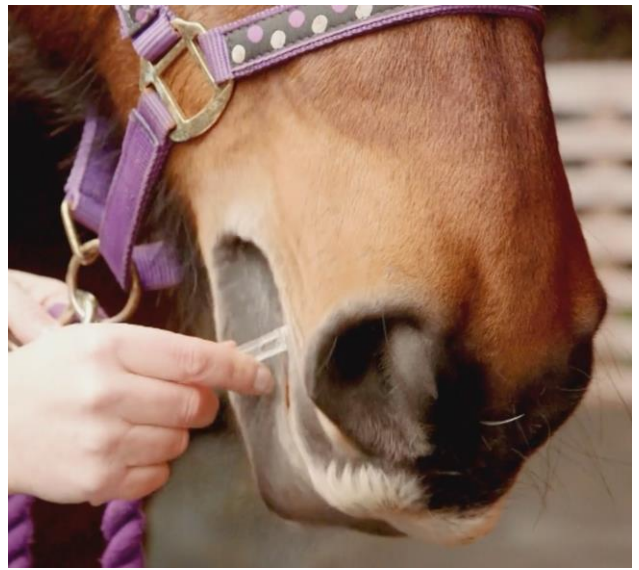


EQUISAL TAPEWORM TESTING:

FREQUENTLY ASKED QUESTIONS FOR PROFESSIONALS

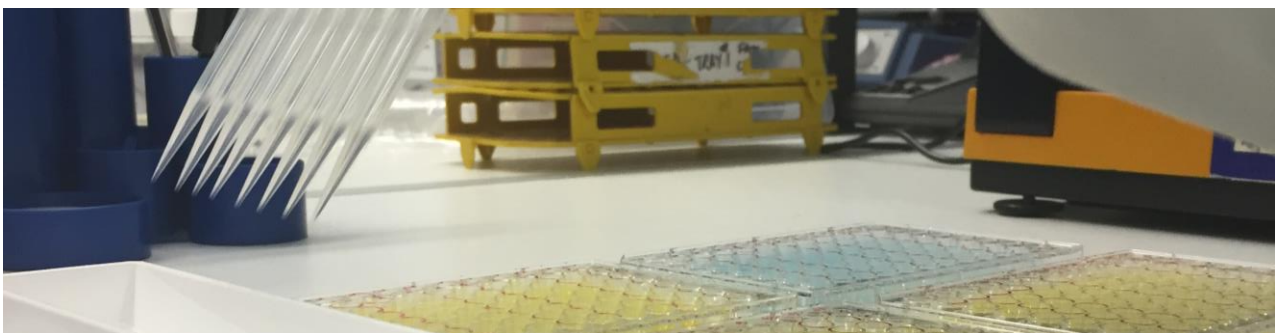
What is the EquiSal Tapeworm test?

The EquiSal Tapeworm test (patent granted) is a simple-to-use horse saliva test for detecting harmful tapeworm infections in horses. It was developed by family business, Austin Davis Biologics Ltd, who provide a testing service to diagnose tapeworm burdens and recommend treatment. The EquiSal Tapeworm test works like a blood test but, instead, uses saliva that horse owners collect themselves using a specially designed saliva collection swab. The saliva swab is posted back to the laboratory in a preservative solution which keeps the sample stable for at least three weeks.



How does the test work?

