Strongylosis in equines: biology, diagnosis and future needs

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discuss clinical signs of cyathostominosis and strongylosis in horses, as well as various techniques that can be adopted for aiding identification

Summary

Over the past two decades, infection with small strongyles (causative agent of cyathostominosis) has become increasingly recognised as a major clinical problem of horses in the UK and worldwide. The implementation of sustainable control strategies for equine cyathostome infection is dependent on a greater insight into epidemiology and pathogenicity of these organisms, together with an enhanced ability for accurate diagnosis.

Horses that have the encysted stage of cyathostomes don’t exhibit any clinical signs until spring. When this happens, it is always associated with severe clinical manifestations and even death of affected horses. The lack of a reliable diagnostic test to measure the level of cyathostomiasis infection and detect larval stages in individual horses is a major gap in the efforts to manage and treat this disease. The presence of diagnostic markers to larval cyathostominosis in horses will enable early detection of the disease; establishment of level of parasitism in an individual horse; identification of horses in the prepatent stage of infection; and early treatment before the occurrence of recrudescence.

Key words

cyathostomins, **cyathostominosis**, **horses**, **diagnosis**

CYATHOSTOMIN nematodes live as adults in the large intestine of virtually all horses. The life cycle of these parasites is direct and horses are infected following the ingestion of infective third stage larvae on contaminated pasture (Figure 1).

Cyathostomins have been reported in horses in the UK and worldwide. Fifty-one species of cyathostomins belonging to 13 genera have been recognised in equids, and approximately 10 of these species comprise the majority of the total cyathostome burden.

Cyathostomes can undergo arrested development in the large intestinal mucosa for more than two years. Occasionally, these nematodes cause larval cyathostominosis, associated with weight loss and severe, persistent diarrhoea and enteritis, which results from mass reactivation of inhibited cyathostomin larvae residing in the intestinal mucosa and the sudden release of large numbers of the larval stages of small strongyles.

The clinical signs of cyathostominosis may be seen seasonally in autumn and spring, or may be brought about by the administration of anthelmintics to remove the luminal stages of small strongyles. Drug resistance of small strongyles to all classes of available antiparasitic compounds – except the macrocyclic lactones (ivermectin and moxidectin) – is a limiting factor in controlling these parasites and may result in an increased prevalence of clinical cyathostominosis over time.

Despite enormous investments in the control of horse parasites, progress on the development of a diagnostic test has been very limited. Indeed, diagnosis of prepatent stages of cyathostomins in horses has always been difficult. Faecal worm egg count is available for determining the level of infection in individual horses. However, faecal testing can be negative, despite the presence of adult and larval stage four (L4) larvae in the faeces. A serological test based on immunoglobulin G (IgG) responses to larval antigen complexes has been reported. However, this test doesn't allow prediction of a horse with cyathostomes.

Enhancement of our current understanding of a horse's metabolic response to cyathostome infection is a promising approach for biomarker identification. However, its potential for diagnosis and parasite disease surveillance in horses has not been investigated.

Strongyle and strongylosis

The term strongyle (commonly known as redworm) refers to nematodes of the family Strongylidae. The two key sub-families are Strongylinae (large strongyles) and Cyathostominae (small strongyles, cyathostomins or small redworm). Strongylosis and cyathostomosis can affect all

equids (horses, donkeys, zebras and mules) of any breed or gender.

Infection can occur in animals of any age (Love et al, 1999), but the clinical signs are more noticeable and severe in younger and unexposed animals. The reduction in sensitivity to strongyle infection in adults suggests the development of a degree of acquired immunity (Klei and Chapman, 1999). However, the immune response can take a long time to develop and, even in cases of prolonged parasite exposure, remains incomplete (Coles et al, 2003).

Equine strongylosis is the most common parasitic infection seen in horses worldwide, having a massive impact economically and on animal welfare. The prevalence in the UK is around 80 per cent (Beugnet et al, 2006). Small strongyle burdens consist of more than 90 per cent larvae and more than 50 per cent of the larval population consists of inhibited early larval stage three (EL3; Duncan et al, 1998; Bairden et al, 2006; Beugnet et al, 2006).

