

A NEW HOST OF *TRYPANOSOMA CRUZI* FROM JUJUY, ARGENTINA:
OCTODONTOMYS GLIROIDES (GERVAIS & D'ORBIGNY, 1844)
(RODENTIA, OCTODONTIDAE)

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To identify wild hosts of Trypanosoma cruzi, surveys were conducted in the subandean valleys of Jujuy Province, Argentina, between June 1986 and March 1987. Seventy two mammals from 13 different species were examined by xenodiagnosis. Fifty two of them were mostly rodents trapped at the localities of Maimará, León and Tilcara, and the remainder had been kept in captivity at the Estación Biológica Experimental, in Jujuy.

Trypanosoma cruzi infection was detected only in 2 Octodontomys gliroides (2 pos./8 exam. 25%) from all 72 examined mammals. Isolates were called Octodontomys Argentina 1 and 2 (OA1 and OA2). Both infected animals were caught at the archaeological ruin of Pucará, at Tilcara. Repeated searches for triatomines in the ruin itself and in neighbour houses rendered negative results.

Groups of mice inoculated with either OA1 or OA2 isolates became infected between 7 (OA1) to 12 days (OA2) postinoculation PI. Parasitemia peaks were observed between day 12th-14th PI. Scarce amastigote nests were found in myocardium and skeletal muscle. Mortality was observed only for mice inoculated with OA1.

Isoenzyme patterns of OA1 and OA2 were identical to one found in dogs and slightly different from that of human parasites in Argentina.

Bones from Octodontomys sp., were recently found in a cave, dated 10200-8600 BC, in Pumamarca, near Tilcara, Jujuy. There are evidences that O. gliroides cohabited with man in ancient times and was associated to the domestic cycle of T. cruzi transmission, playing a role like that of domestic cavies in Bolivia.

Key words: *Trypanosoma cruzi* – natural infection – wild hosts – Rodentia – Argentina

Ancient foci of American Trypanosomiasis were presumably located in the andean valleys of Bolivia, where the parasite circulated among wild *Triatoma infestans* and cavies (Schofield, 1988). Infection could have been introduced to human dwellings via the domestic culture of guinea pigs (*Cavia porcellus*), a practice which is still common in the region, including northern Argentina. At present truly sylvatic colo-

nies of *T. infestans* have only been found in the Cochabamba valley of Bolivia associated with those Caviidae (Dujardin et al., 1987).

In this paper, we report results of several surveys conducted in the northern subandean valleys of Argentina, between June 1986 and March 1987 in order to identify sylvatic hosts of *T. cruzi*, specially rodents.

MATERIALS AND METHODS

Animals were captured in the Province of Jujuy (23° 24' SL and 65° 67' WL) at the localities of Maimará, León, Tilcara and Tres Cruces, placed between 1620 m and 3500 m above sea level.

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Most small rodents were caught using Sherman traps baited with corn grains. At Tilcara, animals were trapped at the Quebrada de Huichairas and at the precolombian ruin of Pucará, using National traps baited with succulent stems and fruits of a local cactacea (*Opuntia* sp.).

Other mammals previously collected in different places of the province and kept in captivity at the Estación Biológica Experimental of the city of Jujuy, were also studied.

All mammals were examined by xenodiagnosis using five 3rd instar nymphs of *T. infestans* for each animal. Rectal contents of triatomines were microscopically examined at 30 and 60 days postfeeding as reported previously, (Wisnivesky-Colli et al., 1985).

To isolate trypanosomes, positive insects were dissected aseptically and their intestines were macerated in 1 ml of brain-heart-tryptose liquid medium (BHT) (Cazzulo et al., 1985) plus 100 U/ml of penicillin and 100 µg/ml of streptomycin. The suspension then was mixed with 10 ml of foetal bovine serum (FBS), centrifuged at 1,500 g for 10 min to remove bacteria and resuspended in BHT plus 10% v/v FBS and 1% hemin. Parasite population was allowed to increase by repeated subcultures in the same medium, and trypanosomes were harvested by centrifugation at 3,000 g for 15 min.

Biochemical identification of trypanosomes was performed by Dr Enrique Montamat at the Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, and involved electrophoretic zymograms of the following isoenzymes: aspartate aminotransferase (ASAT. EC 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PD. EC 1.1.1.49), malate dehydrogenase (decarboxilating) (NADP) (malic enzyme, ME. EC 1.1.1.40), glucose phosphate isomerase (GPI EC 5.3.1.9), phosphoglucomutase (PGM. EC 2.7.5.1) and alcohol dehydrogenase (NADP) (ADH. EC 1.1.1.2) (Montamat et al., 1987).

