-98.4% efficacy in dose confirmation studies, while praziquantel achieved 99.7–100% efficacy when used at 1.0-2.0 mg/kg (Reinemeyer et al. 2006; Owen & Slocombe 2006). Although FEC methods have relatively low sensitivity for detecting tapeworm infection, historical field studies demonstrated substantial reductions in egg shedding following treatment, as assessed by changes in egg detection status or quantitative egg counts (Table 1). For example, Höglund et al. (1998) found that only 1 of 18 foals remained egg-positive one month after pyrantel treatment, while Roelfstra et al. (2006) reported that 32 of 33 horses (97%) were tapeworm egg-negative 10 days after praziquantel treatment. Rehbein et al. (2003) reported that 95-96% of previously egg-positive horses became egg-negative following ivermectin-praziquantel treatment, with cestode egg counts at least 98% lower than in untreated or placebo-treated controls. Similarly, Grubbs et al. (2003) reported a 99.85% reduction in *Anoplocephala* egg counts following moxidectin–praziquantel treatment, while Marchiondo et al. (2006) reported reductions of 92-96% at different time points following pyrantel pamoate treatment. Collectively, these studies provide useful historical benchmarks for assessing changes in *A. perfoliata* egg shedding following anthelmintic treatment.

In contrast, Nielsen (2023) observed substantially smaller reductions in egg shedding (Table 1). Following praziquantel treatment, only 3 of 17 horses (17.6%) that were egg-positive before treatment became egg-negative afterwards, while five horses changed from egg-negative to egg-positive. Following pyrantel pamoate treatment in the same population, none of the 14 horses that were egg-positive before treatment became egg-negative. Quantitative analysis of paired pre- and post-treatment FECs gave overall FECR estimates of 23.5% for praziquantel and 50.9% for pyrantel pamoate. Similarly, Finnerty et al. (2024) reported persistent tapeworm egg shedding following treatment with ivermectin–praziquantel, moxidectin–praziquantel and pyrantel pamoate in 24 Thoroughbred yearlings.

Table 1. Historical and contemporary studies of anthelmintic efficacy against *Anoplocephala* spp. assessed by coprological methods. '-': not calculated.

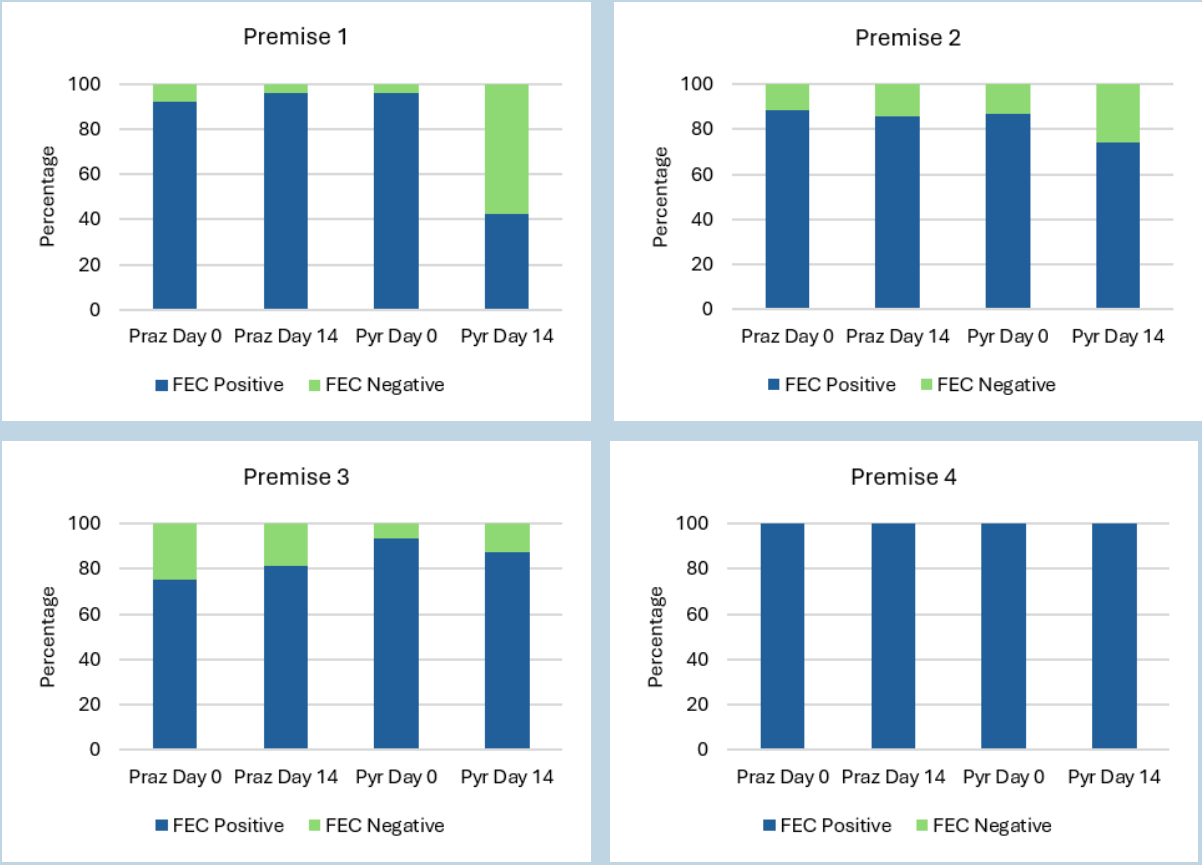
Authors	Year	Compound	No. horses tested (No. positive pre-treatment)	Horses that became egg-negative (%)	Reduction in FEC
Höglund et al.	1998	Pyrantel pamoate	18 (18)	94.4%	-
Rehbein et al.	2003	Ivermectin–praziquantel	273 (~208)	95–96%	≥98%
Grubbs et al.	2003	Moxidectin–praziquantel	400 (70)	95.7%	99.85%
Roelfstra et al.	2006	Praziquantel	33 (33)	97.0%	-
Marchiondo et al.	2006	Pyrantel pamoate	241 (126)	-	92–96%; overall 95%
Lyons et al.	2017	Ivermectin–praziquantel	143 (not reported)	96%*	-
Nielsen	2023	Praziquantel	56 (17)	17.6%†	23.5%
Nielsen	2023	Pyrantel pamoate	56 (14)	0%	50.9%

*reported efficacy estimate. †Five of 56 horses (8.9%) changed from egg-negative to egg-positive following praziquantel treatment.