Strongylus vulgaris was once considered to be the most important parasite in horses, although its significance has reduced since the introduction of treatment regimens with current anthelmintic compounds (Beugnet et al, 2006). The prevalence of strongylosis caused by *S vulgaris* is now much lower than cyathostomosis. For the same reasons there has also been a reduction in prevalence of other large strongyles (*S edentatus* and *S equinus*), with large strongyle eggs now representing a small proportion of total strongyle eggs found in faecal analysis.

Diagnosis

Clinical signs

Clinical signs of strongylosis are variable between individuals and are non-specific to this disease. However, these signs can be used to formulate a strong suspicion of strongylosis when combined with knowledge of the horse's recent history and management routine. Further tests are required for definitive diagnosis.

Traditional methods of identification

Basic identification of parasitic elements in faeces include the following:

• Faecal smears are the basic method used for morphological identification of eggs (Figure 2) and larvae. They are not recommended for analysis of strongyle burdens (Zajac and Conboy, 2006). False negatives occur frequently as eggs are easily missed among faecal debris, while adults and larvae are usually only seen with very high burdens.

• Faecal cultures allow time for eggs and larvae in faeces to develop to an appropriate stage for identification (usually L3; Figure 3). These can be harvested for microscopic examination using the Baermann technique.

• Floatation techniques are based on the principle that all parasitic elements less dense than the floatation solution (FS) used will float to the surface of the liquid, concentrating them here for analysis. They are the most commonly used methods for faecal examination (Zajac and Conboy, 2006). Selection of an appropriate FS is an important aspect of floatation technique as it can affect the method's sensitivity and reliability of results (Broussard, 2003; Cringoli et al, 2004).

Physical properties of the solution, including viscosity, have a role in its efficacy as an FS, but the key factor is its specific gravity (SG). As the density of parasitic elements to be floated increases, the SG of FS must also be higher. However, high SG solutions carry a greater risk of cell distortion due to osmosis and of an obscured view of parasitic elements due to more debris also floating. Strongyle eggs float well in solutions with SG 1.10 to 1.20, so saturated sodium chloride solution (SG 1.20) is commonly used.

• Faecal worm egg counts. Floatation is frequently combined with faecal egg count (FEC) techniques to allow quantifiable analysis of parasitic burdens. These methods are commonly used to assess burdens for strategic anthelmintic administration. Efficacy of the anthelmintic regime and parasite resistance can also be monitored using a faecal egg count reduction test (FECRT). This involves performing an FEC before treatment and two weeks later to assess if the worm burden has been affected and to what extent. Sensitivity of an FEC technique can be improved via selection of an appropriate FS and utilisation of centrifugation steps (Vadlejch et al, 2011). Centrifugation also reduces the floatation time (the time it takes for eggs to float to the surface of the solution after introduction to the slide), making the technique less time consuming (Broussard, 2003).

The key to a more reliable FEC technique is to reduce the amount of extrapolation required when calculating the number of eggs per gram of faeces (EPG). Alterations that have this effect include increasing the volume of the slide/sample volume used and using an appropriate ratio of faeces to solution (Cringoli et al, 2004). Studies show the ideal ratio is between 1:10 and 1:30 (Dunn and Keymer, 1986; Cringoli et al, 2004; Vadlejch et al, 2011). If the concentration of faeces is lower the results are less reliable due to increased dilution. If it is too high, excessive faecal debris may trap eggs and make sample visualisation more difficult (Dunn and Keymer, 1986).

The McMaster FEC technique is commonly used in practice. The technique involves mixing a measured amount of faeces with a known volume of FS before filtering the mixture to remove debris. The filtrate is homogenised by stirring before a small amount is taken to fill the chambers of the McMaster slide with a Pasteur pipette. The slide is left for a set period of time to allow eggs to float up, before being examined microscopically to count the eggs present. A simple calculation is then applied to determine EPG.

Egwang and Slocombe (1982) devised the Wisconsin floatation FEC technique. A set amount of faeces is combined with water. The mixture is strained and centrifuged. The supernatant is removed and the resultant pellet is re-suspended in Sheather's sugar solution (an FS). This is

centrifuged with a coverslip on top of the liquid. Parasitic elements float up and attach to the coverslip, which can then be removed for microscopic examination.

