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ORIGINAL PAPER

Effect of temperature on activity of third-stage larvae of *Angiostrongylus vasorum*

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Abstract To evaluate the effect of temperature on the activity and mortality of the L3 of *Angiostrongylus vasorum*, 1,500 L3 were isolated from experimentally infected snails and distributed into five equal groups. Three groups were incubated at 37°C, 27°C, and 5°C. The remaining two groups were incubated at 27°C and 5°C for 10 days, at which time the temperature for the 27°C group was reduced to 5°C and the 5°C group increased to 27°C. Larva activity was observed daily and inactive larvae were removed. At 37°C, larvae survived up to 8 days. At 27°C, larvae were active until day 6. When subjected to a reduction in temperature from 27°C to 5°C beginning on day 10, the number of active larvae increased until day 13. Only on day 17 did the number of active larvae decline to zero. At 5°C, larvae remained active until day 15, surviving to 24 days. When temperature was increased from 5°C to 27°C beginning on day 10, larvae were found active until day 12 and maintained an intermediate level of activity to day 21. Survival of larvae was greater at lower temperatures, while high temperatures were associated with higher mortality.

Introduction

Angiostrongylus vasorum (Baillet 1866) Kamensky 1905 is a nematode parasite of the right ventricle and auricle, and pulmonary artery of dogs and wild canids. This parasite has a cosmopolitan distribution (Morgan et al. 2005), but is enzootic in dogs in Europe, especially in southeast France (Guilhon and Cens 1973), UK and Ireland (Dodd 1973). Random cases have been reported in the USA, Canada (Williams et al. 1985) and Brazil (Lima et al. 1985, 1994; Duarte et al. 2007). The life cycle of the parasite (and other metastrongyloids) begins with the elimination of the first larva (L1) in the feces of the definitive host into the environment, where they do not feed, relying on energy reserves. Consequently, their survival is dependent on a range of environmental factors. These L1 infect several species of terrestrial and aquatic snails (intermediate hosts) by ingestion and/or penetration (Rosen et al. 1970; Guilhon and Cens 1973; Thiengo 1996; Grewal et al. 2003; Morley 2010). After two molts, the third (L3) infective stage remains in the tissues of the snail or can be actively released into the environment, especially under light and/or thermal stimulus (Barçante et al. 2003). The infection of dogs occurs with the ingestion of intermediate, or paratenic, hosts, such as rodents and amphibians (Bolt et al. 1994), or by the ingestion of infective larvae free in the environment (Barçante et al. 2003).

Changes in temperature, salinity, UV light, and oxygen level can all affect the viability of nematode infective larvae in water (Thurston et al. 1994; Grewal et al. 2002). The level of infectivity of these larvae varies considerably among species, possibly related to foraging strategies, which incur different metabolic demands. Survival is related to declining energy reserves. Metabolic rates increase dramatically after a few weeks in water (Morley 2010). *A. vasorum* have free-living and intermediate host stages, the development and survival of which are strongly affected by temperature, water availability, and, consequently, local climate, and L3 may survive for many days or even months in fresh water (personal observation; Barçante et al. 2003). The main aim of this study was to evaluate the

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effect of temperature on *A. vasorum* L3 (infective stage) activity/mobility and mortality.

Material and methods

The life cycle of *A. vasorum* was reproduced in the Laboratory of Veterinary Helminthology (Departamento de Parasitologia, Universidade Federal de Minas Gerais). *A. vasorum* was isolated from two domestic dogs (*Canis familiaris* Linnaeus, 1758) from Caratinga municipality, Minas Gerais State. *Biomphalaria glabrata* Say, 1818 were experimentally infected with *A. vasorum* L1 derived from feces of the infected dogs (Ethical Committee approval no. 060/03). At 30 days post-infection, snails were crushed and L3 were isolated using the Baermann technique modified by Barçante et al. (2003).

Five groups of 300 L3 were each distributed in culture plates of six wells (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ), with 50 L3 per well supplemented with 3 ml of distilled water. The groups were subjected to controlled temperatures: (A) 37°C, (B) 27°C, and (C) 27°C, with a change to 5°C from day 10 until the end of the experiment, and (D) 5°C and (E) 5°C, with a change to 27°C from day 10 until the end of the experiment. The maintenance of temperature was made in different incubators (37°C and 27°C) or refrigerator (5°C), and temperature changes were made after counting larvae at day 10.

The degree of activity of the larvae was assessed by microscopic examination at 24-h intervals according to Richinitti et al. (1999). The larvae were classified as active (intense movement), intermediate (some movement), or inactive (dead larvae, C-shaped), which were removed from the experiment.

Results

In group A (37°C), the number of active *A. vasorum* larvae dropped to zero on day 3 of observation, while the number of inactive larvae rose during the same period. On day 8, the number of intermediate larvae reached zero (Fig. 1).

