# Title: Prevalence and associated risk factors of trematode infections in equids from selected practices in Gauteng, South Africa

**by Summer Maitland-Stuart**

**Supervisor: Adrienne Viljoen**
**Co-supervisor: Ernst Volker Schwan**

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# DECLARATION OF ORIGINALITY

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Declaration:

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# ETHICS APPROVAL

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Faculty of Veterinary Science

Research Ethics Committee 01 October 2020

**CONDITIONALLY APPROVAL**

| | |
|---|---|
| **Ethics Reference No** | **REC116-20** |
| **Protocol Title** | **Prevalence of patent gastrodiscosis in equids presenting to an equine referral hospital and surrounding practices** |
| **Principal Investigator** | **Dr S Maitland-Stuart** |
| **Supervisors** | **Dr EV Schwan** |

**Dear Dr S Maitland-Stuart,**

We are pleased to inform you that your submission has been conditionally approved by the Faculty of Veterinary Sciences Research Ethics committee, subject to other relevant approvals.

Please note the following about your ethics approval:

1. Please use your reference number (REC116-20) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application for post graduate studies (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. **Applications using Animals:** FVS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

NOTE: Conditionally approved (pending obtaining other relevant approvals).

We wish you the best with your research.

Yours sincerely

**PROF M. OOSTHUIZEN**
**Chairperson: Research Ethics Committee**

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

**4 November 2020**

**Approval Certificate**
**New Application**

**AEC Reference No.:** REC116-20
**Title:** Prevalence of patent gastrodiscosis in equids presenting to an equine referral hospital and surrounding practices

**Researcher:** Dr S Maitland-Stuart

**Student's Supervisor:** Dr A Viljoen

Dear Dr S Maitland-Stuart,

The **New Application** as supported by documents received between 2020-09-03 and 2020-10-30 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-10-30.

Please note the following about your ethics approval:

1. The use of species is approved:

| **Species** | **Number** |
|---|---|
| Horses (Private clients) | 206 |
| **Samples**<br>Faeces (passive collection) | 206 |

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-11-04.
3. Please remember to use your protocol number (REC116-20) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. As part of your approval, the committee requires that you record a **short video footage** of major animal procedures approved in your study. The committee may request them for monitoring purposes at any later point.

**Ethics approval is subject to the following:**

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

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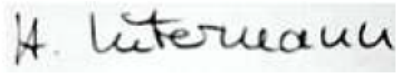
We wish you the best with your research.
Yours sincerely



**Dr. Heike Lutermann**
**DEPUTY CHAIRMAN: UP-Animal Ethics Committee**

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UNIVERSITY OF PRETORIA
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Faculty of Veterinary Science

**Research Ethics Committee** 9 February 2023

**APPROVAL CERTIFICATE**

| | |
|---|---|
| **Ethics Reference No** | **REC116-20** |
| **Protocol Title** | **Prevalence of patent gastrodiscosis in equids presenting to an equine referral hospital and surrounding practices** |
| **Principal Investigator** | **Dr S Maitland-Stuart** |
| **Supervisors** | **Dr EV Schwan** |

**Dear Dr S Maitland-Stuart,**

We are pleased to inform you that your submission has been conditionally approved by the Faculty of Veterinary Sciences Research Ethics committee, subject to other relevant approvals.

Please note the following about your ethics approval:

1. Please use your reference number (REC116-20) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application for post graduate studies (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. **Applications using Animals:** FVS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

We wish you the best with your research.

Yours sincerely

**PROF M. OOSTHUIZEN**
**Chairperson: Research Ethics Committee**

UNIVERSITEIT VAN PRETORIA
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YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

8 November 2021

## Approval Certificate
**Annual Renewal**
**(EXT1)**

| | |
|---|---|
| **AEC Reference No.:** | REC116-20 |
| **Title:** | Prevalence of patent gastrodiscosis in equids presenting to an equine referral hospital and surrounding practices |
| **Researcher:** | Dr S Maitland-Stuart |
| **Student's Supervisor:** | Dr A Viljoen |

Dear Dr S Maitland-Stuart,

The **Annual Renewal** as supported by documents received between 2021-09-20 and 2021-10-25 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2021-10-25.

Please note the following about your ethics approval:

1. The use of species is approved:

| **Species** | **Number** |
|---|---|
| Horses (Private clients) | 206 |
| **Samples** | |
| Faeces (passive collection) | 206 |

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2022-11-08.

3. Please remember to use your protocol number (REC116-20) on any documents or correspondence with the AEC regarding your research.

4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

5. **All Incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.

6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

**Ethics approval is subject to the following:**

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

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We wish you the best with your research.

Yours sincerely

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# ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| CI | Confidence interval |
| BP | Boerperd |
| ELISA | Enzyme-linked immunosorbent assay |
| TB | Thoroughbred |
| OVAH | Onderstepoort Veterinary Academic Hospital |
| WB | Warmblood |

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# ABSTRACT

**Prevalence and associated risk factors of trematode infections in equids from selected practices in Gauteng, South Africa**

| | |
|---|---|
| Supervisor: | Dr A. Viljoen |
| Co-supervisor: | Dr E.V. Schwan |
| Department: | Companion Animal Clinical Studies |
| Degree: | MSc (Companion Animal Clinical Studies) |

**Reasons for performing study:** Prevalence of infection with *Gastrodiscus aegyptiacus* and *Fasciola hepatica* has not been established in equids presenting with the relevant clinical signs. Various environmental and management practices with regards to equid husbandry are hypothesised to place equids at greater risk of infection, but no link has been established. There are currently two testing techniques for patent gastrodiscosis in equids, however the difference in accuracy between the tests has not yet been established.

**Objectives:** To determine the prevalence of patent gastrodiscosis and fasciolosis in equids presenting with clinical signs of infection, to determine the difference in sensitivity and specificity between the two currently available testing techniques and to establish which management and environmental factors place equids at a greater risk of infection.

**Study design:** Analytical cross-sectional study design.

**Methods:** Samples were obtained from equids presenting to the Equine Clinic of the Onderstepoort Veterinary Academic Hospital, as well as three equine private practices in the Gauteng region (Fourways Equine Clinic, Glen Austin Equine Clinic and Pierre van Ryneveld Large Animal Clinic) and these samples were categorised according to their reason for seeking veterinary care, namely:

1) equids presenting with a gastrointestinal complaint, namely chronic weight loss and/or diarrhoea and/or recurrent colic,

2) equids presenting for elective procedures and/or non-gastrointestinal reasons

Two fresh faecal balls were collected from each equid and each sample underwent testing using both the Sedimentation after Benedek and the Visser-filtration techniques. A questionnaire was completed by the primary caretaker for each sampled equid, with questions relating to the equids management and environment in which they are kept.

**Results:** The overall prevalence of infection with *G. aegyptiacus* was found to be 11.6%. The prevalence of horses which tested positive and showed clinical signs of gastrointestinal dysfunction was 8.9% whereas the prevalence of horses which tested positive and were healthy at the time of testing was 15.5%. The overall prevalence of infection with *F. hepatica* was found to be 4.3%. The prevalence of horses which tested positive and showed clinical signs of gastrointestinal dysfunction was 4.9% whereas the prevalence of horses which tested positive and were healthy at the time of testing was 3.6%. One horse had co-infection of both trematodes. No statistically significant difference in detection sensitivity was found between the sedimentation method compared to the filtration method. Access to a water body showed a greater risk of infection with gastrodiscosis, whilst extensive management and grazing had a greater risk for infection with fasciolosis. Exposure of horses to livestock was found to increase risk of infection of horses with gastrodiscosis. Previous treatment of a horse for gastrodiscosis, or treatment with a product containing activity against this specific trematode, had an increased risk of infection with fasciolosis.

**Conclusions:** Testing for gastrodiscosis should not be restricted to horses showing signs of gastrointestinal dysfunction. The prevalence of fasciolosis infection in horses in South Africa may be underestimated. The use of either faecal testing technique (Visser filtration and sedimentation after Benedek) is acceptable for trematode egg detection. Furthermore, access to a water body has a greater risk of infection for gastrodiscosis whilst extensive management practices and previous treatment with an anthelmintic targeted against gastrodiscosis had a greater risk of infection for fasciolosis.

**Key terms:** Equids, *Gastrodiscus aegyptiacus*, *Fasciola hepatica*, Visser filtration, sedimentation after Benedek.

# INTRODUCTION

Whilst nematode infections of equids have been a thoroughly researched topic (Pfister & van Doorn, 2018; Rendle *et al.*, 2019; Gehlen *et al.*, 2020) very little is known about the prevalence and clinical significance of infection of equids with trematodes; specifically, *Gastrodiscus aegyptiacus* and *Fasciola hepatica.*

It has been speculated that trematode infection may remain undetected due to a lack of clinical signs of infection (Bracegirdle, 1973; Applewhaite & Ruiz, 1983; De Kock *et al.*, 2005; Malik *et al.*, 2006; Getachew *et al.*, 2010b; Saeed *et al.*, 2010; Mezgebu *et al.*, 2013; Khan *et al.*, 2020) and as such, the true prevalence of infection may be underestimated. The role of these trematodes in equine gastrointestinal pathology; most notably in cases of recurrent colic episodes and/or diarrhoea and/or weight loss, has also not been determined although numerous case reports have documented gastrointestinal pathology ranging from mild to severe, or sometimes fatal, clinical signs (Bracegirdle, 1973; Azzie, 1975; Roberts *et al.*, 1976; Applewhaite and Ruiz, 1983; Gratwick *et al.*, 2018). Also, it is important to bear in mind that infection of an equid with intestinal parasites does not always lead to clinical disease as a result thereof.

No validated information exists regarding the difference in sensitivity using the Visser filtration technique versus the sedimentation after Benedek technique for the detection of patent gastrodiscosis or fasciolosis in equids (Demelash *et al.*, 2016; Quigley *et al.*, 2017). The use of a coprological ELISA test for fasciolosis specifically (as is utilized in ruminants) has been shown to be an unreliable test in equids (Palmer *et al.*, 2014) and coprological examination for the presence of trematode eggs is still the preferred method of detection in equids (Ward *et al.*, 1997).

Very little is understood regarding the epidemiology of gastrodiscosis and fasciolosis and the relationship to different environmental and managemental practices under which equids are kept. When considering the lifecycle of both these trematodes and the requirement of an intermediate host (in the form of a freshwater snail) and therefore an appropriate environment to sustain these intermediate hosts, it can be presumed that certain management styles would lead to a greater risk of infection (Malek, 1971; Mukaratirwa *et al.*, 2004; De Kock *et al.*, 2005; Ayana *et al.*, 2017; Ghoke & Thorat, 2019). There have been reports of equids kept in conditions which are unsuitable for the survival of these intermediate hosts (and by extension

these trematodes) which have been shown to have patent infections (Azzie, 1975; Malik *et al.*, 2006). This highlights the need for further investigation and understanding into the epidemiology surrounding infection of equids with *G. aegyptiacus* and *F. hepatica*.

This study aimed to investigate the prevalence of trematode infection in equids which present with signs of gastrointestinal disease versus those considered healthy, as well as to establish whether there are management or environmental factors which could lead to an increased risk of infection. Additionally, this study aimed to determine which of the two commonly utilized faecal tests (Visser filtration or sedimentation after Benedek) may have a greater sensitivity when testing for patent trematode infections in equids.

# LITERATURE REVIEW

## Introduction to helminths in equids

Helminths are found naturally throughout the world and gastrointestinal parasites of the equid are thus a normal global finding (Pfister & van Doorn, 2018). Vast research spanning decades has been conducted on the topic of equine internal parasitism and the negative effects in terms of associated morbidity and mortality which a marked helminth infestation can cause upon affected equines (Rendle *et al.*, 2019).