The Wisconsin technique is commonly used for cattle FEC. It is more sensitive than the McMaster technique so is good for detecting low numbers of EPG (Bello and Allen, 2009) and produces fewer false negatives. However, the technique is unsuitable for high EPG counts as the lack of a counting grid makes it easier to miss some eggs or count them twice.

Mes et al (2001) developed a semi-automated sugar-salt isolation to reduce the time taken to analyse a sample by using a computer to determine the egg count. This increases throughput. Reassessment of samples at a later time is also possible as images of samples can be stored on the computer. Additional floatation and concentration steps increase technique sensitivity and reduce the amount of extrapolation required to obtain the EPG results.

The salt-sugar isolation method produces less variation between results than the McMaster technique. This means studies using this method can have a smaller number of samples for statistical differences between groups to be noticed, as small variations are less likely to be missed (Mes, 2003).

FLOTAC is a centrifugal floatation technique designed to detect all parasitic elements (eggs, cysts and larvae) simultaneously (Cringoli, 2006). Most techniques only assess the presence of one type of parasitic element at a time. FLOTAC results are likely to be more reliable than those obtained from other FEC techniques as a much lower multiplication factor is used to calculate EPG (Bauer et al, 2010). Despite the pros of this technique, the product is not available commercially. It is unlikely this technique, without adaptations, will become commonly used in practice as a certain level of laboratory infrastructure (such as a particular type of centrifuge) is required (Cringoli et al, 2010).

Even with the advancements mentioned previously, there are factors caused by parasite biology and life cycles that limit the reliability of these FEC techniques. These include the following:

• They do not measure the actual parasitic burden – EPG figures only correlate with the number of adults likely to be present in the host.

• Eggs are only produced by mature adult females, so an FEC does not allow identification of larval burdens or male worms.

• Recent anthelmintic treatment can reduce EPG.

• Egg production may be suppressed by host immunity. This means older animals may have a low egg count, even if they have the same level of infection as a younger animal that has not yet developed a level of resistance.

• Some nematode species only produce eggs periodically, so eggs might not always be present in faeces dependent on stage in cycle.

• No eggs will be present if the initial infection is within the pre-patent period (PPP).

Molecular diagnosis

Antibody (Ab) detection in blood samples is commonly used to assess tapeworm (*Anoplocephala perfoliata*) burden in horses using an ELISA. Although this technique could be adapted to assess strongyle burden, there are some limitations of the technique that reduce its value as a diagnostic technique. Accurate differentiation between low infection and no infection is not possible. False positives can be obtained as antibodies may circulate in the blood for three to six months postinfection. Mast cells and their proteinases are known to have a role in the mammalian tissue inflammatory response.

The intestinal immune response is poorly understood, but an increase in mast cell count and proteinase expression is noticed with cyathostomin infection (Stratford et al, 2011). A technique able to detect mast cell proteinases has been developed by du Toit et al (2007). Work is still in progress to see if mast cells have a specific, detectable role in the equine immune response to cyathostominosis.

Dowdall et al (2002) identified two antigen (Ag) complexes only present serum after cyathostomin infection. The antilarval Ab IgG(T) was found in samples at five and seven weeks postinfection using ELISA and western blotting techniques, respectively. However, high levels of non-specific binding to other Ag components were observed. A further study by Dowdall et al (2003) enhanced the technique to reduce levels of cross-reactivity. The technique was found to be valuable for future research and diagnostics in a study by Dowdall et al (2004) evaluating the accuracy of results in both experimentally and naturally infected horses.

PCR-based techniques allow amplification of tiny amounts of DNA fragments to be detected in a sample (tissue, blood, urine or faeces). These techniques are both highly specific and sensitive.

One of the most promising techniques for strongyle diagnosis involves the identification of internal transcriber space (ITS) and intergenic spacer (IGS) regions in nuclear ribosomal (r)DNA (Hodgkinson, 2006). rDNA is a repetitive element in the parasite’s genome. When combined with a PCR component, to amplify the target region, the technique is both highly specific and sensitive.

Campbell et al (1995) assessed the extent of DNA sequence variation in the ITS-two region and found low intraspecific variation (less than 0.9 per cent), but a higher level of interspecific variation was present (13 per cent to 29 per cent).