The marked reduction in cestocidal treatment responses recently reported in the US, together with anecdotal reports of reduced efficacy in the EU and UK (Matthews & Peachey 2026), prompted further field investigations in the UK in 2026 (Matthews et al., in preparation). Four Thoroughbred stud farms were selected following veterinary concerns about persistently high tapeworm antibody scores and/or clinical signs consistent with tapeworm-associated disease despite recent cestocidal treatment. Groups of horses at these premises were screened by antibody testing, with high scores indicating a greater likelihood of substantial *A. perfoliata* infection. All groups were initially treated with praziquantel, with pyrantel embonate assessed where egg shedding persisted. FECs were performed on Days 0 and 14 using the EquiStatus™ Flotation FEC method. Tapeworm egg shedding persisted in horses following praziquantel treatment on all four farms and

following pyrantel embonate treatment on three farms (Figure 2). These findings provide field evidence of persistent *A. perfoliata* egg shedding following treatment with both praziquantel and pyrantel, raising concerns about potentially reduced cestocidal efficacy across multiple anthelmintic classes in UK tapeworm populations.

Figure 2. Assessment of *A. perfoliata* egg shedding before and after treatment with praziquantel (Praz) and then double-dose pyrantel embonate (Pyr), using the EquiStatus™ Flotation method. Both treatments were administered at weight-based dose rates. Premises 1–4: n = 26, 35, 16 and 20 horses, respectively.



The application of faecal egg count testing for efficacy investigations

The detection of *A. perfoliata* eggs in faeces 10–14 days after anthelmintic treatment provides evidence of a reduced treatment response. However, interpreting post-treatment FEC results requires consideration of the limitations of coprological detection. FEC has limited sensitivity for detecting tapeworm infection (Matthews et al. 2023), as eggs are shed intermittently and distributed unevenly in faeces, increasing the risk of false-negative results (Gasser et al. 2005). Furthermore, immature or non-gravid worms do not produce eggs and may constitute a substantial proportion, or even the entirety, of the worm burden (Nilsson et al. 1995; Tomczuk et al. 2014). Comparisons of FEC results with post-mortem worm counts have detected eggs in only 22.5–54% of infected horses (Nilsson et al. 1995; Williamson et al. 1998; Meana et al. 1998). Detection is more likely at higher infection intensities; for example, Kjær et al. (2007) reported an overall FEC sensitivity of 46%, increasing to 89% in horses harbouring more than 20 tapeworms, while Williamson et al. (1998) also found greater egg detection in horses with higher worm burdens. Consequently, a negative post-treatment FEC does not reliably exclude persistent infection or establish treatment success. Identifying horses or populations more likely to harbour substantial tapeworm burdens before FEC testing will increase the likelihood of detecting egg shedding and improve the value of FECs as a tool for investigating treatment efficacy.

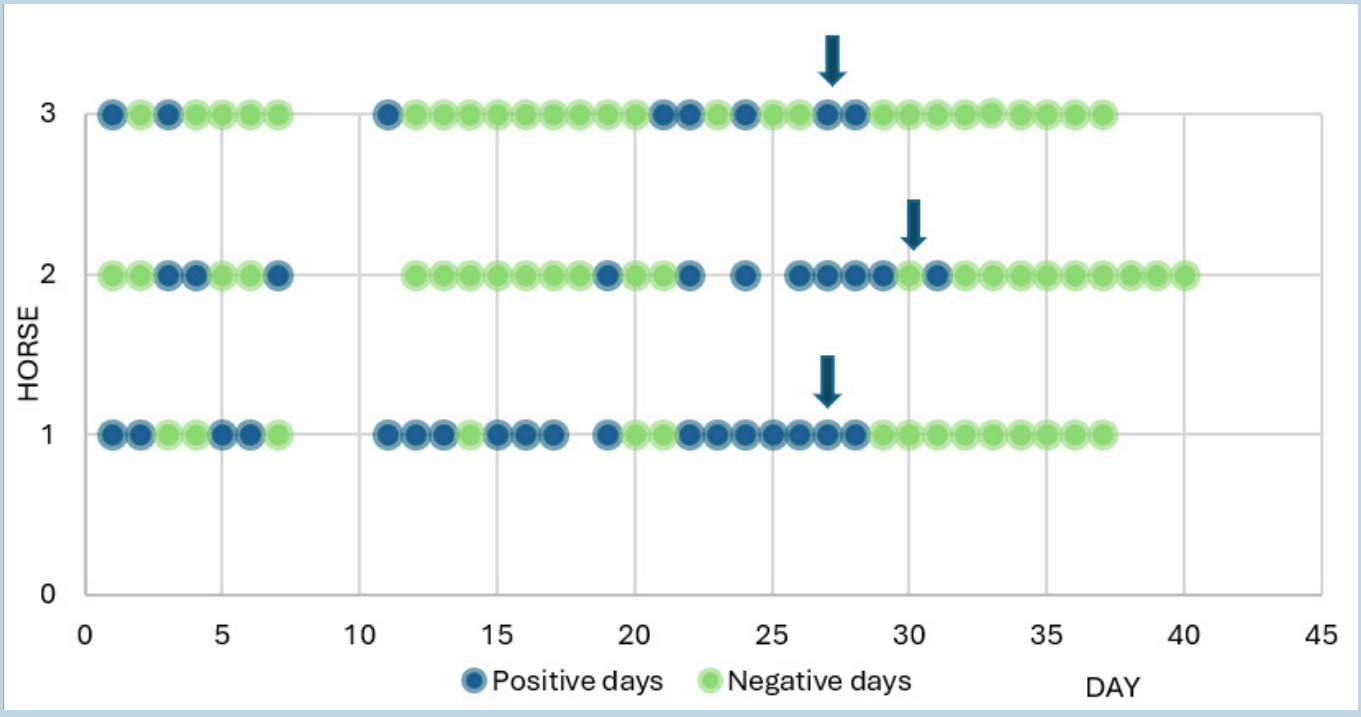
The relationship between infection intensity and FEC detection provides a rationale for using *A. perfoliata*-specific antibody testing to identify horses more likely to harbour higher tapeworm burdens before further FEC investigation. Serum- and saliva-based ELISAs provide indirect measures of infection intensity. In validation studies, Spearman's rank correlations with tapeworm burden were 0.78 for serum and 0.74 for saliva, with sensitivities of 85% and 83% and specificities of 78%