The most commonly reported, and clinically significant, intestinal helminths of importance in equids are the roundworms and tapeworms (Gehlen *et al.*, 2020). Gastrointestinal helminths of equids have been well documented to cause a variety of pathological issues, but the degree of damage caused is often linked to the number and type of helminth present, the immune status of the equid and the overall nutritional health (including body condition score) of the equid (Mezgebu *et al.*, 2013). Clinical signs associated with infection with these helminths is most commonly reported to be diarrhoea, unthriftiness and colic (Balzan *et al.*, 2017) although the connection between colic episodes and the presence of intestinal parasites had not yet been established. Interestingly the results of a study by Gehlen *et al.* (2020) found that despite what was commonly believed no association was found between intestinal worm burden and the occurrence of colic. This was despite the fact that many of the colic patients had reportedly shown signs associated with a high helminth burden; for example, unthriftiness and recurrent colic episodes (Gehlen *et al.* 2020).

Trematodes are a class of helminth within the Platyhelminthes phylum and are commonly referred to as flukes. Whilst there are not many Trematodes which affect equids, *Gastrodiscus aegyptiacus*, *Fasciola spp.* and *Dicrocoelium spp.* are the most commonly found parasites (Ayana *et al.*, 2017). Studies on the prevalence and significance of Trematode infection in equids is sparse. A 2017 study into the prevalence of Trematode infection in central Ethiopia reported prevalence to be 34.9% (134 infected/384 equids), with there being an overall similar prevalence split between donkeys and horses (Ayana *et al.*, 2017).

A lesser investigated intestinal parasite of equids is *G. aegyptiacus,* an amphistome of the class Trematoda (Malek, 1971). This fluke has been found to infect horses, mules, donkeys, zebras, pigs and warthogs (Stunkard *et al.*, 1929; White *et al.*, 1955; Condy 1963;

Munang'andu *et al.*, 2012; Van Laaren, 2014). A case of infection of a rhinoceros infected with *G. aegyptiacus* has also been documented (Stunkard *et al.*, 1929).

Whilst it can be said that almost all equids are infected with internal parasites to a lesser or greater degree, it is not as common to find pathological infections which cause clinical manifestation of disease (Pfister & van Doorn, 2018). Infection with *G. aegyptiacus* has historically been considered to be non-pathogenic (Condy 1963; Azzie, 1975; Roberts *et al.*, 1976; Applewhaite & Ruiz, 1983; Mukaratirwa *et al.*, 2004; De Kock *et al.*, 2005), however there have been an increasing number of case reports describing pathological conditions in equids infected with this fluke (Condy, 1963; Bracegirdle, 1973; Azzie, 1975; Roberts *et al.*, 1976; Applewhaite & Ruiz, 1983; Haq *et al.*, 2017; Gratwick *et al.*, 2018; Ghoke & Thorat, 2019).

Any equids which present with abnormal clinical signs of the gastrointestinal tract should always be assessed for intestinal parasite infection, regardless of history of prior anthelmintic treatment (Pfister & van Doorn, 2018). From a study performed in Ethiopia, it was found that poor body condition was linked to a 3.47 times greater risk of infection with Trematodes, however it was not established whether the body condition was a result of, or caused by the infection (Ayana *et al.*, 2017).

**Prevalence of *Gastrodiscus aegyptiacus* in equids**

*Gastrodiscus aegyptiacus* is a trematode with a predominantly Afro-tropical distribution and cases have been identified extending from South Africa (Azzie, 1975; Wells *et al.*, 1998; Gratwick *et al.*, 2018) and Zimbabwe (Roberts *et al.*, 1976) up to Ethiopia (Bracegirdle, 1973; Getachew *et al.*, 2010b; Mezgebu *et al.*, 2013). Cases of infection have also been identified in Pakistan (Saeed *et al.*, 2010; Malik *et al.*, 2006), as well as in Guyana, South America (Applewhaite & Ruiz, 1983) and India (Ghoke & Thorat, 2019). Von Maker (1989, cited by Malik, 2006) had recorded the presence of *G. aegyptiacus* in the United Kingdom with a prevalence of 5.8%.

The prevalence of infection of this fluke within equid populations has only been determined in a small number of studies whose primary focus was to determine the helminth profiles in those populations (Table 1). In Ethiopia, the prevalence of *G. aegyptiacus* infection from a sample group of 215 donkeys was determined to be 30% (Getachew *et al.*, 2010b) whilst the prevalence within a specific group of working donkeys in the Moretele district of South Africa was up to 63% (Wells *et al.*, 1998). In a study conducted in the semi-arid Sub-Saharan region of Sudan, a prevalence of 4.3% was found within the population of working donkeys (Ismail *et al.*, 2016). A study profiling the prevalence of gastrointestinal helminths of horses kept on a government farm in Pakistan, noted that the most commonly occurring Trematode detected in the equids was *G. aegyptiacus* with a prevalence of 12.82% (Khan *et al.*, 2020). Only one research article (Mezgebu *et al.*, 2013) has compared the prevalence of *G. aegyptiacus* infestation between donkeys and horses around Gondar Town, Ethiopia and found that donkeys appear more susceptible to infection than horses; with a prevalence rate of infestation of 3.56% compared to 2.86%. In a study investigating 400 cases of diarrhoea in horse and donkey foals in Punjab, Pakistan, the most common cause which was isolated was intestinal helminths (isolated from 85% of the cases) of which *G. aegyptiacus* was the most prevalent helminth identified (Haq *et al.*, 2017).

**Table 1. Reported prevalence profile of *Gastrodiscus aegyptiacus***

| Country | Prevalence (%) | Number of equids sampled | Type of test utilised | Reference |
|---|---|---|---|---|
| South Africa (3 different regions) | 14<br>18.4<br>63 | 93 donkeys | Flukefinder | Wells *et al.* 1998 |
| Pakistan | 1.5 | 133 horses | Baermann technique | Saeed et al. 2010 |
| Ethiopia | 30 | 215 donkeys | Sedimentation | Getachew et al. 2010b |
| Zambia | 62.5 | 8 zebra | Sedimentation | Munang'andu *et al.* 2012 |
| Ethiopia | 2.86<br>3.58 | 384 horses<br>105 donkeys | Sedimentation | Mezgebu *et al.* 2013 |
| Sudan | 4.3 | 92 donkeys | Not specified | Ismail et al. 2016 |
| Ethiopia | 10.6<br>13.7 | 79 horses<br>205 donkeys | Sedimentation-centrifugation | Ayana *et al.* 2017 |
| Pakistan | 12.82 | 39 horses | Direct smear and salt flotation | Khan *et al.* 2020 |

**Life cycle of *Gastrodiscus aegyptiacus***

Malek (1971) described the life cycle of *G. aegyptiacus.* The life cycle of this fluke follows a similar life cycle which all Amphistome fluke follow, as is shown in Fig. 1. The fluke requires an intermediate host in the form of the freshwater snail, *Bulinus forskalii,* in which the *G. aegyptiacus* develops up to the mature cercarial stage. Thereafter the mature cercariae of *G. aegyptiacus* encyst on vegetation and flotsam in a suitable water-body or they might become detached, drifting in the water. Once these cercariae encyst they become metacercariae and are the source of infection upon ingestion of infected plant material for definitive hosts such as equids. Malek (1971) stated that it was likely that other planorbid snails could act as an intermediate host for the fluke and it was subsequently shown that other species of freshwater snails might act as intermediate hosts for *G. aegyptiacus,* which could account for detection of the fluke within areas not suitable for survival of *B. forskalii* (Mukaratirwa *et al.,* 2004).

The presence of a suitable water-body, which can be both natural or man-made, as well as an area where the mean yearly temperature intervals fall within the optimal range, allows for survival of the freshwater snail in a broad number of habitats (De Kock *et al.,* 2005). It may also be possible that in *G. aegyptiacus,* as has been noted in another Trematode species *Gastrodiscoides hominis* (which does not affect equids), that there appears to be a wide prepatent period of infection in the snail of between 28-152 days which is affected by season – i.e. the period is shorter in summer months and longer in winter (Dutt & Srivastava, 1972). Drinking from a *B. forskalii* infested water body could present another mode of infection of the equine. In a case report documenting the death of a Thoroughbred filly due to a massive *G. aegyptiacus* infection, it was found that stud farms neighbouring the location of this incident had reported the presence of *G. aegyptiacus* after deworming and both farms had utilised the same river as a water source (Azzie, 1975). Dams and brooks, as well as areas of standing water have been shown to have a higher number of *B. forskalii* snails and thus can be considered as high-risk areas for the presence of *G. aegyptiacus* (De Kock *et al.,* 2005). A heavy infestation with *G. aegyptiacus* was found on post mortem examination of a zebra from a national game reserve and this was attributed to recently constructed dams which were made to limit migration of wildlife through the park, which then provided an ideal environment for survival of freshwater snails (White *et al.,* 1955). Le Roux (1958) speculated that the eggs of the *G. aegyptiacus* Trematode could also be moved vast distances on the feet

of animals which have come to water bodies to drink or graze on vegetation surrounding them.

Extensive management conditions were found to increase risk of Trematode infection by 1.72 times, most likely due to the increased grazing opportunity and time spent in wetter pastures or marshy areas, both of which are known to be favourable for the snails which are intermediate hosts of both *Gastrodiscus aegyptiacus* and *Fasciola hepatica* (Ayana *et al.*, 2017). The results of a study determining the helminth profiles of working donkeys in the Moretele district in the North-West province of South Africa showed that the highest number of donkeys which tested positive for *G. aegyptiacus* were donkeys which originated from a district known as Transactie and were kept under a management system where they were enclosed in an area surrounding the homestead and received no supplemental grazing or roughage (Wells *et al.,* 1998). This Transactie district is situated in a marshy area which frequently flooded during the rainy season and if the lifecycle of *G. aegyptiacus* is considered, this finding corresponds to what is commonly understood regarding management and environment practices and risk of infection of equids with this fluke. This corresponds to the findings made in Gondar Town, Ethiopia, where equids fed only off the pasture and those kept under poor management and housing conditions had a higher risk of infection (Mezgebu *et al.*, 2013).