A PCR-linked restriction fragment length polymorphism (RFLP)-based technique was developed to

identify the DNA sequences and enable identification of the worms and eggs of *S vulgaris*, *S edentatus* and *S equinus*. Identification of more species of Strongylinae and Cyathostominae using the PCR-RFLP technique to assess both ITS-one and two regions was achieved by Gasser et al (1996).

Gasser and Monti (1997) suggested the use of PCR-linked single strand conformation polymorphism (PCR-SSCP) to improve the specificity of the technique to a level that allowed detection of different strains of parasite in a species.

Research into the ITS region has progressed to a level that demonstrates its potential in the use of diagnosing strongyle burdens – particularly large strongyles.

Specific oligonucleotide primers have been developed based on differences in ITS-one and two regions of different species (Hung et al, 1999). These can be used as part of a PCR-based technique to allow species-specific amplification of rDNA obtained from faecal samples.

Amplification and identification of the IGS regions of 16 cyathostomin species was achieved by Kaye et al (1998). This information was used to develop short oligonucleotide probes for detection of the relevant species (Hodgkinson et al, 2001). Southern blotting was used to test the ability of the probes to identify species specifically. Although results were promising, southern blotting is very time-consuming and expensive. Therefore, Hodgkinson et al (2003) developed and optimised an IGS PCR-ELISA, which increases the potential throughput of the technique. The study data also suggested mixed cyathostomin burdens are responsible for causing clinical disease, rather than one specific species. Further work is required in this area to identify more species and develop a quantitative technique.

Quantification of individual species is not yet possible as there is inconsistent repetition of the rDNA sequence between and in species (Hodgkinson et al, 2001). Nielsen et al (2008) developed a semi-quantitative fluorescence-based PCR assay that allows better assessment of burden level with high specificity.

Haematological parameters

Evidence indicates larval cyathostominosis in horses induces biochemical and haematological changes in affected horses. Growth of all parasitic nematodes, including cyathostome, requires energy and macromolecular precursors derived from the metabolic network of the host animal. This means during infection and development of the cyathostome the horse’s body undergoes certain metabolic changes. This indicates larval cyathostominosis can alter the horse’s metabolic profile and this can be associated with specific metabolic biomarkers.

Most infected animals have neutrophilia and hypoalbuminaemia. Less consistent findings include anaemia, eosinophilia, and increased serum alkaline phosphatase and ?-globulin concentrations

(Love et al, 1999).

Smets et al (1999) found the reduced albumin to globulin ratio (less than 0.7) is a more significant, and, perhaps more reliable, alteration to be attributed with cyathostominosis. However, most of these findings are not specific to cyathostominosis or strongylosis, so are not diagnostic on their own. They require the validation of the presence of clinical signs or positive results from other diagnostic techniques.

Serum protein electrophoresis is a technique that can be used to identify and quantify proteins in serum from blood. Using this method, proteins are analysed in groups dependent on their electrophoretic zones. Although this could mask a change in an individual protein level, it is unlikely only one protein level would alter in response to parasitism, so any significant alterations should be noticed. Abbott et al (2007) did not detect significant changes in serum protein levels between infected animals and those that received treatment, so further work in this area is required.

Lipid analysis

Limitations of the diagnostic techniques used in practice mean improvement in this area is essential. The use of molecular techniques is at the forefront of advancements in strongyle identification. However, other areas with the potential for strongyle diagnosis should not be ruled out. Lipidomic is one of these areas.

Lipid metabolites are known to have many key roles in the mammalian body. Lipids have many functions in mammalian tissues. They have important structural and functional roles in biological membranes. They can also be involved in intracellular signaling, control of cell growth, platelet activation and as toxin receptors (Boisseau et al, 2009).

Lipids also have key roles at various stages in host-parasite interactions, including initial recognition and invasion of host cells, replication, persistence and immunomodulation. However, changes in the host’s lipid profile in response to strongyle infection are not known. Advancements in lipidomic analysis have provided molecular information enabling improved understanding of the importance of lipids in host-parasite interactions. Identifying specific roles of different lipid compounds will allow the development of more effective, and species or stage-specific, anthelmintic treatment (Wenk, 2006).

