GUIDELINES FOR USE:

SMALL REDWORM BLOOD TEST

Note: For the purposes of simplifying nomenclature for horse owners, this test has been named 'small redworm blood test'. For the avoidance of doubt, small redworms are also known as small strongyles and cyathostomin species.

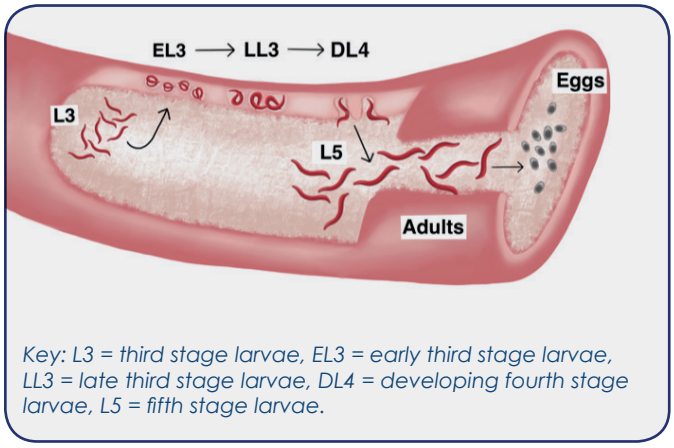
TECHNICAL INFORMATION

The small redworm blood test diagnoses cyathostomin infection in horses.

The test is an ELISA format which detects IgG(T) antibodies specific to three carefully selected cyathostomin antigens, representing the common species as well as all intra-horse stages of the life cycle *(Figure 1)*, including the clinically important ***encysted*** larval phase (Dowdall *et al.*, 2002, 2003, 2004; McWilliam et al., 2010; Mitchell *et al.*, 2016; Geyer *et al.*, submitted).

The small redworm blood test has been optimised for commercial use and has been validated against serum samples obtained from horses at post-mortem for which cyathostomin burdens were determined (n=124). Total worm burdens (TWB) were derived from enumeration of encysted larvae and luminal larvae/adults. The test has high sensitivity and specificity, with area under the curve (AUC) values of Receiver Operator Characteristic (ROC) curves of 0.96 (detailed in Table 1; Matthews, Austin *et al.*, publication in preparation).

Figure 1. Intra-horse stages of the cyathostomin life cycle



Key: L3 = third stage larvae, EL3 = early third stage larvae, LL3 = late third stage larvae, DL4 = developing fourth stage larvae, L5 = fifth stage larvae.

Table 1. Small redworm blood test sensitivity and specificity

TWB[1] threshold	Serum Score cut-off[2]	AUC[3]	Sensitivity	Specificity
1,000	14.37	0.96	98%	81%

[1] Total worm burden (TWB) derived from enumeration of encysted larvae and luminal larvae/adults. The TWB threshold is the number of worms at which the test categorises samples with the stated sensitivity and specificity.

[2] Serum Score cut-off was determined by assessing trade-off of diagnostic sensitivity against specificity over a range of values using GraphPad Prism software.

[3] Receiver operator characteristic (ROC) curve analysis was performed to indicate diagnostic accuracy of the test relating to cyathostomin infection (postive or negative) to total worm burden (TWB). As an estimate of test accuracy, area under the curve (AUC) may be interpreted such that; AUC=0.9-1.0 demonstrates excellent discrimination between positive and negative results; AUC=0.8-0.9, good discrimination; AUC=0.7-0.8, fair discrimination; AUC=0.6-0.7, poor discrimination and AUC=0.5-0.6, no discrimination.