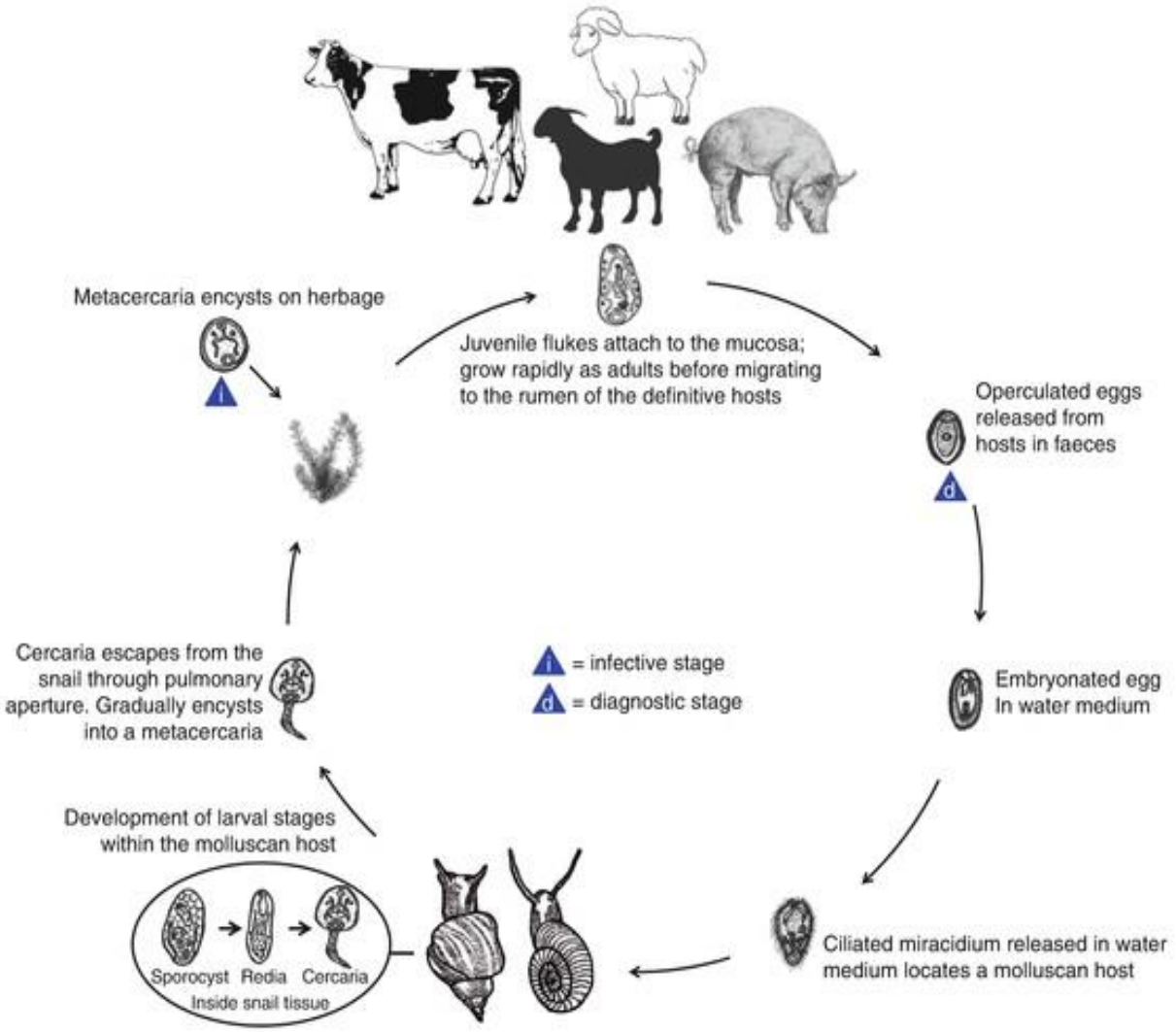


**Figure 1. Schematic representation of the life cycle of Amphistome flukes**

Figure courtesy of Tandon V., Roy B., Shylla J.A., Ghatani S. (2014) Amphistomes. In: Toledo R., Fried B. (eds) Digenetic Trematodes. Advances in Experimental Medicine and Biology, vol 766. Springer, New York, NY. https://doi.org/10.1007/978-1-4939-0915-5_11 (Used with permission)

**Infection of equids with *Gastrodiscus aegyptiacus***

Infection of equids occurs after ingestion of the metacercariae through either grazing or drinking from an infected area. It has been hypothesized that metacercariae, despite being sensitive to desiccation, may remain infective on harvested and baled forage and infection could thus be spread widely (Malik *et al.*, 2006). In drier areas, it has been thought that there is a greater risk of infection during the drier months as grazing is limited and is usually concentrated around water bodies where the intermediate snail host is more likely to be found (Ghoke & Thorat, 2019).

In a case report described by Azzie (1975), a 4-year old Thoroughbred mare died peracutely due to a severe colitis and infection with thousands of immature *G. aegyptiacus* flukes. This mare was stabled and did not have access to grazing for three years prior to her death. Investigation into her roughage and water supplies were inconclusive. Subsequently, yearling horses which had been bred at the same stud farm from which this filly originated, reportedly passed strange "bot-like" worms in the faeces after deworming. It is highly likely in this case that infection in this filly had occurred at the stud and remained undetected until three years after initial infection.

Predilection sites of the adult *G. aegyptiacus* in equids are primarily in the caecum and ascending colon (Matthee *et al.*, 2002) however there was a reported case where adult flukes were found in the stomach and small intestine whilst immature flukes were found in the caecum and colon (Azzie, 1975) (Figures 2 and 3). A study conducted in Sudan on working donkeys found that there was a 15:1 greater prevalence of infection of the colon of these donkeys, compared to infection of the caecum (Ismail *et al.*, 2016).

Whilst historically the detection of *G. aegyptiacus* eggs during a coprological examination was largely considered to be an incidental finding, there have been a number of documented cases over the years of infected equids showing a range of clinical disease. Commonly reported clinical signs of infection range from general malaise, lethargy and unthriftiness (Bracegirdle, 1973; Azzie, 1975; Roberts *et al.*, 1976) to fatal haemorrhagic colitis (Bracegirdle, 1973; Azzie, 1975; Applewhaite and Ruiz, 1983; Gratwick *et al.*, 2018). A recently published case series described 7 horses with severe *G. aegyptiacus* infection which all presented with caecal intussusceptions and typhlocolitis (Gratwick *et al.*, 2018). At least two cases of horses with caecal intussusceptions have been seen at a private equine referral

practice where concurrent infection with *G. aegyptiacus* was diagnosed (S. Higgerty, personal communication). A wide range of clinical manifestations have been reported with infections with high numbers of these trematodes such as oedema, anaemia, poor muscular development and stunted growth (Azzie, 1975; Applewhaite and Ruiz, 1983). The clinical signs most commonly reported with *G. aegyptiacus* infection are recurrent colic signs, chronic weight loss and/or diarrhoea (Azzie, 1975; Applewhaite and Ruiz, 1983; Van Laaren, 2014; Gratwick *et al.*, 2018). A case report of infection of two horses in India with *G. aegyptiacus* was described where each of the horses presented with a history of profuse diarrhoea, and in one of the horses a history of recurrent mild colic episodes (Ghoke & Thorat, 2019). These horses originated from a geographically similar location which bordered on a permanent water body (lake).

Previously, infection with *G. aegyptiacus* was thought to only cause disease in younger horses (Wells *et al.*, 1998), but a recent case study documenting 7 cases of caecal intussusceptions and typhlocolitis, had affected horses ranging in age from 8 to 18 years of age (Gratwick *et al.*, 2018). In another case report from Guyana, a 5-year old gelding was found during postmortem examination to be infected with gastrodiscosis (Applewhaite and Ruiz, 1983). A report by Azzie, 1975, described the presence of adult *Gastrodiscus aegyptiacus* in a 4-year old Thoroughbred mare. Research conducted in working donkeys in the North-West province of South Africa showed that older donkeys had both a higher prevalence of infection with *G. aegyptiacus,* as well as higher egg counts when compared to their younger counterparts and that jennies showed a higher egg count compared to jacks or geldings (Wells *et al.*, 1998). The lack of signs of infection in these animals was attributed to the fact that older animals are presumptively able to tolerate a higher exposure to the fluke without the development of clinical signs and this is due to the fact that older animals have developed some resistance to reinfection and the development of acute clinical signs (Wells *et al.*, 1998). This appears to be contradictory to the findings from a prevalence study of trematodes in equids from Central Ethiopia where there was an 11-fold increase in risk of infection with a trematode parasite in older horses (Ayana *et al.*, 2017).

Conversely, there are multiple reports of seemingly healthy, asymptomatic horses testing positive for infection with *G. aegyptiacus*. This was demonstrated by Roberts (1976) who described apparently normal and healthy horses passing large numbers of adult flukes after anthelmintic treatment (Figure 4). A similar finding was reported by Azzie (1975) where *G.*

*aegyptiacus* flukes were found in faeces of asymptomatic horses which were preventatively dewormed using treatments against the fluke. Numerous studies record the presence of the fluke in donkeys with no apparent clinical signs of infection (Bracegirdle, 1973; Applewhaite & Ruiz, 1983; De Kock *et al.*, 2005; Malik *et al.*, 2006; Getachew *et al.*, 2010b; Saeed *et al.*, 2010; Mezgebu *et al.*, 2013; Khan *et al.*, 2020). Currently, the factors which cause *G. aegyptiacus* to be pathogenic in certain horses, but not others, is yet to be determined (Gratwick, 2018).

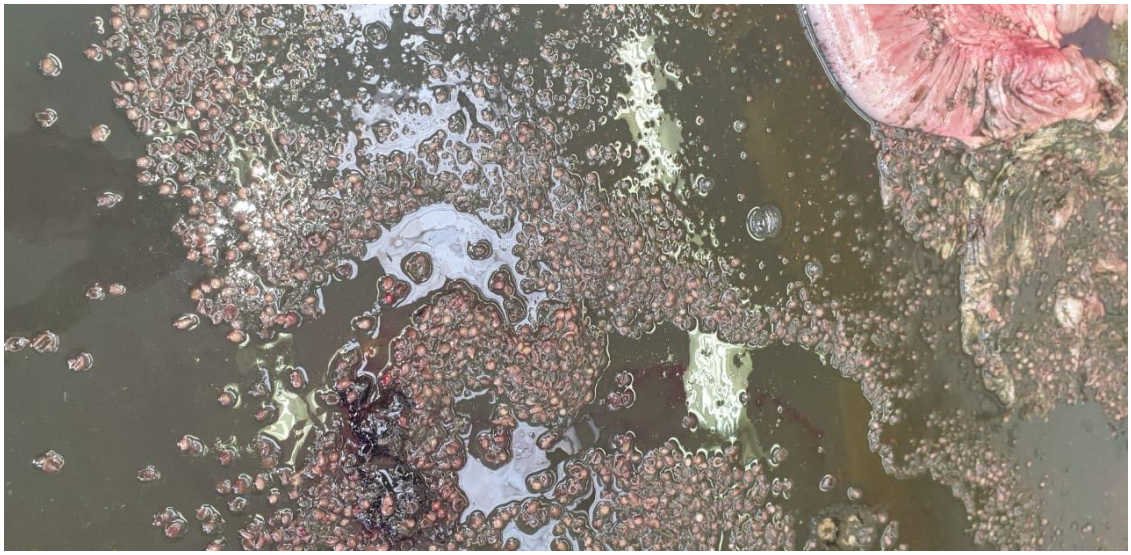

**Figure 2. *Gastrodiscus aegyptiacus* found on post mortem of a heavily infected horse**

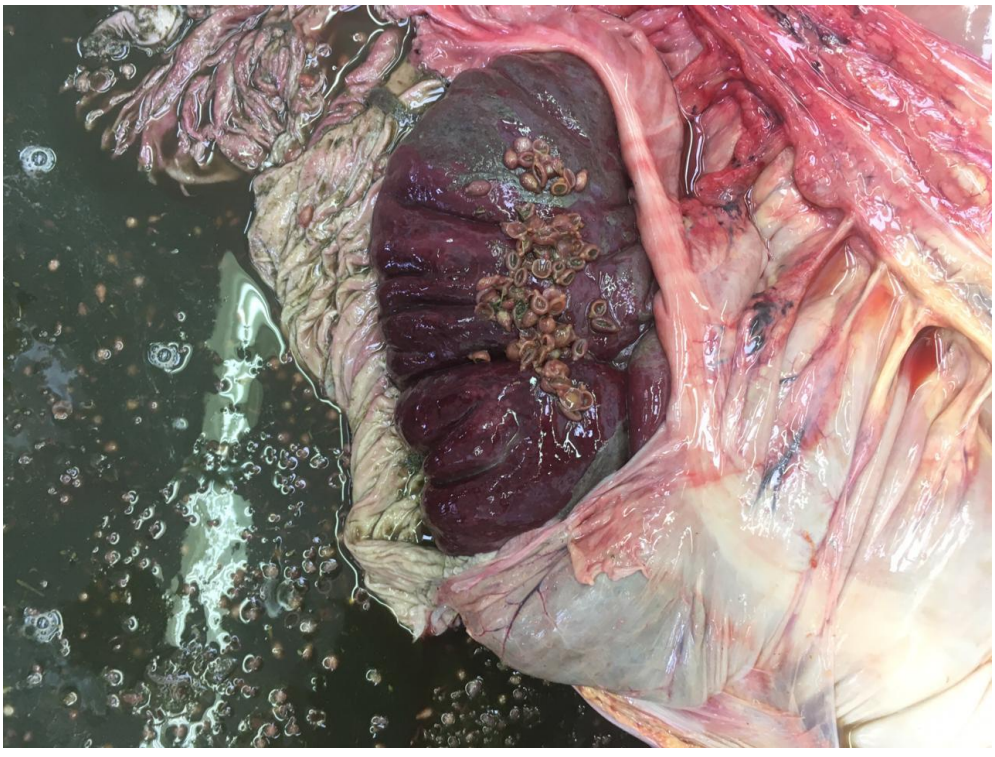

**Figure 3. *Gastrodiscus aegyptiacus* in the colon of a horse at post mortem**

Anthelmintics belonging to the group of salicylanilides have efficacy in a wide number of hepatic and intestinal trematodes (Swan, 1999). Treatment of infection of equids with *G. aegyptiacus* is by administration of a halogenated salicylanilide containing deworming product – either with a product containing the active ingredient oxyclosanide or resorantel (Roberts *et al.*, 1976; Swan, 1999). The currently recommended treatment dosage is 7.5mg/kg of an oxyclosanide-containing product which is administered orally (Roberts *et al.*, 1976, Gratwick *et al.*, 2018).