To test the infectivity of isolated trypanosomes, groups of 7-8 inbred C3H mice (10 day old) were inoculated with approximately 1.5×10^5 parasites from cultures (7.5×10^3 trypomastigotes). Parasitemia counts were performed by Brener's method (Brener, 1962) using tail blood, from day 7 postinoculation

(PI) onwards. Mice were killed between day 12 and 36 PI and thin smears were performed with heart blood. Tissues from heart, muscle, spleen, liver, brain, lung, kidney, bladder, esophagus, stomach and small and large intestines were removed and fixed in 10% neutral formalin solution. Sections of 6 µm were stained with Hematoxyline-Eosin and examined for amastigote nests and histopathology (Ruiz et al., 1986). Xenodiagnosis using 5-6 third instar *T. infestans* nymphs were performed on positive mice prior to sacrifice.

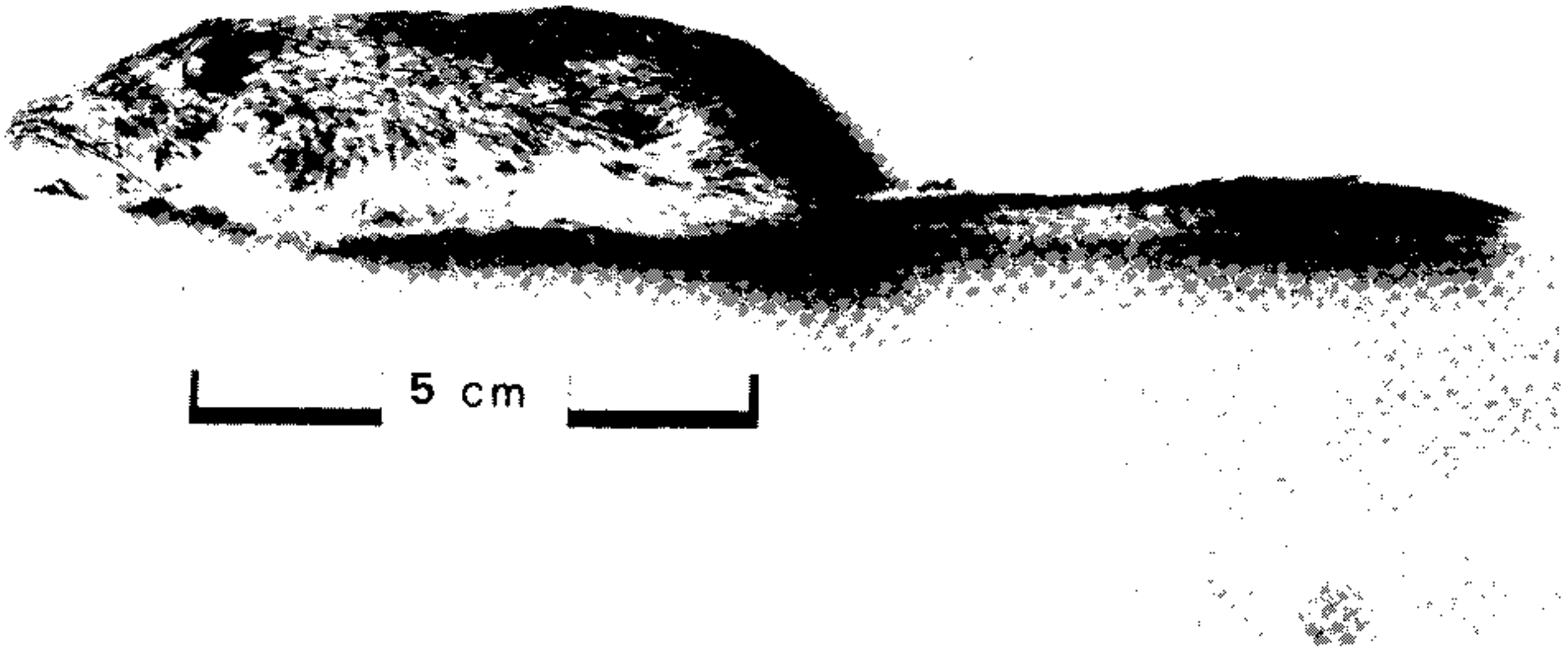
RESULTS

Fifty two captured mammals of the following species (number in brackets) were examined: *Akodon*, sp., country mouse (18), *Thilamys* sp., mouse opossum (13), *Octodontomys gliroides*, "choz-choz" (8), *Ctenomys opimus*, "tuco-tuco" (6), *Galea musteloides*, "cuis" (3), *Oxymycterus* sp., burrowing mouse, (3), *Microcavia australis*, mountain cavy (1). At the Estación Biológica Experimental of Jujuy, the following 20 mammals admitted between March 1984 and October 1986 were studied: *Cebus apella*, weeping capuchin (6), *Felis geoffroyi*, Geoffroy's cat (4), *Dusycion gymnocercus*, south american fox (3), *Dasyprocta punctata*, agouti, (2), *Galictis cuja*, ferret (2), *Euphractus sexcinctus*, six-banded armadillo (1).

Seventy two animals were examined by xenodiagnosis but *T. cruzi* infection was detected only in 2 *O. gliroides* (Fig.) (2 pos./8 exam. 25%). Isolates were called *Octodontomys* Argentina 1 and 2 (OA1 and OA2). Both infected animals were caught at the ruin of Pucará, at Tilcara. However, repeated searches for triatomines in the ruin itself and in local houses at Huichairas valley did not reveal the presence of triatomines.

All mice (7) inoculated with OA1 parasites were found already infected at 7-8 days PI. Parasitemia peak (mean 1.5×10^5 trypomastigotes/ml) was observed on day 12th PI. Cumulative mortality at sacrifice on day 36th PI, was 3/7 (43%) mice.

Seven out of eight mice inoculated with OA2 parasites became infected. Prepatent period was 9-12 days PI. Parasitemia values were much lower than those obtained in animals infected with OA1, mean peak value observed on day 12-14 PI was 3.1×10^3 tripomastigotes/



Octodontomys gliroides caught at Pucará ruin, Tilcara, Province of Jujuy, Argentina.

ml. Mice with highest parasitemias were killed on day 12 PI and the remainder were sacrificed on day 21 PI since no mouse has died up to that time.