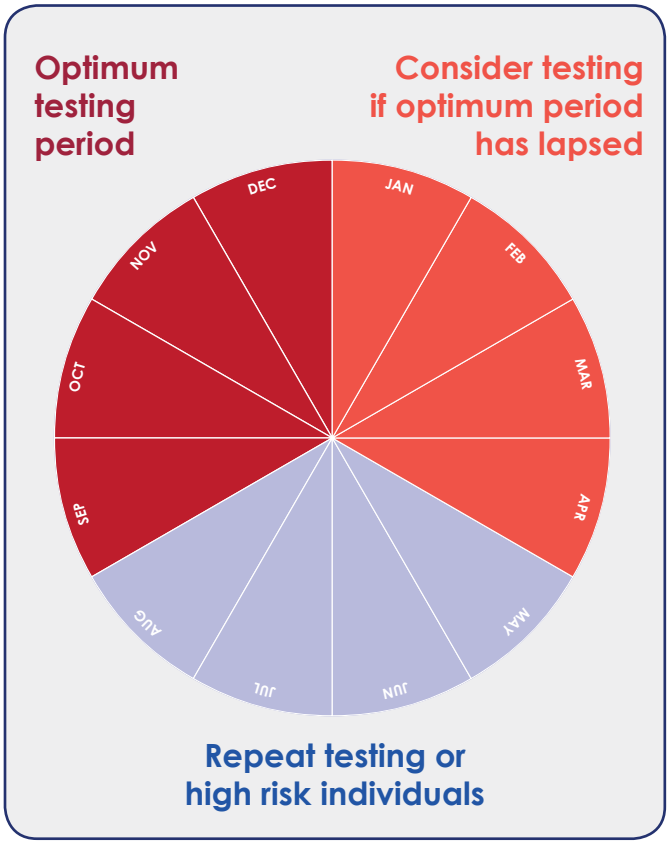
WHEN TO TEST

To inform on moxidectin treatment

We recommend the test is utilised when moxidectin administration would normally be considered for its larvicidal effect against small strongyles. The consensus deworming guidelines published by UK Vet (Rendle *et al.*, 2019) state that moxidectin should be administered during autumn/early winter of each year. However, it is common to utilise moxidectin into the winter and, if the autumn dose is missed, then treating later is advised rather than missing the annual dose. In addition, high risk individuals may receive a repeated dose at other times of the year. Note: moxidectin is the only effective larvicidal drug. Although 5-day fenbendazole treatment has licensed larvicidal properties, there is widespread resistance to this drug so, without knowing the worm population sensitivity status, its use is not recommended.

As a result, we recommend that the optimum testing period is between September and December each year, but if this period is missed, then testing should still be considered until April. *Figure 2* highlights the blood testing schedule based on current UK expert recommendations for applying cyathostomin larvicidal treatments.

Figure 2. Recommended testing schedule for the small redworm blood test



Differential diagnosis of suspect clinical cases

The small redworm blood test is invaluable for the differential diagnosis of larval cyathostominosis in horses with weight loss and/or diarrhoea. Larval cyathostominosis is a rapidly deteriorating disease with a poor prognosis. Since cases commonly lack specific clinico-pathological features, previously diagnosis has been based on an individual's history and exclusion of other conditions. A more rapid and robust diagnosis of the condition via this test will aid a more positive disease outcome for such cases and should be carried out at any time of the year.

Additional information:

Testing should **not** be conducted within 4 months of the last **moxidectin** treatment (based on current knowledge of the decline of serum antigen-specific IgG(T) derived from tapeworm research, Proudman and Trees, 1996).

Please read the following sections 1 2 3 4 with reference to the Decision Flow Chart provided (Appendix 1)

1 SELECTING HORSES SUITABLE TO TEST

It is important to assess whether individual horses and herds of horses are suitable candidates for testing with the small redworm blood test. Factors to consider include the age of the horse, the likelihood of cyathostomin exposure/infection, and previous FEC results. *Table 2* details *an* ***example*** to aid with risk assessment, categorising horses into low, moderate or high risk levels.

Horses with previous FEC results >200 eggs/g are showing evidence of a significantly active cyathostomin life cycle and should therefore receive a routine moxidectin treatment.

Table 2. Examples of considerations to assess cyathostomin infection risks in horses

EXAMPLE: RISK ASSESSMENT FOR CYATHOSTOMIN INFECTION			
RISK LEVEL	**LOW RISK**	**MODERATE RISK**	**HIGH RISK**
Previous FEC results	- always below <50 epg	- usually below <200 epg	- recent results >200 epg
Environmental conditions (combination of conditions to derive risk level)	- closed herd - all herd <200 epg - good paddock management - effective quarantine - low stocking density - frequent poo picking	- closed herd - low proportion of herd >200 epg - good paddock management - medium stocking density - sporadic poo picking	- high herd turnover - high proportion of herd >200 epg - poor paddock management - presence of youngstock - high stocking density - no poo picking - anthelmintic resistance identified
TEST WITH SMALL REDWORM BLOOD TEST	✓	✓	✗ **Treat with moxidectin**

Age

Horses and ponies over the age of 3 months should be tested. Research has shown that foals' serum IgG(T) responses to cyathostomin infection occur within 6-12 weeks of birth (Murphy and Love, 1997; Matthews, Austin *et al.*, publication in preparation) and, after this time, the maternal antibodies derived from colostrum have diminished.

