Vol. 51, n. 5 : pp.923-928, September-October 2008
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
AN INTERNATIONAL JOURNAL

Evaluation of the Possible Role of Ants (Hymenoptera: Formicidae) as Mechanical Vectors of Nematodes and Protists

Fabio Villani[1], Maria Santina de Castro Morini[1]*, Marco Antonio Franco[1] and Odair Correa Bueno[2]

[1]Laboratório de Mirmecologia; Universidade de Mogi das Cruzes; morini@umc.br; 08780-911; Mogi das Cruzes - SP - Brasil. [2]Centro de Estudos de Insetos Sociais; Universidade Estadual Paulista; 13506-900; Rio Claro - SP - Brasil

ABSTRACT

Nematodes and protists can be transmitted to humans in many ways and little concern has been given to the mechanical transmission by ants. This study aimed at analysing how the eggs of Ascaris lumbricoides *and cysts of* Entamoeba coli *could be mechanically transmitted to the man by Formicidae. Through the experiments using nests of* Tapinoma melanocephalum, Linepithema humile *and* Monomorium pharaonis *reared in the laboratory allied to observations of some 17 ant species in an urban park area in Mogi das Cruzes (SP), it was found that* L. humile *was capable of carrying eggs of* A. lumbricoides *both in the field and laboratory conditions (1 worker), as well as was* Camponotus rufipes *(2),* Solenopsis saevissima *(1) and* Acromyrmex niger *(1). The cysts of* Escherichia coli *were found over three workers of* C. rufipes*. Although the frequency of the workers found transporting pathogens was low, the capacity of common household species in carrying pathogens like nematodes and protists was demonstrated.*

Key words: *Ascaris lumbricoides*, *Entamoeba coli*, ant, mechanical vector

INTRODUCTION

Several studies have demonstrated the capacity of insects in transmitting the pathogens to the humans (Bueno and Fowler, 1994; Paraluppi et al., 1996; Iwasa et al., 1999), either as mechanical or biological vectors. A mechanical vector usually contaminates itself when in contact with infected materials, transporting the parasites around and eventually into human food (Leser et al., 2000). The principal insects as the mechanical vectors of pathogens belonged to the orders Diptera, Blattodea and Hymenoptera (Oliveira et al., 2002; Thyssen et al., 2004). Some ant species known as 'tramp ants', belonging to the Formicidae have drawn particular attention in this regard. Commonly established in urban environments, these ants are responsible for much nuisance, usually nesting inside the electrical systems and crevices while foraging into filthy and contamined areas, thus bringing risk of contamination and disconfourt to people in the food industries, bakeries, restaurants, houses and hospitals (Bueno and Campos-Farinha, 1999). In the hospitals, ants may actively carry some bacteria around, causing many problems. *Monomorium pharaonis* has been observed over cirurgy instruments, intravenous fluid holders used for blood transfusions and over

* Author for correspondence

bandages of recently operated patients (Edwards and Baker, 1981). Beatson (1972) detected the presence of *Salmonella* sp. and *Pseudomonas* sp. on the workers of this species. Chadee and Lê Maitre (1990) observed *Klebsiella pneumoniae*, *Streptococcus* sp. (enterococcus group), *Proteus mirabilis* and *Pseudomonas* sp. on the workers of the ants *Tapinoma sessile*, *Solenopsis* sp., *S. molestus* and *M. pharaonis*. Also, *T. melanocephalum* and *Camponotus atriceps* acted as mechanical vectors of bacteria in urban environments (Marcolino, 2004). In spite of the wide geographical distribution in Brazil of nematode parasites known as responsible for deficiencies in intellectual and physical development, mainly of children (Pedrazzani et al., 1989), the mechanical transmission of their infectious forms by insects has been generaly overlooked (Thyssen et al., 2004). Thus, this study was carried out to evaluate the capacity of the Formicidae, including common household species, as the mechanical carriers of *Ascaris lumbricoides* (Nematoda) and *Entamoeba coli* (Protista). Although being harmless to humans, cysts of *E. coli* was used for two reasons: 1) They are similar in shape and form to *E. histolytica*, a common pathogenic species in urban and rural areas of Brazil, and 2) because cysts are more easily obtained from health clinics, together with the eggs of *A. lumbricoides.*

