- 9 Gutsmann, T. et al. (2003) Interaction of amoebapores and NK-lysin with symmetric phospholipid and asymmetric lipopolysaccharide/ phospholipid bilayers. *Biochemistry* 42, 9804–9812
- 10 Leippe, M. et al. (1994) Amoebapores, a family of membranolytic peptides from cytoplasmic granules of *Entamoeba histolytica*: isolation, primary structure, and pore formation in bacterial cytoplasmic membranes. *Mol. Microbiol.* 14, 895–904
- 11 Bruhn, H. et al. (2003) Amoebapores and NK-lysin, members of a class of structurally distinct antimicrobial and cytolytic peptides from protozoa and mammals a comparative functional analysis. Biochem. J. 375, 737–744
- 12 Nickel, R. et al. (1999) Pore-forming peptides of Entamoeba dispar: similarity and divergence to amoebapores in structure, expression and activity. Eur. J. Biochem. 265, 1002–1007
- 13 Andrä, J. et al. (2003) Amoebapores, archaic effector peptides of protozoan origin, are discharged into phagosomes and kill bacteria by permeabilizing their membranes. Dev. Comp. Immunol. 27, 291–304
- 14 Anderson, D.H. et al. (2003) Granulysin crystal structure and a structure-derived lytic mechanism. J. Mol. Biol. 325, 355–365
- 15 Carson, M. (1991) Ribbons 2.0. J. Appl. Crystallogr. 24, 946–950
- 16 Nicholls, A. et al. (1991) Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins* 11, 281–296

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Can wild *Triatoma infestans* foci in Bolivia jeopardize Chagas disease control efforts?

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The expected success of Chagas disease control programs in the Southern Cone countries relied on the assumption that *Triatoma infestans*, the main domestic vector, did not maintain silvatic foci except in the Cochabamba valley in Bolivia. Recent fieldwork revealed that wild populations of this vector are much more widespread throughout Bolivia than previously thought. Therefore, it is important to find out whether these silvatic populations could jeopardize control efforts in Bolivia, and to investigate their possible occurrence in neighboring regions of Paraguay and Argentina.

Control of Chagas disease by elimination of *Triatoma infestans* is being successfully pursued in most of the Southern Cone countries where this triatomine species is the main domestic vector [1]. At the beginning of the late 1990s, the incidence of Chagas disease was an estimated 500 000 new cases per year [2]. Control interventions have been estimated to reduce the incidence of Chagas disease in the Southern Cone region by 60% (in Paraguay) and by up to 99% (in Uruguay and Chile; http://www.who.int/ctd/ chagas/epidemio.htm). In Bolivia, recent Chagas disease control activities have made substantial progress. However, the major obstacle to the elimination of *T. infestans*

Corresponding author: Noireau, F. (noireauf@ioc.fiocruz.br). Available online 28 October 2004 mestic triatomine populations because of the reduced effectiveness of pyrethroids in peridomestic habitats [2]. The expected success of interruption of Chagas disease transmission by vector control relied on some of the triatomine vector's biological behavioral traits. Particularly important was the almost exclusively domestic nature with the exception of restricted areas in Cochabamba valley (in the Bolivian Andes) where a few wild populations have been reported [3]. The idea of an absence of wild insect populations (which would preclude the recolonization of treated areas by insects coming from silvatic foci) was a pivotal argument used for launching large-scale control campaigns against this particular vector. The recent reports providing evidence that wild populations of T. infestans are much more widespread throughout Bolivia than previously thought (Figure 1) draw attention to the need of further research on this important and neglected issue, particularly on the role that silvatic populations play in the process of recolonization of insecticide-treated villages.

in several Southern Cone countries derives from perido-

The Bolivian wild foci of T. infestans

Although previous reports indicated that T. *infestans* isolates (Figure 2a) were occasionally found in silvatic areas in Argentina, Paraguay and Brazil, it was assumed that T. *infestans* did not maintain silvatic foci in these



Figure 1. The distribution of wild *Triatoma infestans* foci in Bolivia. Silvatic foci of *T. infestans* have been found in three distinct Andean departments (Cochabamba, La Paz and Chuquisaca) and in the Boreal Chaco, Santa Cruz department [6–9].