and 85%, respectively, when compared with post-mortem worm counts (Lightbody et al. 2016). Notably, neither test misclassified horses harbouring more than 20 tapeworms (Lightbody et al. 2016). Field studies further demonstrate substantially higher detection rates with antibody testing than with FEC. In Germany, tapeworm eggs were detected in 3/484 horses (0.6%), compared with positive results in 78/481 (16.2%) by serum ELISA and 108/365 (29.5%) by saliva ELISA (Jürgenschellert et al. 2020). Similarly, in Slovakia, eggs were detected in 8/401 horses (2.0%), compared with positive results in 167/424 (39.4%) by serum ELISA and 168/295 (57.0%) by saliva ELISA (Burcáková et al. 2023). In a recent Serbian study, eggs were detected in 19/173 horses (11.0%) by sedimentation–flotation and 3/173 (1.7%) by Mini-FLOTAC, compared with positive serum ELISA results in 61/173 horses (35.3%) (Kukurić et al. 2025). Taken together, these findings indicate that antibody testing detects substantially more horses as positive than coprological methods and can help identify horses or groups more likely to harbour higher tapeworm burdens. By prioritising these groups for subsequent FEC investigation, antibody screening will increase the likelihood of detecting egg shedding and improve the value of FEC as a tool for investigating treatment efficacy.

Detection of tapeworm eggs can vary substantially according to the coprological method used. In a preliminary study involving 18 horses sampled at five time points, Anderson et al. (2024) found that 16%, 47% and 70% of the 90 faecal samples were positive for tapeworm eggs using Mini-FLOTAC (Cringoli et al. 2017), the Cornell–Wisconsin method (Owen & Slocombe 2004) and the Proudman and Edwards (PE) method (Proudman & Edwards 1992), respectively. The PE method yielded higher egg counts, potentially reflecting differences in sample processing, including an additional washing step. Similarly, Hughes et al. (2026) demonstrated that centrifugation duration affected both egg detection and egg counts when using the PE method. Together, these findings demonstrate that FEC results are influenced not only by intermittent and variable egg shedding, but also by specific steps used for egg recovery and detection.

Recent work at Austin Davis Biologics further illustrates the variability of tapeworm egg detection within individual horses over time. In a preliminary study, three horses with evidence of *A. perfoliata* infection, based on EquiSal® saliva antibody scores, were sampled daily before and after anthelmintic treatment. Their saliva scores were not exceptionally high and their infections were responsive to praziquantel. Faecal samples collected at each time point were analysed using the PE method to assess changes in egg detection throughout the sampling period (Figure 3). Egg detection varied across sampling days, with the frequency of positive FEC results before treatment broadly reflecting the corresponding saliva antibody scores. Following treatment, eggs were detected on Day 1, but all subsequent FECs were negative, consistent with an effective treatment response. This sampling approach demonstrated day-to-day variation in FEC results within individual horses, highlighting how a single faecal sample may provide an incomplete picture of tapeworm egg shedding.

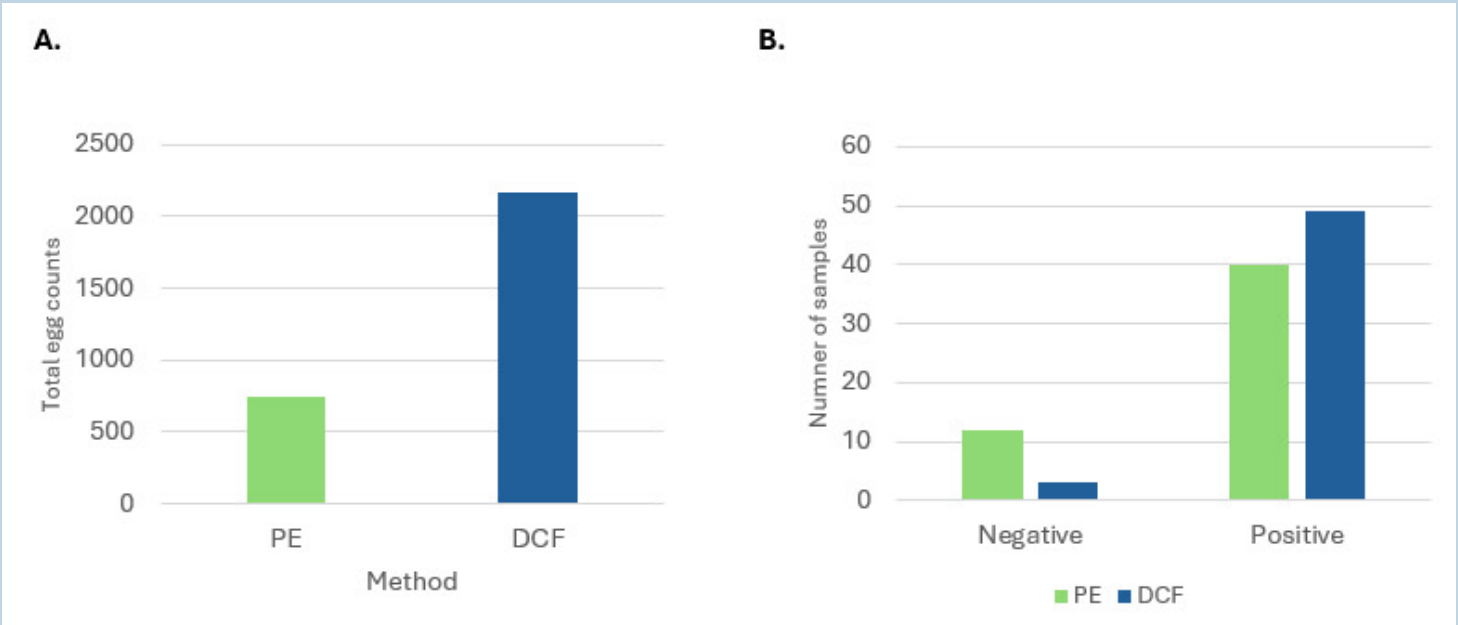
Figure 3. Intermittent tapeworm egg detection over time. Faecal samples were collected regularly from three horses for 26-29 days before and 10 days after anthelmintic treatment (blue arrows). Samples were analysed using the PE method. All horses had moderate/high EquiSal® saliva antibody scores at the start of sampling (Horse 1: 5.78; Horse 2: 2.93; Horse 3: 1.31). No cestocidal treatments had been administered during the preceding 6 months.



