Meerkats (Suricata suricatta), a new definitive host of the canid nematode Angiostrongylus vasorum

Gillis-Germitsch, Nina; Manser, Marta B; Hilbe, Monika; Schnyder, Manuela


Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-141634
Published Version

Originally published at:
Meerkats (*Suricata suricatta*), a new definitive host of the canid nematode *Angiostrongylus vasorum*

Nina Gillis-Germitsch\(^a\), Marta B. Manser\(^b\), Monika Hilbe\(^c\), Manuela Schnyder\(^b,\)\^\* 

\(^a\) Institute of Parasitology, Vetsuisse-Faculty, University of Zurich, Winterthurerstrasse 266a, 8057 Zurich, Switzerland 
\(^b\) Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland 
\(^c\) Institute of Veterinary Pathology, Vetsuisse-Faculty, University of Zurich, Winterthurerstrasse 268, 8057 Zurich, Switzerland

**A R T I C L E   I N F O**

Keywords:
*Angiostrongyulus vasorum*
Cardiopulmonary nematode
Meerkat
*Suricata suricatta*
New definitive host

**A B S T R A C T**

*Angiostrongylus vasorum* is a cardiopulmonary nematode infecting mainly canids such as dogs (*Canis familiaris*) and foxes (*Vulpes vulpes*). Natural infections have also been reported in mustelids and red pandas (*Ailurus fulgens fulgens*). We report the occurrence of natural *A. vasorum* infections in a group of captive meerkats (*Suricata suricatta*), housed at a university facility in Switzerland. *A. vasorum* first-stage larvae (L1) were initially identified in a pooled faecal sample. Individual samples, investigated with the Baermann-Wetzel technique, revealed that 41% (7/17) of the meerkats were infected, with ranges of 2–125 L1/g faeces. PCR and sequencing of part of the ITS-2 region resulted in 100% identity with *A. vasorum*.

1. Introduction

*Angiostrongylus vasorum* is a cardiopulmonary nematode increasingly diagnosed across Europe. Canids such as dogs and foxes represent the most common definitive hosts and slugs and snails the most common intermediate hosts (Koch and Willesen, 2009). Frogs were reported to be intermediate and paratenic hosts (Bolt et al., 1993) and chickens paratenic hosts (Mozer and Lima, 2015) under experimental conditions. Definitive hosts usually become infected by deliberate or accidental ingestion of intermediate hosts containing infectious third stage larvae (L3) (Guilhon and Cens, 1973; Schnyder, 2015). Ingestion of grass, food or water contaminated with secretions of infected gastropods may also lead to infection (Barcante et al., 2003; Morgan et al., 2005). Clinical manifestation in infected dogs range from mild and unspecific signs, such as exercise intolerance and inappetence, to severe respiratory signs and bleeding disorders which can lead to fatal outcomes (Staebler et al., 2005; Sigrist et al., 2017). In addition to dogs (*Canis familiaris*) and foxes (*Vulpes vulpes*) natural *A. vasorum* infections were observed in wolves (*Canis lupus*) (Segovia et al., 2001), coyotes (*Canis latrans*) (Bourque et al., 2005), golden jackals (*Canis aureus*) (Takács et al., 2013), crab-eating foxes (*Cerdocyon thous*) (Duarte et al., 2007), hoary foxes (*Dusicyon vetulus*) (Lima et al., 1994), red pandas (*Ailurus fulgens fulgens*) (Patterson-Kane et al., 2009; Bertelsen et al., 2010), Eurasian badgers (*Meles meles*) (Torres et al., 2001), stoats (*Mustela erminea*), a weasel (*Mustela nivalis*) (Simpson et al., 2016) and an otter (*Lutra lutra*) (Madsen et al., 1999).

Meerkats (*Suricata suricatta*), also known as suricates, belong to the Herpestidae family and originate from arid regions of southern Africa; they are mainly insectivores but also feed on small vertebrates (Doolan and Macdonald, 1996). Suricates are popular animals held in zoos worldwide and are frequently studied for their social behaviour (Clutton-Brock and Manser, 2016). In this study we present the first
report of infections with *A. vasorum* in a group of captive meerkats.