areas because most specimens were found in ecotopes relatively close to human dwelling [4,5]. However, the existence of true Bolivian silvatic foci is now beginning to be documented. Wild-type *T. infestans* was first reported more than 50 years ago in a stony hill situated in the immediate outskirts of Cochabamba [2600 m above sea level (asl)], an important Andean city [6]. During the 1980s, this observation was extended to some limited sites of the Cochabamba region and near other valleys [7]. More recently (beginning in the late 1990s), several new foci were found in three Andean departments: (i) Cochabamba [the new prospected site of Cotapachi located in Quillacollo district (Figure 2b), (2750 m asl; 17°26'S, 66°17'W); (ii) La Paz (Caracato district, 2500 m asl; 17°06'S, 67°41'W); and (iii) Chuquisaca [Mataral district (Figure 2c), 1750 m asl; 18°36'S, 65°07'W] [8]. These wild populations always occur among rock-piles [7], and seem to be associated with small rodents and marsupials as indicated by a recent survey carried out at the Cotapachi site. In this site, T. infestans were collected from 30% of the burrows where Bolomys and Philotys rodent species, and Thilamys marsupials find shelter. Both the hosts and insect vectors presented high level of natural infection (>60%) with the T. cruzi parasite. Silvatic T. infestans populations have also been found outside the Andes, in the lowlands of the Bolivian Chaco (Figure 2d) [9]. The observation that these insects were chromatically different from the typical T. infestans (overall darker coloration with small yellow markings on the connexivum) led to the idea that they could represent a different species, but allozyme, mitochondrial DNA (mtDNA) and cytogenetic analyses confirmed its status as a population of T. infestans [10,11]. The capture of numerous immature forms and adults in hollow trees enabled the characterization of these T. infestans 'dark morphs' main habitat. The infection of this vector by *T. cruzi* was low (2.5%) and the animal hosts are still unknown [12].

T. infestans center of origin

The Andean mesothermic valleys in Bolivia have long been regarded as the geographical center of origin for T. infestans. This was mainly because wild populations could only be found in these valleys. More recently, this view has also been supported by genetic analysis [13]. The introduction of T. infestans to the domestic environment would have been a consequence of the domestication by the pre-Columbians of wild guinea pig, a natural host. After this first step of adaptation to the domestic environment, T. infestans would then have dispersed to other countries of South America, apparently in association with human migrations, ultimately reaching Brazil during the 20th century [14]. The recent detection of wild *T. infestans* foci in the Bolivian Chaco challenges this view. This discovery prompted the suggestion that the most ancient populations would be those of the dry subtropical Chaco forest in southeastern Bolivia, Paraguay and the north of Argentina [15]. However, recent findings based on allozymes, nuclear ribosomal DNA (rDNA) and genome size favor the first hypothesis [16,17]. Indeed, the wild

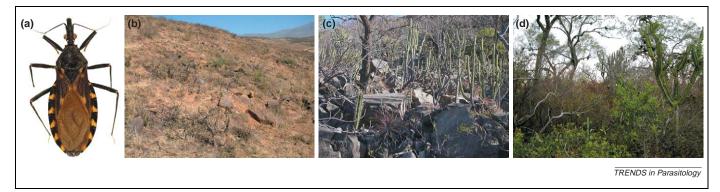


Figure 2. Silvatic habitat of *Triatoma infestans*. (a) *Triatoma infestans* is the main vector of Chagas disease in the Southern Cone countries. (b) *Triatoma infestans* is rupicolous (encountered among rocks) in the Andes within high-altitude regions, where there is a low diversity of vegetation. (c) In areas of middle altitude in the Andes, *T. infestans* can be found among rocks in the middle of xerophytic plants. (d) In the boreal Chaco, the vector is found in hollow trees and where thorn shrubs, bromeliads and cacti predominate in the lowest stratum. Photographs taken by F. Noireau.

T. infestans named 'dark morphs of the Chaco' seem genetically closer to the domestic forms from the same region than to the Andean silvatic forms [16]. But if the Andean hypothesis is accepted, it implies that at least one event of recolonization of silvatic habitat by a domestic population would have occurred in the Chaco. There is still another hypothesis that requires examination: that the Chaco population is a consequence of a straight expansion of a silvatic Andean population that gradually lost heterochromatic DNA during the process. This hypothesis could be tested with an Andes–Chaco transect and subsequent quantification of total DNA content per cell of collected specimens [15].

Distribution of T. infestans silvatic populations

Andean silvatic T. infestans populations are certainly more widespread throughout the mesothermic valleys of Bolivia than was previously accepted. Indeed, our field research shows that efficient investigations in potential sites (e.g. stony hills) have been repeatedly successful in detecting silvatic populations of the vector (F. Noireau et al., unpublished). With regard to other silvatic melanic populations (such as the dark morph population), a much wider distribution throughout the Chaco ecosystem in Argentina, Bolivia and Paraguay is also expected, as indicated by a recent field collection of dark specimens carried out by our group in southeastern Bolivia at the Paraguayan border, and by the record of one specimen in Salta, Argentina (R. Carcavallo, unpublished). The application of ecological landscape approaches (with the use of tools such as geographical information systems and remote sensing) on the currently known T. infestans silvatic foci would enhance our ability to predict the geographic distribution of other silvatic populations for this species [18].