To further investigate factors affecting *A. perfoliata* egg detection, a modified double centrifugal flotation (DCF) method developed at Austin Davis Biologics was compared with the PE method used above. The analysis used 163 matched faecal samples from randomly selected horses on leisure yards that had not received tapeworm treatment for at least 6 months, together with a selected population with reduced cestocidal treatment effectiveness. In the leisure horse population, both methods identified the same number of positive faecal samples (18/163; 11%), with no significant difference in total egg counts (DCF 178 eggs vs PE 101 eggs; P=0.90). In the reduced efficacy population, however, the DCF method recovered substantially more eggs. Across 52 samples collected before (Day 0) and after (Day 14) anthelmintic treatment, the DCF method recovered 2,165 eggs compared with 741 eggs using PE (P<0.001) (Figure 4a). This difference was significant at both individual time points, with higher total egg counts using the DCF method at Day 0 (P<0.001) and Day 14 (P=0.01). The DCF method also identified significantly more positive samples when the two time points were combined (49/52 vs 40/52; P=0.03) (Figure 4b). The modified DCF preparations generally had clearer slide visibility, while PE preparations were more frequently represented in poorer-visibility categories. Egg counts were significantly associated with slide visibility (P<0.001), whereas no significant association was found with faecal consistency.

These findings supported the selection of the modified DCF method for the EquiStatus™ Flotation test, as it provided greater egg recovery in the reduced-efficacy population at both pre- and post-treatment sampling points, with slides generally clearer for egg identification and counting. Maximising egg recovery is important to increase the likelihood of detecting persistent egg shedding following anthelmintic treatment.

Figure 4. Comparison of tapeworm egg counts and egg-positive horses using the PE and DCF methods in the reduced efficacy population. (A) The total number of tapeworm eggs counted across all 52 horse samples using the DCF and PE methods. (B) The proportion of horses assessed as egg-positive or egg-negative by each method.

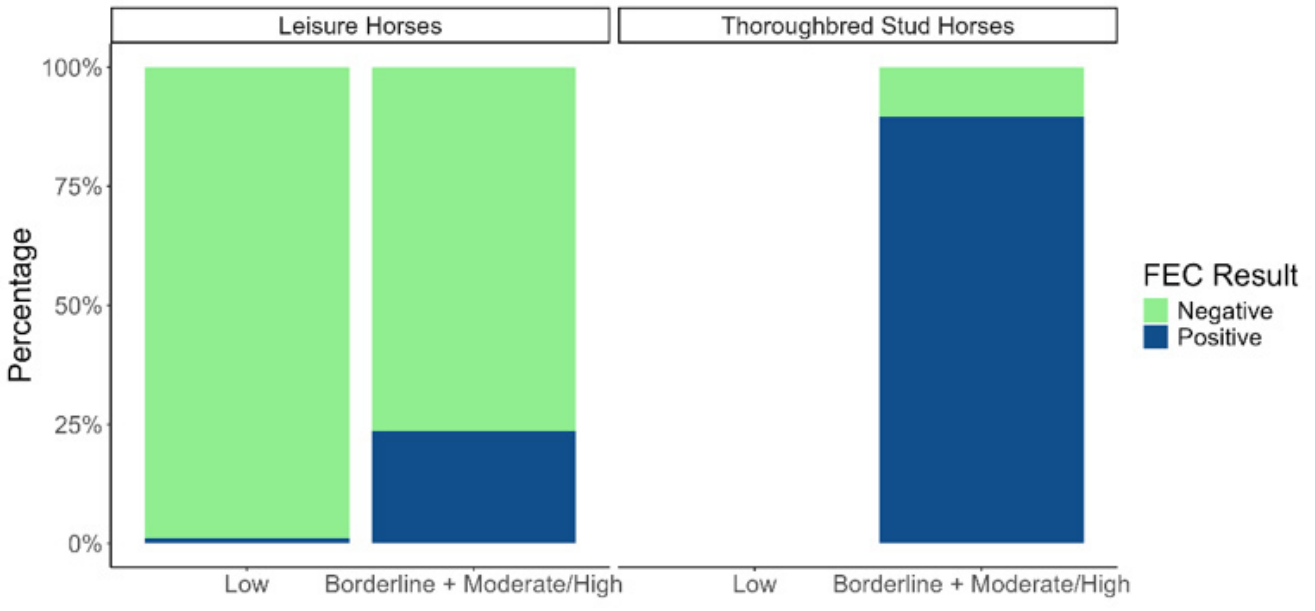


When assessing anthelmintic efficacy, faecal egg shedding should be measured as close to treatment as possible and again at a defined post-treatment interval. The same sampling and egg-count methods should be used at each time point to minimise methodological variation and ensure that changes in egg counts reflect treatment response. Based on the intervals used in the studies summarised in Table 1, a 14-day post-treatment sampling point is advised to allow sufficient time for treatment effects to become apparent.