2. Material and methods

2.1. Meerkats

The group of meerkats consisted of 17 individuals (9 females and 8 males) between 2.5 and 8.5 years of age. All meerkats were born in captivity and kept for behavioural studies. The enclosure is located at a facility at the University of Zurich, Switzerland, and comprised of a heated indoor area which can be fully closed off, and an outdoor area with a partial open roof and walls, consisting of concrete or wire mesh fencing. The indoor and outdoor area measure 61 m² and 262 m², respectively. Substrate in both areas is sandy and between 20 and 180 cm deep, containing some larger rocks and branches. General hygiene principles are applied when entering the enclosure or feeding. Meerkats are fed three meals daily with a variety of fruit, mealworm, crickets, eggs, chicks and/or pellets. Occasionally, meerkats were observed to catch and eat birds, which eggs, chicks and/or pellets. Meerkats undergo routine faecal examination three to four times a year.

2.2. Laboratory analyses

During a routine faecal analysis of a pooled sample of the whole group performed at the Institute of Parasitology, University of Zurich, *A. vasorum* first stage larvae (L1) were microscopically identified (Fig. 1) based on their length and tail morphology (Guilhon and Cens, 1973; Deplazes et al., 2016). Meerkats were subsequently fed non-harmful glitter of different colours and shapes for individual differentiation of faecal matter and individual samples were collected for three consecutive days. Pooled three days faecal samples of each individual were quantitatively analysed by the Baermann-Wetzel technique (Deplazes et al., 2016) based on their length and tail morphology (Guilhon and Cens, 1997). DNA was extracted from the isolated larvae using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer’s instructions, followed by PCR analysis for detection of part of the ribosomal internal transcribed spacer (ITS) 2-region of *A. vasorum* (Jefferies et al., 2011). Two days after the individual faecal testing, one positive meerkat died. The animal was necropsied at the Institute of Veterinary Pathology, University of Zurich.

![Fig. 1. Angiostrongylus vasorum first stage larva isolated by the Baermann technique from a collective faecal sample of a meerkat group. Average size: 332 μm in length and 14.1 μm in width.](image-url)

2.3. Examination of potential intermediate hosts

Potential intermediate or paratenic hosts for *A. vasorum* were collected and examined in order to identify potential sources of infection in the surrounding area of the meerkats enclosure.

Seven months after diagnosing *A. vasorum* infection in meerkats (skipping the cold winter months when gastropod collection is virtually impossible), slugs and snails were collected from the ground, field and bushes around a small periphery (maximum 20 m) from the meerkats enclosure: slugs and snails were collected monthly from May to October, except for June, at dawn on a rainy day. Slugs and snails were euthanized by freezing at −20 °C. Collected specimens were identified to the family level according to Boschi (2011). For further processing the posterior end of larger slugs was cut off with a clean scalpel and transferred to a 2 ml Eppendorf tube, smaller snails and slugs were processed entirely. Each sample was weighed and 500 μl distilled water was added. Samples were processed with the TissueLyser II (Qiagen, Hilden, Germany) with a 5 mm stainless steel bead at 30 beats per second for 10 min. Five hundred mg of 16 pooled samples, consisting of 100 μl of 9–15 gastropods each, were digested with proteinase K and lysis buffer overnight. DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Samples were directly analysed by PCR detecting part of the ITS 2-region of *A. vasorum* (Jefferies et al., 2011). Individual sample analysis of the positive pooled samples was subsequently performed in order to identify single positive slugs and snails.

During the time of the investigation one dead thrush (*Turdus philomelos*) and one bird wing (species unknown) were found in the meerkats enclosure. Tissue samples of muscle and/or organs from the animals were chopped finely and digested in 1% HCL solution with 2.4 g pepsin (800–2500 U mg, Sigma P700, Sigma-Aldrich, Missouri, USA) per litre in a 45 °C water bath rotary shaker for 1 h. Samples were washed with water and centrifuged for 3 min at 500 G and this step was repeated twice. The sediments were then examined under a stereo-microscope for *A. vasorum* L3.

