# New Survey for the Fire Ant Microsporidia *Vairimorpha invictae* and *Thelohania solenopsae* in Southern South America, with Observations on Their Field Persistence and Prevalence of Dual Infections

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**ABSTRACT** The exploration for the fire ant diseases *Vairimorpha invictae* Jouvenaz and Ellis (Microsporidia: Burenellidae) and *Thelohania solenopsae* Knell, Allen, and Hazard (Microsporidia: Thelohaniidae) was conducted from 2001 to 2005 in Argentina, Paraguay, Chile, Bolivia, and Brazil. A total of 2,064 colonies were sampled from 262 sites. Three sites with high prevalence of pathogens were monitored periodically for the persistence of the infections. *V. invictae* occurred at 12% of the sites and in 10% of the colonies. Except for one infected colony in Bolivia, its distribution was restricted to the eastern part of the region surveyed. The highest occurrence was in Santa Fe Province. *T. solenopsae* presented a much wider distribution. It occurred at 25% of the sites and in 13% of the colonies. The highest occurrence was in Buenos Aires Province. This is the first report of *T. solenopsae* in the northwest, in the west, in central Argentina, and in Bolivia, and infecting *S. interrupta* (Santschi). Simultaneous infections were found at 4% of the sites and in 2.2% of the colonies. The periodical examination revealed high infection levels in most occasions. The prevalence of *T. solenopsae* ranged from 10 to 90% of the colonies, *V. invictae* from 0 to 60%, and dual infections from 0 to 50%. Each microsporidium exhibited a characteristic enzootic/epizootic wave. Successive epizootic levels observed in both infections provide a more constant pressure against fire ant populations. These diseases are promising classical biological control agents of the imported fire ants in the United States.

**KEY WORDS** *Vairimorpha invictae*, *Thelohania solenopsae*, fire ants, dual infections, biological control

The classical biological control approach of the red and black imported fire ants, *Solenopsis invicta* Buren and *S. richteri* Forel (Hymenoptera: Formicidae), with the pathogens, *Thelohania solenopsae* Knell, Allen, and Hazard (1977) (Microsporidia: Thelohaniidae) and *Vairimorpha invictae* Jouvenaz and Ellis (1986) (Microsporidia: Burenellidae), has been considered since the 1970s (Allen and Buren 1974, Williams and Whitcomb 1974, Jouvenaz 1983, 1990, Wojcik 1986, Wojcik et al. 1987). Both microsporidia are obligate intracellular microorganisms specific to fire ants (Briano et al. 2002) that were originally discovered infecting the red imported fire ant in Mato Grosso, Brazil, and later found in other species of South American fire ants such as *S. richteri*, *S. quinquecuspis* Forel, and *S. macdonaghi* Santschi (Jouvenaz et al. 1980, Wojcik et al. 1987, Briano et al. 1995a, Briano and Williams 2002, Briano et al. 2002). Surveys conducted in the 1970s revealed the absence of these diseases in North American populations of fire ants (Jouvenaz et al. 1977). However, *T. solenopsae* was discovered infecting colonies of the red imported fire ant in the United States in 1996 (Williams et al. 1998) and now is being used as one of the beneficial organisms in the Areawide Suppression of Fire Ants Program conducted by USDA with the collaboration of several U.S. universities (Pereira 2004).

Since 1988, surveys for these pathogens have been conducted intensively in Argentina (Briano et al. 1995a, Briano and Williams 2002, Briano et al. 2002) to select field locations for long-term studies on their pathobiology, ecology, efficacy, and specificity. Although most of these studies have been concluded (Briano et al. 1995a,b,c,d, 1996, 2002, Briano 2005), there is a continued need to expand explorations to find fire ant colonies with high prevalence of infection, mainly to be used for additional testing and as a source of the infected colonies to be shipped to quarantine facilities in the United States. A secondary objective of this work was to study the field persistence of both microsporidia.