Comparison of the DCF faecal egg count test method with EquiSal® test results

The diagnostic performance of the optimised DCF FEC method was evaluated in 163 leisure horses that had not received cestocidal treatment in the preceding six months. Matched saliva and faecal samples were analysed using the EquiSal® saliva test and DCF method, respectively. Among horses with Borderline or Moderate/High EquiSal® results, 76% tested negative by FEC (Figure 5). The same tests were then applied to matched samples from 97 Thoroughbred horses across four stud farms where reduced cestocidal efficacy had been confirmed. All 97 horses had Moderate/High EquiSal® results, and 90% tested positive by FEC (Figure 5).

Figure 5. FEC results by EquiSal® results category in leisure horses where reduced cestocidal efficacy was not suspected and Thoroughbred horses from four stud farms where reduced efficacy was confirmed. Matched saliva and faecal samples were tested using the EquiSal® saliva test and EquiStatus™ Flotation FEC test, respectively.



These findings highlight the limitations of relying on flotation FEC alone to screen for *A. perfoliata* infection and support the use of antibody screening to identify populations more likely to harbour higher infection prevalence and burdens. Horses with higher tapeworm burdens are more likely to harbour mature, egg-producing worms, increasing the likelihood of egg shedding and detection by FEC.

Antibody screening can therefore help target FEC testing to populations where it is most informative for assessing treatment efficacy.

Using tapeworm antibody screening to select groups for faecal egg count testing

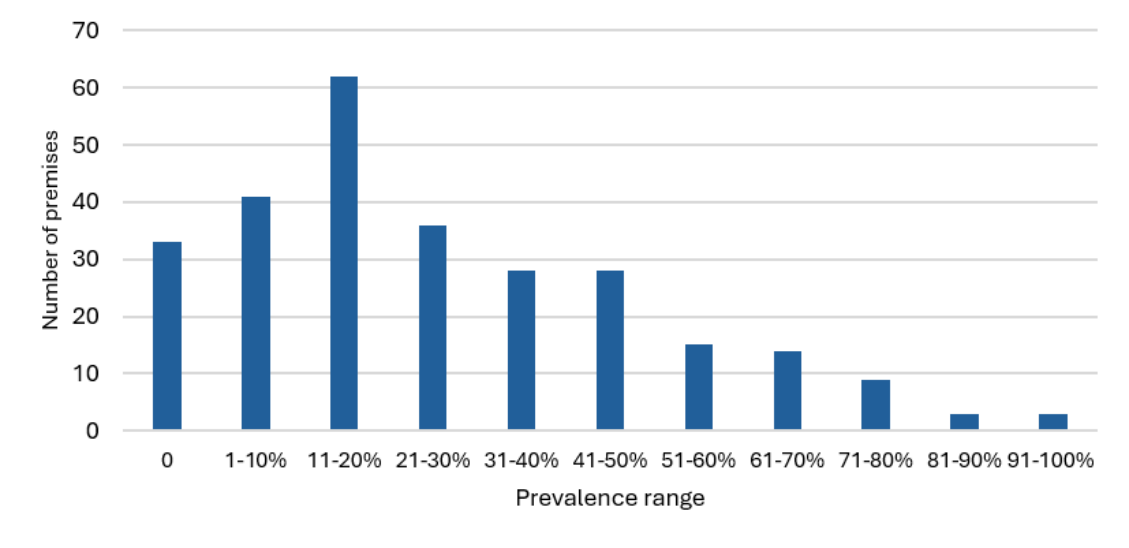
Analysis of faecal egg count versus EquiSal® test results

As indicated above, FEC testing is most informative where horses are more likely to harbour higher tapeworm burdens and shed detectable eggs. Antibody screening can therefore help identify groups in which FEC testing is most likely to provide meaningful evidence of treatment efficacy. Data from over 350,000 UK EquiSal® tests demonstrate the importance of population-level screening as over two-thirds of these horses have Low results, suggesting that *A. perfoliata* infection is absent or occurs at low levels in many populations.

Published UK studies report substantial variation in antibody positivity between populations, ranging from approximately 15% on some premises (Lightbody et al. 2018) to over 80% on others (Wickenden et al. 2025). Similar variation has been reported in Germany and Slovakia (Jürgenschellert et al. 2020; Burcáková et al. 2023), likely reflecting differences in pasture management (Matthews et al. 2026) and environmental conditions affecting transmission via the oribatid mite intermediate host (Wickenden et al. 2025).

To further characterise this variation, commercial EquiSal® data were analysed at premise level (Figure 6). The analysis included 272 premises, each with results for 7-60 horses. Premise-level *A. perfoliata* prevalence was calculated as the proportion of tested horses with Borderline or Moderate/High antibody results. Prevalence varied markedly between premises, ranging from 0% to 100%, but was below 50% in 83% of populations.

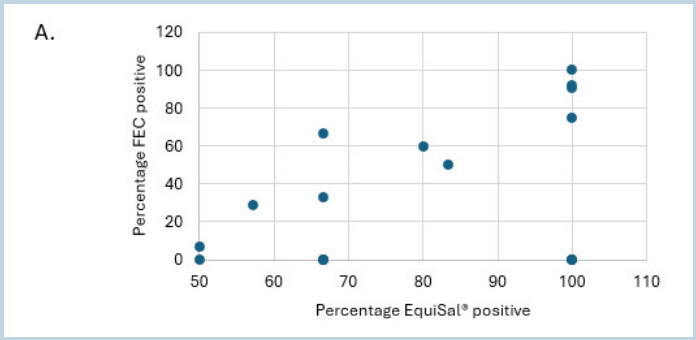
Figure 6. Distribution of EquiSal® test positivity across 272 premises. Test positivity (prevalence) was defined as the proportion of horses with EquiSal® results in the Borderline and Moderate/High categories.

