Case series: periocular habronemiasis in five horses in the Netherlands

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In tropical and subtropical climates, infection of periocular tissue by *Habronema* larvae is a recognised cause of conjunctivitis or blepharitis. To the authors' knowledge, only a few cases of habronemiasis have been described in Western Europe, and it has not been documented previously in the Netherlands. The objective of this report is to describe the occurrence of five cases of (peri)ocular habronemiasis in the Netherlands, of which four date from the past few years. The diagnosis was based on the history, clinical signs and histopathologic examination of biopsy specimens. A granulomatous conjunctivitis/dermatitis and sulphur-like granules were present in all cases. Histopathology showed an eosinophilic granulomatous inflammation, and three out of five (60 per cent) samples revealed one or more nematodes on section. Treatment combinations with surgical excision, local corticosteroid and/or anthelmintic drugs were used. Furthermore, all horses received ivermectin or moxidectin. Treatment resulted in healing of the lesions in four horses. One case, which was refractory to treatment, resolved spontaneously after the onset of colder weather. This case series suggests an increased prevalence of (peri)ocular habronemiasis in the Netherlands. This diagnosis should therefore be considered when being presented with a horse with granulomatous conjunctivitis/dermatitis in Western Europe, especially during the summer months.

Introduction

Infection of periocular tissue by *Habronema* larvae is a well-known cause of infectious conjunctivitis, blepharitis or dermatitis in tropical and subtropical climates.[1] Three nematode species, *Habronema microstoma* (synonym *H majus*), *H muscae* and *Draschia megastoma*, have been identified as the cause of this disease.[2 3] The adult stages live in the equine stomach (gastric habronemiasis), which is usually asymptomatic.[3] The adult female produces eggs in the stomach, which are passed in the faeces from where they are ingested by the maggots of various flies. Once the flies mature, they deposit the infective L3 larvae on mucous membranes of equidae, or in an area of skin that is moist or pathologically altered and therefore attractive for flies. If the larvae are swallowed by the horse, they complete their life cycle by maturing to the adult stage in the stomach.[2 3] If the larvae remain on a cutaneous or mucous membrane level, a local hypersensitivity reaction occurs, commonly referred to as 'summer sores'.[1–4]

Habronemiasis is most frequently seen during spring and summer, when fly populations are most active. In the ocular presentations, lesions typically occur in the medial canthus, and the gross appearance is ulcerative and granulomatous, with so-called sulphur-like granules within and around the lesion.[1 3 4] These granules consist of necrotic, caseous or calcified material surrounding the larvae or their dead remnants.[3]

A presumptive diagnosis can be made based on the clinical presentation; the sulphur-like granules are considered pathognomonic. The diagnosis is confirmed through histopathologic examination, which typically reveals an eosinophilic granulomatous inflammation with nematodes within the histological specimen.[1 3–5] For the molecular diagnosis, a seminested PCR assay was developed and is established for the two species of *Habronema* (*H microstoma* and *H muscae*).[6] For the detection of *Habronema* DNA in the faeces of horses, the PCR assay achieved a diagnostic specificity of 100 per cent and a sensitivity of 97 per cent.[7] This PCR was developed for the diagnosis of gastric habronemiasis, but was also validated for the use in the detection of *Habronema* species in skin samples with cutaneous infection.[8] In that survey, all 12 animals with suspected cutaneous

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habronemiasis tested positive for the DNA of *H microstoma* or *H muscae*.

On cytology from conjunctival scrapings and impression smears eosinophils are abundant. Many treatments have been described for habronemiasis, but combinations of anthelmintic therapy, removal or surgical debulking and anti-inflammatory drugs are recommended by most authors.[1 3–5 9] Lesions can undergo spontaneous remission during winter.[3 4 10]

The condition has been very rarely reported in Western Europe, and to the authors' knowledge it has not been documented previously in the Netherlands. The objective of this case series is to report the recent occurrence of five cases of (peri)ocular habronemiasis and to discuss the diagnosis and treatment of this disease.