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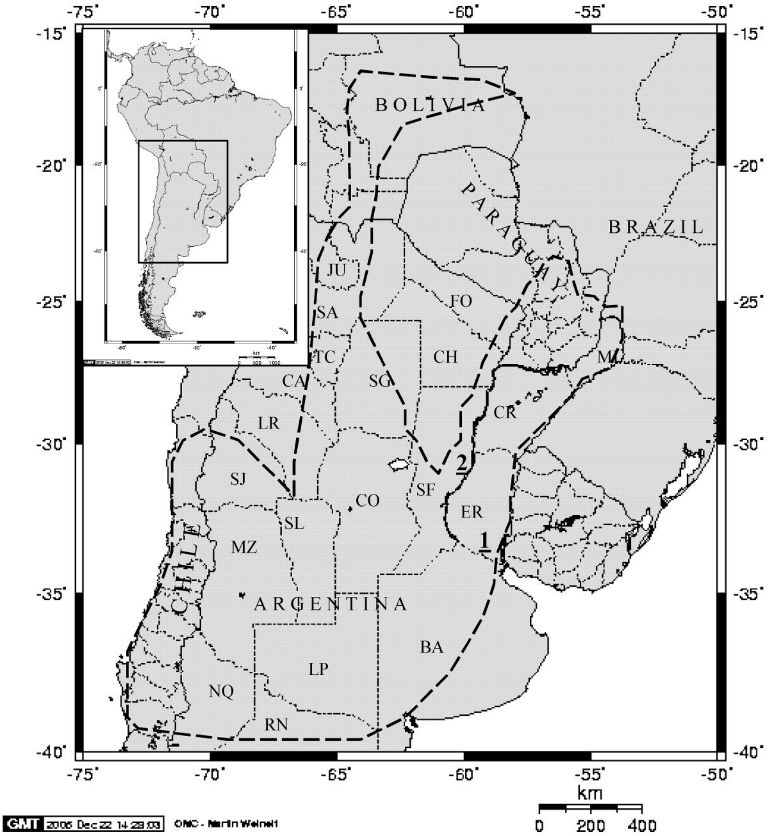


**Fig. 1.** Area explored (within dashed line) for the fire ant microsporidia *V. invictae* and *T. solenopsae* from July 2001 to December 2005 in southern South America. BA, Buenos Aires; CA, Catamarca; CO, Córdoba; CR, Corrientes; CH, Chaco; ER, Entre Ríos; FO, Formosa; JU, Jujuy; LP, La Pampa; LR, La Rioja; MZ, Mendoza; MI, Misiones; NQ, Neuquén; SA, Salta; SJ, San Juan; SL, San Luis; SF, Santa Fe; SG, Santiago del Estero; RN, Río Negro; TC, Tucumán; site 1, Médanos; site 2, San Javier.

## Materials and Methods

**Surveys.** The exploration was conducted from July 2001 to December 2005, mostly in Argentina in the provinces of Buenos Aires, Catamarca, Córdoba, Corrientes, Chaco, Entre Ríos, Formosa, Jujuy, La Pampa, La Rioja, Mendoza, Misiones, Neuquén, Salta, San Juan, San Luis, Santa Fe, Santiago del Estero, Río Negro, and Tucumán, but also in several sites in southeastern Paraguay, southcentral Bolivia, central Chile, and in a small area of Brazil close to the Argentinian border in Misiones (Fig. 1). The area surveyed west of meridian 62° was previously unexplored. A total of 2,064 fire ant colonies were sampled in 262 sites mostly along roadsides, but also in pastures and recreation areas. In all cases, the ants were sampled by introducing a 7-ml vial into the mounds for several minutes. The vials were dusted with talc to prevent the ants from escaping. Samples containing from several hundred to several thousand workers were preserved in 70 or 96% ethanol for transportation to the laboratory. Once in the laboratory, ≈50% of the workers were macerated and a drop of the liquid extract was examined under a phase-contrast microscope (×400) for the presence of spores of *V. invictae* and *T. solenopsae.* The remaining 50% of the workers was kept for identification and as voucher specimens at the SABCL collection. The microsporidia were identified to species based on morphology (Knell et al. 1977, Jouvenaz and Ellis 1986), and identification was confirmed by amplification of a subset of samples by multiplex polymerase chain reaction (PCR) using species-specific oligonucleotide primers as described by Valles et al. (2004).

