

## SCIENTIFIC COMMUNICATION

ANTS' ROLE (HYMENOPTERA: FORMICIDAE) AS  
POTENTIAL VECTORS OF MYCOBACTERIA DISPERSION

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## ABSTRACT

Ants are found worldwide playing an important environmental role. Some species are considered as agricultural pests and potential risk to human life and public health acting as pathogens carriers. Ants as *Paratrechina longicornis* and *Camponotus* spp. have been found inside hospitals. The aim of this study was the research of mycobacteria in 138 samples of ants (137 *Paratrechina longicornis* and only one *Camponotus* spp.) which got into the laboratories of tuberculosis diagnosis. These ants were suspended in sterile saline solution and inoculated into Petraghani and Stonebrink media, incubated at 37° C until 90 days and the isolates were identified as environmental mycobacteria (1 *Mycobacterium fortuitum peregrinum*, 1 *Mycobacterium smegmatis*) and 1 *Mycobacterium tuberculosis* complex. These results showed that ants should also act as mechanical vectors of mycobacteria dissemination in risk environments, reinforcing their significance in public health.

KEY WORDS: Ants, *Mycobacterium*, tuberculosis, insect vector, public health.

## RESUMO

COMPORTAMENTO DE FORMIGAS (HYMENOPTERA: FORMICIDAE) COMO VETORES POTENCIAIS DE DISPERSÃO DE MICOBACTÉRIAS. As formigas têm uma distribuição mundial e representam importante papel no ecossistema. Algumas espécies são consideradas pragas para a agricultura e um risco potencial à vida humana e à saúde pública veiculando mecanicamente agentes patogênicos. Formigas como *Paratrechina longicornis* e *Camponotus* spp. têm sido encontradas em ambientes hospitalares. O foco do presente estudo foi a identificação de micobactérias em 138 amostras de formigas (137 *Paratrechina longicornis* e apenas uma *Camponotus* sp.), que tiveram acesso a áreas de laboratórios de diagnóstico de tuberculose. Essas formigas foram suspensas em solução salina estéril que foi semeada em meios de Petraghani e Stonebrink, incubadas a 37° C por até 90 dias e as estirpes de micobactérias isoladas foram identificadas pelas técnicas clássicas como micobactérias ambientais (sendo 1 *Mycobacterium smegmatis*, 2 *Mycobacterium fortuitum peregrinum* e 1 *Mycobacterium tuberculosis*). Esses resultados mostram que as formigas podem também se constituir vetores de dispersão de micobactérias em ambientes de risco, reforçando sua importância em saúde pública.

PALAVRAS-CHAVE: Formiga, *Mycobacterium*, tuberculose, insetos vetores, saúde pública.

In spite of beneficial to the environment and humans, some insects are considered pests for transmitting diseases and causing structures or agricultural goods damages. They pollinate flowering plants, produce medicines and can be used as human food. On the other hand, they can become annoyances by invading homes, yards, gardens and fields, but usually they are not associated to the transmission of

diseases (CAMPOS, 2008; CAMPOS-FARINHA et al., 1997; UC IPM, 2007).

Ants spread in practically all continents, live together human beings and animals, and play an important role in the ecosystem by recycling nutrients and helping the agricultural plagues control. However, some species can be considered plagues for agriculture, and ones can also imposing risk to human

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and livestock by their bites or abilities to mechanically propagate pathogenic agents, acting as dispersion vector of animal and human beings illness (BIET *et al.*, 2005; ZARZUELA, 2008). Some studies refer to *Paratrechina longicornis*, *Solenopsis* spp. and *Camponotus* spp. as the most frequent ant species found in hospital environment, with high potential as mechanical vectors for dispersion of pathogenic bacteria such as clostridia, salmonelas and mycobacteria (BUENO; CAMPOS-FARINHA, 1998; BUENO; CAMPOS-FARINHA, 1999; SILVA, 1999). The *P. longicornis* usually make their nests in places of high temperature and suitable to their reproduction and development, mainly in sidewalks or roofs, consuming a great variety of foods (meat, candy, fruits etc.). The *Camponotus* spp. constructs their nests inside houses, as in structure imperfections, beam wooden, electric devices, etc. and eject formic acid and generally eat eggs, meat and sugar.

The interactions between insects and bacteria in the environment may significantly contribute to the spread and evolution of human diseases. Many insects can transmit microbes directly into the bloodstream by their bites acting as a reservoir of diseases (BABOUR; RESTREPO, 2000; BIET *et al.*, 2005; COSTA *et al.*, 2006; SCHULLER, 2004; ZARZUELA, 2008).

Beyond pathogenic mycobacteria like *M. tuberculosis* complex, which is the causal agent of tuberculosis in man and animals, there are also known environmental mycobacteria that can be pathogenic for human beings and some animal species. The environmental mycobacteria are easily found on the ground, sand, grass, mud, garbage and in aquatic environment as waste water, sea, lake, river, sewer, swimming pool and watering holes, tap water, aquarium and current waters (PORTAELS, 1973). Many other mycobacteria not belonging to the *M. tuberculosis* complex had been isolated from flies insects, larvae, mosquitoes and cockroaches (FISHER *et al.*, 2001; FISHER *et al.*, 2003; PAI *et al.*, 2003).

Studies in Lower-Zaire found that 44.5% of 332 samples of environmental matrix as mud, water, fish, leech, swamp, sugarcane plantation, plants and ants were positive for Runyon Groups II, III and IV mycobacteria, confirming the significant risk factor of different mycobacteria biotypes in ecosystem to human (PORTAELS, 1973).

This work describes the results of some ant species accidentally present in tuberculosis diagnosis laboratory as dispersion vector of mycobacterial species mainly the *M. tuberculosis*.

