

VECTOR

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CRITERIOS DE NULIPARIDAD Y DE PARIDAD EN *LUTZOMYIA TOWNSENDI* (ORTÍZ, 1959) DEL OCCIDENTE DE VENEZUELA.

Margarita Márquez y José V. Scorza.

RESUMEN

Lutzomyia townsendi (Ortíz, 1959) es un flebótomo antropofílico con concordancia gonadotrófica, en las zonas endémicas de *Leishmania braziliensis* y *L. garnhami* en el occidente de Venezuela.

Mediante el estudio de hembras colonizadas en el laboratorio, nulíparas o paridas, de hembras silvestres alimentadas sobre voluntarios y de hembras silvestres mantenidas con sacarosa, se establecen criterios de nuliparidad con un 56,6% de confianza. En cambio, signos de paridad aparecen en un 82% de las hembras consideradas como tales.

La digestión de un ingesta sanguínea inicia un ciclo gonadotrófico con oogénesis abortiva y acumulación de fosfolípidos en las células epiteliales del estómago y en las glándulas accesorias. La ovoposición completa produce un caliz ovárico grueso e irregular con restos de oocitos abortivos y acumulación de pigmentos. Estos caracteres son suficientes para precisar un criterio de paridad.

La presencia de fosfolípidos en el estómago y en las glándulas accesorias de hembras nulíparas es una indicación de ingesta incompleta de sangre.

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DINÁMICA POBLACIONAL DE *LUTZOMYIA TOWNSENDI* (ORTÍZ, 1959)
(DÍPTERA:PSYCHODIDAE) Y SU PARIDAD EN TRUJILLO, VENEZUELA.

Margarita Márquez y José Vicente Scorza

RESUMEN

Dos años de observaciones sobre las variaciones de densidad de *Lutzomyia townsendi* (Diptera: Psychodidae), junto con estimaciones de los índices de paridad, fueron hechas en la localidad de Calderas, próxima al área endémica de leishmaniasis urbana, en la ciudad de Trujillo, en el occidente de Venezuela.

El régimen pluvial de la zona es bimodal con dos períodos de lluvia, el inicio de las cuales eleva la densidad de flebotomos hasta que la precipitación alcanza más de 200mm, produciéndose entonces un efecto catastrófico sobre las mismas poblaciones.

El porcentaje de paridad es regularmente alto durante todo el año, elevándose más durante el último trimestre, coincidiendo ello con un alza de densidad de mosquitos. Esta alza de paridad y de densidad debe elevar la incidencia de leishmaniasis cutánea en la región.

SUMMARY

In the locality of Calderas near the city of Trujillo in western Venezuela, endemic for urban cutaneous leishmaniasis, the density and parous rates of *Lutzomyia townsendi* main suspected vector, were made along two years altogether climatic estimations. The area shows a bimodal regime of rains with two peaks of heavy rainfall. As soon as rains beging, increases the density of sandflies until 200mm of precipitations when density suddley drops. The parous rates are uniformely high along the year with the exception of the last three months when it increases paralleled to an increases of sandfly density. These last phenomena would have to do with the incidence of cutaneous leishmaniasis in this region.

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HALLAZGO DE *LUTZOMYIA TOWNSENDI* (ORTÍZ, 1959) NATURALMENTE INFECTADA CON *LEISHMANIASIS BRAZILIENSIS*, EN EL ÁREA SUBURBANA DE TRUJILLO, VENEZUELA.

José Vicente Scorza, Margarita Márquez y Julio C. Márquez

RESUMEN

Se comprueba la presencia de *Lutzomyia townsendi* (Ortíz, 1959) naturalmente infectados con *L. braziliensis* en una localidad suburbana de la ciudad de Trujillo, Venezuela. La especie es frecuente durante todo el año, con altas densidades durante los períodos anuales de lluvias; las mayores tasas relativas de paridad ocurren durante la época seca, entre diciembre y febrero, cuando aparecen naturalmente infectadas. Junto con *Lu. townsendi* se ha identificado como especie de importancia secundaria a *Lu. ovallesi* (Ortíz, 1952), esta especie exhibe ritmos densitarios regulares que sugieren un período corto de vida adulta.