Materials and methods

Five cases of habronemiasis were recorded at our faculty during the last 15 years (2001–2016). Patient characteristics, history, results of clinical examination and further diagnostic investigations, treatment and outcome were recorded. Long-term follow-up (>1 year) in the three oldest cases was obtained by telephone conversation with the owner. Histological evaluation was performed of routinely formalin-fixed, paraffin-embedded samples which were stained with haematoxylin and eosin.

Results

Five cases of (peri)ocular habronemiasis were found over the last 15 years (table 1). Horses were of various breeds and the age range was 8–18 years. None of the horses had travelled outside the Netherlands. All horses had been dewormed following an egg count-based anthelmintic treatment strategy. The cases showed a wide variation in the extent and severity of the lesions. Only case 4 was bilaterally affected. All horses presented with sulphur-like granules. No other abnormalities were seen during the clinical and ophthalmic examinations. Complete healing of the lesions occurred in all cases. No recurrence was seen in case 1 (follow-up 10 years) and cases 2 and 3 (follow-up one year). A short-term follow-up of six months was available for case 4 in which the lesions had completely resolved. In case 5, the lesions had healed almost completely at the time that the horse was lost to follow-up.

Case 1

Clinical signs and diagnosis

The ophthalmic examination of the left eye revealed profuse mucopurulent discharge, blepharospasm and chemosis. An elevated granulomatous mass of 1.5 cm in diameter was visible on the palpebral conjunctiva of the medial canthus.

Treatment

A single dose of 0.4 mg/kg BW Moxidectin (Equest(Zoetis, Capelle a/d IJssel, The Netherlands)) was administrated orally, and antibiotic ointment with 0.5 per cent oxytetracycline and 10.000 IU/g polymyxin B (Terramycin(Pfizer, Capelle a/d IJssel, The Netherlands)) was applied topically every six hours for five days. Initially, there was an improvement in clinical signs, but after one week the ocular discharge and chemosis recurred. Since parasitic disease was suspected, the nasolacrimal ducts were flushed once daily with levamisole (Levamisole 10%(Eurovet Animal Health B.V., Bladel, The Netherlands)) for four days, and topical eye treatment with the same ointment was instituted every 12 hours during the same period. For this purpose, levamisole 1 per cent (10 mg/ml) eye drops were custom-made by diluting Levamisole 10%(Eurovet Animal Health B.V) in artificial tears (Lacriforte(AST Farma, Oudewater, The Netherlands)).

Case 2

Clinical signs and diagnosis

Moderate blepharospasm and chemosis of the left eye were present. There was mild ectropion of the lower eyelid, severe epiphora and mucopurulent discharge. In the palpebral conjunctiva of the medial canthus an

TABLE 1: Overview of patient characteristics and history

	Breed	Age	Sex	Coat colour	Month of referral	Duration of complaints at admittance (weeks)	Treatment by referring vet	Drug and time of most recent anthelmintic treatment
Case 1	Shetland pony	9	Mare	Bay	July 2001	3	Antibiotic eye ointment with 0.5% oxytetracycline and 10.000 IU/g polymyxin B was applied topically three to four times daily for an unknown period of time.	0.2 mg/kg BW ivermectin oral, six days previously.
Case 2	Arabian	13	Stallion	Chestnut	July 2015	9	Antibiotic eye ointment with 0.3% gentamicin and 0.1% polymyxin B three to four times daily for seven days and the nasolacrimal duct was flushed repeatedly.	0.2 mg/kg BW ivermectin oral, three months previously.
Case 3	Warmblood	8	Mare	Bay	September 2015	6	Antibiotic eye ointment with 1% chlortetracycline two to three times daily for 10 days.	0.2 mg/kg BW ivermectin oral, four months previously.
Case 4	Warmblood	18	Gelding	Bay	July 2016	8	Antibiotic eye ointment with 2% chloramphenicol and vitamin A two to three times daily for seven days.	Unknown, two months previously.
Case 5	Draft horse	16	Gelding	Roan	July 2016	8	Trimethoprim sulphadiazin 30 mg/kg BW every 12 hours for two weeks orally.	0.2 mg/kg BW ivermectin oral, one year previously.