Younger animals are more susceptible to higher cyathostomin burdens and horses up to the age of 3 years-old need to be carefully monitored.

Previous FEC results

Together with an assessment of environmental conditions, faecal egg count (FEC) results from within the last 6 months should be considered before conducting a test.

Horses with previous FEC results >200 eggs/g are showing evidence of a significantly active cyathostomin life cycle and should therefore receive a routine moxidectin treatment.

All horses over 3 months of age, considered to be at low or moderate risk with recent FEC results of <200 eggs/g can be tested prior to considering moxidectin use.

Determining environmental conditions

Environmental conditions for each horse should be assessed to determine whether the horse/herd has a high likelihood of exposure/infection. If the risk is high (based on environmental factors and FEC history – see above), then a routine moxidectin treatment is more appropriate than testing.

Horses new to a herd in quarantine

It is recommended that horses should be routinely treated with moxidectin during quarantine, especially if the horse was previously unknown to the veterinarian. Reliable and complete history of a new horse should be assessed before considering submitting a sample for testing due to the importance of risk assessment in interpreting the results. A tapeworm serum or saliva antibody test should be conducted to determine whether moxidectin-only or moxidectin/praziquantel combination products should be administered.

2 RESULTS & DECISION TO TREAT

Results are reported as 'Serum Scores' which are relative concentrations of specific IgG(T) derived from ELISA absorbance and the use of ELISA calibration curves.

Serum scores are reported together with statistically derived probabilities (using logistic regression models) that a horse is infected with a cyathostomin burden greater than a given threshold. The veterinarian will be provided with the Serum Score for each horse, together with a set of probabilities for the level of burden. Examples can be seen in *Table 3*.

Table 3. Examples of Serum Scores with associated probabilities of infection above given TWB thresholds

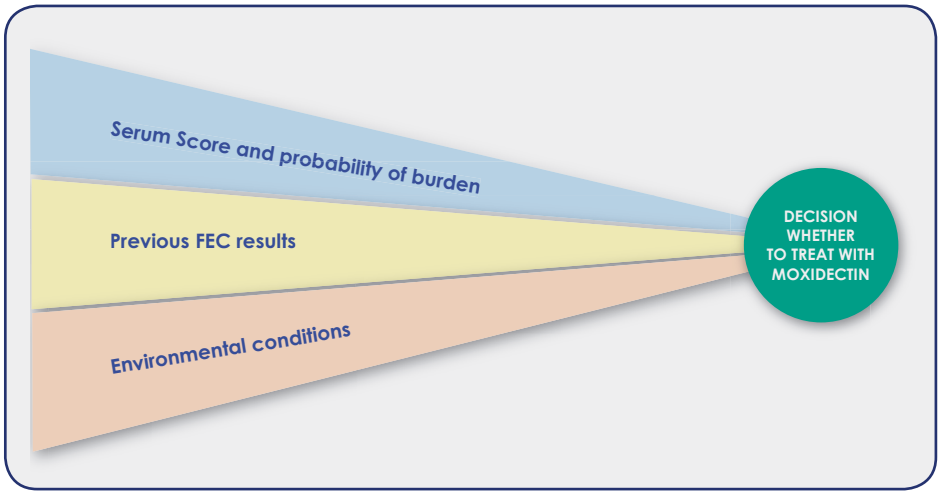
Example Serum Score	Probability of >1,000 TWB	Probability of >5,000 TWB	Probability of >10,000 TWB
0.6	11%	11%	7%
5.2	16%	14%	10%
15.0	33%	25%	18%
22.1	51%	36%	27%
31.6	73%	53%	41%
69.9	99%	95%	91%

AS A ROUGH GUIDE, ANY HORSE HAVING A PROBABILITY OF MORE THAN 25% SHOULD BE CONSIDERED FOR MOXIDECTIN TREATMENT.

Probabilities that TWB levels exceed 1000, 5000 or 10,000 were calculated from three separate logistic regression models developed by categorising positives having TWB>1000, 5000 and 10,000 respectively. Probabilities were calculated from the logit value generated by each model at the selected Serum Scores shown in the first column.

