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

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ABSTRACT

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, whereas none of the bird samples were positive. Meerkats kept in captivity in areas where A. vasorum is endemic and with potential contact to intermediate hosts are at risk of infection. Regular faecal examinations including Baermann-Wetzel technique should be considered.

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 Willeisen, 2009). Frogs were reported to be intermediate and paratenic hosts (Bolt et al., 1993) and chickens paratenic hosts (Mozzer 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 (Barçante 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. Occasionally, meerkats were observed to catch and eat birds, which eggs, chicks and/or pellets. Occasionally, meerkats were observed to catch and eat birds, which eggs, chicks and/or pellets. Occasionally, meerkats were observed to catch and eat birds, which eggs, chicks and/or pellets. Occasionally, meerkats were observed to catch and eat birds, which eggs, chicks and/or pellets.

#### 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 day faecal samples of each individual were quantitatively analysed by the Baermann-Wetzel technique (Deplazes et al., 2016) by determining the number of L1 per gram of analysed faeces. Larvae were morphologically confirmed. DNA was extracted from the isolated larvae using the QIAmp 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). 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. Previous studies showed that *A. vasorum* has a low intermediate host specificity (Guilhon and Bressou, 1960; Eckert and Lämmler, 1972; Patel et al., 2014). Our findings instead, represent a further testimony that *A. vasorum* has also a low definitive host specificity as it not only infects canids but also mustelids (Torres et al., 2001; Simpson et al., 2016) and ailurids (Patterson-Kane et al., 2009).

At the time of diagnosis none of the infected meerkats showed clinical signs: it can be argued that the infections may have been of recent origin, since with a supposed patency of approximately seven weeks and faecal examinations routinely performed three to four times

### Table 1

| ID | sex | LPG |
|----|-----|-----|
| 1  | F   | 0   |
| 2  | M   | 0   |
| 3  | F   | 0   |
| 4  | F   | 66.7|
| 5  | F   | 0   |
| 6  | F   | 0   |
| 7  | M   | 0   |
| 8  | M   | 33.3|
| 9  | M   | 0   |
| 10 | M   | 125 |
| 11 | F   | 2.4 |
| 12 | F   | 10  |
| 13 | F   | 3.8 |
| 14 | F   | 0   |
| 15 | M   | 0   |
| 16 | M   | 0   |
| 17 | M   | 22.7|

* This individual died two days after diagnosis, due to a ruptured spleen.

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.

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. (Hematoxylin and eosin staining, original magnification x 100 (A), x 400 (B)).
against 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* larvae and eggs similar to that seen in infected canid and non-candid species (Poli et al., 1991; Patterson-Kane et al., 2009; Schnyder et al., 2009; Magi et al., 2009; Guardone et al., 2013; Lurati et al., 2014; Lurati et al., 2015; Gillis-Germitsch et al., 2017). The area of Zurich, where the meerkats enclosure is located, has been shown 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 infective 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

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| Month of collection | Total number of collected gastropods (n) | *A. vasorum* PCR positive (n, %) | Families of the collected gastropod species |
|---------------------|------------------------------------------|-------------------------------|------------------------------------------|
| May                 | 35                                       | 0 (0%)                        | Arionidae (n) 11 0 0 22 0 2            |
| July                | 82                                       | 3 (4.4%)                      | Limacidae (n) 0 13 1 0 0              |
| August              | 39                                       | 0 (0%)                        | Agriolimacidae (n) 34 4 1 0 0         |
| September           | 33                                       | 1 (12.5%)                     | Clausiliidae (n) 8 2 3 19 1 0         |
| October             | 4                                        | 0 (0%)                        | Oxychilidae (n) 0 0 2 2 0 0           |
| Total               | 193                                      | 4 (2.1%)                      | Unidentifiable (n) 121 6 19 44 1 2     |

* Only gastropods belonging to the Arionidae were found positive by PCR.

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**Table 2**

Number of slugs and snails collected between the months of May and October around the enclosure of *Angiostrongylus vasorum* infected meerkats.

| Month of collection | Total number of collected gastropods (n) | *A. vasorum* PCR positive (n, %) |
|---------------------|------------------------------------------|-------------------------------|
| May                 | 35                                       | 0 (0%)                        |
| July                | 82                                       | 3 (4.4%)                      |
| August              | 39                                       | 0 (0%)                        |
| September           | 33                                       | 1 (12.5%)                     |
| October             | 4                                        | 0 (0%)                        |
| Total               | 193                                      | 4 (2.1%)                      |

**Fig. 4.** Agarose gel (1.5%) stained with GelRed<sup>®</sup> showing PCR products of part of the internal transcribed spacer (ITS) 2-region of *Angiostrongylus vasorum* of 37 gastropod samples: samples no. 8, 23, 29 and 35 were positive. M: DNA ladder, N: negative control, P: positive control.
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).

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