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Research paper

Use of a commercial serologic test for *Angiostrongylus vasorum* for the detection of *A. chabaudi* in wildcats and *A. daskalovi* in badgers

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ABSTRACT

Three species of the genus *Angiostrongylus* are known to infect European carnivores: *A. vasorum* (mainly in canids but also in other carnivores), *A. chabaudi* (in felids) and *A. daskalovi* (in mustelids). *A. vasorum* is responsible for clinically severe disease in domestic dogs, most commonly diagnosed based on fecal examination and serological detection of circulating antigens. Considering the poorly known host range and the challenging larval differentiation in the feces between the three species of *Angiostrongylus* infecting European carnivores, our aim was to evaluate the cross-reactivity of *A. chabaudi* and *A. daskalovi* with *A. vasorum* using a commercial serologic test developed for domestic dogs. Badgers (*Meles meles*) (n = 10) and wildcats (*Felis silvestris*) (n = 8) were examined between 2015 and 2016 by full parasitological necropsy with subsequent morphological and molecular identification of nematodes and by serology, using IDEXX Angio Detect™ tests. Five out of the ten badgers and two out of the eight wildcats were harboring nematodes in the pulmonary arteries. All nematodes were identified morphologically as *A. daskalovi* in badgers and *A. chabaudi* in wildcats, respectively. Serological examination of the plasma samples revealed the positivity of the same animals as found in necropsy. None of the animals negative at necropsy was positive at serology. The 100% correlation between the necropsy results and the serologic positivity to IDEXX Angio Detect™ in badgers infected with *A. daskalovi* and wildcats infected with *A. chabaudi* suggest that these rapid tests are able to identify circulating antigens of all species of *Angiostrongylus* found in European carnivores: *A. vasorum*, *A. daskalovi* and *A. chabaudi*. The possibility for future in-clinic use of this test in domestic cats should be further investigated.

1. Introduction

The genus *Angiostrongylus* (superfamily Metastrongyloidea) includes 21 species with global distribution. The members of the genus are parasitic in a wide variety of mammalian hosts (rodents, insectivores, treeshrews, and carnivores) (Spratt, 2015). Among these, five species have been reported in carnivores: *A. vasorum* in dogs and foxes (Anderson, 2000); wolves (Segovia et al., 2001); coyotes (Bourque et al., 2005); red pandas (Patterson-Kane et al., 2009); badgers (Torres et al., 2001), in Europe, Americas and Africa; *A. chabaudi* in wildcats (Varcasia et al., 2014) and domestic cats (Dias et al., 2008) in Europe; *A. daskalovi* in mustelids in Europe (Janchev and Genov, 1988); *A. gubernaculatus* in badgers in North America (Ubelaker, 1986) and *A. felineus* in pumas from South America (Vieira et al., 2013). Among the three species identified in carnivores from Europe, only *A. vasorum* has been studied in detail, mainly due to its significant clinical impact on domestic dogs. The other two European species known from carnivores, namely *A. chabaudi* and *A. daskalovi* are poorly known (Gherman et al., 2016a,b).

The diagnosis of canine angiostrongylosis is based on a combination of clinical and laboratory findings, including hematology, biochemistry, coprology, immunologic tests and molecular tools (as reviewed by Elsheikha et al., 2014). The infection with *A. vasorum* may cause a severe disease in dogs, with a heterogenic clinical picture, consisting mainly of cardiopulmonary symptoms, coagulopathy and neurological signs (as reviewed by Helm et al., 2010). Although some clinical signs are more common while oth-

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ers are relatively rare, it is not known which factors define the different clinical manifestations of the disease (Elsheikha et al., 2014). The most common method used for the diagnosis of canine angiostrongylosis is the Baermann migration-sedimentation technique, by which L1 from feces are identified based on morphological features (Elsheikha et al., 2014). However, after the recent description of the fecal larval stages of *A. chabaudi* (Diakou et al., 2016; Gherman et al., 2016b) and *A. daskalovi* (Gherman et al., 2016a) it is evident that the L1 of *A. vasorum* are morphologically indistinguishable from those of the other two species infecting European carnivores. Hence, at least in theory, the larvae found in the feces of dogs could belong to any of the European *Angiostrongylus* species associated with carnivores. *A. vasorum* is known to infect a broad range of carnivore hosts belonging to four families, including mustelids (Torres et al., 2001; Magi et al., 2010), which are at the same time the typical hosts for *A. daskalovi*. Due to the lack of extensive host specificity studies for *A. chabaudi* and *A. daskalovi*, the possibility that dogs could be infected with these species, cannot be ruled out.