Can wild insects recolonize insecticide-treated areas?

The two silvatic T. infestans populations studied so far have been considered to be isolated from neighboring domestic populations [19-21]. Would this evidence be enough to ensure optimism for unimpeded elimination of domestic populations of this vector [22,23]? The lack of gene flow between silvatic and domestic vectors could be alternatively explained by the extremely high infestation rates of dwellings in these particular areas, which would hamper the colonization attempts by these wild insects. Moreover, because of the ecological variety of the wild foci (and our limited knowledge of them), we do not have enough arguments to guarantee that they do not pose any epidemiological risk to nearby villages. For example, to date, no study to assess gene flow between domestic and wild populations has been performed in the Chaco. Unlike Mataral and the Chaco, where the silvatic sites are relatively far away from the nearest huts (from 500 m up to 30 km), in Cotapachi, they begin a couple meters away from the huts. In such a scenario, it is hard not to envisage insects moving freely from rock-piles to huts and vice-versa, as if the whole area was a single panmictic unit. Further research is needed to clarify this important issue, and to determine the role that silvatic populations play in the process of recolonization of insecticide-treated villages. Recolonization could be tested genetically by comparing silvatic and domestic insect populations with the use of polymorphic markers such as mtDNA and microsatellites, to determine whether gene flow is occurring. Alternatively, in Cotapachi, because of the proximity of wild and domestic insect populations, more traditional approaches of estimating dispersal capabilities such as mark-release-recapture studies might be more suitable to investigate this issue. Finally, if T. infestans has undergone a domestication process in the past, why could it not happen again in the future? Because these silvatic T. infestans foci are located in Bolivia, which is in the middle of the Southern Cone region, it is important to investigate if they have spread to neighboring regions of northern Paraguay and Argentina. In conclusion, it is essential to determine the role that these silvatic T. infestans populations have as potential source of reinfestation of treated areas, and thus jeopardize current control efforts.

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References

- 1 Schofield, C.J. and Dias, J.C. (1999) The Southern Cone Initiative against Chagas disease. Adv. Parasitol. 42, 1–27
- 2 Schofield, C.J. (1994) Triatominae: Biology and Control, Eurocommunica Publications
- 3 Gurtler, R.E. et al. (2004) Effectiveness of residual spraying of peridomestic ecotopes with deltamethrin and permethrin on *Triatoma* infestans in rural western Argentina: a distinct-wide randomized trial. Bull. WHO 82, 196–205
- 4 Bejarano, J.F.R. (1967) Estado selvático de *Triatoma infestans* y otros aspectos a tener en cuenta para la eliminación de la enfermedad de Chagas 2das Jorn. Entomoepidemiol. Arg. 3, 171–196
- 5 Usinger, R.L. et al. (1966) The biosystematics of Triatominae. Annu. Rev. Entomol. 11, 309–330
- 6 Torrico, R.A. (1946) Hallazgo de *Eratyrus mucronatus*, infestación natural de vinchucas de cerro y *Eutriatoma sordida* en Cochabamba. *An. Lab. Central Cochabamba* 1, 19–23
- 7 Bermudez, H. et al. (1993) Identification and characterization of sylvatic foci of Triatoma infestans in Central Bolivia. Am. J. Trop. Med. Hyg. 49(Suppl), 371
- 8 Noireau, F. et al. (1999) La realidad de los focos selváticos de Triatoma infestans en Bolivia. In La Enfermedad de Chagas en Bolivia – Conocimientos científicos al inicio del Programa de Control (1998–2002) (Cassab, A.J. et al., eds), pp. 151–155
- 9 Noireau, F. et al. (1997) Detection of sylvatic dark morphs of Triatoma infestans in the Bolivian Chaco. Mem. Inst. Oswaldo Cruz 92, 583–584
- 10 Monteiro, F.A. et al. (1999) Mitochondrial DNA variation of Triatoma infestans populations and its implication on the specific status of T. melanosoma. Mem. Inst. Oswaldo Cruz 94, 229–238
- 11 Noireau, F. et al. (2000) Sylvatic population of Triatoma infestans from the Bolivian Chaco: from field collection to characterization. Mem. Inst. Oswaldo Cruz 95, 119–122
- 12 Noireau, F. et al. (2000) Natural ecotopes of Triatoma infestans dark morph and other wild triatomines in the Bolivian Chaco. Trans. R. Soc. Trop. Med. Hyg. 94, 23–27
- 13 Dujardin, J.P. et al. (1998) Population structure of Andean Triatoma infestans: allozyme frequencies and their epidemiological relevance. Med. Vet. Entomol. 12, 20–29
- 14 Schofield, C.J. (1988) Biosystematics of the Triatominae. In Biosystematics of Haematophagous Insects Systematics Association Special 37 (Service, M.W. ed.), pp. 284–312, Clarendon Press
- 15 Carcavallo, R.U. et al. (2000) Phylogeny of the Triatominae (Hemiptera: Reduviidae). Proposals for taxonomic arrangements. Entomol. Vect. 7, 1–99