The EquiSal Tapeworm test detects tapeworm-specific antibodies in saliva to diagnose burdens. It is comprised of three different ELISA formats, the Specific ELISA, the NSB (non-specific binding) ELISA and the Total ELISA; the results of each ELISA are interpreted through an algorithm/calculation to generate a Saliva Score. The Specific ELISA detects the presence of horse IgG(T) antibodies to *A. perfoliata* 12/13kDa antigens, whereas the Total ELISA and the NSB ELISA are essential to negate the effects of the inherent variability between saliva samples (i.e. high versus low NSB score or dilute versus concentrated samples). The rate of saliva production in a horse can vary greatly and is affected by a variety of factors. High salivation rates can result in relatively dilute samples and, if unaccounted for, could lead to false negatives. By measuring the total IgG(T) concentration in each sample and including this in the final Saliva Score calculation, the Total ELISA overcomes saliva flow rate variability issues and reduces the occurrence of false negatives. The use of the NSB ELISA accounts for variable, non-specific binding effects of saliva, therefore acting as a control for the Specific ELISA. Unlike serum or plasma, the presence of which tends to reduce background IgG(T) binding, some saliva components can adsorb to solid-phase surfaces resulting in ELISA wells becoming 'sticky' for other proteins to a variable extent. Additionally, incorporation of the NSB ELISA ensures that a negative value Saliva Score is obtained for a horse with no tapeworms present. This is especially important with dilute saliva samples, as a positive Specific ELISA score would be artificially exaggerated when interpreted alongside a low Total ELISA score.



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When should you test for tapeworm?

- We recommend that horses are tested every 6 – 12 months depending on the individual's risk factor (see table below) as **also recommended by experts in the UK-Vet equine deworming consensus.**⁽¹⁾
- The first EquiSal test should be carried out four months after the last deworming treatment for tapeworm.
- Horses with a borderline or moderate/high diagnosis and those judged to be in higher risk environments should be retested more frequently to determine whether additional treatment is required; a retest can be carried out two to three months after worming treatment for tapeworm.
- It is common for horses to be diagnosed with tapeworm infections in their first test despite regular anticestode use. In these cases, annual or 6 monthly treatments have not been effective at maintaining a healthy balance, resulting in chronic infection broken briefly by anthelmintic dosing, only for infection to reoccur. It is important to be diagnostic-led and consider targeting doses more often to help break the tapeworm life cycle, decrease pasture contamination, and therefore reduce the horse's risk of reinfection. This strategy, together with good pasture management, can help to prevent chronic infection and enables more efficient management of tapeworm infections.

Effective use of saliva testing for tapeworm

EquiSal Tapeworm diagnosis	Horses with low diagnosis history	Horses diagnosed as low	Horses diagnosed as low	Horses diagnosed as borderline or moderate/high	Horses diagnosed as borderline or moderate/high
Environmental risk	Low risk - closed herd - none with infection - good paddock management	Low risk - closed herd - none with infection - good paddock management	High risk - high herd turnover - other horses with infection - poor paddock management	Moderate risk - closed herd - other horses with infection - good paddock management	High risk - high herd turnover - other horses with infection - poor paddock management
Testing frequency	Once a year	Initially every 6 months	Every 6 months	Initially test every 6 months: if burden levels are not reduced then move to high risk strategy	Retest 2-3 months after treatment: determine if additional treatment required to reduce burden levels*

*Saliva IgG(T) levels reduce more quickly than IgG(T) in blood with 73% of horses reducing to below the treatment threshold within 5 weeks (Lightbody, K. L. et al. (2016) Vet Clin Path, 45: 335–346).



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Where is the validation paper published that supports the accuracy of the EquiSal Tapeworm test?

The validation paper has been published by the journal of Veterinary Clinical Pathology⁽²⁾ and is available as free access at Wiley Online Library.

To summarise the paper, we validated the EquiSal Tapeworm test using samples from horses at post-mortem where the tapeworms present were counted. The data was used to set cut-off values for diagnosing low, borderline, or moderate/high tapeworm burdens. We also carried out a serological test for each of the horses, allowing direct comparisons between the two tests and with tapeworm numbers determined at post mortem. The only 100% accurate way to identify if a horse has a tapeworm burden is to visually count the number within the intestines at post mortem but, as with any tests developed to diagnose infection, there are a small number of false negatives and positives. However, in our validation studies, the EquiSal Tapeworm test only misdiagnosed horses as having no burden when there were less than 20 tapeworms present (and mostly considerably less than 20 tapeworms). This number of tapeworms are considered to be non-pathogenic by parasitologists, therefore EquiSal Tapeworm test successfully identified all pathogenic burdens.