A veterinarian should assess the reported Serum Score and probability of infection together with environmental conditions and previous FEC results for each horse/herd to determine whether moxidectin treatment should be administered or not *(Figure 3)*.

Figure 3. Diagram illustrating evaluations required for decision whether to treat



3 NEXT STEPS: Consider other parasites

After decision NOT to treat with moxidectin

Tapeworm infections should be diagnosed using serum or saliva antibody tests and treated with praziquantel or double dose pyrantel if necessary.

Where large strongyles or bot infestation is suspected, ivermectin may be administered in place of moxidectin.

After decision TO treat with moxidectin

A tapeworm serum or saliva antibody test should be conducted to determine whether moxidectin-only or moxidectin/praziquantel combination products should be administered.

4 NEXT STEPS: When to retest

Low risk horses:

Carry out the small redworm blood test annually. FEC testing **must** be carried out throughout March to October for indication of exposure risk/infection and to inform the need to treat to reduce egg shedding. See Table 2 for an example of risk assessments.

Moderate risk horses:

If the horse has previously had positive FEC results and exposure is considered to be significant (such as a high percentage of horses in the herd with confirmed infection) then conduct blood testing every 6 months, otherwise test annually. FEC testing **must** be carried out routinely throughout March to October for indication of exposure risk/infection and to inform the need to treat to reduce egg shedding.

ADDITIONAL INFORMATION

Serum antibody half-life following moxidectin treatment

Potential residual IgG(T) from past infection could have confounding effects on the accuracy of the assay and this needs to be taken into account in applying the test in practice. If the test is used as directed (once or twice a year for low or moderate risk horses and not within 4 months of the last moxidectin treatment), antigen-specific IgG(T) half-lives are unlikely to affect diagnosis. The test result must be interpreted alongside the clinical and treatment history of the individual or population under assessment.

Research into antigen specific IgG(T) half-life is ongoing and guidelines will be updated as data becomes available.

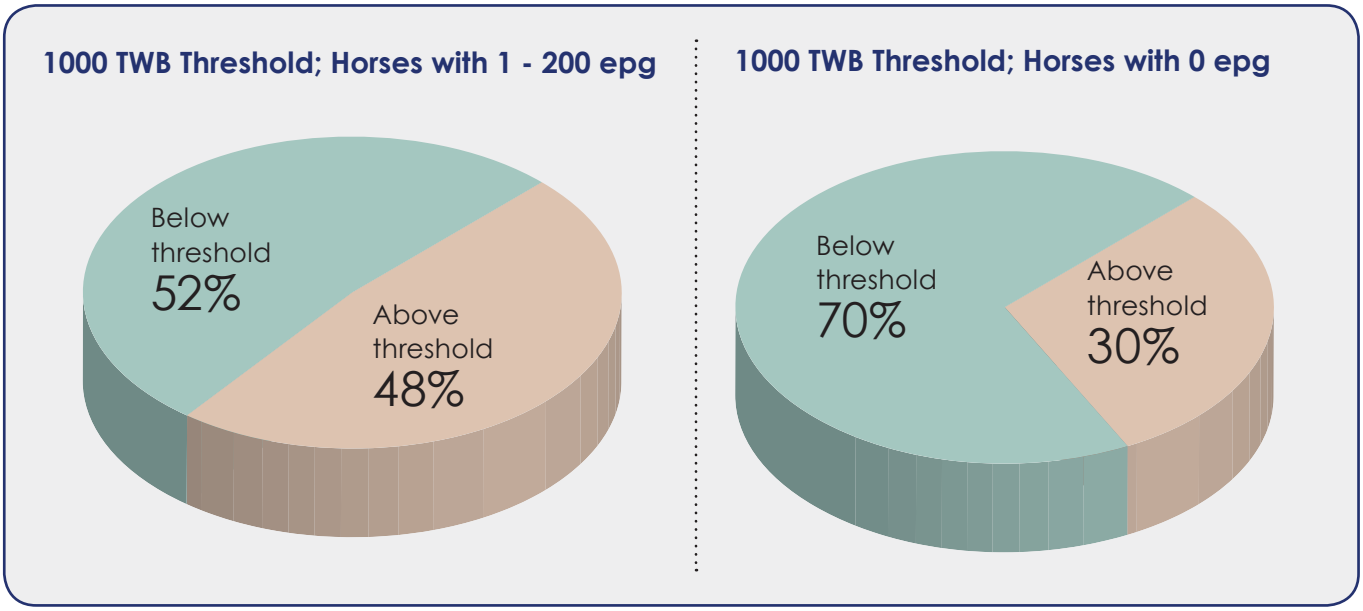
Factors to consider when testing horses with clinical symptoms of cyathostominosis