**Persistence of the Infections.** Three locations with high microsporidian prevalence, two in Médanos, Entre Ríos (Fig. 1, site 1), and one in San Javier, Santa Fe (Fig. 1, site 2), were sampled to monitor the field persistence of both pathogens. The sites in Médanos were sampled 9–10 times at different intervals from July 2001 to March 2005 and the site in San Javier was sampled 5 times from October 2002 to June 2005. The monitoring consisted of vial sampling as described above. On average, 18 colonies (range: 7–70 colonies) were sampled per site and monitoring date. According to biochemical taxonomy studies (Vander Meer et al. 1985) by gas chromatography (GC) performed at the

**Table 1. Prevalence of *V. invictae* as a single infection in the area surveyed of South America**

| Country and province | No. sites examined | Percent (*n*) of sites infected | No. colonies examined | Percent (*n*) of colonies infected |
|---|---|---|---|---|
| Argentina | | | | |
| Santa Fe | 32 | 31 (10) | 669 | 20 (136) |
| Misiones | 16 | 13 (2) | 37 | 8 (3) |
| Corrientes | 14 | 36 (5) | 173 | 7 (12) |
| Entre Ríos | 47 | 15 (7) | 556 | 7 (40) |
| Subtotal | 109 | 22 (24) | 1435 | 13 (191) |
| Brazil | 3 | 33 (1) | 4 | 25 (1) |
| Paraguay | 24 | 21 (5) | 56 | 9 (5) |
| Bolivia | 26 | 4 (1) | 129 | <1 (1) |
| Others | 100 | 0 | 440 | 0 |
| Total | 262 | 12 (31) | 2,064 | 10 (198) |

USDA-ARS-CMAVE, Gainesville, FL, the fire ant species present in the area of Médanos was *S. richteri.* Using the same chemical tool, the fire ant present in San Javier was identified as *S. invicta;* however, the GC profiles differed from those of *S. invicta* in the United States, and it was considered *S. invicta* non-U.S. type. Also, according to PCR studies conducted at CMAVE by the method of Valles and Porter (2003), the proportion of the multiple-queen form (polygyne colonies) was 37% in Médanos and 33% in San Javier. The identification of the social form of the imported fire ants by PCR is based on the fact that it is genetically determined. Monogyne queens are homozygous for the responsible gene (*Gp-9*), whereas polygyne queens are heterozygous. The PCR method is able to accurately discriminate alleles in a single and quick reaction (Valles and Porter 2003).

## Results and Discussion

**Surveys.** Single infections with the pathogen *V. invictae* naturally occurred at 12% (31/262) of the sites surveyed and in 10% (198/2,064) of the colonies sampled (Table 1). Except for one fire ant colony with a very light infection found in eastern Bolivia (first report), the distribution of *V. invictae* was restricted to the more temperate and subtropical eastern part of the region, which included Santa Fe, Misiones, Corrientes, Entre Ríos, Paraguay, and Brazil. Clearly, the highest overall occurrence in Argentina was found in Santa Fe Province, where 20% (136/669) of the colonies were infected (Table 1). In *S. invicta* sites, infection prevalence was highest in San Javier (54%) and La Brava (50%). The highest prevalence in *S. richteri* (U.S. type) sites was found in Médanos, Entre Ríos, with up to 60% of the colonies infected. Although the prevalence of infection was also high in Brazil, with 25% of the colonies being infected, insufficient sites and colonies were examined to accurately estimate *V. invictae* prevalence. The prevalence of *V. invictae* was low in Paraguay and very low in Bolivia, with 9 and <1% of the colonies infected, respectively (Table 1).