Scarce and small amastigote nests were found in myocardium and skeletal muscle of mice infected with both OA1 and OA2 *T. cruzi* isolates. Tissues did not show inflammatory lesions.

Live trypanosomes were recovered by xenodiagnosis of inoculated mice.

The electrophoretic isoenzyme patterns of OA1 and OA2 were identical to one found in dogs and slightly different from that of human parasites in Argentina (Montamat & De Luca, 1990).

DISCUSSION

Octodontomys gliroides is found in the Andean and sub-Andean zones of southwestern Bolivia, in northwestern Argentina from Jujuy to La Rioja and in the Chilean Tarapacá Province (Contreras et al., 1987).

It inhabits dry areas characterized by cacti and rock piles where it digs short burrows con-

nected by superficial runways. This species has diurnal habits and eats succulent plants and the bark of resinous shrubs (Contreras et al., 1987). According to local people, it visits peridomestic areas, getting into abandoned houses. Infected individuals were found in an archaeological area uninhabited at present, the Pucará ruin, where stone houses have been rebuilt following their original features. Probable source of *T. cruzi* infection remain unknown since we could not find triatomines either in the rebuilt houses of the archaeological ruin, or in mud bricked dwellings in the neighbour Huichairas valley. The area had been periodically treated with insecticides in the last eight years and domestic populations of *T. infestans* were considered under control by the local Public Health Service. However, the presence of domestic *T. infestans* populations of low density near the ruin, cannot be ruled out.

Other member of the family Octodontidae, *Octodon degus* is endemic of Central Chile and it has been found infected with *T. cruzi* (Whiting, 1946) reaching a prevalence rate of 2.2% (9/412) (Schenone et al., 1980). *O. degus* is associated with *T. spinolai* in rocky ecotopes and they integrate a separate wild *T. cruzi* cycle, as it is supported by identity of isoenzyme

profiles of trypanosomes isolated from both of them, that differ in turn, from those of domestic stocks, (Apt et al., 1987).

On the contrary, in our case isoenzyme profiles of *T. cruzi* isolated from *O. gliroides* at Tilcara fit with the domestic pattern of the parasite. There are evidences that this rodent cohabited with man in ancient times. Bones from *Octodontomys* sp. were recently found in a cave (Huachichocoana site) dated 10,200-8,600 BC, in Pumamarca, Jujuy, near Tilcara. Other animal and plant remains as well as several cutting litic instruments suggest that the cave was temporarily inhabited by nomadic hunter-gatherers probably coming from the high Puna of Atacama, (Yacobaccio, 1983-85). Adult specimens of *T. infestans* have been found in caves of live *O. gliroides*, accidentally exposed during archaeological excavations performed in 1940-41 (Torres Aparicio, pers. com.).

