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In vitro efficacy of *Metarhizium anisopliae* sensu lato against unfed *Amblyomma parvum* (Acari: Ixodidae)

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Abstract

Amblyomma parvum Aragão (Acari: Ixodidae) is a tick species found with wide distribution in the Neotropical region. Even though it is a wildlife-related tick, it is also a frequent parasite of domestic animals, is aggressive to human beings and may harbor pathogenic microorganisms. Therefore, it is a target species for control on domestic animals, particularly those at the rural–wildlife interface. Herein, the efficacy of two isolates (E9 and IBCB 425) of an entomopathogenic fungus, *Metarhizium anisopliae* sensu lato, already evaluated for ticks that parasitize domestic animals, was tested against unfed *A. parvum* adults. Both isolates displayed high acaricidal efficacy after immersion in fungal conidial suspensions for 5 min. Isolate E9 killed all ticks by the 7th day post-treatment, and isolate IBCB 425 did so by the 11th day. Tick mortality of 80 and 90% was achieved as early as the 3rd and 4th days, respectively, with both treatments. Thus, if a commercial *M. anisopliae* s.l. acaricide against domestic animal ticks is developed, it would also be effective against *A. parvum*.

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Introduction

Amblyomma parvum Aragão (Acari: Ixodidae) is a Neotropical tick species found in a range from Argentina to Mexico, and three distinct lineages can be distinguished within its broad geographic distribution (Lado et al. 2016). Within its range, it is associated with dry and hot environments where adults and immature stages parasitize medium- and large-sized mammals and rodents, respectively (Nava et al. 2008). Importantly, *A. parvum* is frequently found, together with livestock ticks, on several domestic animals, including cattle, horses, dogs and goats (Szabó et al. 2007; Nava et al. 2008; Ramos et al. 2016).

Furthermore, under experimental settings, several domestic and laboratory animals can sustain their entire parasitic life cycle alone (Olegário et al. 2011). In fact, parasitism of domestic animals by *A. parvum* may be enhanced by the mandatory conservation of natural resources within rural properties throughout Brazil and the increased intermingling of domestic and wild animals. Importantly, *A. parvum* ticks, particularly adults, were shown to be aggressive to humans (Ramos et al. 2014) as well as potential vectors of several pathogens. In this regard, this tick species has been found to be infected with *Coxiella burnetii, Ehrlichia chaffeensis* and *Candidatus* Rickettsia andeanae (Nieri-Bastos et al. 2014; Pacheco et al. 2013; Tomassone et al. 2008).

A relevant feature of *A. parvum* is that it is a small brown tick lacking ornamentation, and adults are, at first sight, easily confused with similarly small brown ticks such as *Rhipicephalus sanguineus* or with nymphs of other *Amblyomma* species. Therefore, it is prone to go unnoticed or confused with other tick species, and thus, domestic animal and human infestations are probably underreported.

Tick control on domestic animals relies heavily on acaricides, but with drawbacks. The resistance of tick populations to several acaricidal compounds is a common occurrence after massive use (Higa et al. 2015), and environmental toxicity, with effects on nontarget organisms, is of great concern (De Meneghi et al. 2016). This last aspect is especially important if one considers the abovementioned proximity of wildlife and domestic animals and mixed infestations with domestic and wildlife ticks. Among more environmentally friendly alternative tick control measures, entomopathogenic fungi such as *Metarhizium anisopliae* sensu lato were shown to be effective against ticks in laboratory trials. However, these trials were overwhelmingly conducted with ticks from domestic animals such as *Rhipicephalus microplus* and *R. sanguineus* (Bittencourt et al. 1999; Garcia et al. 2005, 2011). Herein we tested the acaricidal efficacy of *M. anisopliae* s.l. on unfed adult *A. parvum* ticks to evaluate whether a wildlife tick with a great potential to parasitize domestic animals exhibits similar sensitivity to this fungus under laboratory conditions as shown for other tick species.

Materials and methods

Metarhizium anisopliae s.l. fungus

Isolates E9 and IBCB 425 of *M. anisopliae* s.l. fungus, previously shown to have pathogenic effects in vitro against ticks from domestic animals (Monteiro et al. 1998; Garcia

et al. 2004, 2005), were used. Isolates for the experiments were acquired from the stock culture of the Microbiology Laboratory of the Plant Production Department, Faculty of Agrarian and Veterinary Sciences (FCAV)-São Paulo State University (Unesp), Jaboticabal City, São Paulo State, Brazil. These isolates were cultivated in Petri dishes containing a potato–dextrose–agar medium (PDA) at 27 °C in the absence of light for 12 days. Conidia from the surface of colonies were transferred to test tubes containing 20 mL of Tween 80®, 0.1% solution (v/v). The number of conidia per gram of rice in the suspensions was determined using a Neubauer chamber. The same suspensions described by Francisco et al. (2006) were used to determine conidial viability. Overall viability was shown to be 95%.

Amblyomma parvum ticks

Unfed adult ticks were acquired from a laboratory colony of *A. parvum* ticks kept in the Ixodology Laboratory of the Veterinary Faculty of the Federal University of Uberlândia, Minas Gerais, Brazil. Ticks from this colony originated from Araguapaz, Goiás Brazil, and were maintained by feeding them on tick-bite naïve New Zealand rabbits as described previously (Olegário et al. 2011). Laboratory tick colony maintenance procedures were approved by the ethics committee for animal usage of the Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil (protocol number 97/11).