The percentage of sites with colonies infected with *V. invictae* during this survey (12%) was similar to that reported previously for some of the same provinces surveyed (Briano and Williams 2002) but more than two-fold higher than the one reported for the province of Buenos Aires (5%) for *S. richteri* (Briano et al. 1995a). Compared with some of our previously reported surveys, the prevalence of infected colonies is 5- to 10-fold higher (10 versus 2.3 and 1%, respectively). However, we attribute this discrepancy, at least in part, to the fact that our sampling effort was somewhat biased toward these areas of known infection. In one of the sites where Briano and Williams (2002) reported epizootic levels, such as San Justo, Santa Fe, very few fire ant colonies were found during our surveys in that area for this study. This decline in the density of fire ant colonies had been observed for other sites with an initial high prevalence of *V. invictae* such as Saladillo and Isla Talavera (Briano and Williams 2002).

The microsporidium *T. solenopsae* presented a much wider distribution. As a single infection, it occurred at 25% (65/262) of the sites surveyed and in 13% (270/2,064) of the colonies sampled (Table 2). We found it in the Argentine provinces of Buenos Aires, Entre Ríos, Jujuy, La Pampa, Tucumán, Misiones, Salta, Mendoza, Corrientes, Santa Fe, and in Paraguay and Bolivia, ranging from 2 to 34% of the colonies. This is the first report of the occurrence of *T. solenopsae* in several provinces in the northwest (Tucumán, Salta, and Jujuy), in the west (Mendoza), and in central Argentina (La Pampa) and in Bolivia. *T. solenopsae* was found infecting fire ants at altitudes up to 2,280 m above sea level (Tafí del Valle, Tucumán), in arid regions (<106 mm of annual rainfall), and in cold areas (4.2°C average temperature during July, Uspallata, Mendoza) (De Fina 1992). Its distribution range suggests that *T. solenopsae* is more cold-tolerant, can survive under low-moisture conditions, and presents a wider field host range than *V. invictae.* This is also the first report of *T. solenopsae* infecting *S. interrupta* (Santschi), and it is consistent with previous reports stating that this microsporidium is restricted to *Solenopsis* ants (Briano et al. 2002).

The highest overall occurrence of *T. solenopsae* was found in Buenos Aires, with 68% of the sites and 34% of the colonies infected (Table 2), and the site with the highest prevalence at a given time was Médanos, Entre Ríos, with up to 90% of the colonies infected. High prevalence was also found in other localities of Entre Ríos, such as Rio Ibicuicito (50%), Gualeguay (40%), Victoria (30%), and Camps (30%); in localities

Table 2. Prevalence of *T. solenopsae* as a single infection in the area surveyed of South America

| Country and province | No. sites examined | Percent ($n$) of sites infected | No. colonies examined | Percent ($n$) of colonies infected |
|---|---|---|---|---|
| Argentina | | | | |
| Buenos Aires | 22 | 68 (15) | 152 | 34 (51) |
| Entre Ríos | 47 | 26 (12) | 556 | 28 (153) |
| Jujuy | 9 | 44 (4) | 40 | 23 (9) |
| La Pampa | 9 | 44 (4) | 28 | 18 (5) |
| Tucumán | 7 | 29 (2) | 27 | 15 (4) |
| Misiones | 16 | 13 (2) | 37 | 11 (4) |
| Salta | 22 | 27 (6) | 80 | 10 (8) |
| Mendoza | 6 | 17 (1) | 18 | 6 (1) |
| Corrientes | 14 | 21 (3) | 173 | 3 (6) |
| Santa Fe | 32 | 31 (10) | 669 | 3 (21) |
| Subtotal | 184 | 32 (59) | 1780 | 15 (262) |
| Paraguay | 24 | 17 (4) | 56 | 9 (5) |
| Bolivia | 26 | 8 (2) | 129 | 2 (3) |
| Others | 28 | 0 | 99 | 0 |
| Total | 262 | 25 (65) | 2,064 | 13 (270) |

in Santa Fe, such as Rafaela (25%); and in localities in Buenos Aires, such as Ramallo (25%). The occurrence of *T. solenopsae* found during this survey (25% of the sites) was in agreement with Briano et al. (1995a).