MATERIAL AND METHODS

Rearing the ant nests in the laboratory

Nests of *M. pharaonis, T. melanocephalum* and *Linepithema humile* were placed in the plastic trays containing plastic bottle caps with wet cotton inside. They were everyday provided with fresh heart of steer, bits of cake, larvae of *Tenebrio molitor* (Coleoptera) and 50% honey solution. The nests were kept at room temperature.

Forraging experiments in the laboratory

Three nests of each ant species, only ones presenting high foraging activity were chosen**.** The ants were conditioned into foraging for food in the plastic chambers measuring 2.0 cm wide x 10.0 cm long x 1.5 cm high and closed at one end. Before the experiment, they were not given any food for two days. The outer portion of these chambers was covered with vaseline so the ants were forced inside seeking for food that was placed near the closed end. For the experiments, the ants were conditioned into foraring for food at the same place, while passing over a fine layer of soil -- previously sifted and sterilized at 150^0 C -- applied to the bottom of the chambers. Some of this soil was inoculated with a suspension of inactivated eggs of *A. lumbricoides* and cysts of *E. coli* in 10% formaldehyde solution at the ratio of 1-2 mL for every 1.5-2.5 g of sterilized soil. Then two of the three chosen ant nests were presented with chambers containing pseudo-contamined soil while the third nest was presented with a chamber containing crude soil, inoculated with clean 10% formaldehyde to serve as control. The chambers were then placed in the plastic trays and the ants were observed. As the ants crawled over the soil towards the food, they were collected and individually placed in sterilized plastic recipients. A total of 25 ants were collected from each nest during each experiment (N=10). The soils used were saved for later analysis.

Forraging experiments in the field

The same forraging chambers were used during the experiments in the field. The chosen area was a particularly disturbed area in Parque Municipal Francisco Affonso de Mello, a public park in Mogi das Cruzes, SP, Brazil. The chambers with sterilized soil were placed directly on the ground. When a constant flow of foraging ants was obtained, some of them were individually collected in sterilized recipients to serve as controls. Then the chambers were replaced by others containing pseudo-contaminated soil. Ants forraging on the pseudo-contaminated soil were then collected in the same manner. This was repeated 10 times, as was in the laboratory, collecting 250 ants. Due to difficulties in obtaining constant flows of forraging ants in the field, the same control ants were used for all repetitions (N=100). The used soils were saved for later analyses.

All collected ants were mounted on small paper triangles and pinned for identification using the key for genera of Bolton (1994). Within the genera, species and morphospecies were assigned through comparison to identified ant specimens from the official collection of Universidade de Mogi das Cruzes, SP, which comprises the myrmecofauna of this region (Alto Tietê). Vouchers specimens were deposited in this same collection.

Analysis of the pseudo-contaminated soil and controls

To each of the recipients with ants, 0.5 mL of distilled water was added, followed by vigorous shaking. A drop of 10% formaldehyde was then added to conserve the sample during the observations. The liquid from the recipients was then prepared for analysis under optical microscopy as described by Cimerman and Cimerman (2000); cysts and eggs were identified based on Cimerman and Franco (2000). The samples of the pseudo-contaminated soil and control soil were prepared for the examination according to the method of Faust (Cimerman and Cimerman, 2000) under an optical microscope.

RESULTS AND DISCUSSION

Of the ten experimental repetitions conducted in the laboratory, four were made using the species *M. pharaonis*, four with *L. humile* and two with *T. melanochephalum*, due to the difficulty of maintaining nests of this species in the laboratory. *Tapinoma melanocephalum*, along with *M. pharonis*, were considered by Fowler et al. (1992) the most predominant tramp ant species in Brazil, while Harada (1990) refered to these species as urban pests of the tropical regions.