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elevated granulomatous mass of 1.5 cm^2 was visible, and sulphur-like granules were adhered to the surface. A sample for cytology (Cytobrush(CooperSurgical Inc., Trumbull, CT, U.S.A)) was taken, which revealed benign epithelial cells and many eosinophils.

Treatment

In the standing sedated horse the mass was surgically debulked. Before the surgical procedure 0.015 mg/kg BW detomidine (Domosedan(Zoetis)), 0.02 mg/kg BW butorphanol (Dolorex(MSD Animal Health BVBA, Brussel, Belgium)) and 2.2 mg/kg BW flunixin-meglumine (Cronyxin(Eurovet Animal Health B.V)) were administrated intravenously. Nerve blocks of the nervus auriculopalpebralis, nervus lacrimalis and nervus infratrochlearis were performed with 2 ml of 2 per cent mepivacaine (Mepivacaïne HCl 2% (B. Braun Medical BV, Oss, The Netherlands)) each, and 0.5 ml of 1 per cent tetracaine eye drops (Tetracaïne-hydrochloride 1% hour) was applied topically. All granulomatous tissue was excised without compromising the eyelids. The lacrimal puncta were not involved in the lesion. The centre of the mass appeared to contain yellow necrotic material and sulphur-like granules. The surgery site was left to heal by second intention. A single dose of 0.2 mg/kg BW ivermectin (Equimectin(AST Farma)) was administered orally and 1 per cent chlortetracycline eye ointment (Aureomycin(AST Farma)) was applied topically every 12 hours for three days. Subsequently, the horse was treated with 1 per cent levamisole eye drops (Lacriforte(AST Farma), Levamisole 10% (Eurovet Animal Health B.V) topically every 12 hours for four days. Follow-up of two weeks later showed that the clinical signs had not resolved completely, and a granulomatous mass of 3×5 mm was present at the site of excision. Triamcinolone-acetonide (4 mg, Kenacort(Bristol-Myers Squibb, Utrecht, The Netherlands)) was injected subconjunctivally, and moxidectin (Equest(Zoetis)) was administered orally. Two weeks later, the lesion was reduced to an area of 1 mm^2 and the subconjunctival administration of triamcinolone-acetonide was repeated once more. Complete healing occurred within two weeks after the last injection.

Case 3

Clinical signs and diagnosis

Ophthalmic examination of the right eye revealed moderate epiphora and a large amount of mucous discharge. Mild blepharospasm, ectropion and chemosis were present. There was an elevated, round, granulomatous mass of 1 cm in diameter on the palpebral conjunctiva of the medial canthus, with sulphur-like granules adhered to the surface.

Treatment

The granulomatous mass was surgically debulked according to the procedure as described for case 2. Differences in this case were that the ventral lacrimal punctum was affected, and that it was possible to suture the conjunctiva for primary closure (simple continuous pattern, polyglactin 910 5-0 USP). Systemic treatment with a single dose of 0.4 mg/kg moxidectin (Equest(Zoetis)) orally and two days of 0.6 mg/kg BW Meloxicam (Metacam(Boehringer Ingelheim International GmbH, Ingelheim am Rhein, Germany)) every 24 hours orally was implemented. Antibiotic eye ointment with 1 per cent chlortetracycline (Aureomycin(AST Farma)) was applied topically every six hours for four days. The lesion healed without any complications. The horse did not suffer from epiphora and the nasolacrimal duct was flushed without any problems by the referring veterinarian some weeks after the procedure. This indicates that the nasolacrimal duct was patent, probably by drainage through the dorsal lacrimal punctum.