**Figure 4. Adult *Gastrodiscus aegyptiacus* present in equine faeces post deworming**

**Testing for *Gastrodiscus aegyptiacus* in equids**

The use of coprological testing to determine and evaluate gastrointestinal helminth infection is known to be simple, cost effective and efficient (Ward *et al.*, 1997). At present, there is no testing technique to detect *G. aegyptiacus* eggs in faeces which has been validated against the gold standard of using a postmortem examination technique to determine the presence or absence of the helminth (Demelash *et al.*, 2016).

In terms of coprological testing and examination, faeces can be collected and stored for up to 6 months at a temperature of less than 10 degrees Celsius and Trematode eggs will remain unhatched (Palmer, 2013). It must be noted however that the eggs are sensitive to desiccation and do not tolerate an overgrowth of fungus, if storage time is prolonged.

Trematode eggs are not reliably diagnosed using faecal floatation methods as the eggs are generally too heavy to float (Palmer, 2013). Currently, there are two quantitative faecal testing techniques which can be utilised to test for the presence of trematode eggs; these are the sedimentation technique (after Benedek) and the Visser filtration technique (Visser & Pitchford, 1972). Sedimentation techniques for recovery of fluke eggs are currently the method of choice (Demelash *et al.*, 2016). The use of a modified sedimentation technique, such as that described by Benedek, allows for greater removal of faecal material and better sinking and thus recovery of trematode eggs (Demelash *et al.*, 2016). The Visser filtration technique is described to be a rapid technique which is cost effective and allows for the recovery of viable ova without requiring any centrifugation or processing of the faecal sample (Visser & Pitchford, 1972). Whilst both techniques are relatively cheap to perform and provide a rapid result, the sensitivity of either test has yet to be established.

Faecal egg counts have been long known to be unreliable indicators of worm burdens in animals (Demelash *et al.*, 2016). The use of coprological testing for egg detection has numerous influencing factors, including length and intensity of infection, seasonal variations, difficulty performing adequate serial tests under field conditions and variations between laboratory testing methods and techniques (Ward *et al.*, 1997). A limitation with the use of faecal testing for detection of patent infection, as described in ruminants, is that there is variable shedding of eggs day-to-day (Owen, 1977). These factors may cause discrepancies in test accuracy, such as was demonstrated when a horse tested negative using the Visser filtration technique and was then later confirmed on post-mortem to have a prolific infection

of adult *G. aegyptiacus* (Gratwick *et al.*, 2018). Reliability of test results are confounded by the fact that the *G. aegyptiacus* fluke has a relatively long prepatent period of 115 days (Malek 1971) and only mature flukes produce eggs. Older horses were thought to have some form of protection against massive infection of the fluke (Wells *et al.*, 1998) and there may be intermittent shedding of the fluke eggs, as is seen with *F. hepatica* (Owen, 1977). With *F. hepatica* infection it has also been hypothesised that flukes may not always reach maturity within the equid and therefore may not shed eggs in the faeces, leading to difficulties when using faecal sedimentation as a reliable testing technique (Howell *et al.*, 2020). A low level of infection with the *G. aegyptiacus* fluke may also lead to low egg counts, as described in a study conducted on *F. hepatica* (Alves *et al.*, 1988). Adult flukes may also have a protracted lifespan and have been reported to be able to survive up to 4 years within the gastrointestinal tract of equids (Roberts *et al.*, 1976).

In a study involving the determination of the gastrointestinal parasite profiles of working donkeys in Ethiopia, the Baermann technique was used as a Trematode egg recovery test (Getachew, 2010b). During this research, seven of the donkeys which either died or were euthanased were examined post mortally and the helminth profile noted (Getachew, 2010b). Unfortunately, notes as to what was detected on the Baermann technique and the findings post mortally were not compared. The Baermann technique is not the method of choice for detection of flukes and is more commonly utilised for detection of lungworm (*Dictyocaulus arnfieldi)* larvae.

The helminth profiles of working donkeys in the Moretele district of the North-West province were determined and the trematode population was identified using the 'Flukefinder' (Wells *et al.*, 1998). This 'Flukefinder' technique uses differential filtration followed by differential sedimentation and according to the manufacturer has a reported efficiency and accuracy of 100% (Flukefinder®, 2020).

**Prevalence of *Fasciola hepatica* in equids**

Whilst clinical disease caused by liver fluke is shown to be rare in equids, a prevalence of infection of 1.8% was found from a sample of 224 horses at an abattoir in the United Kingdom according to unpublished data by Hodgkinson, cited by Rendle (2019). This is a notably lower prevalence of infection with *Fasciola hepatica* than that of 9.5% which was found in an abattoir study of 200 horses in Ireland (Quigley *et al.*, 2017). Another recent study on *F. hepatica* in horses in the United Kingdom, showed a prevalence of 2.2% of infection in a population of horses sampled from an abattoir (Howell *et al.*, 2020) (Table 2).

In a study comparing the prevalence of gastrointestinal helminths of horses and donkeys in Ethiopia, it was found that both groups of equids were positive for *G. aegyptiacus*, however only donkeys were found to be concurrently infected with *F. hepatica,* with a prevalence of 5.7% (Mezgebu *et al.*, 2013). A high prevalence of fasciolosis infection of working donkeys in Ethiopia was detected with a prevalence of 44.4% in donkeys examined coprologically and 41.9% in donkeys examined at post mortem, with age of the donkey seeming to be unrelated (Gethachew *et al.*, 2010a). Data from the study performed by Getachew *et al.* (2010a) showed that of the 112 donkey livers which were evaluated at post mortem a prevalence of infection with either *F. hepatica* or *F. gigantica* was 41.9% and a significant portion of these livers showed pathology to the bile ducts (including thickening of the bile ducts, thickened portal areas and fibrotic and irregular bile ducts). A study published in South Africa identified the infection of a horse with *F. hepatica,* as well as the incidental post mortem finding of two horses in the same study being infected with *G. aegyptiacus* (Alves *et al.*, 1988). No fluke eggs were detected from infected horses using coprological examination during that study and they remained asymptomatic despite infection being confirmed post mortally.

Alves *et al.* (1988), hypothesized that horses appeared to have a high level of resistance to *F. hepatica* infection as all 10 horses in the study failed to become infected after oral administration of metacercariae. They concluded that this, along with different management and grazing practices may lead to lower overall prevalence of infection of equids with *F. hepatica*.

**Table 2. Reported prevalence profile of *Fasciola* spp. in equids**

| Country | Prevalence (%) | Number of equids sampled | Type of test utilised | Reference |
|---|---|---|---|---|
| Ethiopia | 44.7<br>41.9 | 803 donkeys coprologic<br>112 donkeys post mortem | Sedimentation-centrifiguation-flotation<br>Post mortem | Getachew *et al.* 2010a |
| Ethiopia | 80 | 215 donkeys | Sedimentation | Getachew et al. 2010b |
| Uruguay | 54 | 368 horses | ELISA | Sanchis *et al.* 2015 |
| Ethiopia | 24<br>25.4 | 79 horses<br>205 donkeys | Sedimentation-centrifugation | Ayana *et al.* 2017 |
| Ireland | 9.5 | 200 horses | Post mortem (12), post mortem + FEC (4), FEC (2), histopathology (1) | Quigley *et al.* 2017 |
| United Kingdom | 1.8 | 224 horses | Necropsy | Rendle *et al. 2019* |
| Italy | 14.3 | 29 donkeys | Sedimentation/flotation | Gianfaldoni *et al.* 2020 |
| United Kingdom | 2.2<br>8.7 | 183 horses | Necropsy<br>Serum ELISA | Howell *et al.* 2020 |

**Life cycle and infection of equids with *Fasciola hepatica***

Similarly to infection with *G. aegyptiacus*, *F. hepatica* infection is a largely unknown entity in equids and their impact on equid health and welfare is uncertain (Getachew *et al.*, 2010a; Quigley *et al.*, 2017). *Fasciola hepatica* has a worldwide distribution, whereas infection with *Fasciola gigantica* is limited to a mainly Asian and African distribution (Mera Y Sierra *et al.*, 2020). Whilst *F. hepatica* is a commonly known trematode fluke which infects cattle and sheep, there have also been numerous reports of donkeys having been found to be infected (Owen, 1977). Historically, infection of equids with *F. hepatica* was considered a rare finding and it was thought that equids had resistance to infection (through elimination or inactivation of the fasciolosis in the early stages of infection) as was demonstrated by Nansen *et al.* (1975) when oral doses of metacercariae only resulted in infection in one out of ten horses. In the study by Getachew *et al.* (2010b) fasciolosis infection of donkeys in Ethiopia was found to be of both *F. hepatica* and *F. gigantica.* Infections with *F. hepatica* would appear to be mostly undiagnosed and untreated as the majority of infections are subclinical (Quigley *et al.*, 2017). In donkeys, it has been noted that despite adult flukes being found within the bile ducts of the livers, these donkeys had not shown any clinical signs of infection (Matthews & Burden, 2013).

As with *G. aegyptiacus*, fasciolosis in equids follows a similar lifecycle involving an intermediate lymnaeid snail host (Figure 1). Pastures with water bodies predispose to infection with *Fasciola* spp. as the environment is ideal for the snail hosts and allows for completion of the lifecycle of the trematode (Getachew *et al.*, 2010a). Once the cercariae have left the intermediate snail host, they become metacercariae and encyst on vegetation around marshy areas (Marchiondo *et al.*, 2020). Considering the lifecycle of the fluke, environmental control and prevention of equids from grazing or drinking in wet habitats is important in management and prevention of liver fluke infection (Matthews & Burden, 2013). Grazing ruminants, i.e. cattle and sheep, are the most common definitive hosts for this fluke.

Alves *et al.* (1988), put forward the theory that equids grazing alongside ruminant livestock which are considered more common hosts of liver fluke, may unintentionally ingest eggs whilst grazing and that this may be detected coprologically despite there being no patent infection present. In a case report, Owen (1977) reported that of 6 horses which were found to be positive for fasciolosis, all of the horses were found to cohabit grazing pastures with cattle. Howell *et al.* (2020) stated that horses and ruminants grazing on the same pastures which

were considered high risk for liver fluke infestation (i.e., boggy grounds and around water bodies) had a higher infection rate of this trematode. Whilst alternating or co-grazing pastures between equids and ruminants is a well known helminth control practice, if the equids are to be grazed in pastures where flukes are likely to be more prevalent (i.e., wet pastures), the fluke status of ruminants should be assessed prior to cohabitation of an area (Matthews & Burden, 2013). The access of wild animals to pastures utilized by equids has also been implicated in the increased risk of infection with *F. hepatica* (Gianfaldoni *et al.*, 2020).