The presence of statistical differences in certain lipid molecules in sera from infected versus uninfected horses (Lamb et al, 2012) validated the potential of lipidomics in the development of diagnostic techniques for strongyle infections in horses.

Conclusions

The effects of strongylosis and cyathostominosis can be devastating. To improve the welfare of

equids it is important to prevent the onset of clinical disease. This can be achieved through reliable and quantifiable diagnosis of strongyle infections, which, in turn, allows the development of specific and targeted treatment, and prevention regimes. The selection pressure for development of anthelmintic resistance could also be reduced through the improved diagnosis of strongyle burdens.

The classical faecal examination techniques will remain the cornerstone of equine parasitology diagnosis and surveillance. However, more sensitive and specific techniques capable of diagnosing prepatent stages of strongyle infection would allow targeted and species-specific treatment, and prevent the unnecessary use of anthelmintic compounds in horses with low or no infection.

References

- Abbott J B, Mellor D J and Love S (2007). Assessment of serum protein electrophoresis for monitoring therapy of naturally acquired equine cyathostomin infections, *Veterinary Parasitology* **147**(1-2): 110-117.
- Bairden K, Davies H S, Gibson N R, Hood A J and Parker L D (2006). Efficacy of moxidectin two per cent oral gel against cyathostomins, particularly third-stage inhibited larvae, in horses, *Veterinary Record* **158**(22): 766-767.
- Bauer B U, Pomroy W E, Gueydon J, Gannac S, Scott I and Pfister K (2010). Comparison of the FLOTAC technique with the McMaster method and the Baermann technique to determine counts of *Dictyocaulus eckerti* L1 and strongylid eggs in faeces of red deer (*Cervus elaphus*), *Parasitology Research* 107(3): 555-560.
- Bello T R and Allen T M (2009). Comparison of two faecal egg recovery techniques and larval culture for cyathostomins in horses, *American Journal of Veterinary Research* **70**(5): 571-573.
- Beugnet F, Fayet G, Guillot J, Grange E, Desjardins I and Dang H (2006). *Guide to Clinical Parasitology in Horses*, Kalianxis.
- Boisseau P, Houdy P and Lahmani M (2009). *Nanoscience: Nanobiotechnology and Nanobiology*, Springer.
- Broussard J D (2003). Optimal faecal assessment, *Clinical Techniques in Small Animal Practice* **18**(4): 218-230.
- Campbell A J, Gasser R B and Chilton N B (1995). Differences in a ribosomal DNA sequence of *Strongylus* species allows identification of single eggs, *International Journal for Parasitology* **25**(3): 359-365.
- Coles G C, Eysker M, Hodgkinson J, Matthews J B, Kaplan R M, Klei T R and Sangster N C (2003). Anthelmintic resistance and use of anthelmintics in horses, *Veterinary Record* **153**(20): 636.
- Cringoli G (2006). FLOTAC, a novel apparatus for a multivalent faecal egg count technique, *Parassitologia* **48**(3): 381-384.
- Cringoli G, Rinaldi L, Maurelli M P and Utzinger J (2010). FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in

