



THE SALIVA TEST FOR TAPEWORM

EquiSal® Tapeworm detects tapeworm-specific IgG(T) antibodies in horse saliva to provide a tapeworm burden diagnosis of low, borderline or moderate/high. Treatment is recommended for borderline or moderate/high results. The test has the benefit that horse owners themselves can collect the sample to send back to the laboratory for analysis.

- Proven accuracy, equivalent to blood testing
- Salivary IgG(T) antibodies have shorter half life than that reported for IgG(T) antibodies in blood
- Easy sample collection and postage for laboratory testing
- Results reported to horse owners through veterinary surgeons and SQP stockists
- Enables effective targeted deworming for tapeworm
- Three quarters of horses tested do not need deworming for tapeworm.

**EQUISAL TAPEWORM SALIVA TESTING RECOMMENDED BY EXPERTS
IN THE UK-VET DEWORMING GUIDELINES**

SCIENTIFIC VALIDATION of the EquiSal Tapeworm test published by
Veterinary Clinical Pathology

TARGETED TREATMENT STRATEGY for tapeworm published by Equine
Veterinary Journal (voted by EVJ as most clinically relevant paper of the month)

SCIENTIFIC VALIDATION OF EQUISAL® TAPEWORM TESTING PUBLISHED BY VETERINARY CLINICAL PATHOLOGY.

<http://onlinelibrary.wiley.com/doi/10.1111/VCP.12364/FULL>

Serum and saliva samples were collected from horses at a UK abattoir where tapeworms had been counted. Samples were then analysed in the EquiSal® Tapeworm test or a blood test. EquiSal® saliva scores had strong correlations with both tapeworm numbers and the blood test results. The majority of horses with one or more tapeworms at post-mortem were correctly identified by the test. The remaining few were diagnosed as being low but these horses had burdens considered by experts to be not pathogenic – less than 20 tapeworms present. Most importantly, no high burden (more than 20 tapeworms) horses were misdiagnosed by the test.

The EquiSal® Tapeworm Test can be relied upon to correctly identify the majority of horses with one or more tapeworms and correctly identify all horses with pathogenic burdens.

The test has both high sensitivity (83%) and specificity (85%).

1+ tapeworm cut-off 83% sensitivity 85% specificity	TREATMENT RECOMMENDED	MODERATE/ HIGH	Diagnoses <ul style="list-style-type: none"> • most horses with 1+ tapeworms present • all horses with a high burden (>20 tapeworm)
	NO TREATMENT NEEDED	LOW	Diagnoses <ul style="list-style-type: none"> • horses with no tapeworms present • some horses with a non-pathogenic burden (<20 tapeworms)

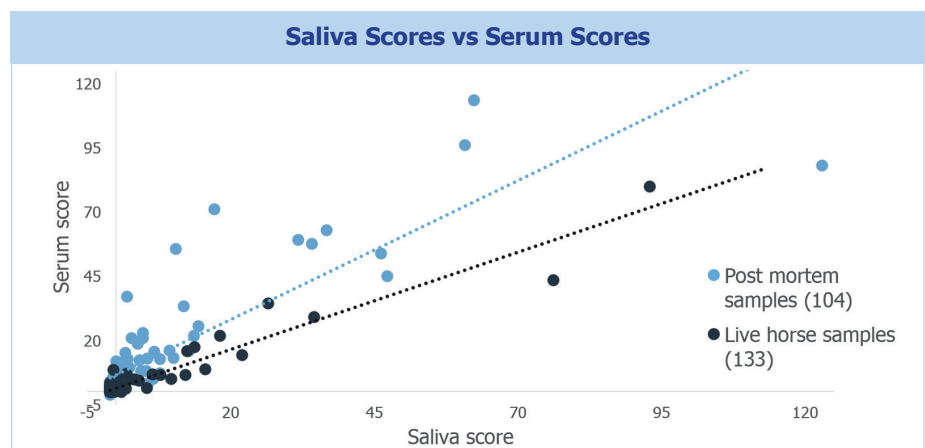
EQUISAL® TAPEWORM TESTING COMPARISON TO BLOOD TESTING

Post mortem samples

Data from validation experiments demonstrated that EquiSal® Tapeworm saliva scores had strong positive correlation with the blood test serum scores (104 samples), with a Pearson's correlation of 0.86. The only occasions that the saliva and serum test did not result in the same diagnosis was when there were less than 20 tapeworms present. This number of tapeworms is considered to be non-pathogenic by experts.

Live horse samples

Matched saliva and blood samples, collected from horses within the normal population (133 samples), also demonstrated a strong positive correlation with a Pearson's correlation of 0.96.