As has been found in various studies regarding infection of *G. aegyptiacus*, there appears to be no definitive trend with regards to prevalence of infection of *F. hepatica* amongst different age groups, breeds or sexes of equids (Quigley *et al.*, 2017), although in the study performed by Getachew *et al.* (2010a), there appeared to be an increase in infection intensity with increasing age of the donkeys. In a study conducted into the parasite profile from donkeys kept for organic dairy farming in central Italy, a prevalence of 14.3% was reported for *F. hepatica* and this was only found in jennies from this population (Gianfaldoni *et al.*, 2020).

In adult ruminants, clinical signs of adult liver fluke infection range from weight loss to pale mucous membranes, anaemia and hypoalbuminaemia (Marchiondo *et al.*, 2020). A case report of a pony in Scotland described the pony as having overt clinical signs of infection (weight loss, anorexia and ventral oedema) along with marked increases in hepatobiliary enzymes as well as histopathological evidence of an eosinophilic cholangiohepatitis (Raftery *et al.*, 2017). There was also evidence of a marked systemic eosinophilia and peritoneal fluid eosinophilia found in this pony. Owen (1977) published a report documenting 38 cases of equines presenting with clinical signs of pathological infection with *F. hepatica.* Signs of infection which have most commonly been noted are poor performance, weight loss, diarrhoea and lethargy (Roberts *et al.*, 1976; Owen, 1977; Quigley *et al.*, 2017).

As well as being uncertain as to the pathogenicity and epidemiology of fasciolosis infection in equids, it has also not been determined whether there is potential for spread of *Fasciola* spp. by equids to other animals and even humans (Getachew *et al.*, 2010a).

Treatment of *F. hepatica* in equids is in the form of off-label use of ruminant deworming products and presently the use of either a triclabendazole containing product at 15mg/kg once off (this treatment targets both immature and mature flukes) or a closantel (20mg/kg) or oxyclosanide (10mg/kg) containing product in two doses given at 8-10 weeks apart (Howell,

2019). Oxyclosande has been shown to only be effective against adult *F. hepatica* (Coles & Stafford, 2001). Anthelminthic resistance to flukicides, most notably triclabendazole, has been noted in the United Kingdom due to its widespread use in ruminants (Matthews & Burden, 2013).

**Testing for *Fasciola hepatica* in equids**

Utilizing and comparing two different faecal testing techniques for the identification and diagnosis of fasciolosis in bovines, it was found that in comparison the use of a modified faecal sieving and staining method (described by Girão and Ueno) was superior to the use of a faecal sedimentation method (using a modified version of the Shore García sedimentation technique) (Kleiman *et al.*, 2005). The percentage of agreement between the two techniques was 41% and the faecal sedimentation method had a false negative rate of 60%. The faecal sieving and staining method was found to be 2.5 times more sensitive in detecting *Fasciola* eggs and is considered to be a more reliable method for diagnosing this fluke in bovines (Kleiman *et al.*, 2005). During the sedimentation method, eggs could be trapped within faecal debris and on the sides of the laboratory receptacles, thus leading to a greater chance of losing eggs out of the sample. The modified faecal sieving and staining method could be used to process large quantities of samples with relative ease under field conditions and the use of a greater number of sieves (up to three in this study) meant that there was a greater chance of finding eggs, especially if there was a low level of infection and associated egg shedding (Kleiman *et al.*, 2005). This study concluded that for the detection of *Fasciola* eggs in faecal samples, the use of a testing method which utilised stacked screens/sieves was a more efficient diagnostic technique than sedimentation methods. This is in contrast to a study performed by Happich *et al.* (1969), which stated that the sedimentation technique for detection of *F. hepatica* was a simple technique which could be easily modified to suit field conditions and was successful as a quantitative test for this trematode. As with the study performed by Kleiman *et al.* (2005), Happich *et al.* (1969) stated that the sedimentation technique was a viable technique to use for detection of "light" levels of infection.

In a report by Alcaino *et al.* (1982), as cited by Alves *et al.* (1988), the limitations of the reliance on only coprological testing for identification of *F. hepatica* eggs was highlighted when 16 out of 100 confirmed positive equids had a false negative faecal test result when 5g of faeces was tested. In another study, 500g of faeces was tested from a horse which was confirmed positive at necropsy for liver fluke infection and a total of only 8 eggs were detected – therefore highlighting the increased chances of missing the eggs on coprological examination considering the low egg numbers shed (*Alves et al.*, 1988). In a case report describing a severe eosinophilic cholangiohepatitis in a pony from Scotland, despite having chronic severe hepatic changes due to liver fluke infection, there was a negative finding for

fluke eggs using a modified sedimentation test (Raftery *et al.*, 2017). Limitations of utilizing faecal egg detection for diagnosing infection with fasciolosis are, amongst others, the fact that not all infective stages will reach patency within the host and that there is a prolonged period of approximately 14-15 weeks from infection to faecal egg shedding (Sanchis *et al.*, 2015). In a recent publication from the World Association for the Advancement of Veterinary Parasitology, it recognized that there is currently no reliable way to diagnose or quantify *F. hepatica* infection (Nielsen *et al.*, 2022).

As with *G. aegyptiacus* infection, infection with *F. hepatica* is most likely under reported as there is no rapid and reliable method of diagnosis (Quigley *et al.*, 2017). A long prepatent period, intermittent shedding and routine faecal examinations which exclude tests for liver fluke in equids, are some factors described by Alves *et al.*, (1988) which may lead to liver fluke being undiagnosed in coprological testing.

As is the case with many trematode infections, faecal egg detection methods have low diagnostic sensitivity (around 60-70% as reported by Quigley *et al.*, (2017)). The prevalence of infection determined in the study by Howell *et al.* (2020) was determined using a modified cattle *F. hepatica* excretory secretory antibody detection Enzyme-Linked Immunosorbent Assay (ELISA). The use of ELISA for detection of coproantigens has been described in cattle and whilst this is a more sensitive testing technique, it does not provide any information with regards to animals which are actively shedding eggs nor numbers of eggs shed (Kleiman *et al.*, 2005). There also appears to be low sensitivity using a coproantigen ELISA test in order to detect patent infection in equids, as was determined in a study conducted in Australia comparing the efficacy of the use of an ELISA test in cattle, sheep and horses (Palmer *et al.*, 2014). This study found an overall sensitivity of only 9% when using the commercial ELISA kit cut-off values and this only increased to 28% when a custom set of cut-off values was implemented. The researchers hypothesized that as horses have no gall bladder (therefore antigen concentration levels may be lower) and the process of hindgut fermentation possibly lowering the antigen concentration by partially destroying them, this could lead to a lower overall sensitivity and reduced value as a diagnostic tool (Palmer *et al.*, 2014). They concluded that although coproantigen ELISA is a cheaper and more efficient diagnostic tool in ruminant species, its value in equids is limited and that faecal sedimentation remains the test of choice in equids (Palmer *et al.*, 2014).

## STUDY OBJECTIVE

1. Determine the prevalence of equids which test positive on faecal examination for *G. aegyptiacus* and *F. hepatica* which present with gastrointestinal signs namely; recurrent colic, weight loss and/or diarrhoea, compared to equids which present for non-gastrointestinal reasons.

2. Determine the difference in the sensitivity and specificity of the two diagnostic techniques (sedimentation after Benedek and Visser filtration) for recovery of *G. aegyptiacus* and *F. hepatica* eggs.

3. Determine which environmental and management practices place equids at greater risk of becoming infected with *G. aegyptiacus* and *F. hepatica.*

# STUDY HYPOTHESIS

1. A higher prevalence of *G. aegyptiacus* positive test results is expected in equids presented for gastrointestinal reasons compared to equids presented for non-gastrointestinal reasons. *F. hepatica* is not expected to be found in either category of equid tested.

2. No significant difference between the sensitivity and specificity of the two faecal tests is expected.

3. Equids presenting from areas with access to free standing water, in the form of dams, rivers, marshes are expected to have a greater chance of testing positive for *G. aegyptiacus*. *F. hepatica* is not expected to be recovered.

# MATERIALS AND METHODS

## Animals

A total of 206 equids were required for sampling for this study. This was statistically determined using data analysed from the results of trematode testing performed at the Helminthology Department of the Faculty of Veterinary Science, University of Pretoria, over the 2018 – 2019 period which suggested a 15% prevalence (14/93) of *G. aegyptiacus.* It was desired to estimate this proportion with a precision of 5% and at the 95% level of confidence. 196 horses would be required based on these assumptions and this number was increased by 5% to account for possible laboratory errors or subsequent data exclusions for a total sample size of 206 horses.

Equids presenting to the Equine Clinic at the OVAH were assigned to one of two groups:

1) those presenting with a complaint of a gastrointestinal nature; namely weight loss and/or diarrhoea and/or recurrent colic episodes

2) those presenting for veterinary care for elective surgeries or non-gastrointestinal complaints.

Equids which presented with gastrointestinal complaints (recurrent colic/weight loss/diarrhoea) were also sampled from designated sole equine private practices.

If there was any reason for concern, the equid was not included in the study.

## Sample collection

The name of the equid and category under which they present was recorded on receipt of a faecal sample. A minimum of two fresh faecal balls were passively collected from the stable of each equid presented to the OVAH. The faecal balls were placed in a clearly marked, sealed double-plastic sample bag. Faecal samples were assigned random identification numbers to ensure that the laboratory technician performing the testing was blinded to which

category of presenting complaint each submitted sample was assigned to. If faecal samples could not be submitted for immediate testing, the sample was stored in a designated sample refrigerator and submitted to the helminthology laboratory at the University of Pretoria's Onderstepoort Veterinary Faculty for testing as soon as possible.

Faecal samples were also collected and submitted from equids from three sole equine private practices (Fourways Equine Clinic, Pierre Van Ryneveld Clinic, Glen Austin Equine Clinic) which provide veterinary services to a wide geographical area of the Gauteng province. Equids that met the requirements for sampling and inclusion in the study were those which were considered to be under the higher risk category for *Gastrodiscus* infection, i.e., those with a history of recurrent colic signs, weight loss and/or diarrhoea. Faecal samples were collected passively from each horse sampled and utilised the same packaging and storing protocols as described for samples collected from equids which were referred to the OVAH.

Dr Summer Maitland-Stuart, the principal investigator, was responsible for the collection, transport and identification of the samples by allocation of randomised identification numbers.

The owners and primary caregivers of the sampled equids were asked to consent to completing a short questionnaire pertaining to the management practices and environment in which the equid/s is/are kept.

**Consent and questionnaire form**

DEPARTMENT OF COMPANION ANIMAL
CLINICAL STUDIES

FACULTY OF VETERINARY SCIENCE

UNIVERSITY OF PRETORIA

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Project title: Prevalence and associated risk factors of trematode infections in equids from selected practices in Gauteng, South Africa.

*Gastrodiscus aegyptiacus* is a Trematode fluke which affects equids. Infection of equids with this fluke was once considered to be an incidental finding, however clinical signs associated with infection (i.e. recurrent colic episodes, weight loss and/or diarrhoea) have become more commonly reported. Various environmental and management practices with regards to equid husbandry has been hypothesised to place equids at greater risk of infection, but these have

not yet been established. There are currently two testing techniques for patent gastrodiscosis in equids, namely the sedimentation after Benedek and Visser filtration test. The difference in accuracy between the tests has not yet been established. The aims of this research study are to determine the prevalence of patent gastrodiscosis in equids presenting with clinical signs of infection, to determine the difference in sensitivity and specificity between the two currently available testing techniques and to establish which management and environmental factors place equids at a greater risk of infection.

All equids admitted to the Onderstepoort Veterinary Academic Hospital, or to one of three pre-selected surrounding equine veterinary practices, may be included in the study. A faecal sample will be collected on admission and submitted for testing for *Gastrodiscus aegyptiacus*. No additional tests will be performed or samples obtained for purposes of this study, only data documented in the patient's record will be used. No treatment will be administered as part of this study.