SUMMARY

Lutzomyia townsendi (Ortíz, 1959) has been found naturally infected with *L. braziliensis* in an suburban place of the city of Trujillo, Venezuela, where cutaneous leishmaniasis is endemic. The species is found along the whole year with higher densities during the two rainfall yearly peaks. Larger number of parous sandflies appear during the dry season immediately before the rainy period when infected flies were detected. Besides *Lu. townsendi*, *Lu. ovallesi* (Ortíz, 1952) is found showing rhythmic variations in density which suggest a short adult expectancy of life.

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ACTIVIDAD INTRADOMICILIAR DE **LUTZOMYIA YOUNGI** (DIPTERA, PSYCHODIDAE)
EN VENEZUELA *.

José Vicente Scorza y Elina Rojas.

RESUMEN

Un estudio sobre la actividad picadora intradomiciliar y nocturna de *Lutzomyia youngi* en una localidad endémica próxima a la ciudad de Trujillo (Venezuela), realizada en el segundo semestre de 1985, revela un aumento progresivo de la tasa de picadura por noche, un mes después de iniciadas las lluvias y aumentadas las temperaturas mínimas y máximas con elevación de la tasa de baja humedad relativa. La actividad de picadura cesa súbitamente cuando la precipitación acumulada se aproxima a los 500mm. Estas observaciones concuerdan con estudios similares sobre la dinámica poblacional de la misma especie en ambiente extradoméstico, donde la densidad para el mismo período aumenta con las lluvias y disminuye cuando la precipitación acumulada pasa los 200mm.

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Vol XXXV. Nº2. Agosto – Diciembre, 1995.

INHIBICIÓN DEL SITIO ESPECÍFICO DE ADHESIÓN DE *LEISHMANIA BRAZILIENSIS* DESPUÉS DE LA INGESTIÓN DE MANOSA POR *LUTZOMYIA YOUNGI*.

Leonor Rojas de Castillo y José V. Scorza.

RESUMEN

Hembras silvestres de *Lu. youngi* atrapadas con trampa de Shannon en una localidad no endémica para leishmaniasis cutánea y deplecionadas de azúcares reductores ingeridos naturalmente a las 48 horas, solamente ingieren manosa o galactosa cuando estos azúcares se mezclan con sacarosa. Infectados por lotes, alimentando las *Lu. youngi* sobre hámsteres diferentes inoculados con *L. braziliensis*, han sido alimentados adicionalmente con sacarosa mezclada con colorante artificial Mc. Cormick durante 120 horas, detectándose promastigotos en el píloro de 31% de los insectos ingurgitados y presencia de parásitos como tapones en la válvula estomodeal en el 42% de los infectados. La ingestión de sacarosa - manosa por insectos similarmente infectados aunque produjo el mismo porcentaje de infecciones, redujo el número de flebotomos con infecciones pilóricas e inhibió la fijación de haptomonas a la válvula estomodeal, en tanto que aumentó la frecuencia de infecciones rectales por haptomonadas. La sacarosa - galactosa, sin alterar el porcentaje de infectados, produjo una menor colonización de la válvula estomodeal. Se especula la presencia en la cutícula estomodeal en *Lu. youngi* de actividad lectínica similar a Con A y de actividad similar a la ricina en la cutícula del píloro para explicar estos resultados, que comparten el punto de vista de la producción de un lipofosfoglicano la membrana de promastigotos de la fase estacionaria de desarrollo, cuyos terminales activos de manosa y/o galactosa serían inhibidos competitivamente por la ingestión de los mismos azúcares durante las 120 del desarrollo.

Talleres 4: 65, 1995

BIONOMY OF VECTORS OF AMERICAN VISCERAL LEISHMANIASIS IN TRUJILLO STATE, VENEZUELA VI.- ISOENZYME CHARACTERIZATION OF **LEISHMANIA** PARASITES ISOLATED FROM NATURALLY INFECTED **LUTZOMYIA LONGIPALPIS**.

Moreno G. & Oviedo M.

Keys words: Isoenzyme, identification, **Lutzomyia longipalpis**, **Leishmania infantum**

SUMMARY

Lutzomyia longipalpis and **Lu. evansi** are sympatric in focus endemic of visceral leishmaniasis in the area of El Batatillo at the North of Trujillo State, Venezuela. Here they constitute the highly anthropophilic species and both might play some role vector. Nevertheless **Lu. longipalpis** is the most abundant and was found with spontaneous infections.