Moreover, as the Baermann method is considered to have certain limitations (fresh feces needed, intermittent shedding of larvae, possible misidentification with other parasitic or pseudoparasitic larvae) (Helm et al., 2010), more recently, several immune-based serologic tests have been developed for the detection of *A. vasorum* antigens (Verzberger-Epshtein et al., 2008; Schnyder et al., 2011) or antibodies (Cury et al., 1996; Cury et al., 2002; Schucan et al., 2012) in the blood of domestic dogs. A commercial blood test is also available for the detection of the antigens of *A. vasorum* in domestic dogs (IDEXX Angio Detect™, IDEXX Laboratories, USA). Generally, antigens of *A. vasorum* are detectable around five weeks after experimental infection of dogs, persist after elimination of the parasite and no cross-reactions with other canine parasites were found (Schnyder et al., 2011).

Considering the low host specificity of certain *Angiostrongylus* species, the unknown causes of the variable clinical outcome of infected dogs and the difficulty of larval differentiation in the feces between the three species infecting European carnivores, our aim was to evaluate the cross-reactivity of *A. chabaudi* and *A. daskalovi* with *A. vasorum* using a commercial serologic test developed for domestic dogs and the possibility of its use in other hosts.

2. Materials and methods

Between 2015–2016, during an extensive survey of parasite diversity in wild carnivores from Romania, badgers (*Meles meles*) (n = 10) and wildcats (*Felis silvestris*) (n = 8) collected by hunters or found as roadkills (Fig. 1) were examined by full parasitological necropsy. All the nematodes collected from the pulmonary arteries were identified morphologically and molecularly as described by Gherman et al. (2016a,b).

From each examined animal, a whole blood sample (2 ml) was collected from the heart in EDTA, centrifuged and the plasma preserved frozen at −20 °C. Subsequently, each plasma sample was examined using a commercial test (IDEXX, Angio Detect™) according to the manufacturer's instructions.

3. Results

Out of the ten examined badgers, 5 were harboring nematodes in the pulmonary arteries (Table 1). All nematodes were morphologically and molecularly identified as *A. daskalovi* as described by Gherman et al. (2016a). Serological examination of the plasma samples collected from badgers, using IDEXX Angio Detect™, revealed positivity of the same five samples, as in necropsy (Table 1).

Out of the eight wildcats, at necropsy, two were infected with nematodes in the pulmonary arteries, all identified as *A. chabaudi* (as shown by Gherman et al., 2016b). Serological examination of the plasma samples collected from wildcats, using IDEXX Angio Detect™, revealed positivity of the same two samples, as in necropsy (Table 1).

For all badgers and wildcats, all of the control lines of the IDEXX Angio Detect™ were positive (Fig. 2).

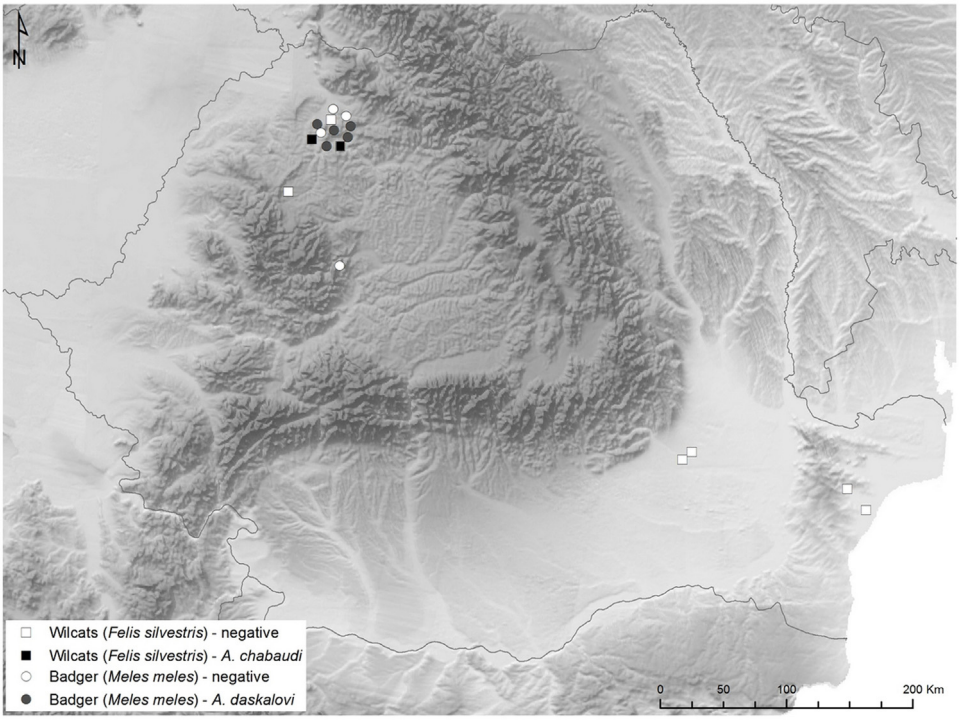

Fig 1. Geographical distribution of the collected samples (n = 10 badgers and n = 8 wildcats).