***(To be completed by the participant's owner / authorised agent)***

**Please encircle Yes or No where necessary**

*("Equid" refers to a horse/donkey/zebra/mule for the purposes of this study)*

Address or GPS coordinates where equid is stabled:

_____

How long has the equid resided on the property mentioned above? _____

Has the equid ever had: Recurrent colic: Y/N Diarrhoea: Y/N
Weight loss: Y/N

What anthelmintic (deworming) has the equid received in the last year (with approximate dates)? _____

Has the equid ever been treated for *Gastrodiscus* specifically? Y/N

If YES, which products were used? _____

What type of roughage/hay is the equid fed? _____

How is the hay stored? _____

Is there a permanent source of water on the property/does the equid have access to any of the following (e.g. during outrides etc.) :

Dam River Brook Pond Irrigated Pastures Marshy Ground

Has the equid in question ever been diagnosed as *Gastrodiscus* positive? Y/N

Have any equids on the property ever been diagnosed with *Gastrodiscus*? Y/N

Do any other animals have access to the same pastures as this horse? Please specify species: _____

1. Do you grant consent that data collected on your animal (history, signalment, physical exam finding, laboratory test results) may be used for this study?

Yes / No

2. Has detailed information regarding the study been discussed with you?

Yes / No

I hereby give permission that my equid (*name*) _____

Breed: _____ Sex: _____ Age: _____

may participate in this study conducted through the Department of Companion Animal Clinical Studies.

*Participant Number allocated to horse:* _____

□ I understand that no compensation will be payable to me.

□ I understand that I will not be liable for any costs of the study.

□ I understand that no personal information will be disclosed but results may be used anonymously in publications.

□ I understand that it is my right to withdraw my animal from the study.

Signed at _____ on the _____ day of _____ 20_____

Signature Owner/Authorised Agent _____

Contact Number: _____

Signature by Dr Summer Maitland-Stuart (Principle Investigator):

_____

We thoroughly appreciate your willingness to participate in this study. If you have any questions, please feel free to contact me (Dr Summer Maitland-Stuart) at summer.maitland-stuart@up.ac.za.

**Sample processing and analysis**

All faecal samples were processed at the Helminthology laboratory at the University of Pretoria's Faculty of Veterinary Sciences. Both the sedimentation (after Benedek), as well as the Pitchford Visser (or Visser filtration as it is sometimes referred to) tests were performed on each sample which was submitted. The laboratory personnel responsible for testing of the samples were blinded to any information pertaining to the sample received.

**Pitchford Visser filtration technique**

Utilising a digital scale, 5 grams of faecal material is weighed out into a container. 100ml of tap water is then added to the faeces and briefly mixed. The faecal suspension is then poured out into the inner filter (95µm weave size) of an assembled Pitchford-Visser Filter, which is suspended from a filter stand. The container is then washed several times with tap water and each time the liquid is transferred to the inner filter, until no faecal material remains in the container.

Using a hosepipe attached to a municipal water supply (in order to achieve high enough pressure) which has an adjustable spray nozzle on the other end, the faecal mixture is thoroughly washed onto the outer (50µm weave size) filter (Figure 5). Washing is continued until the water runs clear from the filter. The inner filter is then removed and all coarse faecal material is washed off.

The stopcock on the outer filter is opened over a 1 litre glass jar and the contents of the filter are washed through, using a gentle stream of water until the jar is almost full. The filtrate is then left to sediment for 5 minutes and thereafter is slowly decanted until approximately 2cm

of filtrate remains in the glass jar – care must be taken not to discard any of the sediment. Tap water is again added to the remaining filtrate in the glass jar until it is almost full. The contents are left for another 5 minutes to allow sedimentation to occur and thereafter the decanting process is performed again. Addition of tap water, subsequent sedimentation and decanting of the supernatant is performed one last time. On the last decant, not less than 10ml of concentrated sediment must be left in the cylinder. The remaining sediment can be transferred to a smaller volume container to aid the collection and examination of the sediment (if this step is performed it is advised to allow the sediment to settle for an additional 5 minutes prior to further processing).

**Figure 5. Apparatus utilised for the Visser filtration test**

The final suspension is mixed by shaking the jar/container and approximately 5ml of the liquid component of the mixture is then poured out into a lined/gridded 50ml petri dish (counting chamber). To enhance contrast between the eggs and debris in the sample, one to two drops of a 1% Methylene blue solution is added to this mixture and is then agitated to allow thorough mixing to occur. The sample is then left to settle for 2 minutes and is then examined at 25X to 30X magnification using a Stereo microscope (Figure 7).

The total number of trematode eggs in the sample is recorded and divided by 5 in order to calculate the total eggs per gram (epg) of faeces.

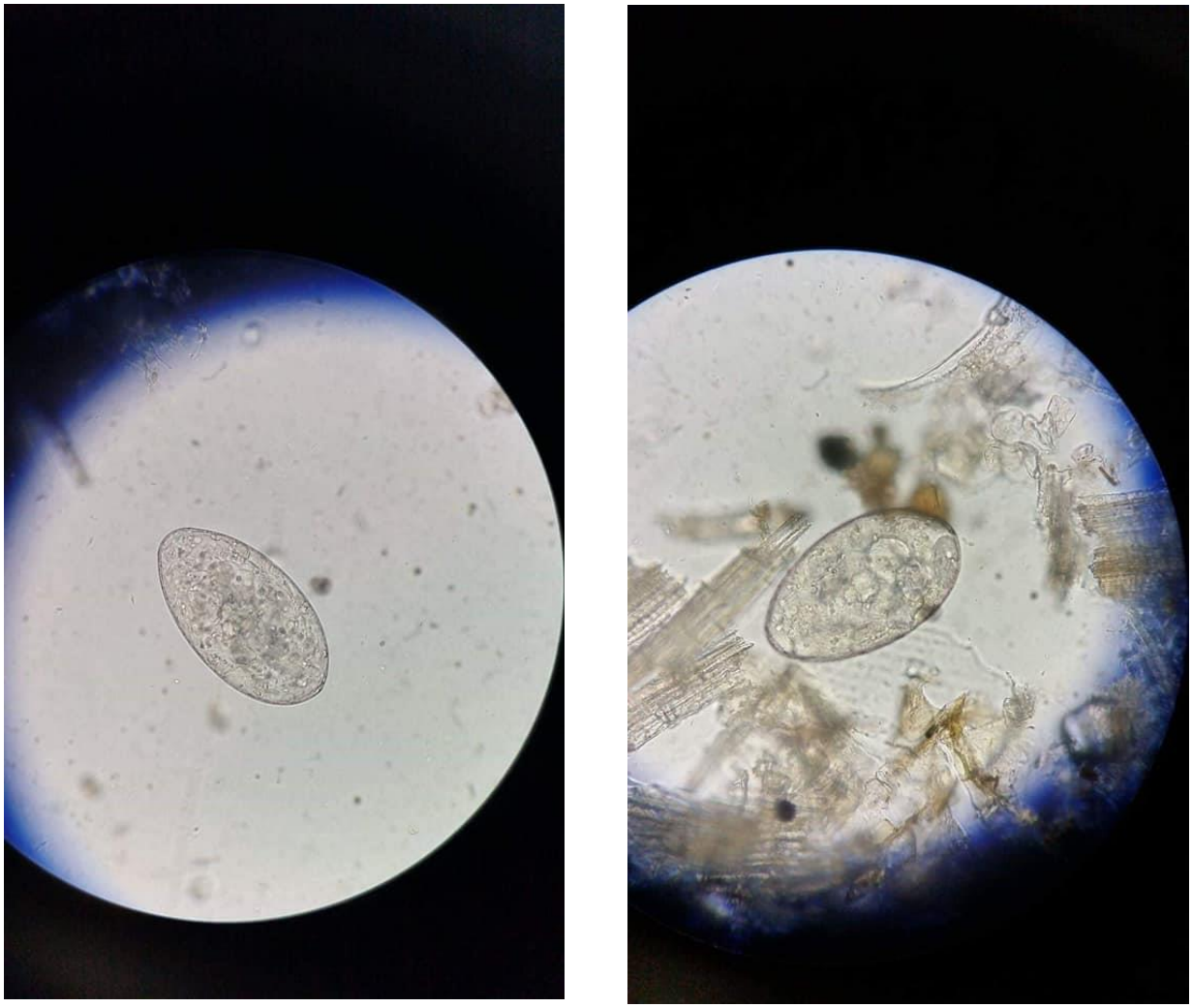

**Figure 6. *Gastrodiscus aegyptiacus* eggs as observed under a microscope**

**Sedimentation after Benedek**

Utilising a digital scale, 5g of faecal sample is weighed out and this is placed in a container and mixed with approximately 100ml of tap water. The faecal suspension is then strained through a tea sieve (which is lined by a 100m x 100mm gauze swab) which is placed over the top of a conical flask (Figure 6). Additional tap water is used to pour over the sieve until the conical flask is almost filled. Finally, a wooden tongue depressor is used to press additional moisture out of the coarse material left on the sieve and gauze swab into the flask.

**Figure 7. Apparatus utilised for the Sedimentation after Benedek test**

The liquid within the flask is left to sediment for 5 minutes and thereafter the supernatant portion is slowly decanted until not less than 30ml of sediment remains within the flask. The flask is then again filled with tap water and the previous step is performed an additional two times.

After the last decanting step, the remaining sediment is transferred to a smaller (approximately 100ml) container and left to sediment for another 5 minutes. The liquid component is then poured off, leaving not less than 10ml of concentrated sediment

The sediment is then well agitated and approximately 5ml of the watery sediment is placed in a gridded 50ml petri dish which is then placed on a stereo microscope for evaluation. The eggs are counted using a 25X to 30X total magnification.

1 to 2 drops of a 1% Methylene blue solution may be added to the sediment prior to counting (Figure 8).

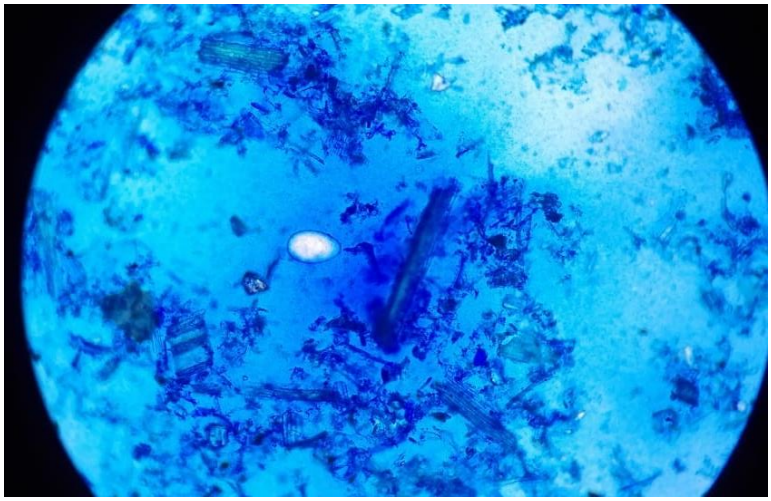

**Figure 8. *Gastrodiscus aegyptiacus* egg as observed under a microscope after addition of methylene blue**

The examined sediment is then discarded, the petri dish well rinsed and the remainder of the sediment is systematically examined as described.

The total number of eggs counted is then divided by 5 to express the result in eggs per gram (epg).

**Monitoring and treatments**

No additional monitoring of hospitalised equids was required as part of this study design and no treatments for *G. aegyptiacus* were administered as part of this study.

**Data analysis**

Prevalence data were presented with 95% mid-P exact confidence intervals calculated using available freeware (OpenEpi: Open Source Epidemiologic Statistics for Public Health, www.OpenEpi.com). The apparent prevalences determined by the sedimentation and filtration methods were compared using McNemar's chi-square tests and agreement between the tests was assessed using kappa statistics.