Case 4

Clinical signs and diagnosis

Epiphora and a mucoid ocular discharge were present in both eyes. A protruding granulomatous lesion was present in the palpebral conjunctiva of the medial canthus bilaterally, measuring 5 mm in diameter in the right eye and 15 mm in the left. The skin adjacent to the medial canthus was ulcerated (Fig 1A). Sulphur-like granules were adhered to the surface. Both nasolacrimal ducts were patent. A sample for cytology

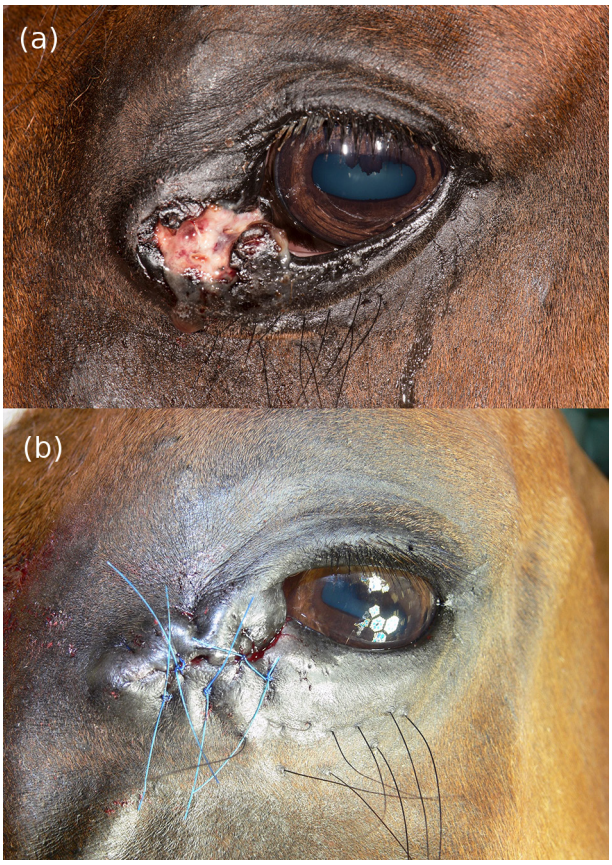

FIG 1: Case 4, an 18-year-old warmblood with a habronemiasis lesion of the left eye prior to treatment (a) and after surgical debulking (b).

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(Cytobrush(CooperSurgical Inc)) was taken that showed the presence of many eosinophils.

Treatment

Due to the extensive nature of the lesions and the character of the horse, surgical debulking was performed bilaterally under general anaesthesia in dorsal recumbency (Fig 1B). The granulomatous tissue was excised as completely as possible but without compromising any more skin. The mass of the right eye contained a considerable amount of necrotic material and sulphur-like granules and both lacrimal puncta were intact. In the left eye, the skin of the medial canthus was more severely affected and the ventral lacrimal punctum was neither visible nor patent when the nasolacrimal duct was flushed intraoperatively. Primary closure of the skin was performed with simple interrupted and vertical mattress sutures (Nylon 4-0 USP). Meloxicam (Metacam(Boehringer Ingelheim International GmbH)) 0.6 mg/kg BW was administered orally before surgery and additionally every 24 hours for four days postoperatively because of the extent and severity of the lesions. Postoperative treatment with eye ointment was not possible due to the uncooperative behaviour of the horse. Oral treatment with a single dose of 0.4 mg/kg BW moxidectin (Equest(Zoetis)) was implemented. As the removal in the left medical canthus might have been incomplete, 4 mg triamcinolone-acetonide (Kenacort(Bristol-Myers Squibb) was administered subconjunctivally at day 7 postoperatively. The sutures were removed at day 10. The right medial canthus had healed completely. The left side had dehisced partially, but healed completely by second intention within two weeks after discharge. Even though the ventral lacrimal puncta of the left eye had been affected, the horse did not show any signs of epiphora afterwards.

Case 5

Clinical signs and diagnosis

An extensive granulomatous ulcerated skin lesion 25×10 cm extending rostrally from the left eye was present with some sulphur-like granules adhered to the surface; the eye itself was not involved (Fig 2A).