For this study, 903 females were dissected and identified by examination of genitalia and parasites from positive sandflies inoculated into culture medium NNN with added antibiotics.

Five of the 770 (0.64%) females dissected of **Lu. longipalpis** were found with promastigotes in the abdominal midgut and thoracic midgut. The isoenzyme characterization on starch gel electrophoresis of three of them isolated, compared with reference strains from around the Mediterranean, showed they were linked to the **Leishmania infantum** complex.

The isoenzyme profiles of these isolates were indistinguishable from those obtained from dogs and humans in the same endemic region. Their similarity, as proved by ten enzymes, confirms the reservoir role of the domestic dog and the role of **Lu. longipalpis** as a vector in this area of visceral leishmaniasis.

This present communication reports the first isolation in Venezuela of **Leishmania infantum** from the sandfly **Lutzomyia longipalpis**.

This work was supported by CONICIT - S1-2513

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BIONOMY OF VECTOR OF AMERICAN VISCERAL LEISHMANIASIS IN TRUJILLO STATE, VENEZUELA II.- LONGITUDINAL STUDY OF *LUTZOMYIA EVANSI* IN ENDEMIC SITUATION.

Moreno G. & Oviedo M.

Keys words: Bionomic, populations dynamic, *Lutzomyia youngi*.

SUMMARY

Sporadic cases of infantile visceral leishmaniasis (LV) had been reported in Los Pajones locality (150msnm) at the North of Trujillo State, Venezuela.

With the purpose of to study the dynamic transmission of LV in this focus area a longitudinal study was carried out to determine the sandfly fauna, its abundance, physiological age, natural infection and its relationship with the macroclime. Active catches of sandflies were conducted once every two weeks in the peridomicil, using Shannon traps and aspiration in sheep corrals between the 19th and 20th hours. The physiological age of females were determined using the criteria of nulliparous and parous, taking the age of the laboratory female as control.

2.264 of sandflies were caught, revealing that *Lutzomyia evansi* is the antropophilic specie predominat (99,9%) in Shannon traps as in sheep corrals, showing two pots-rainy picks (July and December) of population abundance. In the study of physiological age, with the exception of February, March and April, the parous females percent remain above 30% along the year, detecting two periods of highest parous females between July - August (58,3% and 52%), coinciding with decrease the precipitation period.

The predominance of *Lutzomyia evansi* in the focus area, were cases of infantile visceral leishmaniasis had been reported, speaks about the responsibility of this specie in the epidemiological cycle.

This work was supported by CONICIT - S1 - 2513

Talleres 4: 104, 1995

BIONOMY OF VECTOR OF AMERICAN VISCERAL LEISHMANIASIS IN TRUJILLO STATE, VENEZUELA V.- FEEDING PREFERENCES OF **LUTZOMYIA LONGIPALPIS** AND **LU. EVANSI** IN WILD POPULATIONS

Villegas, E., Bendezú, H., Moreno, G. & Oviedo, M.

Keys words: bloodmeals, feeding, **Lutzomyia longipalpis**, **Lu. evansi**.

SUMMARY

The purpose of this study was to determine the importance of domestic animals and man as sources of bloodmeals for **Lutzomyia longipalpis** and **Lu. evansi**, its role in the maintenance of sandfly populations and the transmission of visceral leishmaniasis in the focus areas of El Batatillo and Los Pajones (310 and 150msnm) located at the North of Trujillo State, Venezuela.

Sandflies were caught using Shannon traps and aspiration in sheep corrals between the 19th and 20th hours. Bloodmeals of 150 individuals females were identified by the ELISA test, using four antisera: human, dog, sheep and bovine.

Dog blood was the most frequent bloodmeals found, for **Lu. longipalpis** (37,7%) and **Lu. evansi** (34,3%); sheep blood was the second preference (33,3% and 32,2%); human blood was the third preference (26,6% and 27,08%) while the bovine blood was the less frequent (2,2% and 6,25%).

The results reflect the euriphagic nature of these two sandflies species. Epidemiological implications of sandflies are discussed.

