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Vector Biology
and Control Project

Trip Report

Technical Assistance in Studying Chagas' Vector Feeding Patterns

Bolivian Chagas' Disease Control Program of the USAID Community and Child Health Project

June 13-22, 1992

by

Robert A. Wirtz, Ph.D.

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Acknowledgments

The author would like to thank the staff of the USAID/La Paz Community and Child Health (CCH) Project, and especially those of the Chagas' Disease Component. Special thanks are due to Drs. Faustino Torrico and Hernán Bermudez for their cooperation and assistance in the laboratory and to Dr. Fanor Baldarrama for the insectary and study site tours.

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1. Summary

A direct blood-meal ELISA was established in the laboratory of the Bolivian national Chagas' control team. This ELISA can detect human, bovine, cat, chicken, guinea pig, sheep and swine hosts using peroxidase conjugated anti-IgG (H+L) antisera The host blood-meal source in adult *Triatoma infestans* fed on chickens and held for 25 days, and in adult *T. infestans* fed on humans and held for 10 days, were detected using this assay. *T. infestans* held for longer times post-feeding will be tested when available. The host blood-meal ELISA will be a valuable tool for studies on the domestic and sylvatic cycles of Chagas' in Bolivia.

2. Introduction

A detailed description of the program, study site and importance of the sylvatic cycle of Chagas' in the Cochabamba area is given in VBC Report No. 82236, "Environmental Assessment of the Bolivian Chagas' Disease Control Program of the Community and Child Health Project (511-0594)," August 1, 1991, by Dr. Andrew A. Arata. The scope of work defining this consultant trip was as follows:

In order to study the sylvatic ecology of populations of the Chagas' disease vector, *Triatoma infestans*, in the Cochabamba area of Bolivia, it is necessary to determine the sources of blood meals on which they depend. The enzyme-linked immunosorbent assay (ELISA) is the most appropriate method for determining blood meal sources and the national Chagas' team in Cochabamba needs to have this capability in its laboratory.

- The consultant will spend one week in Cochabamba instructing the local technicians and professional staff in the use of this test and interpretation of results.

- The consultant will transport the necessary reagents to initiate testing and training. He will assist the local staff in producing, or making arrangements to obtain, reagents for future work.

- The consultant will prepare a brief report outlining the work done in Cochabamba and recommending future assistance that may be required to make the national laboratory self-sufficient in this area.

3. Findings

3.1 Host Blood-Meal ELISA

A direct blood-meal ELISA was established in the laboratory of the national Chagas' control team. This ELISA can detect human, bovine, cat, chicken, guinea pig, sheep and swine hosts using peroxidase conjugated anti-IgG (H+L) antisera. Absorbance with heterologous sera, diluted 1:500, was required to eliminate cross-reactivity and ensure the specificity of the assay. The host blood-meal source in adult *T. infestans* fed on chickens and held for 25 days, and in *T. infestans* fed of humans and held for 10 days, were detected using the direct ELISAs. *T. infestans* held for longer times post-feeding were not available for testing. Host IgG in adult insects triturated in 500 μl of phosphate-buffered saline and diluted 1:1000 were easily detected. Blood meals from the above-mentioned hosts should be identifiable in field-collected, engorged triatomes using the direct ELISA.

Attempts to use peroxidase anti-IgG (gamma) reagents to maintain specificity and eliminate the need for heterologous sera were not successful. Comparative testing of the direct versus the double antibody sandwich ELISAs was discussed and planned in detail. Specific antisera against other possible hosts, such as opossums, armadillos and reptiles (snakes/lizards), may be needed. Plans were discussed for the collaborative production of these reagents if they are not available from commercial sources. Other hosts to which antisera should be generated may be identified as the study on field-collected *T. infestans* progresses.

3.2 *T. infestans* Behavior Studies

The most appropriate methods for studying the behavior of domestic and sylvatic *T. infestans* and *T. sordida* were discussed in detail. The use of mark-release-recapture methods in conjunction with baited or black-light traps and sentinel chickens should be pursued. Another valuable tool would be an ELISA to detect and monitor antibody to *T. infestans* feeding secretions. Such an assay could be developed using salivary gland extracts as the ELISA capture antigen. The assay could be used to

screen animals in the study area to identify triatome feeding patterns. This assay could also be used to monitor seroconversion of sentinel animals and humans or in longitudinal epidemiological studies.

Plans are in effect to use electrophoretic isoenzyme patterns of domestic and sylvatic populations to investigate the possibility of a *T. infestans* species complex. The use of restriction fragment length polymorphism to address this question should also be considered.