During the laboratory experiments, *M. pharaonis* workers were not pseudo-infected with *E. coli* cysts or with *A. lumbricoides* eggs (Table 1). However, this species has been reported transporting the following pathogenic microorganisms, especially in the hospitals: *K. pneumoniae*, *Streptococcus*, *Salmonella* and *Pseudomonas* (Beatson, 1972; Edwards and Baker, 1981; Chadee and Lê Maitre, 1990).

The results of the experiments with *T. melanocephalum* were also all negative (Table 1), which might have been caused by the smaller number of repetitions. Nevertheless, *M. pharaonis* and *T. melanocephalum* were relatively small ants, measuring 1.2-2.0 mm (Campos-Farinha et al., 1997) and 2.0 mm, respectively (Schüller, 2004) in length. It was possible that this diminute body size could hinder the transportation of eggs of *A. lumbricoides* (about 70µm in diameter) and *E. coli* cysts (about 30µm in diameter) (Cimerman and Cimerman, 2000), considering that these species were demonstrated to transport the bacteria (Bueno and Fowler, 1994) up to 2µm in size (Tortora et al., 2003).

The eggs of *A. lumbricoides* presented a convoluted shape (Neves, 2000) that facilitates the transportation, but these two ant species had practically no structures such as hairs, spines or other surface sculptures to which the parasite could adhere. Anyway, since the infectious agents were priorly killed with formaldehyde, it was possible that they might have undergone some kind of physical alteration that could have inhibited the adherence to the ants' body surface.

In the experiments using *L. humile* (Table 1), 1% of the ants that marched over the pseudo-contaminated soil presented eggs of *A. lumbricoides* adhered to their bodies. This native species was very common in the hospitals around the southeastern Brazil (Bueno and Fowler, 1994) and could carry pathogenic bacteria (Ipinza-Regla et al., 1994), while being of significant importance in the urban environment for its outstanding adaptive and dispersive capacities and for building extensive nests in the ground (Tsuitsui et al., 2000; 2003).

During the field experiments, the specimens of 17 ant species were collected from the pseudo-contaminated soils, while 12 species were sampled from the control soil (Fig. 1 A and B). In all the experiment, only eight ants from three species were found to bear eggs or cysts: a (0.4%) *Acromyrmex niger* worker with an egg of *A. lumbricoides*; five (2%) *C. rufipes* workers, having three of them cysts of *E. coli* and two with eggs of *A. lumbricoides*. A (0.4%) *L. humile* worker and one (0.4%) *S. saevissima* were found with an *A. lumbricoides* egg. Of these pseudo-infected species, *A. niger* (Attini) was the only species not normally found in human residences. *Camponotus rufipes* is a large ant when compared to the others and bears hairs over the body surface. It is known to nest inside the wooden doors, frames, floors, crevices in walls, in the drawers and in wooden panels. It feeds basically from sweet substances, but can also feed on meat (Bueno and Campos-Farinha, 1999). *Solenopsis saevissima* is an omnivorous species like all *Solenopsis* and as such can eat foods such as oils, meat, butter, cheese, fruit, bread and sweets (Campos-Farinha et al., 1997), besides being active predators and scavengers (Rossi and Fowler, 2004). Workers are polimorphic and range from 2-6 mm in length, differently from *M. pharaonis* and *T. melanocephalum*. A single worker of *L. humile* (2.2 - 2.8 mm long, in this species) was found transporting eggs of *A. lumbricoides*. Due the nesting habits of this species - presenting large numbers of individuals per colony – and also due to their growing proximity to the urban environment, they could act as important carriers of this nematode species here tested. However, Thyssen et al. (2004) concluded that ants were not mechanical vectors of helminthes when collected inside and in the vicinity of residences in Campinas, SP, Brazil, although no ants of this species were recorded in their samples. Results showed that size influence the transportation of pathogens for facilitating adhesion of the parasites. This could explain why only in workers of *C. rufipes* a higher incidence of pseudo-infected ants was found.