When data from the serological tapeworm test and the EquiSal Tapeworm test were compared, they were shown to have strong positive correlation with each other. However, when these tests did not result in the same diagnosis, it was when there were non-pathogenic burdens present (less than 20 tapeworms). Overall, the serological tapeworm test and EquiSal Tapeworm test were found to have the same level of accuracy at diagnosing tapeworm burdens.

Have any studies on the use of EquiSal Tapeworm testing been carried out?

Yes; a targeted treatment strategy using the EquiSal Tapeworm test has been proven to control tapeworm burdens and reduce the use of anti-tapeworm anthelmintics at Bransby Horses, a horse welfare charity in the UK. The study, published in Equine Veterinary Journal,⁽³⁾ reports findings from 237 horses where EquiSal Tapeworm testing was used to inform on anthelmintic administration over the course of a year. The EquiSal Tapeworm diagnostic led approach reduced the use of anti-tapeworm treatments by 86% compared to 6 monthly interval treatment strategies.

Most horses diagnosed below the treatment threshold in the first EquiSal Tapeworm test remained below the threshold in the following two tests and 168 horses (71%) required no anti-tapeworm treatment at all. Importantly, no increase in tapeworm infection prevalence was observed during the study period and only seven horses received treatment following all three EquiSal Tapeworm tests, suggesting that some horses are more susceptible to tapeworm infections.

The patterns of infection and reinfection observed during the study highlight the value of regular monitoring with the EquiSal Tapeworm test. Six monthly testing will identify horses acquiring new tapeworm infections allowing treatment at an early stage, limiting paddock contamination and exposure of the rest of the herd, and will also identify those individuals which may be more prone to reinfection. Incorporation of EquiSal Tapeworm testing into worming schedules will decrease the frequency of anti-tapeworm treatments and reduce the risk of resistance developing in tapeworms in the future.



Surveys and Population Studies

Use of a saliva-based diagnostic test to identify tapeworm infection in horses in the UK

K. L. Lightbody, J. B. Matthews, J. G. Kemp-Symonds, P. A. Lambert, C. J. Austin ✉

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How does the EquiSal Saliva Score relate to burden diagnosis and treatment recommendation?

Validation of the EquiSal Tapeworm test against the gold standard of counting tapeworms present at post mortem, demonstrated that the test identifies 1+ tapeworm burdens (Saliva Score of greater than -0.09) with a sensitivity of 83% and a specificity of 85%. Saliva Scores show strong positive correlation to tapeworm numbers with a Spearman's rank correlation of 0.74. Additionally, statistical analysis identified a very narrow range between Saliva Score -0.09 and 0.6 which is considered borderline.

The table below details the results provided by the EquiSal Tapeworm test service. The test identifies horses with low burdens, borderline results or moderate/high burdens and treatment is recommended as detailed in the table below.

EquiSal Tapeworm Saliva Score	< -0.09	-0.09 to 0.6	> 0.6
Tapeworm diagnosis	Low	Borderline	Moderate/High
Tapeworm treatment recommended	No	Yes	Yes

Why doesn't the EquiSal Saliva Score have units?

The EquiSal Tapeworm test uses three different ELISA formats to test each saliva sample, these measure tapeworm-specific antibodies and also account for individual horse saliva flow rates and the inherent variability of saliva matrices in ELISAs. Each ELISA plate is run with a calibration curve using appropriate standards, therefore results for individual ELISAs exist as a relative concentration rather than just optical density. This ensures results are reproducible and tightly controlled between ELISA tests. The final Saliva Score is generated using an algorithm/calculation to combine the results from each ELISA format. Consequently, the Saliva Score does not have units.

How can the Saliva Score have a negative value?