In cases where there is severe hypoproteinaemia, antigen-specific IgG(T) levels may be low; this should be taken into account when interpreting the results of the test alongside other clinico-pathological parameters. It may be worthwhile determining total plasma protein concentration on horses with clinical symptoms of cyathostominosis to rule out false negatives occurring due to low concentrations of IgG(T) present in the sample.

PREVALENCE OF HORSES ABOVE 1000 TWB THRESHOLD IN THE SMALL REDWORM BLOOD TEST

Initial assessment of cyathostomin prevalence in horses with previous recent FEC data (n=502; Figure 4) has revealed 48% of horses with <200 epg were above the 1000 TWB threshold (Serum Score cut-off 14.37), whereas just 30% of horses with 0 epg were above threshold (Matthews, Austin *et al.*, publication in preparation). The proportion of horses receiving moxidectin will be dependent on veterinary assessment of the horse's probability of infection above given TWB thresholds, together with FEC history and environmental conditions.

Figure 4. Percentage horses above and below 1000 TWB threshold with known FEC results (when 1000 TWB threshold is applied, the test is categorising samples above or below 1000 worms).



HOW TO USE THE SMALL REDWORM BLOOD TEST SERVICE AT AUSTIN DAVIS BIOLOGICS

1. Register veterinary practice with Austin Davis Biologics by emailing **info@austindavis.co.uk**

2. Unique barcoded sample submission sheet pdf's will be sent to each veterinary practice

3. Fully read these guidelines to determine which horses are suitable for testing and how to interpret results

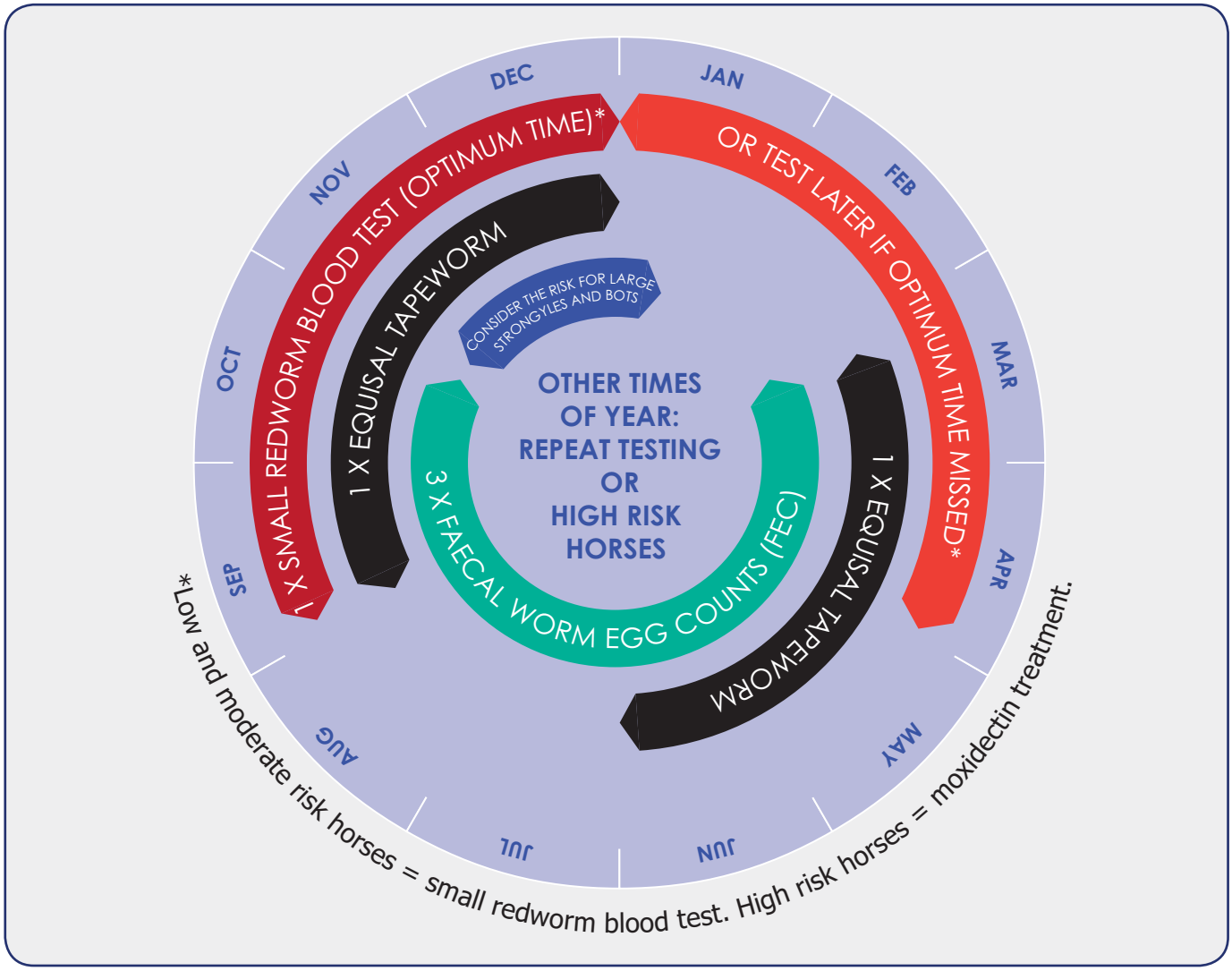
4. Collect blood sample in a red top tube. Submit either the red top tube, or at least 0.5 ml of serum in a 1.5 ml microfuge tube, with a submission sheet per sample. Discounts are available if processed serum samples are submitted.