Table 1 - Number of eggs of *A. lumbricoides* and cysts of *E. coli* per optical microscope field found in the treated soil used for the laboratory and field experiments.

Experiment	Amount of pseudo-contaminated suspension applied (mL)	Quantity of soil (g)	Number of eggs/cysts found in the soil
Laboratory experiments			
Monomorium pharaonis			
Control	1.0/2.0	1.5/2.5	-
1	1.0	1.5	4 - 5
2	1.0	1.5	4 - 5
3	2.0	2.5	7 - 8
4	2.0	2.5	7 - 8
Tapinoma melanocephalum			
Control	1.0/2.0	1.5/2.5	-
1	1.0	1.5	4 - 5
2	2.0	2.5	7 - 8
Linepithema humile			
Control	1.0/2.0	1.5/2.5	-
1	1.0	1.5	4 - 5*
2	1.0	1.5	4 - 5
3	2.0	2.5	7 - 8
4	2.0	2.5	7 - 8
Field experiments			
Control	1.0/2.0	1.5/2.5	-
1	1.0	1.5	3 - 4*
2	1.0	1.5	3 - 4*
3	2.0	2.5	6 - 7
4	2.0	2.5	7 - 8
5	1.0	1.5	4 - 5*
6	1.0	1.5	4 - 5
7	1.0	1.5	4 - 5
8	2.0	2.5	7 - 8*
9	2.0	2.5	7 - 8
10	2.0	2.5	7 - 8

* Experiments in which pseudo-infected ants were found.