In group B (27°C), from day 7, active larvae were no longer observed and the number of intermediate larvae began to decrease until day 19 (Fig. 2). However, when the temperature was reduced to 5°C beginning day 10 (group C), there was an increase in the number of active larvae persisting to day 13 (Fig. 3). On day 17, the number of active larvae once again reached zero. Intermediate larvae were observed until day 24.

Group D (5°C) remained active until day 15 (Fig. 4). Intermediate larvae were found until day 24. When the larvae were exposed to a temperature of 27°C beginning day 10 (group E), active larvae were found up to day 12. Intermediate larvae were observed until day 21 (Fig. 5).

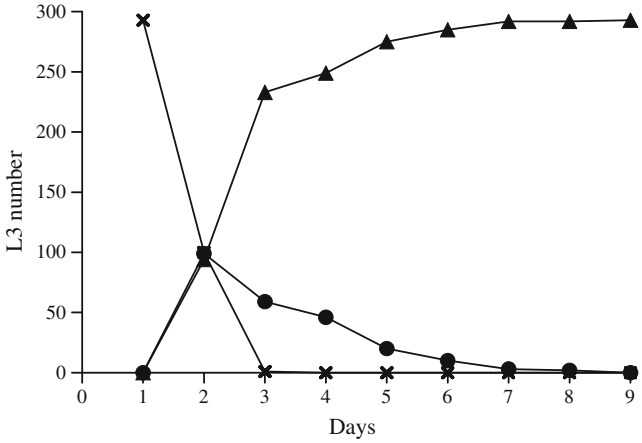

Fig. 1 Number of third-stage larvae of *A. vasorum* at 37°C according to the days of observation. Active larvae = intense movement (*line with x*); intermediate larvae = some degree of movement (*full circle*); and inactive = dead larvae, in the form of C (*full triangle*)

Discussion

Studies of *Angiostrongylus* show that infectivity of L3 is directly related to their motility and that larvae standing in a "c" position would represent inviable larvae (Richinitti et al. 1999). Contrary to what was suggested by Morera (1985) for *Angiostrongylus costaricensis*, our results showed that the L3 of *A. vasorum* survived longer at lower temperatures, increasing the potential for infection of the definitive host. Conversely, survival at high temperatures was greatly reduced. Richinitti et al. (1999) estimate, by mathematic model, that, independent of temperature,

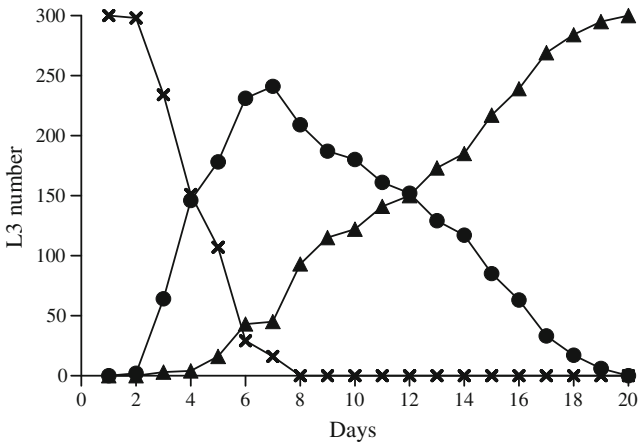

Fig. 2 Number of third-stage larvae of *A. vasorum* at 27°C according to the days of observation. Active larvae = intense movement (*line with x*); intermediate larvae = some degree of movement (*full circle*); and inactive = dead larvae, in the form of C (*full triangle*)

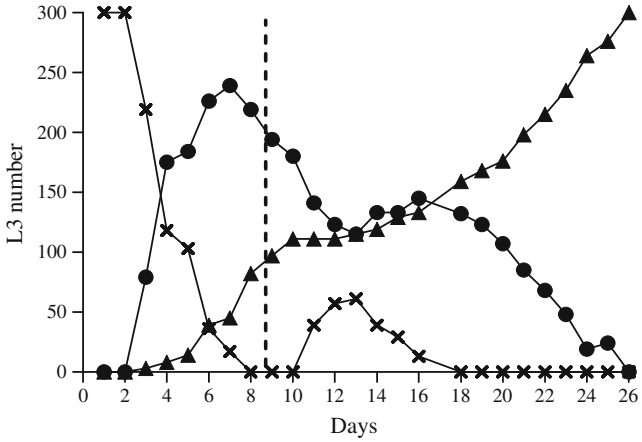

Fig. 3 Number of third-stage larvae of *A. vasorum* to the initial temperature of 27°C reduced to 5°C according to the days of observation. The *dashed line* indicates the date on which temperature was reduced. Active larvae = intense movement (*line with x*); intermediate larvae = some degree of movement (*full circle*); and inactive = dead larvae, in the form of C (*full triangle*)