Salivary tapeworm-specific IgG(T) antibody half life

In an ongoing study, EquiSal® Tapeworm testing is carried out every 2 weeks, following tapeworm treatment, on horses diagnosed with tapeworm but with no access to grazing. Data collected so far (19 horses) shows 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 in grazing horses by the risk of reinfection from tapeworm larvae after deworming treatment. Tapeworm reinfection is evident in studies undertaken using 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 deworming for horses grazing in these circumstances too.

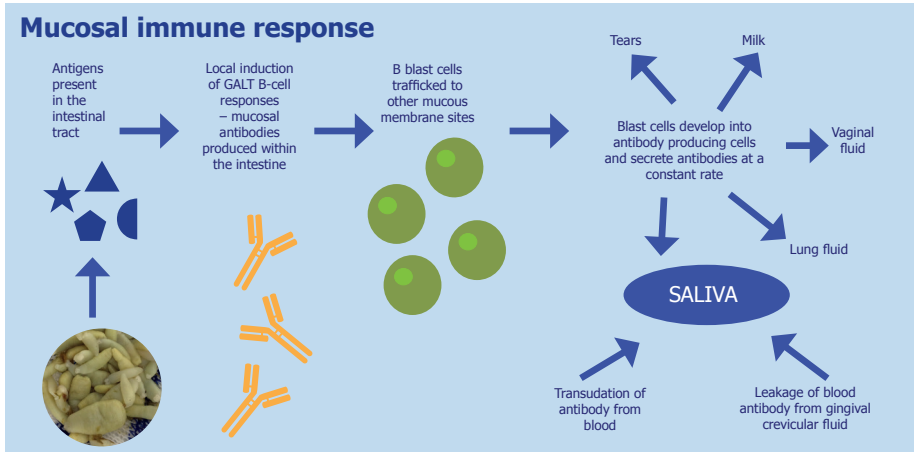
Mucosal versus humoral antibody responses

Salivary tapeworm-specific IgG(T) antibodies are part of the mucosal immune response to tapeworm infection. A previous study showed tapeworm-specific IgG(T) antibodies being produced at the site of infection and secreted as a local mucosal antibody response¹.

In contrast to humoral antibody responses (detected in blood testing), mucosal antibodies detected 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 mucosal antibodies 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 infection².

It remains possible that low levels of humoral antibodies could leak into the saliva by transudation or passage through the gingival crevicular space; however, our data suggests that this is not a significant factor.



1. Pittaway CE, Lawson AL, Coles GC, Wilson AD. Systemic and mucosal IgE antibody responses of horses to infection with *Anoplocephala perfoliata*. *Vet Parasitol*. 2014;199:32-41.
2. McGhee JR, and Fujihashi K. Inside the mucosal immune system. *PLoS Biol*. 2012;10(9): e1001397

EFFECTIVE TARGETED TREATMENT FOR TAPEWORM

<http://onlinelibrary.wiley.com/doi/10.1111/evj.12742/abstract>

A targeted treatment strategy using the EquiSal® Tapeworm saliva 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, recently published in Equine Veterinary Journal, 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 deworming schedules will decrease the frequency of anti-tapeworm treatments and reduce the risk of resistance developing in tapeworms in the future.

Lightbody, K. L., Matthews, J. B., Kemp-Symonds, J. G., Lambert, P. A. and Austin, C. J. (2017), Use of a saliva-based diagnostic test to inform on tapeworm infection in horses in the UK. Equine Vet J. DOI: 10.1111/evj.12742

HELMINTH RESEARCH PROGRAMME

Our research includes:

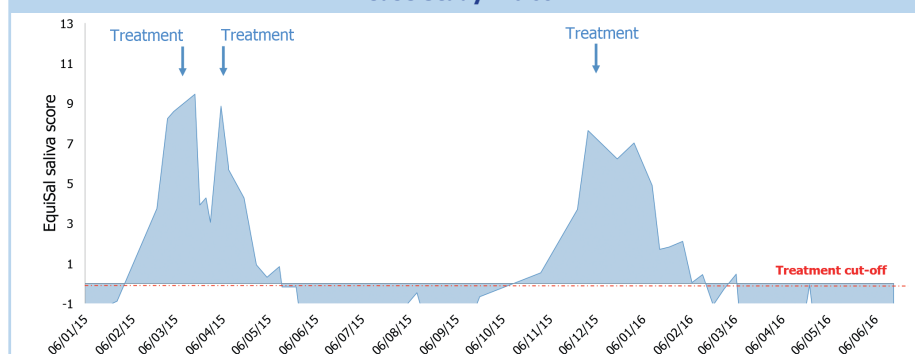
- Studies to investigate salivary antibody response to tapeworm infections in relation to management conditions, treatment strategies and reinfection risks.
- Research into tapeworm prevalence using scientific and questionnaire based data.
- Tapeworm life cycle research including oribatid mite research in conjunction with the Royal Veterinary College and Petplan Charitable Trust.
- Encysted cyathostomin diagnostics in conjunction with the Moredun Institute.
- New saliva-based diagnostic tests within the veterinary sector.

