Veterinary Parasitology 188 (2012) 156–159



Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

# Occurrence of pathogenic fungi to *Amblyomma cajennense* in a rural area of Central Brazil and their activities against vectors of Rocky Mountain spotted fever

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ARTICLE INFO

*Article history:*
Received 27 September 2011
Received in revised form 16 February 2012
Accepted 23 February 2012

*Keywords:*
Ixodidae
Rickettsia
Isolation
*Metarhizium*
*Beauveria*
*Paecilomyces*

ABSTRACT

Two isolates of *Beauveria bassiana* and one of *Purpureocillium lilacinum* (=*Paecilomyces lilacinus*) were found infecting *Amblyomma cajennense* engorged females collected on horses (0.15% infection rate from a total of 1982 specimens) and another two isolates of *P. lilacinum* and one *Metarhizium anisopliae* detected in soils (2.1% from 144 samples) collected in typical pasture habitats of this tick in Central Brazil from October 2009 to March 2011. Fungi were isolated from soils with *Rhipicephalus sanguineus* as surrogate baits. No fungi were found in ticks or soils during the driest months (May to August). Testing pathogenicity of fungi all *R. sanguineus* females were killed regardless of the isolate and fungi sporulated abundantly on the cadavers. *A. cajennense* was less susceptible to infection with *P. lilacinum* within 20 days than *R. sanguineus*. All three fungal species probably act as natural antagonists of *A. cajennense* particularly in the rainy season and have interest for integrate control of vectors of Rocky Mountain spotted fever.



## 1. Introduction

The star tick, *Amblyomma cajennense*, is a heteroxenic ectoparasite that is especially common on horses although this species also infests other domestic and sylvatic animals and is a nuisance for humans. In Latin America *A. cajennense* is one of the main vectors of *Rickettsia rickettsi*, the causal agent of Rocky Mountain spotted fever (Parola et al., 2005). This tick completes only one generation each year and shows a distinct seasonality. In Central Brazil adults predominate in the hot, rainy season (November to March); six-legged larvae hatch in the drier and colder season (March to July) followed by the eight-legged nymphs. Both immature stages can frequently be found in pastures where they avidly attack hosts moving past the vegetation on which the ticks rest. Free-living tick stages distributed in large areas are difficult to control with synthetic acaricides, but pathogenic microorganisms, especially fungi, act as natural antagonists of many arthropod pests and may possibly be particularly valuable for integrated tick control (Samish et al., 2004; Fernandes and Bittencourt, 2008; Tuininga et al., 2009). Both *Beauveria bassiana* and *Metarhizium anisopliae* can infect eggs, larvae, nymphs and adults of *A. cajennense* under laboratory conditions (Lopes et al., 2007; Fernandes and Bittencourt, 2008) but nothing is known about naturally occurring mycoses of this tick in the field. *Rhipicephalus sanguineus*, another important ixodid and potential vector of *R. rickettsii* in the neotropics, mainly attacks dogs but can also affect humans (Parola et al., 2005). Highly virulent fungi adapted to the target tick species and to regional climatic conditions can provide important starting points for developing effective biorational

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0304-4017/
doi:10.1016/j.vetpar.2012.02.016

mycoacaricides. The present study reports the first isolations of pathogenic fungi from field-collected *A. cajennense* or from their natural off-host habitats and demonstrates their pathogenicity to *A. cajennense* and *R. sanguineus*.

## 2. Materials and methods

Live *A. cajennense* ticks and soil samples from their habitats were collected once a month from October 2009 to March 2011 from the privately owned Santa Branca Farm, ca. 40 km NE of Goiânia in Central Brazil (16°23′41″S; 49°04′47″W, WGS 84). *A. cajennense* is frequent in this area and can be found on various hosts but most prominently on horses and capybaras. Humans are also affected by this tick but human incidences of spotted fever have never been reported from the studied area.

Locations where soils were collected were randomly chosen in human-made pastures (*Brachiaria decumbens*, Poaceae) and did not change throughout the study. These sites are protected against continuous sunlight by vegetation and are preferred resting places for horses, livestock and capybaras. From each of eight locations (all separated by at least 100 m), 25 g of mineral soil were scraped to a depth to 2–3 cm after removing leaf litter or other organic matter, transferred to a plastic bag and stored in a polystyrene cooler at 20 °C until being processed in the laboratory within a few hours of collection. On the same dates at least 100 *A. cajennense*, nymphs and adults, were collected from about 10 different acaricide-free horses on the same field and stored individually in sterile plastic tubes (2 ml). Ambient temperature and relative humidity in the local were monitored at the beginning (about 9 am) of each collection throughout the study with a thermohygrometer (Comercial Química Americana Ltda., Paulínia, São Paulo, Brazil).