animals and humans, *Nature Protocols* **5**(3): 503-515.
- Cringoli G, Rinaldi L, Veneziano V, Capelli G and Scala A (2004). The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep, *Veterinary Parasitology* **123**(1-2): 121-131.
- Dowdall S M, Matthews J B, Mair T, Murphy D, Love S and Proudman C J (2002). Antigen-specific IgG(T) responses in natural and experimental cyathostominae infection in horses, *Veterinary Parasitology* **106**(3): 225-242.
- Dowdall S M, Proudman C J, Love S, Klei T R and Matthews J B (2003). Purification and analyses of the specificity of two putative diagnostic antigens for larval cyathostomin infection in horses, *Research in Veterinary Science* **75**(3): 223-229.
- Dowdall S M, Proudman C J, Klei T R, Mair T and Matthews J B (2004). Characterisation of IgG(T) serum antibody responses to two larval antigen complexes in horses naturally or experimentally infected with cyathostomins, *International Journal for Parasitology* **34**(1): 101-108.
- du Toit N, McGorum B C, Pemberton A D, Brown J and Dacre K J (2007). The involvement of mast cells and mast cell proteinases in the intestinal response to equine cyathostomin infection, *Veterinary Immunology and Immunopathology* **115**(1-2): 35-42.
- Duncan J L, Bairden K and Abbott E M (1998). Elimination of mucosal cyathostome larvae by five daily treatments with fenbendazole, *Veterinary Record* **142**(11): 268-271.
- Dunn A and Keymer A (1986). Factors affecting the reliability of the McMaster technique, *Journal of Helminthology* **60**(4): 260-262.
- Egwang T G and Slocombe J O (1982). Evaluation of the Cornell-Wisconsin centrifugal flotation technique for recovering trichostrongylid eggs from bovine faeces, *Canadian Journal of Comparative Medicine* **46**(2), 133-137.
- Gasser R B and Monti J R (1997). Identification of parasitic nematodes by PCR-SSCP of ITS-two rDNA, *Molecular and Cellular Probes* **11**(3): 201-209.
- Gasser R B, Stevenson L A, Chilton N B, Nansen P, Bucknell D G and Beveridge I (1996). Species markers for equine strongyles detected in intergenic rDNA by PCR-RFLP, *Molecular and Cellular Probes* **10**(5): 371-378.
- Hodgkinson J E (2006). Molecular diagnosis and equine parasitology, *Veterinary Parasitology* **136**(2): 109-116.
- Hodgkinson J E, Lichtenfels J R, Mair T S, Cripps P, Freeman K L, Ramsey Y H, Love S and Matthews J B (2003). A PCR-ELISA for the identification of cyathostomin fourth-stage larvae from clinical cases of larval cyathostominosis, *International Journal for Parasitology* **33**(12): 1,427-1,435.
- Hodgkinson J E, Love S, Lichtenfels J R, Palfreman S, Ramsey Y H and Matthews J B (2001). Evaluation of the specificity of five oligoprobes for identification of cyathostomin species from horses, *International Journal for Parasitology* **31**(2): 197-204.
- Hung G C, Gasser R B, Beveridge I and Chilton N B (1999). Species-specific amplification by PCR of ribosomal DNA from some equine strongyles. *Parasitology*, **119**: 69-80.
- Kaye J N, Love S, Lichtenfels J R and McKeand J B (1998). Comparative sequence

analysis of the intergenic spacer region of cyathostome species, *International Journal for Parasitology* **28**(5): 831-836.
- Klei T R and Chapman M R (1999). Immunity in equine cyathostome infections, *Veterinary Parasitology* **85**(2-3): 123-133.
- Lamb J, Hallowell G D, Harris P, Zhu X Q and Elsheikha H M (2012). Lipidomic analysis of serum from horses with strongyle infection, *Journal of Veterinary Science Technology* **3**(6): 122.
- Love S, Murphy D and Mellor D (1999). Pathogenicity of cyathostome infection, *Veterinary Parasitology* **85**(2-3): 113-121.
- Mes T H (2003). Technical variability and required sample size of helminth egg isolation procedures, *Veterinary Parasitology* **115**(4): 311-320.
- Mes T H, Ploeger H W, Terlou M, Kooyman F N, Van der Ploeg M P and Eysker M (2001). A novel method for the isolation of gastrointestinal nematode eggs that allows automated analysis of digital images of egg preparations and high throughput screening, *Parasitology* **123**(3): 309-314.
- Nielsen M K, Peterson D S, Monrad J Thamsborg S M, Olsen S N and Kaplan R M (2008). Detection and semi-quantification of *Strongylus vulgaris* DNA in equine faeces by real-time quantitative PCR, *International Journal for Parasitology* **38**(3-4), 443-453.
- Smets K, Shaw D J, Deprez P and Vercruysse J (1999). Diagnosis of larval cyathostominosis in horses in Belgium, *Veterinary Record* **144**(24): 665-668.
- Stratford C H, McGorum B C, Pickles K J and Matthews J B (2011). An update on cyathostomins: anthelmintic resistance and diagnostic tools, *Equine Veterinary Journal (Suppl)***39**: 133-139.
- Vadlejch J, Petrtýl M, Zaichenko I, ?adková Z, Jankovská I, Langrová I and Moravec M (2011). Which McMaster egg counting technique is the most reliable? *Parasitology Research* **109**(5): 1,387-1,394.
- Wenk M R (2006). Lipidomics of host–pathogen interactions, *FEBS Lett* **580**(23): 5,541-5,551.
- Zajac A M and Conboy G A (2006). *Veterinary Clinical Parasitology* (7th edn), Blackwell Publishing, Ames, Iowa.