Interestingly, in Buenos Aires Province, where *S. richteri* was the predominant fire ant, *T. solenopsae* was the predominant infection. Conversely, in Santa Fe province, where *S. invicta* was the predominant fire ant species, *V. invictae* was the predominant infection. These data are consistent with a report by Briano et al. (2002), who found that *S. invicta* were more susceptible to infection by *V. invictae.* This field host preference would make the future release of *V. invictae* in the United States very promising against *S. invicta,* which is the imported fire ant with the widest distribution.

In our survey of 262 sites, both microsporidia coexisted only in 11 locations, and in 10 (4%), both infections were found simultaneously in at least one colony (dual infections). We found dual infections in 2.2% (46/2,064) of the colonies sampled in Santa Fe, Entre Ríos, Misiones, Corrientes, and Buenos Aires (Table 3). These occurrences were 3- and 11-fold higher than the ones reported by Briano and Williams (2002) (1.3% of the sites and 0.2% of the colonies). The percentage of dual infections (2.2%) was almost twofold higher than the combined probability of finding *V. invictae* (10%) and *T. solenopsae* (13%) in the same colony simultaneously ($0.10 \times 0.13 = 0.013 = 1.3\%$). This probability prediction is not consistent with previous findings (Briano and Williams 2002, Briano 2005) and results from repeated and planned samplings in sites with an unusually high prevalence (epizootic levels) of dual infections.

In sites where both microsporidia co-occurred, the total percentage of colonies infected was 48% (range: 17–77%); *V. invictae* infected 21% (165/788) of the colonies, *T. solenopsae* infected 20% (160/788) of the colonies; and dual infections infected 7% (53/788) of the colonies (Table 4). The percentage of total colonies infected (48%) was higher than the prevalence of each of the infections in sites where they did not co-occur. *V. invictae* was found exclusively in 15 sites (*Vairimorpha* sites), where its prevalence was 32%, and *T. solenopsae* was found exclusively in 33 sites (*Thelohania* sites) where its prevalence was 36% (Table 4). In other words, it seems that the coexistence of both diseases increased the proportion of total infected colonies in local populations of fire ants.

The prevalence of *V. invictae* in *Vairimorpha* sites and in sites of co-occurrence with *T. solenopsae* (including dual infections) was similar (32 versus 28%, respectively; $\chi^2 = 1.21$, df = 1, $P = 0.27$). However, the prevalence of *T. solenopsae* in *Thelohania* sites was significantly higher than that found in sites where it coexisted with *V. invictae,* including dual infections (36 versus 27%, respectively; $\chi^2 = 11.45$, df = 1, $P = 0.0007$). This might be indicating some interference mechanisms of *V. invictae* against *T. solenopsae* that should be further studied.

Both *V. invictae* and *T. solenopsae* were found equally infecting polygyne or monogyne colonies of *S.*

Table 3. Prevalence of dual infections in the area surveyed of South America

| Country and province | No. sites examined | Percent ($n$) of sites infected | No. colonies examined | Percent ($n$) of colonies infected |
|---|---|---|---|---|
| Argentina | | | | |
| Santa Fe | 32 | 9 (3) | 669 | 3.9 (26) |
| Entre Ríos | 47 | 9 (4) | 556 | 2.7 (15) |
| Misiones | 16 | 6 (1) | 37 | 2.7 (1) |
| Corrientes | 14 | 7 (1) | 173 | 2 (3) |
| Buenos Aires | 22 | 5 (1) | 152 | 0.6 (1) |
| Others | 131 | 0 | 477 | 0 |
| Total | 262 | 4 (10) | 2,064 | 2.2 (46) |