Acaricide efficacy tests

Overall, 120 adult ticks (60 males and 60 females) were divided into two experimental groups and one control group. Each treatment was performed four times each with groups of ten ticks (quadruple testing). Treatment consisted of the immersion of ticks for 5 min in the fungal conidial suspension (either *M. anisopliae* s.l. E9 or TBCB test groups) at a concentration of 1.0×10^9 conidia/ml or the conidia suspension vehicle alone (control group). After immersion, the ticks were paper dried and individually stored in plastic tubes at 25 °C and 80% relative humidity under a 12:12 h light regimen. Tick infection and mortality were evaluated until the 11th day post-immersion. Tick infection was determined by visual inspection and mortality by the immobility of ticks stimulated by slight blowing.

Statistical analysis

The analysis of variance was conducted using the Kruskal–Wallis test. The level of significance was set at 5%.

Results

Both *M. anisopliae* s.l. isolates induced 100% mortality in unfed adult *A. parvum* ticks. Isolate E9 killed all ticks by the 7th day post-treatment, and isolate IBCB 425 did so by the 11th day (Fig. 1). Nevertheless, the results did not differ significantly; both isolates induced rapid mortality, and 80% and 90% of tick mortality was achieved as soon as the 3rd and 4th days, respectively, with both treatments. Control ticks began to die on the 5th day, and by day 11 after treatment, more than 70% remained alive.

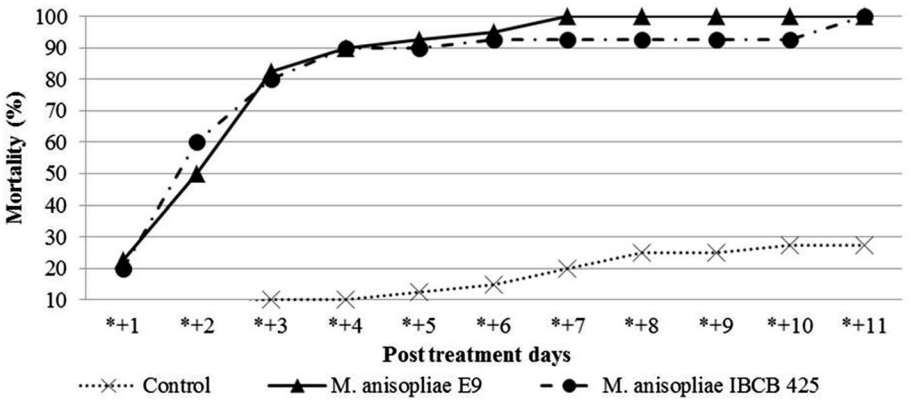

Fig. 1 Cumulative daily mortality rate of unfed adult *Amblyomma parvum* ticks following immersion in either *Metarhizium anisopliae* s.l. E9 or IBCB 425 isolate conidial solutions or control solution

The number of *A. parvum* ticks found dead each post-treatment day after immersion in either *M. anisopliae* s.l. isolate E9 or IBCB 425 conidial solutions or the control solution is displayed in Table 1.

Discussion

A high and fast mortality of unfed adult *A. parvum* ticks was observed after immersion in a conidial suspension of *M. anisopliae* s.l. isolates. This high mortality may have been caused by the high sensitivity of *A. parvum* to fungal infection and/or by the high concentration of the conidial suspension. *Amblyomma parvum* is a tick species found in xeric environments (Nava et al. 2008), and it can be speculated that it has not evolved appropriate defense mechanisms against parasites associated with moist surroundings, such as fungi. At the same time, the conidial suspension used was at a high concentration in relation to some other acaricidal tests (Basso et al. 2005; Polar et al. 2005; Lopes et al. 2007; Garcia et al. 2011) but not to all (Bittencourt et al. 2003). A conidial concentration increase was shown to be directly related to an increase in the tick mortality rate (Lopes et al. 2007). In this regard, conidial concentration in commercial products should ideally be high enough to attain the desired efficacy against several species if mixed tick infestations occur, as found in the case of *A. parvum* on domestic animals (Szabó et al. 2007; Ramos et al. 2016).

Table 1 Number of *Amblyomma parvum* ticks found dead each post treatment day after immersion in either *Metarhizium anisopliae* s.l. isolate E9 or IBCB 425 solutions or a control solution

Isolate	Number of ticks found dead each post treatment day											Total
	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	
E9	9	11	13	3	1	1	2	0	0	0	0	40
IBCB 425	8	16	8	4	0	1	0	0	0	0	3	40
Control	0	0	1	0	1	2	1	2	2	0	0	10

Both *M. anisopliae* s.l. isolates displayed a similar efficacy, although E9 exerted a somewhat faster effect than IBCB 425. This difference in acaricidal activity between the two isolates was nonsignificant because both killed 90% of the ticks in 4 days and both killed 90% of the ticks in 11 days. Nonetheless, the acaricidal activity of *M. anisopliae* s.l. was high when compared to the activity in similar tests against other tick species with the same fungus (Garcia et al. 2004, 2011). Unfortunately, direct comparison with the efficacy of isolates from other studies is hampered by the use of dissimilar fungus stocks, conidial suspension concentrations and tick stages, an issue that will be further scrutinized in the future.

The use of entomopathogenic fungi against ticks has long been pursued, but in vitro efficacy in laboratory tests has not been achieved in corresponding field tests (Garcia et al. 2011). Among other factors, solar UV radiation in the field is harmful to fungi (Braga et al. 2001), and an appropriate formulation in which the conidia are suspended might circumvent such drawbacks (Hedimbi et al. 2008). Considering observations from the results presented herein, if a stable *M. anisopliae* s.l. conidia formulation is achieved, control of ticks on domestic animals, including *A. parvum*, will be feasible.

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