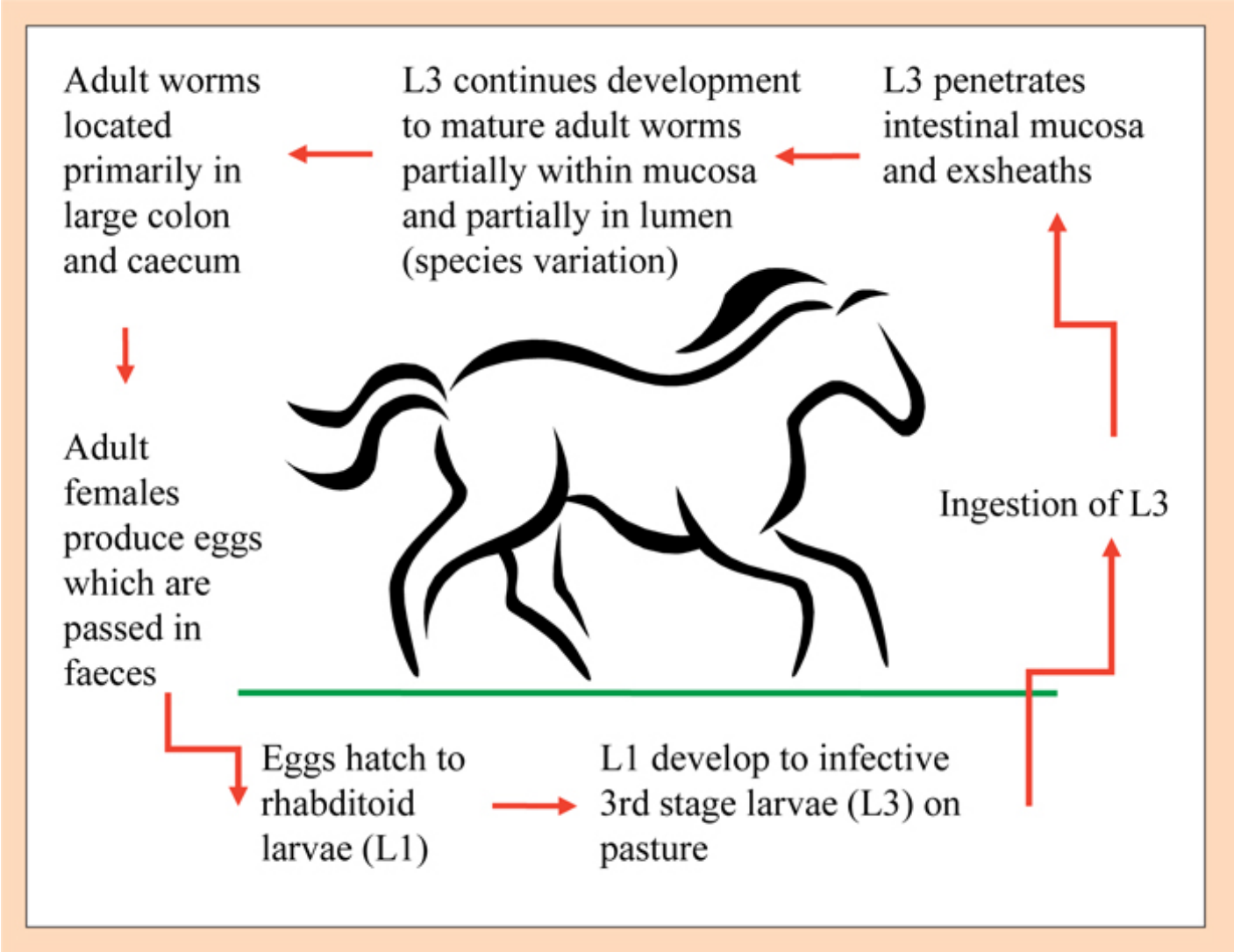


Figure 1. Basic strongyle life cycle in equines.