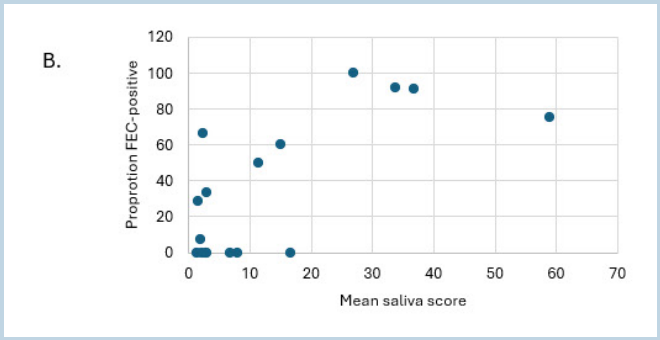


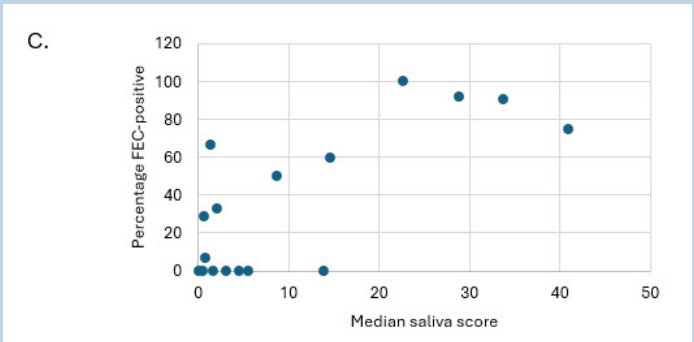
These findings provide further evidence that infection levels are heterogeneous between populations and highlight the value of antibody screening for identifying groups where FEC testing is more likely to generate sufficient positive results for meaningful assessment.

Given the marked variation in EquiSal® positivity between populations, a further analysis examined whether the magnitude of saliva antibody responses could help prioritise populations for FEC testing. Seventeen groups with high EquiSal® positivity (>50% of horses with Borderline or Moderate/High results) were selected to compare saliva antibody responses with FEC positivity (Figure 7). This threshold aimed to identify groups more likely to contain horses with detectable *A. perfoliata* infection, maximising the opportunity to identify FEC-positive horses. The groups were not intended to represent the wider UK population, where lower infection levels would be expected; rather, the analysis explored whether antibody response magnitude could guide the selection of populations for further FEC investigation.

Figure 7. Relationships between population-level EquiSal® antibody results and FEC positivity. Scatter plots showing the proportion of FEC-positive horses at 17 premises in relation to (A) the proportion of horses classified as EquiSal® positive (Borderline or Moderate/High), (B) mean EquiSal® saliva score and (C) median EquiSal® saliva score. Each point represents one premise (n = 17).







The proportion of FEC-positive horses was positively associated with both mean saliva score (Spearman's ρ = 0.57, P = 0.017) and median saliva score (ρ = 0.61, P = 0.009). In contrast, the proportion of EquiSal®-positive horses showed a weaker, non-significant association with FEC positivity (ρ = 0.35, P = 0.166). Population size was also positively associated with FEC positivity (ρ = 0.60, P = 0.010), potentially reflecting greater variability in smaller groups. These findings suggest that, among populations with high EquiSal® positivity, the magnitude of saliva antibody responses will help prioritise populations for further FEC investigation.

The observed association between antibody response magnitude and FEC positivity in these 17 populations prompted a further comparison based on how the populations had been identified. The populations comprised 13 groups selected for high EquiSal® positivity and four in which reduced anthelmintic efficacy had been identified through pre- and post-treatment FEC testing. Among the 68 horses in the EquiSal®-selected groups, 48 (70.6%) were EquiSal® positive and 12 (17.6%) were FEC-positive. In contrast, all 97 horses in the four groups with identified reduced efficacy were EquiSal® positive and 87 (89.6%) were FEC-positive. Mean saliva scores were 5.9 and 39.1, respectively, and median scores were 4.4 and 31.5. At the population level, mean saliva score, median saliva score and FEC positivity were all significantly higher in groups with identified reduced efficacy (Mann–Whitney U, P < 0.01 for all comparisons).

Although these findings are preliminary due to the small number of groups studied, they support the use of antibody testing to prioritise groups for FEC investigation. Both the magnitude of antibody responses and the proportion of EquiSal®-positive horses will help identify populations more likely to yield sufficient FEC-positive horses for further investigation.

The 17 groups were also examined to assess how group size influenced estimates of FEC positivity. In the smallest groups, comprising 3-7 horses, estimates varied widely, from 0% to 67%, highlighting the potential uncertainty associated with small sample sizes.

Taken together, the population-level analyses suggest that both the proportion of EquiSal®-positive horses and the magnitude of antibody responses may help identify groups more likely to yield FEC-positive horses.

To determine whether these relationships were also evident at individual-horse level, paired saliva and faecal results from two datasets were analysed (Table 2). The first comprised 163 horses from groups tested during routine diagnostic activity. Horses were classified by EquiSal® result as Low or Borderline and Moderate/High and FEC positivity compared between categories. FEC positivity was 1.1% (1/91) among horses with Low results and 23.6% (17/72) among those with Borderline or Moderate/High results. FEC positivity differed significantly between categories (Fisher's exact test, P < 0.001). Saliva antibody score was also positively associated with total tapeworm egg count (Spearman's ρ = 0.39, P < 0.001). Thus, although most horses with Borderline or Moderate/High results were FEC-negative, higher antibody scores within this category were associated with a greater likelihood of FEC positivity.