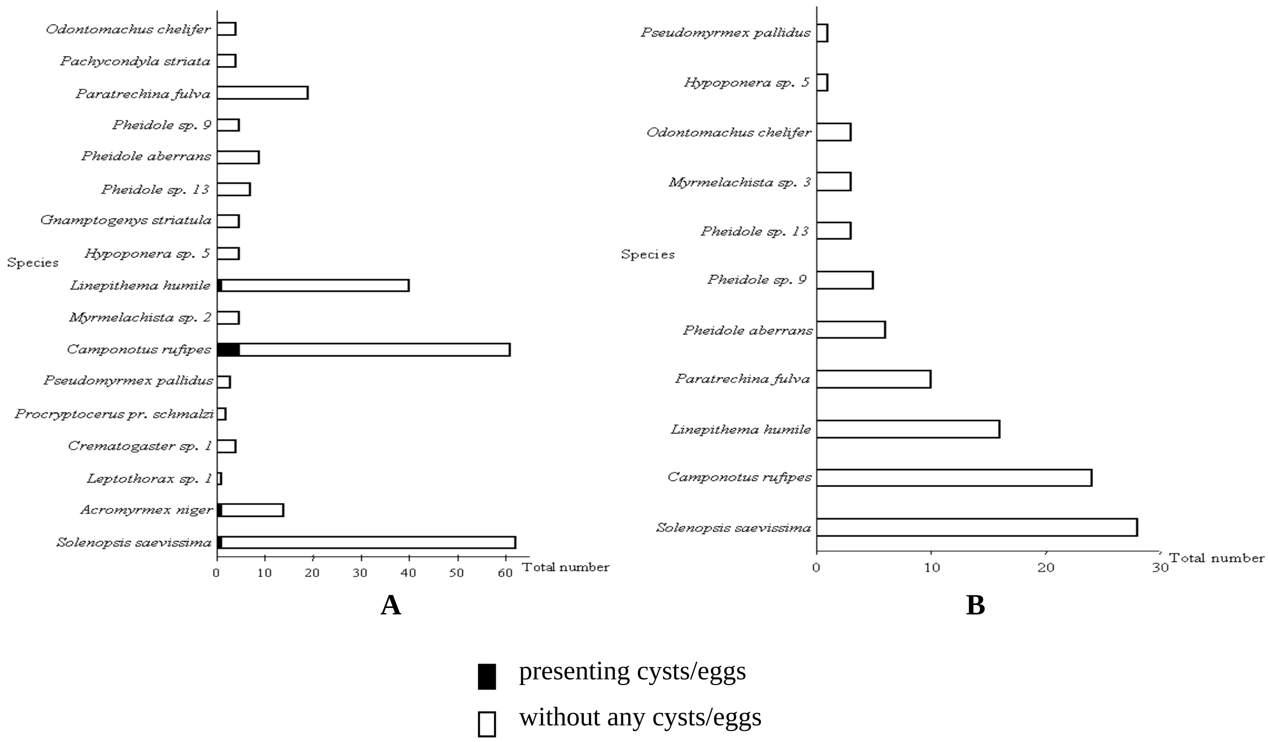

Figure 1 - Total number of collected ants that marched over clean (A) and pseudo-contaminated (B) soil in the field experiments, separated in species.

ACKNOWLEDGEMENTS

We would like to thank the Fundação de Amparo ao Ensino e Pesquisa (FAEP/UMC) for financial support of this project.

RESUMO

Os Nematoda e Protista podem ser transmitidos ao homem de diversas maneiras, mas pouca ênfase é dada para a transmissão mecânica por intermédio de formigas. Assim, esse trabalho procurou investigar a transmissão mecânica de ovos de *Ascaris lumbricoides* e cistos de *Entamoeba coli* pelos Formicidae. Através de experimentos com espécies mantidas em ninhos no laboratório (*Tapinoma melanocephalum*, *Linepithema humile* e *Monomorium pharaonis*) e com 17 espécies de formigas de uma área antropizada na região de Mogi as Cruzes (SP), foi possível constar que os ovos *A. lumbricoides* foram transportados por *L. humile*, tanto no campo (1 operária) como no laboratório (1 operária), por *Camponotus rufipes* (2), por *Solenopsis saevissima* (1) e por *Acromyrmrex niger* (1). Em três operárias de *C. rufipes* foram encontrados cistos de *E. coli*. Apesar da baixa incidência de transporte, as três primeiras espécies pelo fato de viverem muito próximas ao ser humano, podem levar para dentro do ambiente domiciliar patógenos de Nematoda e Protista.

REFERENCES

Beatson, S. H. (1972), Pharao's ants as pathogen vectors in hospitals. *Lancet*, **1**, 425-427.

Bolton, B. A. (1994), *Identification guide to the ant genera of the world*. Harvard University Press: Cambridge. 222 pp.

Bueno, O. C. and Fowler, H. G. (1994), Exotic ants and native ant fauna of Brazilian Hospitals. In: Williams, D.F. (Ed.). *Exotic ants: Biology, impact and control* *of introduced species*. Boulder, Westview Press, pp. 191-198.

Bueno, O. C. and Campos-Farinha, A. E. C. (1999), As formigas domésticas. In: Mariconi, F.A.M. (Ed.) *Insetos e outros invasores de residências*, FEALQ, Piracicaba, pp. 135-180.

Campos-Farinha, A. E. C.; Justi Jr., J.; Bergmann, E. C.; Zorzeno, F. J. and Netto, S. M. R. (1997), *Formigas urbanas*. São Paulo: Instituto Biológico, Boletim Técnico. 20 pp.

Cimerman, B. and Cimerman, S. (2000), *Parasitologia Humana e seus Fundamentos Gerais*. São Paulo: Ed. Atheneu. 390 pp.

Cimerman, B. and Franco, M. A. (2000), *Atlas de Parasitologia – Artrópodes, Protozoários e Helmintos*. São Paulo: Ed. Atheneu. 105 pp.

Chadee, D. D. and Lê Maitre, A. (1990), Ants: Potencial mechanical vectors of hospital infections in Trinidad. *Trans. Royal Soc. Trop. Med. Hyg.*, **2**, 84:297.

Edwards, J. P. and Baker, L. F. (1981), Distribution and importance of the Pharao's ant *Monomorium pharaonis* (L) in National Health Service Hospitals in England. *J. Hosp. Inf.*, **2**, 249-254.