- 16 Panzera, F. et al. (2004) Genomic changes of Chagas disease vector, South America. Emerg. Infect. Dis. 10, 438–446
- 17 Bargues, M.D. *et al.* Phylogeography of the Chagas disease main vector *Triatoma infestans* based on nuclear rDNA sequences and genome size. *Am J Trop Med Hyg* (in press)
- 18 Gorla, D.E. (2002) Variables ambientales registradas por sensores remotos como indicadores de la distribucion geografica de *Triatoma* infestans (Heteroptera: Reduviidae). Ecol. Austral. 12, 117–127
- 19 Dujardin, J.P. et al. (1987) Isozyme evidence of lack of speciation between wild and domestic Triatoma infestans (Heteroptera: Reduviidae) in Bolivia. J. Med. Entomol. 24, 40–45
- 20 Dujardin, J.P. et al. (1997) Metric differences between silvatic and domestic Triatoma infestans (Heteroptera: Reduviidae) in Bolivia. J. Med. Entomol. 34, 544–551
- 21 Carlier, L. et al. (1996) RAPD. In Proceedings of the Ist International Workshop on Population Genetics and Control of Triatominae (Schofield, C.J. et al., eds), pp. 81–83, Instituto Nacional de Diagnostico y Referencia Epidemiologicos
- 22 Guhl, F. and Schofield, C.J. (1996) Population genetics and control of Triatominae. *Parasitol. Today* 12, 169-170
- 23 Dujardin, J.P. et al. (1997) The use of morphometrics in entomological surveillance of silvatic foci of *Triatoma infestans* in Bolivia. Acta. Trop. 66, 145–153

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Letter

Veterinary antiparasitic needs: met and unmet

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I thank Timothy Geary *et al.* for their plea to obtain more support for basic parasitological research [1]. Before they arrive at this correct conclusion, however, there are some arguable statements made in the section headed 'New opportunities' in their article published in *Trends in Parasitology*.

Under the subheading *Cryptosporidium parvum* infections in cattle, it is stated that 'no drug with proven efficacy and safety is commercially available'. I would like to bring readers to the attention that, in the mid-1990s, in several European countries, a product based on halofuginone lactate (Halocur[®]) was introduced, with proven efficacy and safety against *C. parvum* in young calves [2,3]. This product is currently registered and commercialized in most of the European Union countries.

In the section dealing with tissue coccidians, the authors commented that 'conclusive efficacy of evidence in the field even for registered vaccines for horses and cattle remains to be published'. Recently, an article was published describing the effect of a vaccine comprising killed, whole *Neospora caninum* tachyzoites (Bovilis[®] Neoguard) on the crude abortion rate in dairy cows under field conditions [4]. To complete the picture on tissue coccidians, although not for cattle, a live *Toxoplasma gondii* vaccine (Ovilis[®] Toxovax) is registered and

sold in a number of European countries for immunizing susceptible ewes against the effects of *T. gondii* infection, namely early embryonic death and abortion [5,6].

Indeed, there are several unmet veterinary antiparasitic needs, however, for some of these needs, there are solutions already available.

References

- 1 Geary, T. et al. (2004) The changing landscape of antiparasitic drug discovery for veterinary medicine. Trends Parasitol. 20, 449–455
- 2 Villacorta, I. et al. (1991) Efficacy of halofuginone lactate against Cryptosporidium parvum in calves. Antimicrob. Agents Chemother. 35, 283–287
- 3 Naciri, M. et al. (1993) The effect of halofuginone lactate on experimental Cryptosporidium parvum infections in calves. Vet. Parasitol. 45, 199-207
- 4 Romero, J.J. et al. (2004) Effect of a killed whole Neospora caninum tachyzoite vaccine on the crude abortion rate of Costa Rican dairy cows under field conditions. Vet. Parasitol. 123, 149–159
- 5 Buxton, D. et al. (1995) A commercial vaccine for ovine toxoplasmosis. Parasitology 110(Suppl), S11–S16
- 6 Buxton, D. et al. (1991) Vaccination of sheep with a live incomplete strain (S48) of *Toxoplasma gondii* and their immunity to challenge when pregnant. Vet. Rec. 129, 89–93

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