**Table 4. Prevalence of *V. invictae* and *T. solenopsae* in Argentine sites where they did and did not coexist**

| | Percent ($n$) of colonies infected with | | | |
|---|---|---|---|---|
| | *Vairimorpha invictae* | | *Thelohania solenopsae* | |
| | In *Vairimorpha* sites ($n$ = 15) | In sites with both ($n$ = 11) | In *Thelohania* sites ($n$ = 33) | In sites with both ($n$ = 11) |
| Colonies examined | 196 | 788 | 432 | 788 |
| Infected with a single disease (*Vairimorpha* or *Thelohania*) | 32 (62) | 21 (165) | 36 (157) | 20 (166) |
| Dual infected colonies | 0 | 7 (53) | 0 | 7 (53) |
| Total infected colonies | 32 (62) | 28 (218) | 36 (157) | 27 (213) |

*invicta* in Santa Fe and *S. richteri* in Entre Ríos. This ability to infect both social forms is consistent with previous findings on *S. richteri* in Buenos Aires Province (Briano et al. 1995b) and on *S. invicta* in other areas of Santa Fe (Valles and Briano 2004). In the United States, *T. solenopsae* seemed to be restricted to polygyne colonies of *S. invicta* (Oi et al. 2004), but recently, Fuxa et al. (2005) reported the discovery of *T. solenopsae* infecting a small number of monogyne colonies of the red imported fire ant in Louisiana.

Fire ants were found free of infections in Formosa, Chaco, Santiago del Estero, Córdoba, Neuquén, Rio Negro, San Luis, San Juan, and Chile. However, lack of detection should not be construed as an absence of infection; additional surveys should be conducted in these regions to balance sampling efforts in time and space with other positive areas. Only six colonies of *Solenopsis gayi* (Espínola) were found in 4 of the 85 sites surveyed in central Chile. In addition, only a few sites were surveyed in Brazil, and the prevalence of the diseases found is not representative. In January 2003, one colony of *S. invicta* was found infected with the Neogregarine *Mattesia* sp. in Paraguay (Pereira et al. 2002).

**Persistence of Infections.** Repeated examination of the three sites selected in Entre Ríos and Santa Fe revealed a high prevalence of infected colonies, reaching epizootic levels in most occasions (Figs. 2–4). As in Briano and Williams (2002), we considered an epizooty when at least 20% of the colonies were infected (minimum sample size of 10 colonies). The total prevalence of pathogens ranged from 15 to 100% of the colonies, being ≥20% in 23 of the 24 monitoring dates.

In Rt. 11 km 189, Médanos, Entre Ríos, the total prevalence of microsporidian pathogens was 78% (range: 50–100%) of the colonies. On two occasions (November 2002 and April 2003), all fire ant colonies sampled were infected. The predominant infection was *T. solenopsae*, infecting 58% of the colonies (range: 40–90%); *V. invictae* infected 17% (range: 0–60%), and dual infections were observed in 3% of the colonies (range: 0–14%). At Rt. 11, km 203, the total prevalence was 58% (range: 20–90%); *T. solenopsae* infected 36% of the colonies (range: 10–59%), *V. invictae* infected 10% (range: 0–60%) of the colonies, and dual infections were 12% (range: 0–50%). In April 2002, this site showed the highest prevalence of dual infections ever recorded in Argentina (50%).

In San Javier, the total prevalence of pathogens was 46% (range: 15–67%). The predominant microsporidium was *V. invictae*, infecting 29% (range: 0–54%) of the colonies, followed by *T. solenopsae* at 8% (range: 4–14%). Dual infections were observed in 11% of the colonies (range: 0–43%). These infection levels were similar to the ones reported by Briano and Williams