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BINOMIA DE VECTORES DE LEISHMANIASIS VISCERAL EN EL ESTADO TRUJILLO, VENEZUELA. V.- REFERENCIAS ALIMENTARIAS DE POBLACIONES SILVESTRES DE ***LUTZOMYIA LONGIPALPIS*** Y ***LUTZOMYIA EVANSI***

Villegas, E., Bendezú, H., Moreno, G. & Oviedo, M.

RESUMEN

La finalidad de este estudio fue determinar la importancia de los animales domésticos y el hombre como fuentes sanguíneas de ***Lutzomyia longipalpis*** y ***Lutzomyia evansi***, además de su papel en el mantenimiento de estas poblaciones y la transmisión de leishmaniasis visceral en los focos El Batatillo y Los Pajones (310 y 150msnm) al Norte del Estado Trujillo, Venezuela. Las capturas se realizaron utilizando una trampa de Shannon y aspiración directa en corrales de cabars entre las 19 y 20 horas. 150 ingestas sanguíneas fueron identificadas con la técnica de ELISA, utilizando cuatro antisueros: perro, ovejo humano y vaca. La sangre de perro fue la más frecuente ingesta sanguínea encontrada para ***Lutzomyia longipalpis*** (39,58%) y ***Lutzomyia evansi*** (33,33%), sangre de cabra fue la segunda en preferencia (31,25% y 34,31%) seguida de la sangre humano (27,08% y 26,47%) y la sangre de bovino fue la menos frecuente (2,08% y 5,88%). Los resultados reflejan la naturaleza eurifagica de estas dos especies flebotominas. Las implicaciones epidemiológicas son discutidas.

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PP. 1, 1997.

SUSCEPTIBILITY OF FEMALES SANDFLIES (*Lutzomyia youngi*, DIPTERA: PHLEBOTOMINAE) FROM TRUJILLO, VENEZUELA TO DDT, MALATHION, PROPOXUR AND LAMBDAHALOTRIN *

Alvarez, L. , Scorza, J. V.****

SUMMARY

Lutzomyia youngi Feliciangeli & Murillo, 1987 is a vector for Cutaneous Leishmaniasis in the city of Trujillo, Venezuela, where the condition is highly endemic. Information on the susceptibility of this vector species to chemical insecticides currently used in Venezuela, have been obtained after exposition of wild females captured in non endemic place at the outskirts of Trujillo city to papers impregnated with different dilutions of DDT, Malathion, Propoxur and Lambdaalotrin technical grade to estimate CL50, CL95 and doses diagnoses following WHO (1970). Values for CL50, CL95 and doses diagnoses respectively, were obtained for DDT 0.29-1.04 and 3.54%, for Malathion 0.23- 0.64 and 1.9%, for Propoxur 0.0066- 0.011 and 0.026% and for Lambdaalotrin 0.0004- 0.0015 and 0.0056%. Lethal cheats 50 and 95 were determined with papers impregnated with diagnoses doses , excepting with the Lambdaalotrin that went to concentration of 0.0025%. Values of TL50 and TL95 for DDT were 6.10 min. and 13.17 min.; for Malathion 15.6 min. and 38.2 min.; for Propoxur 18.3 min. and 36.7 min. and Lambdaalotrin 11.08 min. and 33.4 min. *Lu. youngi* was sensitive to all the tested insecticides to doses diagnoses. It was observed that the studied population is hypersensitive to the synthetic piretroide Lambdaalotrin in connection with the other rehearsed insecticides, suggesting that this has bigger toxic action on *Lu. youngi*.

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DETERMINACIÓN DE ÉPOCAS DE CAPTURAS DE *Lutzomyia youngi* EN CALDERAS,
TRUJILLO, VENEZUELA.

Alvarez, L. & Scorza, J.V.