A second dataset comprised horses from four Thoroughbred stud farms where elevated antibody scores and reduced anthelmintic efficacy had been identified. Day 0 saliva and faecal samples were collected before praziquantel treatment. Only horses with Moderate/High or Off-scale EquiSal® results were included. FEC positivity was 87.3% (55/63) among horses with Moderate/High results and 94.1% (32/34) among those with Off-scale results. This difference was not statistically significant (Fisher's exact test, odds ratio = 2.31, P = 0.486) but saliva antibody score was positively associated with total tapeworm egg count (Spearman's ρ = 0.34, P < 0.001).

This analysis indicated that in routine diagnostic populations, horses with Moderate/High scores were more likely to be FEC-positive, although many horses with Moderate/High scores were FEC-negative.

Table 2. FEC positivity according to EquiSal® antibody in routine diagnostic populations and selected Thoroughbred stud farms. Results are presented as the number of FEC-positive horses, the total number tested and the percentage FEC-positive. Note: Off-scale results exceed the linear range of the ELISA calibration curve and should not be interpreted as quantitatively comparable with scores within the measurable range.

Population and EquiSal® category	FEC-positive horses (n/n)	FEC-positive (%)
Routine diagnostic populations: Low	1/91	1.1
Routine diagnostic populations: Borderline and Moderate/High	17/72	23.6
Selected stud farm populations: Moderate/High	55/63	87.3
Selected stud farm populations: Off-scale	32/34	94.1

In contrast, FEC positivity was high in horses in both the Moderate/High and Off-scale categories in groups where veterinary concerns had prompted investigation and reduced anthelmintic efficacy had been identified.

These findings support EquiSal® antibody profiling as a risk-stratification tool to identify groups requiring further investigation, although the relationship between antibody scores and egg shedding depends on population context. Antibody scores should not be used alone to predict individual egg shedding. A high proportion of Moderate/High or Off-scale results will support FEC testing, particularly where clinical concerns or questions about treatment efficacy exist. A predominantly Low profile may indicate lower priority for investigation but does not rule out infection or egg shedding. Decisions should consider the antibody profile alongside clinical signs, treatment history and management factors.

To further explore the practical application of this approach, saliva antibody profiles from populations with previously identified reduced anthelmintic efficacy were compared with those from a larger group of commercial service populations:

- Group 1: 54 commercial service exemplar populations with a minimum of 10 horses/group from 2025, selected because more than 50% of horses were EquiSal® positive; and
- Group 2: four populations in which reduced anthelmintic efficacy had been identified using pre- and post-treatment FEC testing.

Table 3. Comparison of group-level EquiSal® antibody profiles across commercial service and reduced efficacy populations. Results are presented as median and interquartile ranges (IQR) of population-level measures for Group 1 and Group 2. No individual horse-level data were used in this analysis.

Parameter	Group 1	Group 2
Mean saliva score [IQR]	2.33 [1.47–4.33]	35.25 [32.02–42.31]
Median saliva score [IQR]	0.44 [0.10–1.25]	31.30 [27.29–35.55]
Minimum saliva score [IQR]	−0.09 [−0.09–−0.09]	7.37 [7.08–8.10]
Maximum saliva score [IQR]	15.14 [8.90–28.21]	94.06 [74.69–138.93]
Percentage off-scale [IQR]	0.0 [0.0–2.21]	35.88 [29.71–41.92]

Note: The % off-scale variable is expressed in percentage points. The commercial group has a median of 0%, despite several premises having non-zero values.

The group with identified reduced anthelmintic efficacy had substantially higher EquiSal® antibody levels than the commercial service exemplar group (Table 3). Pairwise comparisons identified significant differences between Groups 1 and 2 across all five EquiSal® parameters (Table 4).

Table 4: Pairwise comparison of EquiSal® antibody score parameters between groups. Groups were compared using two-sided Mann–Whitney U statistic, with Holm–Bonferroni correction across the five outcomes. The U statistic and adjusted p-value are reported for each parameter; an adjusted $p < 0.05$ was considered statistically significant.

Outcome	G1 vs G2 *U*	Raw *p*-value	Holm-adjusted *p*-value
Population mean saliva score	0	0.000971	0.00291
Population median saliva score	1	0.00108	0.00291
Population minimum saliva score	0	0.0000024	<0.001
Population maximum saliva score	10	0.002773	0.00291
% off-scale	2	0.000078	0.000312

These findings support the use of EquiSal® antibody screening to prioritise populations for FEC-based assessment of anthelmintic efficacy. Previous analysis (Matthews et al., 2023; Figure 5) showed that many populations have fewer than one-third of horses testing EquiSal® positive. These populations may be less suitable for FEC-based assessment, as a greater proportion of horses are likely to have absent or low tapeworm burdens, increasing the likelihood of negative FEC results. Even among populations with >50% EquiSal® positivity, those with previously identified reduced anthelmintic efficacy (Group 2) had significantly different antibody profiles from the exemplar populations (Group 1). Differences across all measures indicated variation not only in the proportion of positive horses, but also in the magnitude and distribution of antibody responses. Although the small number of populations makes this a proof-of-concept analysis, the findings suggest that saliva antibody magnitude and distribution provide additional information beyond positivity alone when prioritising populations for targeted FEC assessment.

Serum antibody analysis

A similar approach was applied to serum antibody data, usually derived from larger groups, including horses on breeding premises. These populations often contain more young horses with potentially higher overall infection levels. Antibody prevalence and distribution may therefore differ from those in the EquiSal® dataset. Three groups were compared:

- Group 1: Horses from 42 premises tested through the commercial service in 2025, with at least 10 horses tested per premise.
- Group 2: A subset of Group 1 in which more than 50% of horses tested antibody-positive.
- Group 3: Four populations in which FEC results indicated reduced anthelmintic efficacy.

For each group, antibody positivity, mean, median and maximum antibody scores and the percentage of off-scale results were calculated (Table 5).