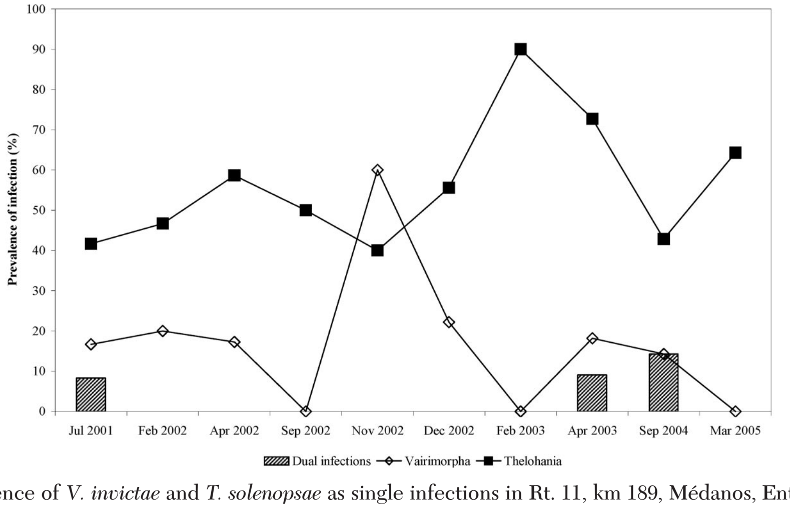


**Fig. 2.** Persistence of *V. invictae* and *T. solenopsae* as single infections in Rt. 11, km 189, Médanos, Entre Ríos Province, Argentina.

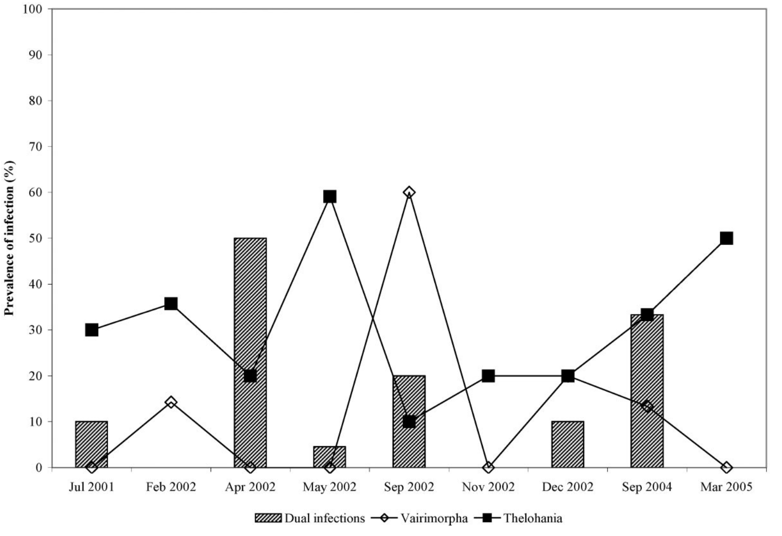


**Fig. 3.** Persistence of *V. invictae* and *T. solenopsae* as single infections in Rt. 11, km 203, Médanos, Entre Ríos Province, Argentina.

(2002) for *V. invictae* for other areas of Santa Fe Province. Again, in *S. richteri*, the predominant infection was *T. solenopsae*, and in *S. invicta*, the predominant disease was *V. invictae.*

Each microsporidium species exhibited a characteristic enzootic/epizootic wave (Tanada and Kaya 1993). While *V. invictae* occurred more sporadically, more limited in time, and with sudden changes in prevalence, *T. solenopsae* showed a sustained (high or low) presence within the fire ant populations with less abrupt fluctuations in prevalence (Figs. 2–4). *T. solenopsae*, as a single infection, was present in the fire ant populations in 100% of the occasions (24/24). The continual occurrence of *T. solenopsae* might be indicating its high ability to be transmitted.