Negative scores, such as -0.09 stated for the 1+ tapeworm cut-off, arise as a result of the algorithm/calculation which is used to combine the results from the three ELISA formats which comprise the EquiSal Tapeworm test. It is not an optical density value.



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What is the average Saliva Score for a moderate/high burden diagnosis?

An extremely high Saliva Score can go up to well over 300 but the average moderate/high score is around 16.0. There is strong positive correlation between tapeworm number present and the Saliva Score, but there are always horses that won't fit this model exactly as each individual horse has its own immune response to a burden.



Why do you recommend treatment for just one or more tapeworms – why not just recommend worming for high burdens only, i.e. 20 or more tapeworms?

The statistical analysis of the data in the validation studies demonstrate that the EquiSal Tapeworm test is equally accurate for diagnosing tapeworm burdens with either a 1+ tapeworm cut-off or a 20+ tapeworm cut-off. We therefore had to make an informed decision on which cut-off to use for recommending treatment and there were a number of factors which brought us to the conclusion that 1+ tapeworm cut-off was the most responsible.

- The validation data highlighted that using a 1+ tapeworm cut-off, none of the high burden horses (20+ tapeworm burdens) were misdiagnosed.
- Although there is a general acceptance that the presence of 20 tapeworms or less is not pathogenic,^(4,5,6) there is no research or clinical data to back this up.
- The prevalence of horses with 1+ tapeworm burdens is low and recent figures from the EquiSal Tapeworm testing service indicate that approximately a quarter have a burden. Therefore even when diagnosing burdens with as low as 1 or 2 tapeworms present, the number of horses receiving anthelmintics is considerably less than when routine worming strategies are used.
- Considering that it has previously been recommended to treat horses every 6 months for tapeworm, it is unrealistic to expect that testing for tapeworm would occur more often than this unless research suggests otherwise. If a 20+ tapeworm cut-off was used as the recommendation for treatment, the effect of leaving a moderate burden untreated for 6 months before the next test is unknown.

Unless additional research data is generated to support the use of a 20+ tapeworm burden cut-off, the EquiSal Tapeworm test will continue to diagnose 1+ tapeworm burdens for treatment recommendation.

How long after worming are tapeworm-specific antibodies present in saliva?

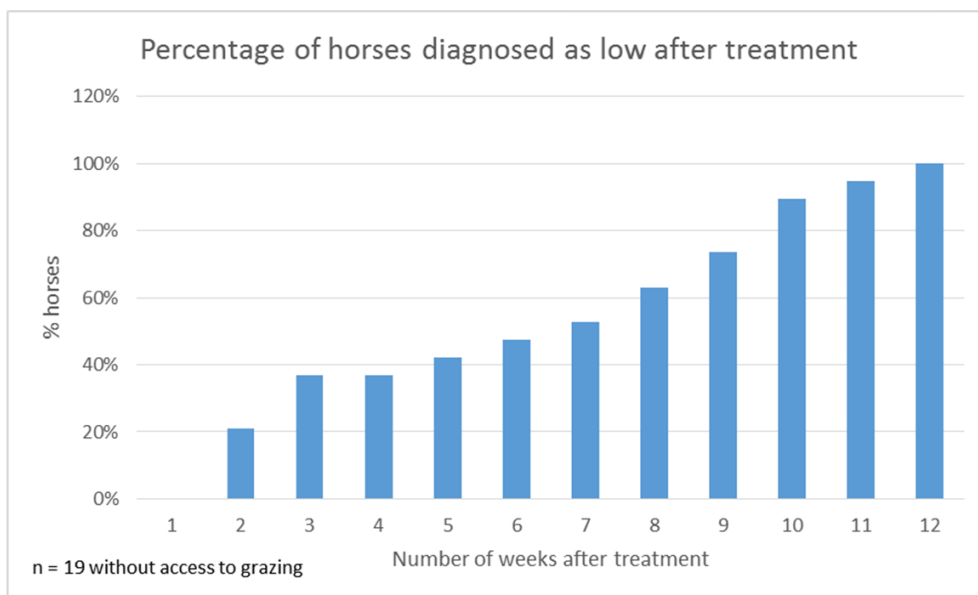
In a study on horses diagnosed with tapeworm but with no access to grazing, EquiSal Tapeworm testing was carried out every 2 weeks following tapeworm treatment. The study data demonstrated that 50% of horses' saliva scores had reduced to low within 6 weeks, 90% had reduced within 10 weeks and the remaining 10% of horses taking a further 2 weeks to drop to low. This suggests that antibody responses in saliva have less memory to tapeworm infection than that reported for antibodies in blood.