The normality assumption of quantitative data was assessed by calculating descriptive statistics, plotting histograms and performing the Anderson-Darling test in commercial software (MINITAB Statistical Software, Release 13.32, Minitab Inc, State College, Pennsylvania, USA). Quantitative data were compared based on faecal detection groups (*Gastrodiscus* detected, *Fasciola* detected and neither detected) using one-way ANOVA or Kruskal-Wallis tests for normally distributed and apparently non-normal data respectively. Horses were classified as *Gastrodiscus* spp. or *Fasciola* spp. positive when either or both of the sedimentation and filtration methods were positive for the respective parasitic infection. Univariate binary logistic regression was used to calculate odds ratios and 95% confidence intervals to evaluate the association between potential risk factors and *Gastrodiscus* and *Fasciola* statuses.

Variables with $P < 0.2$ based on Wald tests were selected for multivariable modeling. Selected variables were ranked smallest to largest based on the univariate Wald P values with the first variable selected to initiate the multivariable model. All other variables were assessed using a manual forward selection process based on P value ranking. Variables were manually added one-by-one to the multivariable model. Variables with $P > 0.05$ after entry were removed at each step and the next variable evaluated. Model building continued until all selected variables were evaluated. Unless stated otherwise, all statistical analyses were performed using commercial software (IBM SPSS Statistics Version 28, International Business Machines Corp., Armonk, New York, USA) with significance set as $P < 0.05$.

# RESULTS

No additional monitoring or further treatment of any equids tested was performed or required throughout the study.

A total of 207 faecal samples were collected over a time period of 10 months (from December 2020 up to October 2021). This included samples from 205 horses, 1 donkey and 1 zebra. Both the donkey and zebra samples tested negative on each test. Of the total samples, 123 samples were evaluated from equids which had one or more clinical signs of gastrointestinal dysfunction (weight loss and/or recurrent colic episodes and/or diarrhoea) and 84 samples were received from equids which were considered to be clinically healthy and showed no signs of gastrointestinal dysfunction.

All faecal samples were tested using both the sedimentation after Benedek, as well as Visser filtration faecal tests in order to evaluate the presence of trematode eggs within the sample.

**Prevalence of Gastrodiscosis**

Overall *Gastrodiscus* detected using either test was 24/207: 11.6% (7.7, 16.5) with a 95% confidence interval.

The total number of *Gastrodiscus* positive cases detected via the sedimentation technique was 23/207: 11.1% (7.4, 16.0) with a 95% confidence interval.

Overall *Gastrodiscus* positive cases detected via the Visser filtration technique was 17/207: 8.2% (5.0, 12.6) with a 95% confidence interval.

The total number of *Gastrodiscus* positive cases which presented with clinical signs was 11/123: 8.9% (4.8, 15.0) with a 95% confidence interval.

The total number of *Gastrodiscus* positive cases which were clinically normal at the time of testing was 13/84: 15.5% (8.9, 24.4) with a 95% confidence interval.

**Table 3. Comparison of *Gastrodiscus aegyptiacus* positive samples by test**

| | *Gastrodiscus* positive (sedimentation) | *Gastrodiscus* negative (sedimentation) | Total |
|---|---|---|---|
| *Gastrodiscus* positive (filtration) | 16 | 1 | **17** |
| *Gastrodiscus* negative (filtration) | 7 | 183 | **190** |
| Total | **23** | **184** | **207** |

The sedimentation method detected more positive horses but the apparent difference in sensitivity was not significant (P = 0.07). Kappa statistics showed that there was good

agreement between the sedimentation and filtration methods for *Gastrodiscus* detection – Kappa (95% CI): 0.779 (0.632, 0.926). This is substantiated by the standards for strength of agreement as proposed by Landis and Koch where a kappa coefficient of 0.61-0.80 was described as substantial (Sim & Wright, 2005).

**Prevalence of Fasciolosis**

An unexpected finding was the presence of *Fasciola* eggs within the sample group which was tested.

Overall *Fasciola* detected using either test was 9/207: 4.3% (2.1, 7.8) with a 95% confidence interval.

The total number of *Fasciola* positive cases detected via the sedimentation technique was 7/207: 3.4% (1.5, 6.6) with a 95% confidence interval.

The total number of *Fasciola* positive cases detected via the Visser filtration technique was 8/207: 3.9% (1.8, 7.2) with a 95% confidence interval.

The total number of *Fasciola* positive cases which presented with clinical signs was 6/123: 4.9% (2.0, 9.9) with a 95% confidence interval.

The total number of *Fasciola* positive cases which were clinically normal at the time of testing was 3/84: 3.6% (0.9, 9.4) with a 95% confidence interval.

Whilst the filtration method detected slightly more horses as *Fasciola* positive compared to the sedimentation method (Table 4), the apparent sensitivity was not different between the two tests (P = 1.0). Kappa statistics showed that there was good (substantial) agreement between the sedimentation and filtration methods for *Fasciola* detection – Kappa (95% CI): 0.793 (0.564, 1.0) (Sim & Wright, 2005).

**Table 4. Comparison of *Fasciola hepatica* positive samples by test**

| | *Fasciola* positive (sedimentation) | *Fasciola* negative (sedimentation) | Total |
|---|---|---|---|
| *Fasciola* positive (filtration) | 6 | 2 | **8** |
| *Fasciola* negative (filtration) | 1 | 198 | **199** |
| Total | **7** | **200** | **207** |

**Comparison of quantitative data between outcome groups**

When the quantitative data (age in years, residence duration in months and time since last anthelmintic treatment in days) was analysed from the responses received via the questionnaire which was completed for each equid, it was found that the results are not significantly different between the three groups however the duration of residence was longer in the group which tested fasciolosis positive. It must be kept in mind that the sample sizes were small in the affected groups.

**Table 5. Comparison of quantitative data (age, residence duration and time since anthelmintic treatment) among the outcome groups**

| **Variable** | **Faecal negative** | | ***Gastrodiscus* positive** | | ***Fasciola* positive** | | **P Value†** |
|---|---|---|---|---|---|---|---|
| | **n** | **Descriptive data*** | **n** | **Descriptive data*** | **n** | **Descriptive data*** | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Age (yr)** | 174 | 12.6 (6.1) | 23 | 14.0 (7.8) | 8 | 13.6 (3.9) | 0.549 |
| **Residence duration (months)** | 170 | 36 (12, 72) | 22 | 36 (16, 75) | 8 | 54 (38, 144) | 0.218 |
| **Time since last anthelmintic treatment (days)** | 114 | 109 (97, 197) | 15 | 110 (109, 197) | 5 | 109 (107, 109) | 0.578 |

* Presented as mean (standard deviation) for normally distributed data and median (interquartile range) for data violating the normality assumption

† Based on 1-way ANOVA for normally distributed data and Kruskal-Wallis tests for data violating the normality assumption

**Univariate and multivariable associations between testing *Gastrodiscus* positive and risk factors for infection**

When evaluated, it was found that Thoroughbred horses appeared to be at greater risk of infection. This factor, as well as the feeding of lucerne, access of the equids to a dam/presence of a dam on the property, and the presence or access of equids to livestock were the only statistically relevant risk factors contributing towards an increased risk of infection (Table 6).

When using a multivariate model to evaluate risk of infection it was found that only the access of equids to a dam/presence of a dam on the property and the presence or access of equids to livestock were the only statistically relevant risk factors for infection (Table 7). However, the multivariate model shows a very poor fit to the data obtained.

**Table 6. Comparison of univariate associations between *Gastrodiscus* faecal detection and potential risk factors for infection**

| Variable | Level | Parameter estimate ($\beta$) | Odds ratio (95% CI) | P value |
|---|---|---|---|---|
| **Age** | | | | 0.888 |
| | <10 years | -0.251 | 0.78 (0.27, 2.22) | 0.639 |
| | 10-15 years | -0.051 | 0.95 (0.34, 2.63) | 0.922 |
| | ≥ 16 years | Referent | | |
| **Sex** | Female | 0.182 | 1.20 (0.50, 2.90) | 0.685 |
| | Male | Referent | | |
| **Breed** | | | | 0.132 |
| | Arabian | 0.029 | 1.03 (0.27, 3.90) | 0.966 |
| | BP | -0.050 | 0.95 (0.28, 3.27) | 0.937 |
| | TB | -1.503 | 0.22 (0.06, 0.89) | 0.034 |
| | WB | -1.030 | 0.36 (0.10, 1.28) | 0.114 |
| | Other | Referent | | |
| **Clinical signs** | Yes | -0.623 | 0.54 (0.23, 1.26) | 0.154 |
| | No | Referent | | |
| **Diarrhoea** | Yes | -1.025 | 0.36 (0.08, 1.60) | 0.178 |

| | | | | |
|---|---|---|---|---|
| | No | Referent | | |
| **Lucerne feeding** | Yes<br>No | -1.107<br>Referent | 0.33 (0.11, 1.01) | 0.051 |
| **Hay covered** | All or some<br>None | -0.782<br>Referent | 0.46 (0.17, 1.20) | 0.112 |
| **Dam present** | Yes<br>No | 1.008<br>Referent | 2.74 (1.08, 6.92) | 0.033 |
| **Marshy ground** | Present<br>Absent | 0.770<br>Referent | 2.16 (0.88, 5.29) | 0.093 |
| **Other equids with *Gastrodiscus*** | Yes<br>No | 0.701<br>Referent | 2.02 (0.85, 4.78) | 0.111 |
| **Fowl present** | Yes<br>No | 0.646<br>Referent | 1.91 (0.81, 4.49) | 0.139 |
| **Livestock present** | Yes<br>No | 2.806<br>Referent | 16.5 (1.44, 190) | 0.024 |

CI = confidence interval

BP = Boerperd, TB = Thoroughbred, WB = Warmblood

**Table 7. Comparison of multivariable associations between *Gastrodiscus* faecal detection and potential risk factors for infection**

| Variable | Level | Parameter Estimate (β) | Odds ratio (95% CI) | P value |
|---|---|---|---|---|
| Dam present | Yes | 0.994 | 2.70 (1.05, 6.95) | 0.039 |
| | No | Referent | | |
| Livestock present | Yes | 2.763 | 15.9 (1.29, 194) | 0.031 |
| | No | Referent | | |

**Univariate associations between testing *Fasciola* positive and risk factors for infection**

Risk factors for infection which were found to be significant on univariate screening were stabling of horses at two different yards (Yard H and Yard L), previous deworming with a product which deworms against *Gastrodiscus* spp., having had previous treatment specifically for gastrodiscosis, access to equids which had been previously diagnosed with gastrodiscosis, access to pasture (veld) and the presence of fowl on the property (Table 8). On further evaluation, the univariate results obtained for fasciolosis positive cases could not be used to fit in to a multivariate model.