In the laboratory, ticks were surface-cleaned by individually rotating them for about 10 s in 2–3 ml distilled sterile water. Ticks were then dried with sterile filter paper, placed in Petri dishes (55 mm × 10 mm) and maintained at 25 ± 1 °C and relative humidities (RH) > 98%. Mortality was monitored daily for 20 days. Dead ticks were surface-sterilized by dipping in 93% ethanol, immersed in 2.5% sodium hypochlorite for 3 min, dipped three times in sterile water, and finally transferred on filter paper in a petri dish. Ticks were then incubated at >98% RH and 25 ± 1 °C for 15 days, and fungal development on the cadavers was evaluated daily. Fungi growing on dead ticks were transferred with a sterile loop directly onto PDA medium (potato dextrose agar; Stevens, 1981) amended with chloramphenicol (0.5 g/L medium).

For isolation of pathogenic fungi from soil samples, *R. sanguineus* adult females collected previously from dogs were used as surrogate baits. This species has no season-dependent development, and adult females are available throughout the year. Three engorged female ticks previously processed as above were permanently exposed in petri dishes (90 mm × 20 mm) to 3 g of each collected and homogenized soil sample and incubated at >98% RH and 25 ± 1 °C for 20 days. Dead ticks were processed for fungal isolation as described above.To evaluate the pathogenicity of the isolated fungi, three engorged females each of *R. sanguineus* and *A. cajennense* were rolled with a sterile forceps for about 10 s each on the sporulating fungal cultures. The inoculated and untreated control ticks were placed in Petri dishes (55 mm × 10 mm) and incubated at 25 ± 1 °C and >98% RH. Mortality was monitored daily for 20 days. Dead individuals were processed as mentioned above, and fungi were reisolated from mycotized cadavers. Tests on pathogenicity were repeated three times with single fungal cultures for each fungus and repetition.

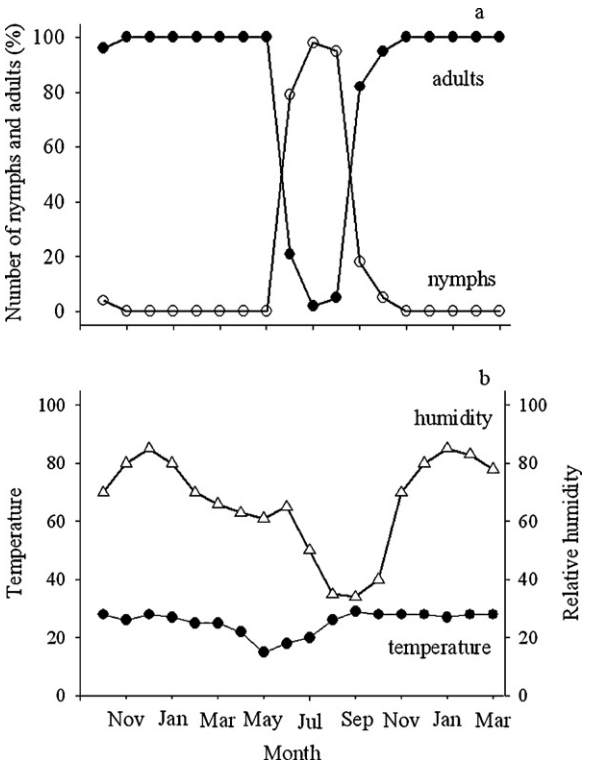


**Fig. 1.** Seasonal relative occurrence of *Amblyomma cajennense* adults and nymphs on horses in a rural area in Central Brazil (a) and temperatures (°C) and relative humidities monitored (b) between October 2009 and March 2011 in the test area.

All fungi that emerged from dead ticks were identified morphologically (Humber, 1997) and stored in the Collection of Entomopathogenic Fungi at IPTSP (Instituto de Patologia Tropical e Saúde Pública) in Goiânia, Brazil.

## 3. Results

A total of 1982 of *A. cajennense* individuals and 144 soil samples were collected between October 2009 and March 2011. Adult ticks prevailed from October to May in both years tested, with totals of 1041 females and 630 males during these periods, while nymphs (total 311) predominated from June to August in 2010 (Fig. 1a). The temperatures and relative humidities measured at the beginning of each collection are presented in Fig. 1b. Pathogenic fungi were detected in *A. cajennense* and soils during months with sporadic to frequent rainfalls (Table 1). Of the six fungal isolates originating from mycotized ticks, three (2.1% from 144 samples) were baited from soils (*M. anisopliae* IP 363

**Table 1**
Fungal isolations from *Amblyomma cajennense* adults or soils between October 2009 and March 2011 in a rural area of Central Brazil and pathogenicity against adult females of *A. cajennense* and *Rhipicephalus sanguineus*.

| Fungus | Isolate | Origin | Month/year of isolation | Cumulative mortality (%)[a,b] | |
|---|---|---|---|---|---|
| | | | | *A. cajennense* | *R. sanguineus* |
| *Beauveria bassiana* | IP 361 | Tick | 01/10[c] | 100 | 100 |
| *B. bassiana* | IP 364 | Tick | 09/10[d] | 100 | 100 |
| *Metarhizium anisopliae* | IP 363 | Soil | 09/10[d] | 100 | 100 |
| *Purpureocillium lilacinum* | IP 362 | Tick | 04/10[e] | 66.6 ± 11.6 | 100 |
| *P. lilacinum* | IP 359 | Soil | 10/09[d] | 66.6 ± 9.1 | 100 |
| *P. lilacinum* | IP 360 | Soil | 01/10[c] | 66.6 ± 11.6 | 100 |

[a] 20 days post-inoculation.
[b] All cadavers showed external sporulation of the inoculated fungus after 15 days of post-mortem incubation in a humid chamber.
[c] Rainy season.
[d] Beginning of the rainy season.
[e] End of the rainy season.

and *Purpureocillium lilacinum* [formerly *Paecilomyces lilacinus*; Luangsa-ard et al., 2011] IP 359 and IP 360) and another three (0.15% infection rate from a total of 1982 specimens) from live, infected engorged females of *A. cajennense* collected from horses (*B. bassiana* IP 361 and IP 364 and *P. lilacinum* IP 362; Table 1).