It is important to understand that the situation is complicated if the horse becomes reinfected by tapeworm larvae after worming treatment. Tapeworm reinfection was seen in some horses kept in poorly managed paddocks where reinfection can obviously happen very easily. But, given that the tapeworm's life cycle requires an intermediate host (an oribatid mite), even well managed paddocks containing horses with high tapeworm burdens could harbour infected oribatid mites within the grass. This means that there is still a reinfection risk after worming for horses grazing in these circumstances too.

Reduction of tapeworm-specific antibodies in saliva after treatment



- Horses without access to grazing tested every 2 weeks following praziquantel dose



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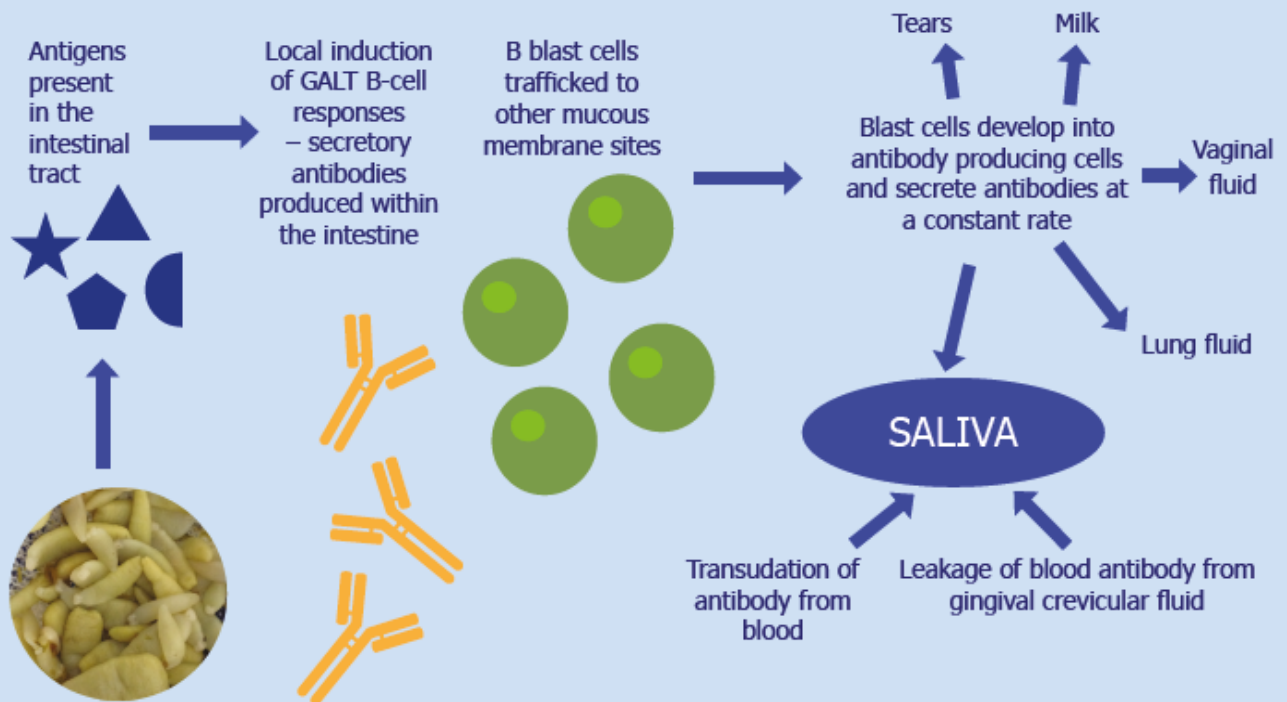
Why do tapeworm-specific antibodies in saliva reduce faster than in blood after treating for tapeworm?