RESUMEN

La zona de Calderas ubicada a 12km. del área endémica de leishmaniasis cutánea en la ciudad de Trujillo, Venezuela, es un área natural de cría de *Lutzomyia youngi*, detectándose en ella altos números de ejemplares por captura con trampa de luz. Con el fin de precisar la dinámica poblacional de las hembras de esta especie, incriminadas como vectoras en de leishmaniasis, durante todo el año 96, realizamos observaciones sobre las variaciones poblacionales de hembras de *Lutzomyia youngi*, atraídas por trampa de Shannon durante 1 hora de captura, en la localidad las Calderas. Registramos dos picos de máximo incremento poblacional en los meses Abril (n=388) y Agosto (n=342), con precipitaciones pluviales de 90 y 31,5mm respectivamente y un mínimo en Diciembre cuando la precipitación pluvial acumulada sobrepasa los 120mm. Con estas observaciones podría sugerirse como mejor época de captura para *Lu. youngi* los meses cuando ocurren menores precipitaciones.

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POPULATION DYNAMICS OF ***LUTZOMYIA EVANSI*** FROM A VISCERAL & CUTANEOUS
LEISHMANIASIS ENDEMIC AREA OF TRUJILLO, VENEZUELA.

Oviedo M., González A., Barazarte R. & Vivenes M.A.

SUMMARY

Epidemiological surveys in the lowlands of Trujillo State at the north of Venezuelan Andes have shown that these areas are endemic of visceral leishmaniasis (LV). In order to study the transmission dynamics in this area, a longitudinal project was begun to study sandfly fauna, abundance, physiological age, natural infection in relation to macroclimatic factors. Sandfly collections were made once every 2 wk for 15 mo using the Shannon trap & aspiration in the houses & tree holes. Sticky paper was also placed in pigpens, sheep corrals & chicken yards. Among 3,466 sandflies collected, ***Lutzomyia evansi*** was the predominant anthropophilic species (85,19%) collected with Shannon trap, indoors, tree holes & animal corrals. Population fluctuations were related to the bimodal occurrence of rains in the study area. After dissection of 835 ***Lu. evansi*** infection with promastigotes were found in 2 (0,23%) of them. One of these was isolated & inoculated intraperitoneally into the hindleg of ***Mesocricetus auratus*** which developed a typical dermal granulomata. The predominance of ***Lu. evansi*** in the focus of natural infection suggests that this species is involved in the local epidemiological leishmaniasis cycle.

Simposio Internacional ISOPS III

Agosto, 1999.

SEASONAL DYNAMICS ON SAND FLIES OF TRUJILLO – VENEZUELA.

Elina Rojas, Carmen Morales and José V. Scorza.

RESUMEN

The new epidemiological pattern of cutaneous leishmaniasis in the state of Trujillo – Venezuela can be the result of the interaction between the domiciliar environment and the incidence in children (0-12 years old), mothers and family group. During 1985 – 1998, 1.075 cases were diagnosed and medically treated in the Center “J.W. Torrealba” – Trujillo, all these patients live in urban conditions.

The seasonal sandfly behaviour was detected, with collections made by Shannon trap from 7 to 8pm. in the peridomicilie. The collection were made twice a month. In 35.295 sandflies. *Lutzomyia youngi* was the predominant species, *Lutzomyia ovallesi*, result the most important species in attention to physiological age or parity (25.8 and 35.8% resp.). Others species collected were: *Lu. scorzai*, *Lu. erwindonaldoi*, *Lu. lichi* and *Lu. trinidadensis* in least opportunities. *Lu. youngi* was found naturally infected with *Leishmania braziliensis* and we expect to demonstrate the vectorial competence of *Lu. ovallesi* and to confirm it in *Lu. youngi*.

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Agosto, 1999.

EFFECT OF A SECOND BLOOD – MEAL ON THE OESOPHAGUS COLONISATION BY
LEISHMANIA MEXICANA COMPLEX ***IN LUTZOMYIA EVANSI***.
Vivenes A., Oviedo M., Márquez J.C., & Montoya-Lerma J.

SUMMARY

The vectorial competence of any leishmaniasis vector relies not only on its ability to develop and harbour parasites in its intestine but it is essential that colonisation of the foregut by infective promastigotes occurs. In the study of vector capacity two assumptions are widely accepted: firstly that host seeking is stopped by a full bloodmeal and, secondly, that a full infecting bloodmeal is sufficient for an appropriate sandfly vector to become infective. However, two recent observations induced us to revisit these aspects in sandflies. Firstly, hungry gravid and semi-gravid ***Lutzomyia evansi*** were collected inside houses and secondly, this sandfly species could experimentally harbour and develop ***Leishmania mexicana*** but parasites did not reach its oesophagus. Hence, we were interested to establish whether a second blood-meal taken by ***Lu. evansi***, subsequent to an infective one, may influence the oesophageal colonisation of ***Le. mexicana*** parasites.