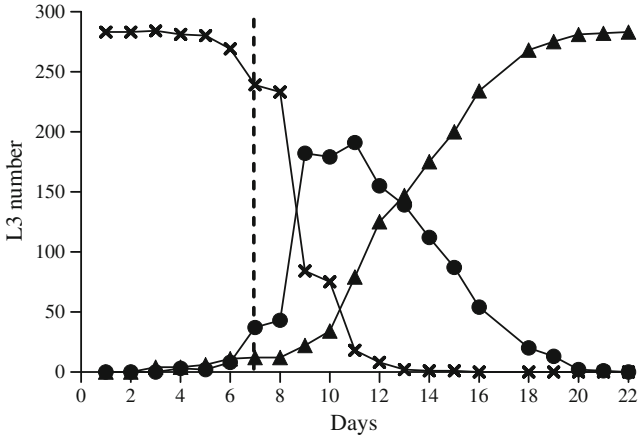

Fig. 5 Number of third-stage larvae of *A. vasorum* with the initial temperature of 5°C increased to 27°C according to the days of observation. The *dashed line* indicates the day on which temperature was increased. Active larvae were those with intense movement; intermediate larvae, those with some degree of movement; and inactive larvae were dead larvae, in the form of "C." *Line with "x"* indicates active larvae; with *full circle*, intermediary larvae; with *full triangle*, inactive larvae

80 days is the minimum length of time required for reaching a 100% reduction of active larvae of *A. costaricensis*. Studies of other Metastrongyloidea have shown that increasing temperature gradually reduces the survival of L1, affecting their viability, although response to temperature varies among species (Skorping 1982; Shostak and Samuel 1984; Lorentzen and Halvorsen 1986; Cabaret et al. 1991).

Although *A. vasorum* is considered a widely distributed parasite, most reports of angiostrongylosis in dogs are from temperate or cold areas, and it is considered enzootic in some areas, such as Europe (Patteson et al. 1993; Bolt et al. 1994; Morgan et al. 2009). Parasites with free-living stages or intermediate hosts exhibit a distribution highly dependent on climate: (1) Although many species of snails and slugs can act as intermediate hosts, slugs are less able than snails to survive cold winters and desiccation, and their activity is severely reduced under such conditions (Morgan et al. 2009). (2) The L3 of *A. vasorum* are able to leave the body of the intermediate host snail and contaminate the environment (vegetation, soil, and water; Barçante et al. 2003). (3) At high temperatures, collections of water, the surrounding environment, and the secretions left by the snails on vegetation and the ground dry up; under these conditions, the infective larvae do not survive, thus halting transmission. (4) High temperatures increase the metabolic rate of L3, negatively affecting survival (Weaver et al. 2010). (5) Areas with cooler climatic conditions allow these larvae to survive longer in the environment (water and/or secretions), increasing the chances of contact with a definitive host.

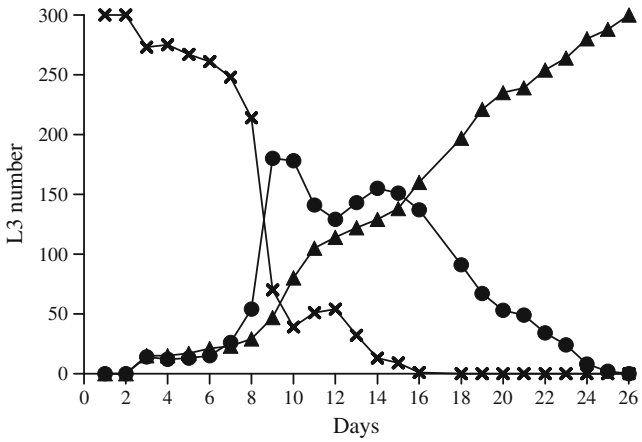

Fig. 4 Number of third-stage larvae of *A. vasorum* at 5°C according to the days of observation. Active larvae were considered those with intense movement; intermediate larvae, those with some degree of movement; and inactive larvae were dead larvae, in the form of "C." *Line with "x"* indicates active larvae; with *full circle*, intermediary larvae; with *full triangle*, inactive larvae

Morgan et al. (2010) shows that the period of higher infection rates by *A. vasorum* occurs in autumn, in temperate weather, an important factor in snail and slug development. Associating these data to our data, a warm temperature is favorable to the development and maintenance of the intermediate host and for the preservation of the active infective larvae of *A. vasorum* in the environment, which increases the risk of infection of the definitive host.

This report is consistent with literature showing that climate and temperature play an important role in aspects of parasite transmission such as dynamics and the activity of the intermediate host and parasite development in the intermediary host (Jenkins et al. 2006; Ferdushy et al. 2010; Morgan et al. 2010; Morley 2010).

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