3. Results

3.1. Detection of first stage larvae of *A. vasorum* and biomolecular confirmation

Seven (4 females and 3 males) of 17 meerkats were shedding live *A. vasorum* L1. The L1 showed the distinct dorsal spine and kink at the tip of the tail of *A. vasorum* (Fig. 1) (Guilhon and Cens, 1973). They measured on average 332 μm in length and 14.1 μm in width (measured on 21 larvae), which is within range of previous size descriptions for *A. vasorum* L1 (Deplazes et al., 2016). The number of larvae per gram faeces (LPG) ranged from 2.4 to 125 (mean: 37.7) per animal (Table 1). Sequencing analysis of the larvae confirmed the presence of *A. vasorum* with a 100% identity (GenBank accession nos. KF270683, GU045376, EU627598) (Fig. 2).

All meerkats, including the negative ones, were topically treated with 10 mg imidacloprid/2.5 mg moxidectin (Advocate*, Bayer Animal Health) per animal shortly after individual diagnosis. One, four and ten months after treatment faeces of all meerkats were negative for *A. vasorum* L1.

3.2. Necropsy and histology of one meerkat

The sudden death of one meerkat was caused by a ruptured spleen with associated blood loss, most likely due to a traumatic event such as a fall. In the lung one adult *A. vasorum* specimen was found upon macroscopic examination after an incision into a diaphragmatic lobe. Histological examination of the lung revealed a multifocal granulomatous pneumonia caused by *A. vasorum* larvae and eggs as well as intima and media hyperplasia and isolated arteriosclerosis of larger
3.3. Potential intermediate and paratenic hosts

A total of 193 slugs (n = 146) and snails (n = 47) were collected (Table 2). The most commonly found gastropods belonged to the family Arionidae (62.7%, n = 121), followed by snails of the Clausiliidae family (22.8%, n = 44). Gastropods from the family Agriolimacidae (9.8%, n = 19), Limacidae (3.1%, n = 6) and Oxychilidae (0.5%, n = 1) were less common. Two small snails could not be identified due to broken shells. The number of gastropods collected each month and tested PCR positive for *A. vasorum* are presented in Table 2.

Three of the 16 pooled gastropod samples were positive for *A. vasorum*. These three pools consisted of 37 individual gastropod samples of which four were positive after individual testing (Fig. 4). Sequencing revealed 100% identity with *A. vasorum* (GenBank accession nos. KF270683, GU045376, EU627598) (Fig. 2) for all four samples based on 78 base pairs (bp). The four positive slugs were all from the Arionidae family, resulting in a prevalence of 3.3% (4/121; 95% Confidence Intervals, CI: 0.9–8.2%) for Arionidae slugs and an overall prevalence of 2.1% (CI: 0.6–5.2%, 4/193) for all collected gastropods. Three of the positive slugs were collected in July and one in September (Table 2). The thrush and the bird wing did not harbour any nematode larvae.

4. Discussion

We report the first case of *A. vasorum* in meerkats and within the Herpestidae family. Meerkats were shown to act as definitive hosts by harbouring an adult specimen and releasing live L1 in their faeces.

![Fig. 2. Sequence alignment of part of the internal transcribed spacer (ITS) 2-region (78 bp) of *Angiostrongylus vasorum* (GenBank accession no. KF270683), of DNA isolated from *A. vasorum* first stage larvae obtained from meerkats and from one slug.](image1)