Laboratory bred ***Lu. evansi*** were fed on hamsters experimentally infected either with one of two ***L. mexicana*** strains (i.e. ***L.m. amazonensis*** and ***L. m. mexicana***). Fed flies were maintained in darkness, under constant conditions (26°C and 85% RH) with sucrose solution. Putatively infected flies were dissected and parasite development and location in their gut assessed at 24 h intervals till 168 h post-infection. At this time, a second meal, on an uninfected hamster, was offered to all survivors. Refed flies were kept as indicated above, receiving sucrose solution. Dissections were continued to 72 hours after re-feeding, following the same 24 h schedule.

Lu. evansi infection susceptibility ranged from 29,4% (n=119) to 21,7% (n=147) for ***L.m. mexicana*** and ***L.m. amazonensis***, respectively. The typical suprapylarian development was observed, though free flagellate forms were present in the Malphigian tubules and posterior gut of very heavy infections. In only 5% of ***L.m. mexicana*** and in none of the ***L. m. amazonensis*** infected flies was there migration and colonisation of the oesophagus by parasites after digestion of the first blood meal. In 75 and 60%, respectively, in both groups after a second blood meal.

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MORPHOLOGY AND GENETIC CONGRUENCE: ***LUTZOMYIA LONGIPALPIS***
(PSYCHODIDAE: PHLEBOTOMINAE) POPULATIONS FROM VENEZUELA.
Arrivillaga J.C., Rangel Y., Feliciangeli D.M. & Oviedo M.

SUMMARY

Lutzomyia longipalpis (Lutz & Neiva), the main vector of visceral leishmaniasis in most countries of tropical America, has been considered by several authors as a species complex based on different genetic and phenotypic studies but no Venezuelan populations have been analyzed. We sampled 4 geographical, geological, ecological and epidemiological different populations from Venezuela. These samples were analyzed by isoenzyme and larval mouthpart morphology – morphometric. The isoenzyme results evidence a strong genetic structure, which was correlated to morphology and morphometric analysis. We suggest the existence of two species of ***Lu. longipalpis*** in Venezuela.

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COMPARISON OF EXPERIMENTAL INFECTIONS OF LUTZOMYIA EVANSI AND LUTZOMYIA LONGIPALPIS WITH LEISHMANIA CHAGASI FROM TWO FOCI OF VISCERAL LEISHMANIASIS IN COLOMBIA AND VENEZUELA.

Montoya-Lerma J., Oviedo M., Cadena H. & Lane R.P.

SUMMARY

Classical studies on the epidemiology of American Visceral Leishmaniasis (AVL), due to *Leishmania chagasi*, indicate an unique and powerful association of this parasite with the sandfly *Lutzomyia longipalpis*, throughout its geographical range, though there are places where *Lu.evansi* acts as alternate or suspected vector. The present study examines and compares the experimental infection rates of these two sandfly species with *Le.chagasi*.

Laboratory bred *Lu. longipalpis* and *Lu. evansi* originally from Colombia and Venezuela were experimentally infected using two Colombian and two Venezuelan *Le. Chagasi* strains, inoculated in hamsters and via artificial membrane (only Colombian assays). Prior to and after infection, caged flies were placed in plastic bags and kept in the shade outside at approx. 28°C and over 90% relative humidity. Cotton pads soaked in sucrose solution and water were provided every day. Although a considerable number of both *Lu. evansi* (395) and *Lu. longipalpis* (310) were fed on infected hamsters, no infection was recorded with either Colombian *L. chagasi* strain. Dissections of blood-fed flies at 4 and 5 day post feeding revealed no detectable changes in the sandfly midgut while digesting the blood meal. Additionally, no alterations in sandfly behaviour and survival rate were observed. In contrast, 2.1% (n=80) *L. longipalpis* developed infections when fed on ILL/VE/94/LL2 infected hamsters. In all cases, infection of the hamsters was confirmed parasitologically.