However, *V. invictae* was detected in 63% (15/24) of the occasions as a single infection and in an extra 17% (4/24) in dual infection with *T. solenopsae.* Dual infections were detected in 50% (12/24) of the occasions. Briano and Williams (2002) also reported high variation in prevalence of *V. invictae* in other sites surveyed such as Isla Talavera, San Justo, and Saladillo. Although *V. invictae* was not detected in the field in 5 of 24 occasions, low, undetected levels of inoculum must have persisted in the field with sufficient density and/or spatial distribution to initiate an epizooty after a few to several months. Also, it might be indicating a reasonable high survival capacity of the spores. This is not consistent with information reported by Briano and Williams (2002) for other areas where *V. invictae*

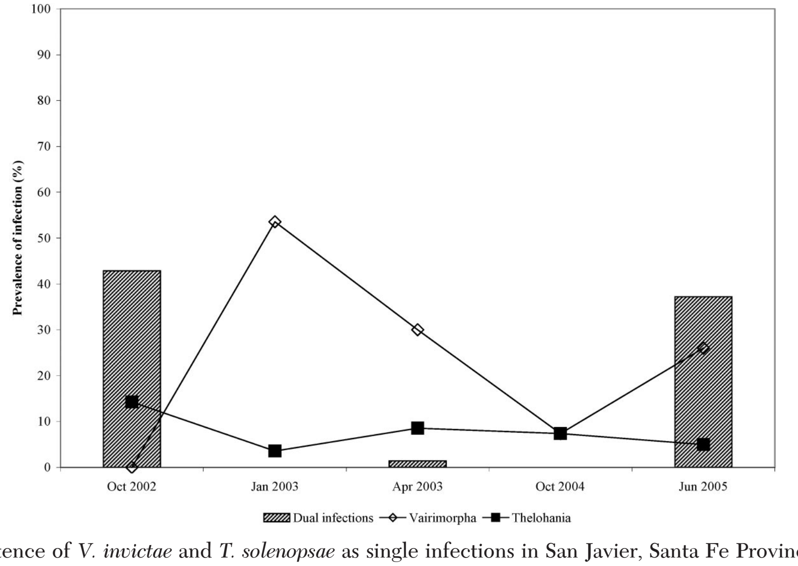


**Fig. 4.** Persistence of *V. invictae* and *T. solenopsae* as single infections in San Javier, Santa Fe Province, Argentina.

showed a continued decline and became undetectable for long periods in populations of *S. richteri* (non-U.S. type) but agrees with previous studies in that *V. invictae* seemed to prefer *S. invicta* as a host (Briano et al. 2002). Host specificity and preference tests should be conducted to confirm this. Although movement of fire ants mounds has been reported (Briano et al. 1995c), the effect of this movement of chronically infected colonies on the dispersal of both diseases has not been determined.

Neither the more sporadic presence of *V. invictae* nor the fluctuations in prevalence of both microsporidia were seasonal. This agrees with Cook (2002). who also reported the lack of relationship between *T. solenopsae* infection prevalence and climatic conditions in the United States. High natural occurrence and fluctuations in prevalence were found for *T. solenopsae* infecting *S. invicta* in Florida (Oi et al. 2004), Texas (Cook 2002, Brain et al. 2004), Oklahoma (Karpakakunjaram and Wright 2004), and Louisiana (Fuxa et al. 2005).

In the three selected sites periodically examined, high peaks in prevalence of *T. solenopsae* seemed to coincide with low peaks in prevalence of *V. invictae*, and high peaks of *V. invictae* seemed to coincide with low peaks of *T. solenopsae* (Figs. 2–4). The reason for the apparent poor coexistence of both diseases in the same site remains unclear and should be further studied with appropriate laboratory tests to check if mutual interference of both microsporidia exists. However, the successive high levels of both diseases, one at a time, would determine a more constant pressure against the host populations of fire ants, as the total number of infected colonies indicated.

The wider distribution of *T. solenopsae*, with high field persistence and high prevalence, the more sporadic but epizootic behavior of *V. invictae* with its field preference for *S. invicta*, the ability of both microsporidia to infect monogyne and polygyne colonies of fire ants, and recent findings (Briano 2005) indicating substantial decline in densities of red imported fire ants infected with both microsporidia suggest that these diseases are highly appropriate for a long-term classical biological control approach. If this is confirmed after the field release of *V. invictae*, it will have important implications in the biological control program of the imported fire ants currently in progress in the United States.

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