**Table 8. Comparison of univariate associations between Fasciolosis faecal detection and potential risk factors for infection**

| Variable | Level | Parameter estimate ($\beta$) | Odds ratio (95% CI) | P value |
|---|---|---|---|---|
| **Age** | | | | 0.403 |
| | <10 years | -1.401 | 0.25 (0.03, 2.26) | 0.215 |
| | 10-15 years | 0.078 | 1.08 (0.26, 4.51) | 0.915 |
| | ≥ 16 years | Referent | | |
| **Sex** | Female | -0.605 | 0.55 (0.11, 2.70) | 0.458 |
| | Male | Referent | | |
| **Breed** | | | | 0.490 |
| | Arabian | 0.742 | 2.10 (0.13, 35.3) | 0.606 |
| | BP | 1.617 | 5.04 (0.50, 51.1) | 0.171 |
| | TB | 0.759 | 2.14 (0.22, 21.2) | 0.517 |

| | | | | |
|---|---|---|---|---|
| | WB<br>Other | -0.214<br>Referent | 0.81 (0.05, 13.3) | 0.881 |
| **Yard H** | Yes<br>No | 1.797<br>Referent | 6.03 (1.54, 23.6) | 0.010 |
| **Yard L** | Yes<br>No | 1.856<br>Referent | 6.40 (1.60, 25.6) | 0.009 |
| **Wormer with *Gastrodiscus* activity** | Yes<br>No | 1.423<br>Referent | 4.15 (1.06, 16.3) | 0.041 |
| **Previous *Gastrodiscus* treatment** | Yes<br>No | 1.833<br>Referent | 6.25 (1.59, 24.5) | 0.009 |
| **Eragrostis feeding** | Yes<br>No | -1.335<br>Referent | 0.26 (0.07, 1.02) | 0.053 |
| **Pasture access (veld)** | Yes<br>No | 1.628<br>Referent | 5.10 (1.31, 19.9) | 0.019 |
| **Other equids with *Gastrodiscus*** | Yes<br>No | 2.447<br>Referent | 11.6 (1.42, 94.2) | 0.022 |
| **Fowl present** | Yes<br>No | 2.574<br>Referent | 13.1 (1.61, 107) | 0.016 |

CI = confidence interval, BP = Boerperd, TB = Thoroughbred, WB = Warmblood

# DISCUSSION

The objective of this study was to determine the prevalence of trematode infection of equids which presented with clinical signs of infection, to determine the difference in sensitivity and specificity between the two currently available testing techniques as well as to establish which management and environmental factors place equids at a greater risk of infection. The study sampling took place over a 10-month period and attempted to include equid samples from a broad range of urban to peri-urban equine concentrated areas in the Gauteng province of South Africa. A total of 207 equine samples were tested and of these 123 equids were classified as showing clinical signs of gastrointestinal dysfunction at the time of testing whereas 84 equids sampled were declared by the primary caregiver to be healthy at the time of testing. One donkey and one zebra were included in the sampling, however these samples tested negative.

In the present study, it was found that the overall prevalence of infection of horses with the trematode *Gastrodiscus aegyptiacus* was 11.6% (Table 3) which is in the range of that described previously in the literature for equids; 14% (Wells *et al.*, 1998), 30% (Getachew *et al.*, 2010b), 1.5% (Saeed *et al.*, 2010), 2.8% in horses and 3.5% in donkeys (Mezgebu *et al.*, 2013),4.3% (Ismail *et al.*, 2016), 12.82% (Khan *et al.*, 2020).

It was interesting to note that there was a higher prevalence of horses which tested positive for gastrodiscosis which were considered to be clinically normal at the time of testing, compared to those which were displaying clinical signs. The prevalence of horses which tested positive and showed clinical signs of gastrointestinal dysfunction was 8.9% whereas the prevalence of equids which tested positive and were healthy at the time of testing was 15.5%. This corresponds to the widely held belief that infection with *Gastrodiscus aegyptiacus* oftentimes goes largely unnoticed due to a lack of corresponding clinical signs of infection (Bracegirdle, 1973; Applewhaite & Ruiz, 1983; De Kock *et al.*, 2005; Malik *et al.*, 2006; Getachew *et al.*, 2010b; Saeed *et al.*, 2010; Mezgebu *et al.*, 2013; Khan *et al.*, 2020). Thus, it cannot be assumed that a lack of clinical signs implies a lack of patent infection.

Contrary to the findings from a study in the North-West province investigating infection of donkeys with gastrodiscosis where it was found that older donkeys and female donkeys had a higher prevalence of infection (Wells *et al.*, 1998), the findings in the current study showed that age and sex of horses were not significant risk factors for gastrodiscosis infection in this study, with a wide spread of age of infection of horses ranging from 4 to 35

years of age (p-values >0.6) and an almost equal distribution between the sexes (p-value >0.6). With only 9 horses positive for fasciolosis, it is important to note that the sample size is much smaller – however there was still a fairly wide age spread between 8 and 18 years of age (p-values >0.2) and sex distributions were not different (p-value >0.4) (Table 6).

Both gastrodiscosis and fasciolosis share a common lifecycle involving an intermediate host in the form of a freshwater snail and therefore the presence of a water body or wet environment in which the snails can survive is an important consideration for completion of the trematode lifecycle (Malek, 1975; De Kock *et al.,* 2005; Getachew *et al.,* 2010a). This was demonstrated as one of the risk factors for infection with *G. aegyptiacus* with horses being 2.74 times at greater risk of infection if they reside in an area, or have access to, a dam on the property (p-value: 0.033) (Table 6 and 7).

Exposure of horses to livestock was found to increase risk of infection of horses with *G. aegyptiacus* by 16.5 times (p-value: 0.024). As there were only three horses which had reported contact with livestock and it was found that two of the three were gastrodiscosis positive, it must be considered that the presence of livestock might be a proxy for another factor (such as extensive grazing systems, less intensive management practices and so on) which might not have been included in the questionnaire rather than the presence of livestock themselves being a risk factor (Table 7). Livestock are not known to be one of the definitive hosts in the lifecycle of *G. aegyptiacus* whereas sheep and cattle are known definitive hosts for *F. hepatica.*

The infection of horses with fasciolosis was an unexpected finding in this study and as with *G. aegyptiacus* infection it has been anecdotally accepted within the equine veterinary industry of South Africa to have a low prevalence and significance associated with infection. Whilst infection of equids with fasciolosis has been reported with both *F. hepatica* as well as *F. gigantica* (Getachew *et al.,* 2010b), the findings from the current study were that fasciolosis infection was only with *F. hepatica*.

In the present study it was found that the overall prevalence of infection of horses with the trematode *F. hepatica* was 4.3% (Table 4) which was generally lower than those previously described in the literature for equids; 41.9% (Getachew *et al.,* 2010a), 5.7% (Mezgebu *et al.,* 2013), 9.5% (Quigley *et al.,* 2017), 14.3% (Gianfaldoni *et al.,* 2020), 2.2% (Howell *et al.,* 2020).

The prevalence of horses which tested positive for fasciolosis and showed clinical signs of gastrointestinal dysfunction was 4.9% whereas the prevalence of horses which tested positive and were healthy at the time of testing was 3.6%. Whilst these results may intuitively

make more sense considering the higher prevalence found in horses showing signs of infection, there was an overall smaller positive sample set and results are therefore less likely to be statistically significant. Only one horse was found to have co-infection with both gastrodiscosis and fasciolosis.

Whilst it was attempted to include samples from as vast a geographical range as possible, it must be considered that the areas selected for testing may not have been representative enough to include a wide variety of environmental and geographical regions. No significant conclusions were found regarding geographical area from which the horse originated and risk of infection, although the 9 horses which tested positive for *F. hepatica* originated from one of two commercial equestrian yards which are located less than 1km apart from each other (Table 8). This would suggest that there is either a shared or common water source which the horses have access to, or perhaps access to livestock when grazing although neither of these risk factors were found to have statistical significance for increased risk of infection in the present study. It has been hypothesized that equids, if they co-graze with livestock, could unintentionally ingest *Fasciola* eggs which have been passed by the livestock and this could lead to detection of *Fasciola* eggs on faecal examination of the equids, despite patent infection not being present (Alves *et. al.*, 1988).

It was found that the risk of infection with fasciolosis was increased 5.10 fold if the horse had access to pasture/veld (Table 8). This aligns with reports in the literature which found that extensive management conditions where equids had extended access to marshy/wet grazing conditions increased the risk of infection with trematodes by 1.72 times (Ayana *et al.*, 2017). Contradictory to the results for *G. aegyptiacus*, the presence of a water body was not found to have a statistically significant increased risk of infection for *F. hepatica.* Unfortunately, due to the small number of horses which tested positive for fasciolosis, it may be that the statistical test has low power. Similarly, a study on liver fluke prevalence in Ireland by Quigley *et al.* (2017) found that there was no statistically significant correlation between *F. hepatica* infection and environmental or management factors and the present study corroborates those findings.

An interesting finding from the present study was that a history of a horse having been previously treated for gastrodiscosis, or having received an anthelmintic treatment with activity against gastrodiscosis (whether the horse had tested positive at the time of treatment or not), increased a horse's risk of infection with fasciolosis by 6.25 times (p-value: 0.009) and 4.15 times (p-value: 0.041), respectively (Table 8). A possible reason for this finding may be that the currently widely utilised anthelmintic Oxyclosanide, which is used to treat

gastrodiscosis in equids, has activity against adult liver fluke only and therefore immature liver fluke may persist within the equid host. Whilst Oxyclosanide may be used to treat both fasciolosis and gastrodiscosis, the dosage when treating for fasciolosis is quite a bit higher (Howell *et al.,* 2020). In addition, there may be a level of competitive inhibition which occurs within equids with the presence of infection with both gastrodiscosis and fasciolosis in an equid acting to suppress infection numbers and elimination of one of these trematodes (in this case gastrodiscosis) may lead to the development of overt infection with the other trematode (fasciolosis in this case). Co-infection with both flukes may occur, as was demonstrated in one horse in the present study.

Two different testing techniques i.e., sedimentation after Benedek and Visser filtration were utilized on each sample in an attempt to determine whether there is a significant difference between the accuracy of each test. In the present study, whilst the sedimentation method had detected more horses positive for either one or both of the trematodes compared to the filtration method, the apparent difference in detection sensitivity was not significant (p-value: 0.07). For the detection of gastrodiscosis positive horses, there was good agreement between the sedimentation and filtration methods (Table 3). For the fasciolosis positive horses, the filtration method detected slightly more positive horses however the apparent sensitivity was not different between tests (p-value: 1.00) and there was good agreement between both tests for fasciolosis detection (Table 4). For the detection of *Gastrodiscus* eggs in faeces, there are numerous factors which hamper the detection, amongst others; the length and intensity of infection, seasonal variations, difficulty performing serial testing in the field, variations between laboratories and testing techniques (Ward *et al.,* 1997), variable/intermittent shedding of eggs (Owen *et al.,* 1977), a long pre-patent period of 115 days (Malek *et al.,* 1971), the shedding of eggs only by mature flukes and the possibility that the trematode might not reach maturity within the host and therefore will not actively shed eggs. The detection of *Fasciola* eggs in faeces face similar obstacles, namely; not all infective stages reach patency in the host, there is a prolonged period from infection to shedding, intermittent/variable shedding (Alves, *et al.,* 1988; Sanchis *et al.,* 2015) and the fact that routine faecal samples are not tested specifically for trematodes (Quigley *et al.,* 2017). Thus, it can be concluded that the detection of either trematode faces similar challenges and that in the present study neither test was established as superior compared to the other. As has been described by Demelash *et al.,* (2016), the gold standard for validation of testing techniques must be performed through post mortem establishment of the presence or absence of infection. This was not performed in this present study. A possible approach to improving the

sensitivity of faecal testing techniques would be to perform sequential faecal sampling to increase sensitivity in order to account for the low or intermittent shedding of trematode eggs.

Limitations in this study include the limited geographical area which was sampled - only peri-urban equine concentrated areas of Gauteng were sampled. There was a relatively small sample size of 207 faecal samples which were evaluated and a small overall number of positive samples which could lead to statistical results having lower power. The results of the faecal sample testing were also not validated at post mortem. Potential bias could exist for sampling of horses which were infected with either gastrodiscosis or fasciolosis based on the inclusion criteria for sampling and equid owner/yard manager bias towards equid selection.

# CONCLUSIONS

In conclusion, the overall prevalence of infection with patent gastrodiscosis coupled with the fact that many asymptomatic horses tested positive for the trematode, suggests that routine faecal testing for this trematode should be performed. The presence of fasciolosis positive horses in the present study was unexpected, but further serves to iterate that routine trematode testing and careful differentiation between the two trematodes should be considered. The sensitivity of neither the Visser filtration nor sedimentation after Benedek faecal testing techniques were statistically significant compared to the other, however the sample size was low and a lack of significant difference cannot infer test sensitivity being similar. Regular access to a waterbody presented an increased risk for infection with gastrodiscosis, whereas extensive management/grazing conditions and a history of previous treatment with an anthelmintic which has properties against gastrodiscosis led to an increased risk of infection with fasciolosis.

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