Treatment

Due to the size of the lesion, surgical intervention was not an option in this case. Neomycin-sulphate and hydrocortisone-acetate ointment (Vetaderm(AST Farma)) mixed with injectable ivermectin solution (Ivomec(Merial B.V., Velserbroek, The Netherlands)) was applied locally once daily for two weeks. The horse was also treated with a single dose of 0.2 mg/kg BW ivermectin (Eraquel(Virbac, Barneveld, The Netherlands) orally. This therapy seemed unsuccessful in decreasing the size of the lesion, and the lesion progressed slightly. Because it is known that habronemiasis lesions can regress with the onset of colder weather, the owner was advised to 'wait and see'. In October, the lesion started to regress spontaneously, without any treatment being instituted at this time. The size of the lesion decreased by around 90 per cent in two months (Fig 2B). After one more month, the lesion had resolved almost completely. After this the horse was lost to follow-up.

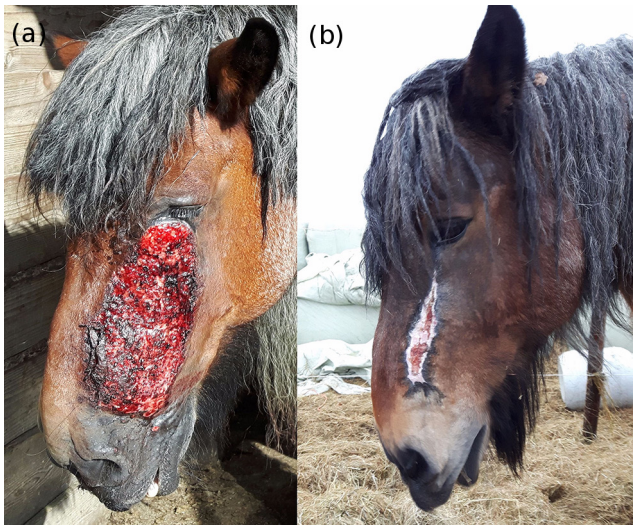

FIG 2: Case 5, a 16-year-old draft horse gelding with an extensive *Habronema* lesion on the left side of the head (a) August 5, 2016; (b) December 9, 2016).

Histopathologic examination

From all cases, tissue samples or biopsies were submitted for histopathology, and all showed a marked infiltration of multifocal to coalescing eosinophilic granulomas.

Frequently, these contained a core of eosinophilic necrotic debris mixed with many degenerate eosinophils surrounded by epithelioid macrophages with few lymphocytes and plasma cells in the adjacent tissue (Fig 3), with occasionally multifocal moderate to large clusters of coccoid bacteria (cases 4 and 5; Fig 4). In cases 1, 4 and 5, the granulomas contained sections of nematodes of up to 60 µm diameter (Fig 4).

PCR

A seminested PCR assay was performed on tissue samples from cases 4 and 5 by the Department of Veterinary Clinical Sciences of the University of Teramo in Italy. In the present study, case 4 tested positive for the DNA of *H microstoma*, and the sample of case 5 was negative for *Habronema* species.

Discussion

Habronemiasis in horses has predominantly been described in tropical and subtropical climates. To the authors' knowledge, this is the first description of a case series of (peri)ocular habronemiasis in North-Western Europe. In Western Europe, to our knowledge, only one confirmed case of ocular habronemiasis has been published thus far.[12] Cutaneous habronemiasis has been documented twice in the UK.[13 14]

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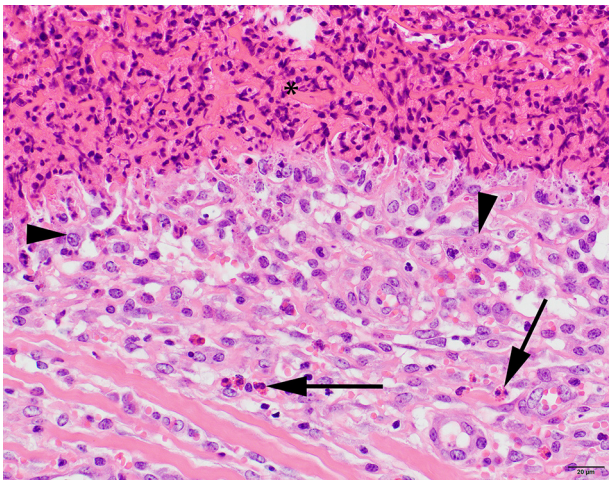

FIG 3: Eosinophilic granuloma: a core of eosinophilic necrotic debris (*), surrounded by epithelioid macrophages (black arrow) and eosinophilic granulocytes (black arrowhead), haematoxylin and eosin stain.