In tests of the pathogenicity of each of these isolates for ticks, 100% of individuals of *R. sanguineus* were infected and died within 20 days of incubation regardless of the fungus. The fungus-induced mortality of *A. cajennense* varied from 66.6% (IP 359, IP 360, IP 362) to 100% (IP 361, IP 363, IP 364) at the same period. No control ticks had died at the same moment. All cadavers showed external sporulation of the inoculated fungus after 15 days post-mortem incubation in a humid chamber (Table 1).

## 4. Discussion

The present study reports the first natural occurrence of *B. bassiana* and *P. lilacinum* on *A. cajennense*. Both *P. lilacinum* and *M. anisopliae* occurred in soils in the same local where *A. cajennense* can frequently be found throughout the year, and pathogenicity tests confirmed that both fungi can infect and kill this tick. All detected fungi are typical soil-inhabiting fungi, and *B. bassiana* and *M. anisopliae* are the most common species isolated from field-collected ticks in previous studies (Chandler et al., 2000; Samish et al., 2004). *P. lilacinum* – which was recently transferred *Paecilomyces* to the new genus *Purpureocillium* by Luangsa-ard et al. (2011) as a continuing step in the reclassification of species now phylogenetically excluded from *Paecilomyces* – is reported for the first time as a natural pathogen of an ixodid tick. Another species, *Isaria fumosorosea* (formerly *Paecilomyces fumosoroseus*), was isolated from *Ixodes ricinus* (Hartelt et al., 2007).

The low proportion (0.1 5%) of ticks with fungal infection and of soil samples with entomopathogenic fungi (2.1%) in the present study was strikingly lower than the values found in other studies where up to 25% of *Rhipicephalus* spp or *Ixodes scapularis* were found to be infected with *B. bassiana* or *M. anisopliae* (Samish et al., 2004; Benoit et al., 2005).

Pathogenic fungi were isolated from soils or ticks during the rainy period but never between the months of May and August when rains are exceptionally uncommon in this part of Brazil. Moreover, fungi were never detected on larvae or nymphs but only on engorged adult females that seemed to be more susceptible to fungal infection than males or immature stages as was also found for *Ixodes* spp (Samish et al., 2004). The probability of fungal contamination and infection of this heteroxenic tick rises with increasing age and exposure to fungal propagules in the off-host environments. Engorged gravid females are key targets for any intentional applications of fungal pathogens since the death of such females also eliminates large numbers of eggs. The post-mortem production of infective conidia on fungus-killed individuals eventually declined during the drier season (when external development and sporulation of the fungus on the infected ticks was prevented by the decrease of moisture), and ticks were less exposed to infection due to both the reduced quantity of infective inoculum and to ambient relative humidities that become too low to support the germination and cuticular penetration required for new fungal infections.

The reduction of pathogenic fungal titers in soils collected in pastures appears to be related to vegetation and abiotic factors (especially sunlight and moisture) since Rocha et al. (2009) isolated *M. anisopliae*, *P. lilacinum*, *Fusarium* sp and *Pochonia chlamydosporia* from soils and slurries collected in a nearby tropical gallery forest and baited with *R. microplus* (10.3%) in the same manner used in this study.

The effectiveness of *M. anisopliae* and *B. bassiana* under laboratory conditions is well established for *R. sanguineus* but only very few studies have demonstrated their activities against *A. cajennense* (Reis et al., 2004; Samish et al., 2004; Fernandes and Bittencourt, 2008; Lopes et al., 2007). *R. sanguineus* seemed to be more susceptible to infection by *P. lilacinum* than *A. cajennense*. Previous findings about the susceptibility of diverse ticks to fungal entomopathogens were corroborated by the demonstrations of high susceptibility in laboratory conditions of *A. cajennense* and *R. sanguineus* in the present study to isolates tested here and of their abilities to recycle by sporulating on fungus-killed ticks.

All three fungal species studied here probably act as natural antagonists of *A. cajennense* populations in the tested area, and particularly during the rainy season. Further investigations will explore the potential of these pathogens for development as the principal active ingredients of mycoacaricides for the control of the vectors of

Rocky Mountain spotted fever and other important tick pests.

## Acknowledgements

The authors thank the National Council of Scientific and Technological Development (CNPq, Brazil) for financial support, Jeremias Lunardelli for kindly permitting to collect fungi at Santa Branca Farm, and Durval R. Ferreira for technical assistance.

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