Salivary tapeworm-specific antibodies are part of a mucosal immune response which is known to have shorter memory than humoral (systemic) immune responses. The decline in tapeworm-specific salivary antibodies we observed in the trial described above, fits well with a previous study where, tapeworm-specific IgG(T) antibodies were produced at the site of infection and secreted as a local mucosal antibody response.⁽⁷⁾ The hypothesis is that these antibodies are secreted through the mucosal epithelial cells by transcytosis with the equine analogue of the neonatal Fc receptor (FcRn), rather than the polymeric Ig receptor.⁽⁸⁾

In contrast to humoral antibody responses (detected in blood testing), mucosal antibody responses have a much shorter persistence and immunological memory. It can, therefore, be reasonably concluded that the tapeworm-specific antibodies measured in the EquiSal Tapeworm test are actually mucosal antibodies against tapeworm antigens produced in the salivary glands by plasma cells that originated as recirculating (via the lymphatic system) B-blasts triggered in the gut submucosa at the site of infection.⁽⁹⁾ It remains possible that low levels of humoral antibodies could leak into the saliva by transudation or passage through the gingival crevicular space; however, the pilot trial data suggest that this is not a significant factor.

Salivary diagnostics for helminths

EquiSal Tapeworm Test detects secretory antibodies specific to tapeworm antigens. It exploits the horse's secretory immune response to antigens within the intestine.



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Under what circumstances would the serological test diagnose a high burden when EquiSal Tapeworm test diagnoses a low burden?

Two differing diagnoses may seem confusing, however, to help make sense of this, we can refer to our validation experiments with samples from horses at post-mortem.

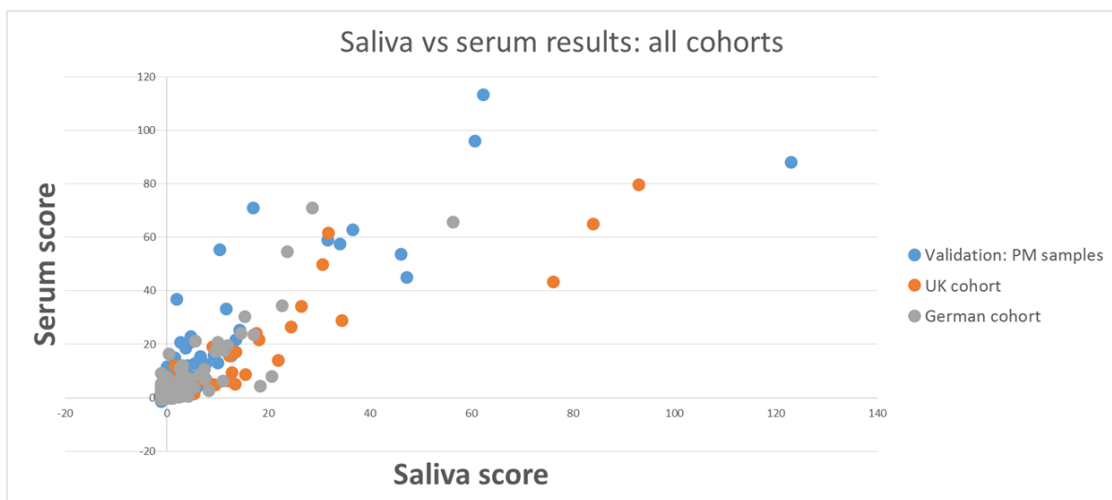
When the EquiSal Tapeworm test was validated using samples from horses at post-mortem where tapeworms present were counted, the test only misdiagnosed horses as having no burden when there were less than 20 tapeworms present (and mostly considerably less than 20 tapeworms). Similarly, when the EquiSal Tapeworm test was compared to the serological test, the only times that the diagnoses did not agree was when there were less than 20 tapeworms present. This number of tapeworms are considered to be non-pathogenic (ie not disease causing) by expert parasitologists.