Several surveys in Western Europe show a wide variation in the occurrence of gastric habronemiasis as revealed by postmortem examination of the stomach: in the Netherlands 4 per cent of the 70 horses examined,[15] in Belgium 55 per cent of 11 horses,[16] in Northern France 8.5 per cent of 410 horses,[11] in Germany 33 per cent of 400 horses[17] and in Sweden 1.1 per cent of 461 horses.[18] This raises questions about the comparability of the test populations or the influence of other factors. No longitudinal information is available to evaluate whether changes over time have occurred in the gastric infestation rates.

We suspect that the prevalence of (peri)ocular habronemiasis in the Netherlands has increased because four of the five described cases were seen in the two last summer seasons. An increased prevalence may be related to a higher fly density, altered anthelmintic strategies and/or increased parasitic resistance. The increase in population of vector species may be related to climate change[19] and/or decreased use of pesticides.

A second possibility for an increasing prevalence of habronemiasis may be the selective anthelmintic treatment policy currently recommended for intestinal parasite control in Western Europe.[20] Some parasites are not well controlled by this anthelmintic strategy, and this may cause an increased occurrence.[21]

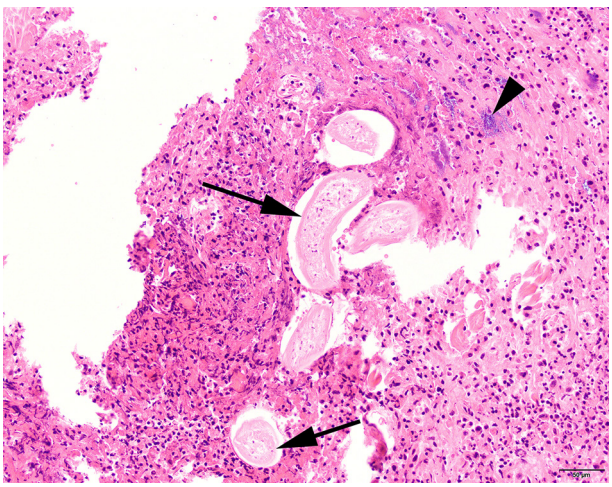

FIG 4: Cross sections of nematodes (black arrow) and deposits of coccoid bacteria (black arrowhead), haematoxylin and eosin stain.

A third possibility is increased resistance to anthelmintic treatment. Currently, there is evidence for the development of increased resistance of the equine cyathostomes and *Parascaris equorum* to macrocyclic lactones including ivermectin and moxidectin.[22] There are, however, no reports on the resistance of *Habronema* species to these drugs. *Habronema* species are not included in the standard faecal egg count reduction tests generally used in surveys documenting parasitic resistance, because the eggs of *Habronema* species are very difficult to demonstrate with coproscopical concentration techniques. Many authors have demonstrated the high efficacy (98–100 per cent) of ivermectin and moxidectin against adult *Habronema* species.[23–25] A more recent survey from Brazil demonstrated an efficacy of 92–95 per cent, 98–100 per cent and 100 per cent of ivermectin, abamectin and moxidectin, respectively.[26] However, recent reports within the geographical area of this study region are lacking, and one cannot exclude the possibility of more recently developed parasitic resistance.

A definitive diagnosis of habronemiasis cannot always be made, because the sensitivity of finding larvae on histopathologic examination of clinical cases appears to be low.[5 9 27 28] This could be explained by the fact that the larvae tend to be few in number, and might be digested or necrotic in the more chronic lesions. *Habronema* larvae live for less than one month in cutaneous tissues, and larval death might cause even more necrosis and calcification than a living parasite.[29] In all our cases, the biopsies were collected at a later stage of the disease. This might have decreased the chances of identifying the larvae on histological examination. The individual cases that yielded positive results, had the shortest duration of clinical signs (three weeks, case 1) or the most severe lesions (cases 4 and 5). In the more severely affected cases 4 and 5, clusters of coccoid bacteria were seen on histology. These bacteria were not further characterised as their presence was interpreted as a secondary bacterial infestation, which was to be expected given the extensive ulceration.