![Fig. 3. Histological image of the lung of an *Angiostrongylus vasorum* infected meerkat. An adult nematode within a thickened artery (A), larvae and eggs surrounded by plasma cells, macrophages, neutrophilic granulocytes and lymphocytes (B) are visible, indicating granulomatous pneumonia as well as intima and media hyperplasia of larger lung vessels.](image2)
A. vasorum has a recognised medical indication for treatment and prophylaxis in non-canid species. The combination moxidectin/imidacloprid is mostly incidental. Little is known on anthelmintic treatment of A. vasorum in non-canids and diagnosis in meerkats was mostly incidental. However, reports of clinical signs and pathological changes due to A. vasorum larvae and eggs similar to that seen in infected canid and non-canid species (Poli et al., 1991; Patterson-Kane et al., 2009; Schnyder et al., 2010; Eleni et al., 2014; Simpson et al., 2016). No evidence of the presence of A. vasorum infective stages was obtained. Similarly, frogs were found in or were sighted around the enclosure and are described as potential intermediate or paratenic hosts under experimental conditions (Bolt et al., 1993), but the meerkats were never observed to eat frogs during the observation period.

A. vasorum is widespread in dogs and foxes in Switzerland and neighboring European countries (Barutzki and Schaper, 2009; Magi et al., 2009; Guardone et al., 2013; Lurati et al., 2015; Gillis-Germitsch et al., 2017; Maksimov et al., 2017). The area of Zurich, where the meerkats enclosure is located, has been previously to be endemic for A. vasorum in definitive hosts (Lurati et al., 2015; Gillis-Germitsch et al., 2017) and is now additionally supported by the identification of positive mollusc intermediate hosts. The meerkats likely acquired the infection by ingestion of an intermediate mollusc host harbouring infectious L3, since 2.1% of gastropods encountered within only 20 m of the meerkats enclosure were harbouring A. vasorum, a higher prevalence than one (1.6%) found in molluscs in hyperendemic areas (Patel et al., 2014). The highest number of gastropods and of A. vasorum positive specimens were collected in July. This reflects previous findings of increasing or high numbers of A. vasorum positive gastropods in summer months (Ferdushy et al., 2009; Jefferies et al., 2009).

Birds were occasionally found in the enclosure and chicken have been experimentally shown to act as potential paratenic hosts of A. vasorum (Mozzer and Lima, 2015), but for birds no evidence of the presence of A. vasorum infective stages was obtained. Similarly, frogs were found in or were sighted around the enclosure and are described as potential intermediate or paratenic hosts under experimental conditions (Bolt et al., 1993), but the meerkats were never observed to eat frogs during the observation period.

Meerkats do not naturally occur in A. vasorum endemic areas. However, they are popular and common zoo held animals worldwide. Enclosures of zoo animals are very rarely hermetically sealed and intermediate or paratenic hosts of A. vasorum may find their way into enclosures.

In a recent report from Italy Angiostrongylus dujardini was reported in meerkats (Eleni et al., 2016): two young meerkats suffering from anorexia and tachypnea died due to infection with this lungworm, a species usually infecting rodents and also transmitted by ingestion of molluscs.

It is therefore essential to regularly check enclosures of meerkats and potentially further animal species susceptible for an A. vasorum infection for intruding of intermediate or paratenic hosts. Furthermore, in susceptible animals living in A. vasorum endemic areas regular faecal examination for gastrointestinal parasites should be complemented by the Baermann technique in order to early identify a potential infection with lungworms and treat infected animals accordingly, preventing complications due to the infection.

Conflict of interest

None.

Funding

The captive meerkat population is funded by the University of Zurich. We would like to acknowledge Bayer Vital GmbH, Business Unit Animal Health, Germany, for the financial support of Nina Gillis-Germitsch in the form of a doctoral fellowship.
Ethics approval

Meerkats were facility-born at the University of Zurich. All institutional and national guidelines for the care and use of laboratory animals were followed upon approval by the Cantonal Veterinary Office of Zurich (animal permission number: 153).

Acknowledgements

The authors would like to acknowledge Francesca Gori and Isabelle Specker for their help with laboratory analyses and Lucienne Tritten and Felix Grimm for providing support with sequence interpretation. We would also like to thank Megan Wyman for gathering additional information about the meerkats and Annakatrin Häni for providing the slug identification book as well as the technical laboratory staff of the Institute of Veterinary Pathology for the preparation of the histological slides.

References