Table 5. Group-level serum antibody profiles across commercial service and reduced efficacy groups. Results are presented as the mean or median of the corresponding group-level measures. 'Prevalence' represents the mean of the percentage of test-positive horses per population. Serum antibody scores and off-scale results were summarised at the group level.

Group	n	Mean prevalence	Mean serum score	Median serum score	Median maximum score	Mean off-scale	Median off-scale
Group 1	42	63.92%	11.61	8.83	38.82	17.98%	12.79%
Group 2	31	75.17%	14.49	11.52	43.19	23.90%	22.5%
Group 3	4	89.45%	25.40	24.26	53.97	49.47%	52.27%

Serum antibody profiles showed a clear gradient across the three groups, with test positivity, antibody scores and the proportion of off-scale results increasing from Group 1 to Group 3. Groups with identified reduced anthelmintic efficacy (Group 3) had the highest antibody responses and greatest proportion of off-scale results. Population-level measures were compared using two-sided Mann–Whitney U tests, with Holm–Bonferroni correction for multiple comparisons (Table 6).

Table 6. Statistical comparison of population-level blood antibody profiles. Holm–Bonferroni correction was applied across the measures tested (mean antibody score, median antibody score and percentage off-scale) to account for multiple comparisons. Statistical significance was defined as an adjusted p value.

Outcome	Group 1 vs. Group 3: Raw *p*	Holm-Bonferroni-adjusted *p*	Group 2 vs. Group 3: Raw *p*	Holm-Bonferroni-adjusted *p*
Mean serum antibody score	0.00065	0.0039	0.0020	0.0085
Median serum antibody score	0.0063	0.0189	0.0197	0.0214
% off-scale	0.0017	0.0085	0.0107	0.0214

Group 3 differed significantly from both Groups 1 and 2 in mean and median serum antibody scores and the proportion of off-scale result. These findings indicate that the magnitude and distribution of serum antibody responses provide useful population-level information beyond antibody-positive/negative classification alone. Serum antibody profiles will therefore help identify populations for which further FEC investigation is most likely to inform anthelmintic efficacy assessment. However, given the small number of reduced efficacy populations and variation between commercial premises, these findings are preliminary. Further data are needed to confirm these patterns and establish criteria for consistent application in the guidelines.

Considerations for FEC sample size

The number of horses sampled is an important consideration when using FEC testing to assess *A. perfoliata* infection at population level. To examine the effect of sample size on egg detection, a random sampling analysis was performed in R using Day 0 FEC results from populations with identified reduced anthelmintic efficacy. All horses had Moderate/High EquiSal® antibody scores, including some Off-scale results, indicating a high likelihood of substantial infection. For each sample size, horses were randomly selected from the dataset and the process repeated 100 times. The likelihood of detecting at least one FEC-positive horse increased with sample size, from 60% with one horse to approximately 95% with two, 99% with three and 100% with four.

These findings demonstrate the value of testing multiple horses but should be interpreted in the context of the high infection levels in these populations, as detection rates will be lower in populations with lower infection levels. The results do not establish a minimum sample size applicable to all populations, but illustrate the general principle that sampling more horses increases the likelihood of detecting egg shedding. This is consistent with the analysis of the 17 populations described above, in which FEC positivity varied considerably among groups of 3–7 horses. FEC results from small groups should therefore be interpreted with caution, particularly when all or most horses test negative. Negative results from a small sample cannot reliably exclude egg shedding across the wider population and the sample size required for a meaningful assessment will depend on the expected level of infection. Based on these analyses, the following provisional guidance is recommended for group-level FEC testing:

- ≥10 horses: Preferred target, particularly where antibody levels or expected infection burden vary.
- 7–9 horses: Acceptable if 10 horses cannot be sampled, but negative findings carry greater uncertainty.
- 5–6 horses: May provide useful evidence of egg shedding, but negative findings offer limited evidence that shedding is absent at population level.
- <5 horses: Testing may detect egg shedding but negative findings should be considered indicative and interpreted alongside the antibody profile and history.

These recommendations should be considered alongside the antibody profile and history-based assessment of expected infection levels. A negative FEC result from a small sample should not, in isolation, be taken as evidence that the wider group is free from tapeworm egg shedding or that anthelmintic treatment has been successful.

In conclusion

The evidence presented in this document supports a targeted approach to investigating *A. perfoliata* infection and anthelmintic efficacy. Infection varies considerably between horse populations, while the sensitivity of FEC is limited by intermittent egg shedding, variation in egg recovery and the presence of immature or non-gravid worms. Consequently, negative FEC results do not necessarily indicate an absence of infection. Antibody profiling provides complementary population-level information, with the magnitude and distribution of responses helping to identify groups in which further FEC investigation is most likely to be informative.

This approach is particularly relevant given increasing concerns about reduced efficacy of available cestocidal anthelmintics. Although historical studies reported consistently high treatment efficacy, more recent investigations, including the UK field investigations described here, have identified persistent egg shedding following treatment with praziquantel and pyrantel. These findings raise concerns about treatment response under field conditions.

For veterinary surgeons and SQPs, the practical objective is to use antibody screening to guide the selection of populations for investigation, followed where appropriate by paired pre- and post-treatment FEC testing using a consistent methodology. Results should be interpreted alongside antibody profiles, treatment and management history and the number of horses tested. Negative findings from small groups require particular caution, as they cannot reliably exclude egg shedding across the wider population.

EquiStatus™ brings together population-level antibody profiling, treatment and management history and standardised FEC testing within a single investigative framework. This supports a risk-based approach to identifying persistent egg shedding and investigating suspected reduced efficacy consistent with UK guidance favouring monitoring-led parasite control over routine treatments (CANTER, 2026). As the available cestocidal options remain limited, improving the identification and investigation of potential treatment failure is important for the responsible long-term management of tapeworm control.

For practical criteria covering population selection, antibody-profile interpretation, sampling numbers and assessment of treatment response, refer to the EquiStatus™ Tapeworm Guidelines [link to be inserted].

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