5. Samples will be tested within 3 working days of receiving the sample into the laboratory and results emailed on the day of testing

DIAGNOSTIC-LED WORM CONTROL PROGRAMME

Conducting testing with optimal timing is key to success with diagnostic-led worm control. *Figure 5* illustrates a suitable seasonal plan for diagnostic-led worm control programmes.

Figure 5. Recommended seasonal testing for a diagnostic-led worm control programme



Notes

These guidelines are subject to change and will be updated when additional information/data becomes available.

References

Dowdall, S.M., Matthews, J.B., Mair, T., Murphy, D., Love, S., Proudman, C.J., 2002. Antigen-specific IgG(T) responses in natural and experimental cyathostominae infection in horses. Veterinary Parasitology 106, 225-242.

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Murphy D, Love S (1997). The pathogenic effects of experimental cyathostome infections in ponies. Veterinary Parasitology. 70: 99-110.

Proudman CJ, Trees AJ (1996). Correlation of antigen specific IgG and IgG(T) responses with Anoplocephala perfoliata infection intensity in the horse. Parasite Immunol. 18:499-506.

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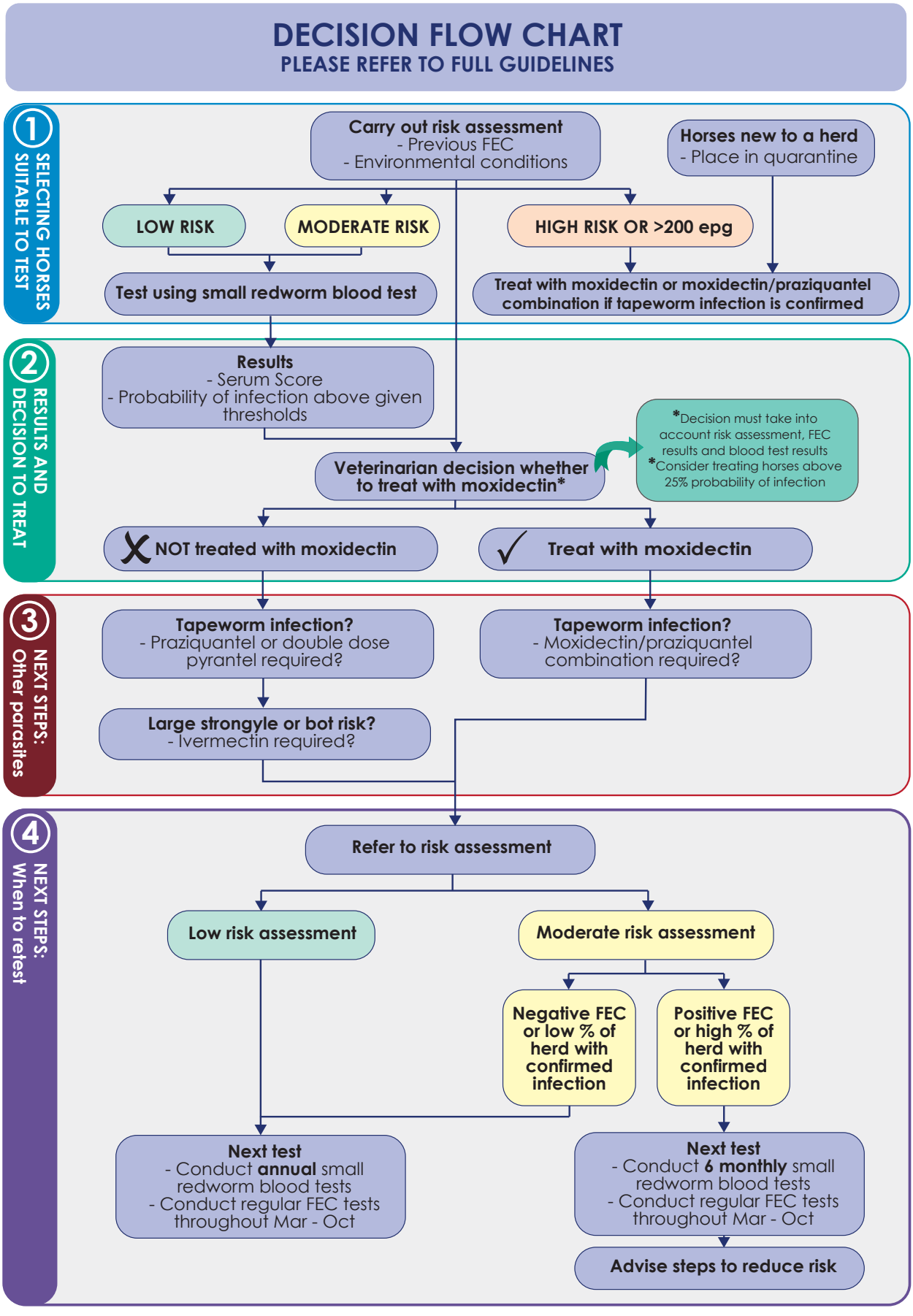
The small redworm blood test was developed in collaboration with Prof. Jacqui Matthews' group at the Moredun Research Institute. This group conducted initial research at University of Liverpool (funded by the Horse Trust), then at Moredun Research Institute (funded by the Horseracing Betting Levy Board, the Thoroughbred Breeders Association and the Horse Trust). The test is covered under patent applications and granted patents originating from patent number PCT/GB 2010/112836.