Fowler, H. G.; Anaruma-Filho, F. and Bueno, O. C. (1992), Vertical and horizontal foraging intra and interspecific spatial autocorrelation patterns in *Tapinoma melanocephalum* and *Monomorium pharaonis* (Hymenoptera: Formicidae). *Ciência e Cultura*, **44**, 395-397.

Harada, A. Y. (1990), Ant pests of the Tapinomini tribe. In: Van der Meer, R. K.; Jaffé, K. and Cedeno, A. (Eds). *Applied Myrmecology: a world perspective* in Insect Biology. Westview Press, Bolder (CO), pp. 298-305.

Ipinza-Regla, J.; Figueroa, G. and Moreno, I. (1984), *Iridomyrmex humile* (Formicidae) y su papel como possible vector de contaminacion microbiana en industrias de alimentos. *Folia Entomol. Mex.*, **62**, 111-124.

Iwasa, M.; Markino, S.; Asakura, H.; Kobori, H. and Morimoto, Y. (1999), Detection of *Escherichia coli* O157:H7 from *Musca domestica* (Diptera: Muscidae) at a cattle farm in Japan. *J. Med. Entomol.*, **36**, 108-102.

Leser, W.; Barbosa, V.; Baruzzi, R. G. and Franco, L. J. (2000), *Elementos de Epidemiologia Geral*. São Paulo: Editora Atheneu. 177 pp.

Marcolino, M. T. (2004), Formiga como vetor de bactérias nosocomiais no Hospital das Clínicas da Universidade Federal de Uberlândia. PhD Thesis, Universidade Federal de Uberlândia, Minas Gerais, Brasil.

Neves, D. P. (2000), *Parasitologia Humana*. São Paulo: Atheneu. 229 pp.

Oliveira, V. C.; Mello, R. P. and D`Almeida, J. M. (2002), Dípteros muscóides como vetores mecânicos de ovos de helmintos em jardim zoológico. Brasil. *Rev. Saúde Pública*, **36**, 614-620.

Paraluppi, N. D.; Vasconcelos, J. C. de; Aquino, J. S. de; Castellon, E. G. and Silva, M. (1996), Calliphoridae (Diptera) in Manaus: IV. Bacteria isolated from blowflies collected in street markets. *Acta Amazonica*, **26**, 93-96.

Pedrazzani, E. S.; Mello, D. A.; Pizzigat, C. P.; Pripas, S.; Fucci, M. and Santoro, M. C. M. (1989), Helmintoses intestinais. III Programa de educação e saúde em verminoses. R*ev. Saúde Pública*, **23**, 189-195.

Rossi, M.N. and Fowler, H.G. (2004), Predaceous ant fauna in new sugarcane fields in the State of São Paulo, Brazil. *Braz. Arch. Biol. Tech*., **47**, 805-811.

Schüller, L. (2004), Microorganismos patogênicos veiculados por "formigas andarilhas" em unidades de alimentação. Tese de mestrado, Faculdade de Saúde Pública da Universidade de São Paulo, São Paulo, Brasil.

Thyssen, P. J.; Moretti, T. C.; Ueta, M. T. and Ribeiro, O. B. (2004), O papel de insetos (Blattodea, Diptera e Hymenoptera) como possíveis vetores mecânicos de helmintos em ambiente domiciliary e peridomiciliar. *Caderno de Saúde Pública*, **20**, 1096-1102.

Tortora, G. J.; Funke, B. R. and Case, C. L. (2003), *Microbiologia*. Porto Alegre: Artmed. 357 pp.

Tsuitsui, N. D.; Suarez, A. V.; Holway, D. A. and Case, T. J. (2000), Reduced genetic variation an the success of an invasive species. *PNAS*, **97**, 5948-5953

Tsuitsui, N. D.; Suarez, A. V. and Grosberg, R. K. (2003), Genetic diversity, asymmetrical aggression and recognition in a widespread invasive species. *PNAS*, **100**, 1078-1083.

Received: May 02, 2006;
Revised: February 20, 2007;
Accepted: June 27, 2008.