Results from membrane infections revealed variable development of two strains of *L. chagasi* in both, its dominant (*Lu. longipalpis*) as well in its occasional (*Lu. evansi*) vector species. However, overall the most complete development of leishmaniae parasites was observed in the gut of the co-evolved sandfly species. On the other hand, variable degrees of refractoriness were observed in the non-related fly species.

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MOLECULAR SYSTEMATICS OF *LUTZOMYIA EVANSI* AND THE VERRUCARUM GROUP
Montoya-Lerma J., Ready P.D., Testa J.M., Oviedo M. & Lane R.P.

SUMMARY

The Verrucarum species group is one of the informal subgeneric within the New World (NW) genus *Lutzomyia* França and, based on male morphological characters, it has been split into 3 series: *serrana*, *townsendi* and *verrucarum* (Young D.G. & Duncan M.A. 1994. Mem. Amer. Entom. Inst. 54, 1-881). Several of its species are incriminated vectors of trypanosomatids of the NW subgenus *Leishmania* (*Viannia*) Lainson & Shaw, but *Lutzomyia evansi* (Nunez-Tovar) is unusual in transmitting *Leishmania* (*Leishmania*) *infantum chagasi* Cunha & Chagas, which has probably been introduced in recent times from the Old World. In Venezuela, *L. evansi* is widespread in a range of ecological zones, except humid forest, whereas in Colombia it is restricted to semi-arid zones in the north of the country. In order to help explore the population structure of *L. evansi* in different regions and habitats, we have undertaken comparative analyses of mitochondrial cytochrome b sequences (mtDNA) of individual flies, using the methods developed for *Lutzomyia* (*Nyssomyia*) *whitmani* (Antunes & Coutinho) (Ready P.D. **et al** 1997. Bull.Entomon.Res. 87, 187-195; Ready P.D. *et al.* 1998. Trans.R.Soc.Trop.Med.Hyg. 92, 159-160; Ishikawa E.A.Y. *et al.* 1999. Mems. Instituto Oswaldo Cruz 94, 1-7).

In the present communication, we report 2 conclusions: (1) Cladistic analysis of mt DNA agreed with morphotaxonomy in placing *L. evansi* in the *verrucarum* series; (2) mt DNA molecular clocks indicated that Venezuelan and Colombian populations form 2 separate lineages that diverged in the Pleistocene (within the last 1.5 million years). We

are following up these findings by investigating the microgeographical genetic variation of *L. evansi*, in order to discover any links with vectorial status.

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STANDARDISATION OF PCR FOR DETECTION OF LEISHMANIA CHAGASI IN SANDFLIES
Cadena H., Labrada L.A., Oviedo M., Lane R.P. & Montoya-Lerma J.

SUMMARY

The isolation and accurate characterisation of *Leishmania* species are fundamental to epidemiological studies of leishmaniasis. Usually, efforts are directed at developing new tools for the detection characterisation of *Leishmania* parasites from patients and reservoirs. In contrast, little emphasis has been given to identifying parasites in the sandfly gut and overcoming the time demanding method of sandfly section and subsequent culturing.

The main objective of this study was to test the usefulness of a PCR assay as an alternative, rapid, sensitive and specific method for detection and characterisation of *Leishmania chagasi* infections in sandflies. Batches of *Lutzomyia longipalpis* and *L. youngi* were experimentally infected with either *Le. chagasi* or *Le. mexicana* (2x10⁶ promastigotes/ml). Twelve hours post-infection, a single putatively infected fly was placed in ETOH 80% with batches of 15, 20 and 30 uninfected flies of the same species. At room temperature flies were macerated in 100 µl of grinding buffer, immediately digested and DNA was extracted and amplified. Results showed that only products coming from *Le. chagasi* infected groups were amplified. The sensitivity of the PCR was tested by adding 1 from 1: 10000 of *Le. chagasi* DNA dilution (i.e. 200 fg or approximately 20 parasites) to macerates of 15, 20, 30 and 40 sandflies in ETOH 80%. Results revealed a full single band in cellulose gel only in the first group (i.e. 15 sandflies), suggesting that the assay can detect a fraction of a single parasite in crude biological samples.

At present, the assay is used to test and compare the natural infection rates of wild caught sandflies from two American Visceral Leishmaniasis foci, due to ***Le. chagasi***, of Venezuela and Colombia.

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