APPENDIX 1


DECISION FLOW CHART
PLEASE REFER TO FULL GUIDELINES
1 SELECTING HORSES SUITABLE TO TEST
Carry out risk assessment
- Previous FEC
- Environmental conditions
Horses new to a herd
- Place in quarantine
LOW RISK
MODERATE RISK
HIGH RISK OR >200 epg
Test using small redworm blood test
Treat with moxidectin or moxidectin/praziquantel combination if tapeworm infection is confirmed
2 RESULTS AND DECISION TO TREAT
Results
- Serum Score
- Probability of infection above given thresholds
Veterinarian decision whether to treat with moxidectin*
*Decision must take into account risk assessment, FEC results and blood test results
*Consider treating horses above 25% probability of infection
NOT treated with moxidectin
Treat with moxidectin
3 NEXT STEPS: Other parasites
Tapeworm infection?
- Praziquantel or double dose pyrantel required?
Tapeworm infection?
- Moxidectin/praziquantel combination required?
Large strongyle or bot risk?
- Ivermectin required?
4 NEXT STEPS: When to retest
Refer to risk assessment
Low risk assessment
Moderate risk assessment
Negative FEC or low % of herd with confirmed infection
Positive FEC or high % of herd with confirmed infection
Next test
- Conduct annual small redworm blood tests
- Conduct regular FEC tests throughout Mar - Oct
Next test
- Conduct 6 monthly small redworm blood tests
- Conduct regular FEC tests throughout Mar - Oct
Advise steps to reduce risk