The aim of this work is to show the public health risk of mycobacterial species been disseminated by ants when living in the surroundings of tuberculosis diagnosis laboratories.

Ants were randomly captured in the non-lab and laboratorial areas of two bovine and human

tuberculosis diagnosis laboratories located in São Paulo, SP, Brazil.

Each insect was identified at genus and specie level categories according to their body parts and structures by using a microscope.

Each ant was directly collected into screw cap tubes during the day, frozen at -20° C for 30 minutes, and washed with 1 mL sterile physiological saline, under agitation, for 10 min. Aliquots of these solutions were inoculated, in duplicate, in Petragnani and Stonebrink culture media and incubated at 37° C, up to 90 days, to evaluate the presence of *Mycobacterium* spp. or *M. bovis*, respectively, on external part of insect body (KANTOR, 1988). For analyses of internal content, each insect was crushed on a slide and cover glass, and transferred to a tube containing 1 mL of physiological saline that was stirred for 10 min. Aliquots of these materials were inoculated in Petragnani and Stonebrink culture media and incubated at 37° C, up to 90 days.

*Mycobacterium* species present in the alcohol-acid resistant bacilli colonies identified by Ziehl-Neelsen method and optical microscopy, were isolated and identified by the classical methods (KANTOR, 1988) as *M. tuberculosis* and submitted to DNA amplification using primers set JB21 and JB22 (RODRIGUEZ *et al.*, 1995).

Among 138 ant samples captured from areas surrounding laboratories of animal and human tuberculosis diagnosis, 137 were identified as being *Paratrechina longicornis* and only one as *Camponotus* spp.

Analyses of samples inoculated in Petragnani or Stonebrink media, that allow the isolation of mycobacterium genus or species showed four positive results for genus and two for mycobacteria species. Among four mycobacteria genus isolated (6.4%), two were found on external body and other two in the crushed ant materials. Further identification by biochemical and molecular assays characterized them as *Mycobacterium fortuitum-peregrinum* (2), *Mycobacterium smegmatis* (1), and *Mycobacterium tuberculosis* (1).

All four ants positive for mycobacteria were belonging to *Paratrechina longicornis* specie. These ants were collected from non-lab areas (1 in water closed and 2 in the kitchen) and into the laboratory incubator.

The Figure 1 shows photomicrography of *M. tuberculosis* colonies grown on Petragnani and Stonebrink culture media inoculated with materials derived from only one ant.

Forty-four samples showed negative results either for the presence of mycobacteria or other contaminants, such as fungus and bacteria, that could affected the reliable evaluation of the presence of mycobacteria in another ninety negative samples.

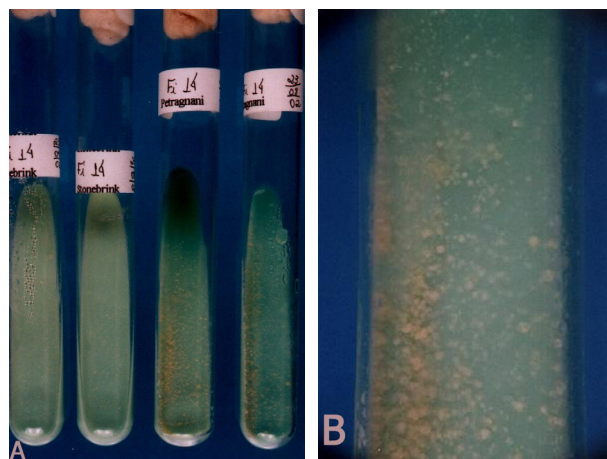


Fig. 1 - Photomicrography of *Mycobacterium tuberculosis* colonies, grown on Petragrani and Stonebrink media inoculated with materials derived from only ant (A) and colonies in detail (B).

As tuberculosis is considered one of the illnesses of high mortality the research of potential vectors of mycobacteria, especially in environments of high risk work in public health, assumes great importance even nowadays.

It is known that ant species such as *Paratrechina longicornis*, *Solenopsis* spp. and *Camponotus* spp. consume great variety of food and therefore, they can be bait and nourished by elk-yolk media, commonly used for mycobacteria isolation (Roxo observation).

As expected, the most prevalent specie observed in areas surrounding laboratories of tuberculosis diagnosis, *P. longicornis* was found as mycobacteria potential vector. These crazy ants are common in hospital areas, being of hard control, and they could accidentally assessed studied places because of their garden soil renewing and building restoration (ZARZUELA, 2008).

SCHULLER (2004) demonstrated, experimentally by ultramicroscopy, that ants in contact with bacterial colonies can carry microorganisms adhered to their legs. COSTA et al. (2006) evidenced a high mutualistic association between bacteria, fungus and ants collected in hospital environment. These contaminants growth can overcome the slow growth and high requirements of mycobacteria development in culture. This fact could explain the high contamination of ant samples and also the high amount of negative results for mycobacteria observed in this study.

Even though, nowadays, optimized isolation procedures and PCR analyses have been useful for evidencing the presence of environment or pathogenic mycobacteria such as *M. fortuitum-peregrinum*, *M. smegmatis*, and *M. tuberculosis*, and to demonstrate the role of ants as vectors carrying microorganisms on their bodies, either internally or outside.

We conclude that two species of *M. fortuitum-peregrinum*, one of *M. smegmatis* and one of *M. tuberculosis* were present in ants captured from two laboratories of tuberculosis diagnosis.

Therefore, these results reinforced the needs of attention with the possibility of direct or indirect transmission of mycobacteria by ants, for example, through their stings or water and food contaminated by them.

As a source of contamination, ants can compromise the environmental quality, and could play high risk to livestock and public health.

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