It would seem that *O. gliroides* was associated to the domestic transmission cycle of *T. cruzi*, playing a role like that of domestic cavies in Bolivia. Tilcara is placed in the Humahuaca valley, an obligate stop in the ancient way connecting the incaic bolivian and chilean settlements with those of northern Argentina. On the other hand, the low pathogenicity and virulence of both OA1 and OA2 *T. cruzi* isolates in mice would indicate an old coevolutionary association between the strain of the parasite and rodents. Further studies are needed to clarify the present epidemiological role of *O. gliroides*.

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REFERENCES

- APT, W.; AGUILERA, X.; ARRIBADA, A.; GOMEZ, L.; MILES, M. & WIDNER, G., 1987. Epidemiology of Chagas' disease in northern Chile: isoenzyme profiles of *Trypanosoma cruzi* from domestic and sylvatic cycles and their association with cardiopathy. *Am. J. Trop. Med. Hyg.*, 37: 302-307.
- BRENER, Z., 1962. Therapeutic activity and criterion of cure on mice experimentally infected with *Trypanosoma cruzi*. *Rev. Inst. Med. Trop. Sao Paulo*, 4: 389-396.
- CAZZULO, J. J.; FRANKE DE CAZZULO, B. M.; ENGEL, J. C. & CANNATA, J. J. B., 1985. End products and enzyme level of aerobic glucose fermentation in Trypanosomatids. *Mol Biochem. Parasit.*, 16: 329-332.
- CONTRERAS, L. C.; TORRES MURA, J. C. & YAÑEZ, L., 1987. Biogeography of Octodontid Rodents: An Eco-Evolutionary Hypothesis. *Filideana Zoology. New Series*, 39: 401-411.
- DUJARDIN, J. P.; TYBAYRENC, M.; VENEGAS, E.; MALDANADO, L.; DESJEUX, P. & AYALA, F. J., 1987. Isoenzyme evidence of lack of speciation between wild and domestic *Triatoma infestans* (Heteroptera, Reduviidae) in Bolivia. *J. Med. Entomol.*, 24: 40-45.
- MONTAMAT, E. E. & DE LUCA D'ORO, G. M., 1990. Polimorfismo enzimático en *Trypanosoma cruzi* de Argentina. Reunión de la Sociedad Argentina de Protozoología, Córdoba, Argentina, p. 41.
- MONTAMAT, E. E.; ARAUZO, S.; CAZZULO, J. J. & SUBIAS, E., 1987. Characterization by electrophoretic zymograms of 19 *Trypanosoma cruzi* clones derived from two chagasic patients. *Compar. Biochem Physiol.*, 87: 416-421.
- RUIZ, A. M.; ESTEVA, M.; RIARTE, A.; A.; SUBIAS, E. & SEGURA, E. L. 1986. Immunoprotection of mice against *Trypanosoma cruzi* with a lyophilized flagellar fraction plus adjuvant. *Immunol Letters*, 12: 1-4.
- SCHENONE, H.; VILLARROEL, F.; ROJAS, A. & ALFARO, E., 1980. Factores biológicos y ecológicos en la epidemiología de la enfermedad de Chagas en Chile. *Bol. Chile. Parasit.*, 35: 42-54.
- SCHOFIELD, C. J. 1988. Biosystematics of the Triatominae, p. 285-312. In M. W. Service, *Biosystematics of Haematophagous Insects*. Clarendon Press, Oxford.
- WHITING, C., 1946. Contribución al estudio de las reservas de parásitos de la enfermedad de Chagas en Chile. I. Primeros hallazgos en Chile de mamíferos silvestres infestados por *Trypanosoma cruzi*. *Rev. Chile. Hig. Med. Prev.*, 7: 69-100.
- WISNIVESKY-COLLI, C.; GURTLER, R. E.; SOLARZ, N. D.; LAURICELLA, M. & SEGURA, E. L., 1985. Epidemiological role of humans, dogs and cats, in the transmission of *Trypanosoma cruzi*, in a central area of Argentina. *Rev. Inst. Med. Trop. Sao Paulo*, 27: 346-352.
- YACOBACCIO, H. D., 1983-85. Explotación complementaria de recursos en sociedades cazadoras recolectoras surandinas. *Cuad. Inst. Nac. Antrop.*, 10: 493-514.