Figure 2. Strongyle egg detected in faeces of a horse. Scale bar = 50?m.

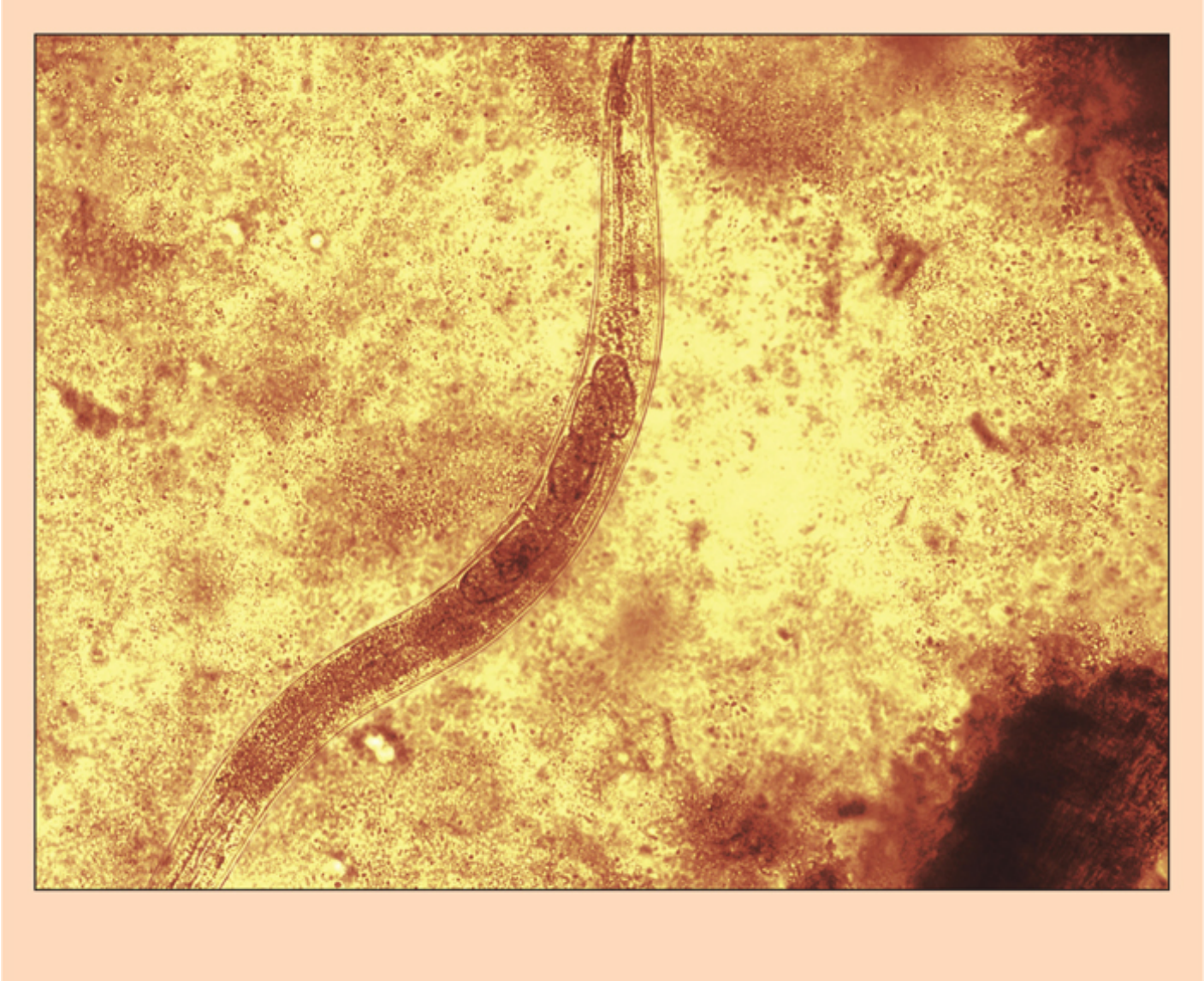


Figure 3. Strongyle larvae recovered via Baermann technique from equine faeces following 20 days of faecal culturing in vitro.