An insight into how tapeworm infections are spread: case study

The case study described here illustrates typical data obtained during studies monitoring tapeworm-specific antibodies in saliva. The figure details EquiSal® saliva scores for Horse A who grazes a well-managed paddock adjacent to Horse C. In February 2015, Horse A was infected by tapeworm, as determined by an increase in saliva score. This finding was unexpected as the horses do not leave the paddock and there are no surrounding paddocks to act as an infection source. However, it was later found that a passing horse had defecated adjacent to the paddock. This is the first anecdotal evidence that intermediate hosts of tapeworm, the oribatid mites, are able to travel. The mites would have ingested tapeworm eggs from the faeces and then moved to a position where Horse A could have ingested them. Horse A's burden was treated with praziquantel and the saliva score reduced to low burden status and remained low for the duration of the summer.

However, in November 2015 Horse A's saliva score increased to moderate/high burden status. The hypothesis is that during Horse A's initial infection, tapeworm eggs released onto the pasture were ingested by resident oribatid mites, where the larvae developed before infecting Horse A. Horse A was treated with praziquantel before again returning to low burden status. During this time, Horse C remained at low burden status until, at the end of January 2016, a moderate/high burden was diagnosed. Horse C was treated with praziquantel and returned to low burden. There are a number of findings evident from this case study; a) the spread of tapeworm infection between horses, b) the reduction of tapeworm-specific salivary antibodies after treatment and c) the dynamic nature of parasite infections. Together with numerous other study data we are gathering, this type of case study is expanding our knowledge of tapeworm infections and associated control strategies.

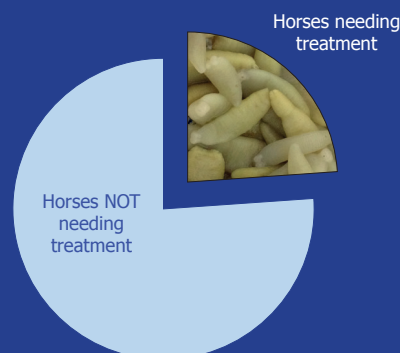
Case Study: Horse A



PERCENTAGE OF HORSES REQUIRING TREATMENT

Saliva score results from the EquiSal® Tapeworm testing service demonstrates that approximately a quarter were borderline or moderate/high and therefore recommended for treatment.

This illustrates that use of a targeted approach to controlling tapeworm burdens results in a significant reduction in anthelmintic usage.



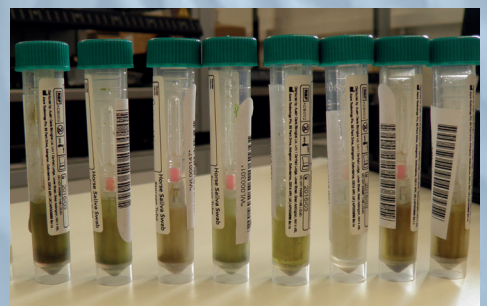
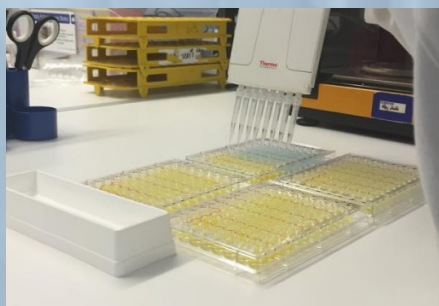
FURTHER DETAIL ON OUR RESEARCH PROGRAMMES CAN BE SEEN ON OUR WEBSITE UNDER NEWS STORIES

The testing process

To provide a reliable diagnosis, the EquiSal® Tapeworm test (patent pending) has to contend with numerous salivary variables such as, saliva flow rate variability, the horse's recent food intake and presence of particulate matter. To overcome these variables, three ELISAs are conducted on each sample; a Total IgG(T) antibody ELISA, a Non-specific binding ELISA, and a Specific ELISA. An algorithm is then used to generate a saliva score for each sample.

Current tapeworm testing recommendations

The first EquiSal® Tapeworm test should be carried out four months after the last deworming treatment for tapeworm. Routine testing should then be carried out every six months. If a horse has a borderline or moderate/high diagnosis, a retest can be carried out two to three months after deworming treatment for tapeworm.



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