Since histopathologic examination has a limited sensitivity, molecular diagnosis (PCR) of habronemiasis may be a useful tool[8] and we included this test in our diagnostic procedure in 2016. For optimal diagnosis, we suggest that clinical findings, cytology, histopathology and PCR should be combined. In case 5, the PCR tested negative, although larvae were found on histopathologic examination. This may be explained by the fact that not all the affected tissue contains larvae. Another possibility is that amplification failure may have occurred due to PCR inhibition. Furthermore, the PCR is designed for the detection of *H microstoma* and *H muscae*, and not *D megastoma*.

The differential diagnoses for (peri)ocular habronemiasis are squamous cell carcinomas, foreign

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body granulomas and phycomycosis,[1 5] and the diagnosis will be confirmed by histology and/or PCR.

Many different treatment options for habronemiasis have been described. In general, the treatment should aim at eliminating the infection, decreasing the inflammation and reducing the size of the lesion.

The role of anthelmintic drugs is debatable as the lesions are thought to be a result of local hypersensitivity to dead or dying larvae.[1 3] In some cases, the administration of ivermectin may worsen the signs of pruritus.[4] Only one study investigated the use of ivermectin as a single treatment for *Habronema* lesions, but this did not provide solid evidence for its efficacy as a sole treatment.[30] However, anthelmintic drugs play an important role in preventing (re)infection.[1 3 4] By eliminating the adult stages of the parasite in the stomach, no eggs will be produced, and this will result in a decrease in the number of infected flies. This effect has been demonstrated as a result of moxidectin therapy by Schuster and Sivakumar.[31] If lowering infection pressure would be the incentive, one could consider treating other in contact horses. Local anthelminthic treatment of the lesions with solutions of ivermectin, echothiophate and trichlorfon has been described.[1 10] However, there is no evidence for its use in the treatment of the lesion itself.

In many cases, surgical debulking and treatment with corticosteroids can reduce the size of the lesion and the extent of the inflammatory response. Corticosteroids can be administered systemically, topically, intralesionally or subconjunctivally.[1] We instituted the latter treatment in two cases because frequent topical treatment of the eye was no longer possible due to the behaviour of the horse. Case 1 was treated with levamisole topically, because a *Thelazia lacrymalis* infection was considered as a possible differential diagnosis at the time. In retrospect, the lesions were not typical for an infection with *Thelazia* species, because those lesions are located in the lacrimal duct and lacrimal gland, and are not associated with parasite migration and a hypersensitivity reaction.[2] The efficacy of levamisole has not been tested against *Habronema* species, and we would not include it in the treatment of future cases. Case 5 did not respond to the initial treatment, and no other treatment options were implemented in this case because of financial constraints. However, the lesions started to regress in October, most likely due to the onset of colder weather. This phenomenon may be attributed to the decreasing number of flies.

Prevention of (re)infection is an important part of the management of habronemiasis. Fly control is essential since the flies serve as intermediate hosts. Elimination of their breeding environment decreases the number of flies. Hence, proper waste and manure management and regular cleaning of stables and paddocks is paramount.[32] Fly nets and blankets can protect specific areas, with face masks being beneficial against (peri)ocular habronemiasis. Horses can be stabled during peak fly hours, and fly repellents may also be an effective management tool.

Conclusions

This case series suggests an increased incidence of (peri)ocular habronemiasis in the Netherlands. The diagnosis should be considered in Western Europe when being presented with a horse with a granulomatous conjunctivitis/dermatitis. The presence of sulphur granules and cytology revealing many eosinophils may aid the tentative diagnosis. Making a definitive diagnosis by demonstrating larvae on histopathologic examination may be difficult, but the PCR for *Habronema* species may be useful. Treatment should be directed at eliminating the infection, decreasing the inflammation and reducing the size of the lesion. Anthelmintic treatment and fly control are important measures in prevention of (re)infection.

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Competing interests None declared.

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