Another important point to consider is that when salivary antibodies were monitored after worming in the antibody reduction trial described above, the data suggested that salivary antibodies reduce more quickly than previously reported for blood antibodies. Together with scientific understanding of the horse's immune system, this suggests that blood tests are more likely to pick up previous infections than the EquiSal Tapeworm test. This is also a possible reason for different diagnoses between the two tests.

Matched serum and saliva samples



Study	#Samples	Pearsons R ²
Validation: PM samples	104	0.738
UK cohort	188	0.859
Germany cohort	363	0.778



Very strong positive correlation



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Why do some horses have persistently moderate/high Saliva Scores despite worming as recommended?

We often see samples from horses who have been tested two or three times, six months apart, and the results are still moderate/high despite worming as recommended. Also some customers who carry out a retest two to three months after worming find that a moderate/high burden is still diagnosed. These scenarios do not necessarily mean that there is evidence of resistance. It is important to remember that there are several reasons for a persistently high Saliva Score, including;

- Poor pasture management, which allows horses to become reinfected after worming. We have seen reinfection occurring many times in a trial carried out on horses kept in poorly managed paddocks.
- Reinfection from other herd members. As the anti-tapeworm drugs are very short lived in the horse's system, all horses in a field (and preferably the whole yard) should be treated for tapeworm at the same time. Best practice would be to test all the herd members to identify high burdened horses to effectively control infection. However, in some cases this is not possible, so at the very least horses should be treated simultaneously. Failure to treat at the same time will result in untreated horses continuing to shed eggs onto the pasture and treated horses will become reinfected immediately after treatment. By deworming all the horses together, the life cycle will be broken briefly due to the absence of adult tapeworms where low levels of eggs are shed onto the pasture. In heavily infected paddocks, the horses will initially become reinfected, but as deworming is repeated, shedding of eggs onto the pasture is reduced until the level of infected mites on the paddock is very low and horses will be less likely to become reinfected.
- Arrival of a new horse(s) with a moderate/high burden to the paddock.
- Reinfection whilst visiting and grazing infected land, such as at club camps or grazing at show grounds.
- Under-dosing of dewormer, which allows infection to persist.
- Reinfection from adjacent fields.

Due to the management issues listed above and that salivary tapeworm-specific antibody reduction is not immediate following deworming, we cannot yet identify whether resistant tapeworms are present. Further research is required before we can use EquiSal Tapeworm results to identify resistance. If you are faced with either a situation of persistently high Saliva Scores or a moderate/high burden present in a horse that has been routinely treated for tapeworm, consider the management of the horse and its worming history. If the same drug has been used consistently, it could be worth altering the drug prescribed as a precaution.



FREQUENTLY ASKED QUESTIONS FOR PROFESSIONALS

If a large proportion of a herd is diagnosed with a tapeworm burden, should all the horses be treated?

It should first be considered what benefit would be gained from treating a whole herd for tapeworm and not just targeting those with a diagnosed infection. Praziquantel has no lasting effect, therefore treating a horse with no tapeworm burden will have no benefit. The drug will soon be out of the horse's system and the horse will have the same risks of picking up a burden from its field companions as it would have done without the treatment. The key to managing tapeworm burdens

is to ensure testing is routinely carried out to monitor for new emerging burdens and to practice good paddock maintenance which is critical to eliminating burdens (or preventing infection in the first place).

Can the EquiSal Tapeworm test be used to test donkeys?

Although it is expected that the test works for all equids, including donkeys, we haven't specifically validated the test for donkeys. All validation experiments were carried out on horses.

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