During our discussions, national team members mentioned two observations that are intriguing and should be pursued. The first was the report that on two occasions, *T. infestans* fed on a sheep and cow died after feeding. These experiments should be repeated using the same sheep and cow if possible, as well as control chickens or guinea pigs. If high mortality is again observed for triatomes fed on the sheep or cow, the possibility of anti-*T. infestans* antibody should be investigated. Similar observations of high tick mortality after feeding on cattle in Australia resulted in the development of an anti-tick vaccine. The second observation focused on a report in the literature of a red-eye tsetse mutant resistant to African trypanosomiasis infections. Since a red-eye *T. infestans* mutant is available in the laboratory colony, its susceptibility to *T. cruzi* and ability to transmit the infection should be compared to those of black-eye *T. infestans* using the mouse model.

4. Recommendations

4.1 A technician should be hired at the Universidad Mayor de San Simon to continue assay development and the testing of field-collected samples. The technicians and professional staff in the laboratory are fully occupied with existing work. It will be imperative to have additional technical support to develop ELISAs for host blood-meal identification, antibody to triatome feeding secretions, and testing of field-collected *T. infestans* and serum samples in a timely manner.

4.2 A vacuum pump, trap system and manual microtiter plate washer should be purchased. A vacuum source is needed to operate the plate washer, the use of which will reduce the potential for contamination and false positive ELISA results (Annex A).

4.3 Reagents and supplies listed in Annex B should be purchased to permit testing of field-collected triatomes. Additional work will be required to optimize the sensitivity of the direct ELISA by determining, for example, optimum reagent concentrations and incubation times. Once this method has been optimized for sensitivity and specificity, a standard protocol should be established for testing field-collected *T. infestans*. The maximum time after feeding at which host IgG can still be detected should be determined using laboratory-reared adult and different instar *T. infestans* fed on chickens and humans.

4.4 A -70° C freezer with racks should be purchased for storing test samples and reagents. The laboratory's -20° C freezer space is fully utilized and is inadequate for longer term, stable storage of valuable field specimens and reagents. A -70° C Revco freezer costs about US$6,000 to $7,000 without storage racks.

4.5 An ELISA to detect antibody to *T. infestans* feeding secretions in human and other animal sera should be developed. This assay would be a very useful epidemiological tool for studying the domestic and sylvatic cycles of Chagas'.

4.6 The team should evaluate the use of luminous paint and powders for mark-release-recapture studies (Annex C).

4.7 The team should determine the feasibility of using black lights for monitoring adult triatome behavior studies. A review of the literature should give the information required to conduct this study.

4.8 If significant numbers of engorged, field-collected *T. infestans* test negative in the blood-meal ELISA, the range of host antisera should be expanded. If reagents are not available for detecting opossum, armadillo and reptile (snake/lizard) IgG, they should be developed. Antisera against other possible hosts may be needed.

4.9 The team should conduct studies on triatome host preference and the effect of anti-triatome antibody in hosts on the fecundity and development of *T. infestans*.

4.10 The team should compare the red-eye mutant's susceptibility to *T. cruzi* and ability to transmit the infection to those of black-eye *T. infestans* using the mouse model.

4.11 The team should consider the use of restriction fragment length polymorphism (RFLP) to investigate the possibility of a *T. infestans* species complex.

4.12 A double antibody sandwich ELISA should be optimized and compared with the direct assay for sensitivity and specificity. Theoretical advantages of the sandwich ELISA are increased sensitivity, an ability to test a sample aliquot for multiple host immunoglobulins, and specificity without the need to use heterologous sera.

5. Itinerary

June 1992

13	Departed Washington, D.C., and arrived Cochabamba, Bolivia.
13-19	Delivered reagents and supplies necessary to conduct ELISAs. Participated in scientific discussions at the CCH administration building. Instructed professional staff at the Universidad Mayor de San Simon in the use of the blood meal ELISA and interpretation of results. Visited the *Triatoma infestans* insectary.
20	Visited the field study site near Punata and participated in scientific discussions.
21	Depart Cochabamba, Bolivia.
22	Arrive Washington, D.C.

6. People Contacted

CCH Chagas' Control Program, Ccchabamba

Dr. Fanor Baldarrama G.
Dr. Hernán Bermudez P.
Dr. Faustino Torrico T.

Annex A

Source for Vacuum Pump, Waste Trap and Manual Plate Washer

Thomas Scientific
99 High Hill Road at I-295
P.O. Box 99
Swedesboro, NJ 08085-0099
202-652-3160 or 800-345-2100

1. 6106-Z25	NUNC Immuno Wash 8, each	$293.75
2. 1048-H05	GAST oiless diaphragm pump*, each	$249.00
3. 4949-J26	Flask, filter, 1,000 ml, each Order 3 flasks at $12.35 each	$37.05
4. 8741-F83	Stopper, No. 8, 1 lb.	$14.50
5. 9561-E16	Tubing, 1/4 x 1/8 x 3/16, pkg. 10 ft.	$34.65

Note: This is the 120 V model: call for the 240 V model. The diaphragm pump is more durable than the rotary vane pumps when used with the plate washer.

Annex B

Reagents for Host Blood Meal ELISAs

June 26, 1992

Kirkegaard & Perry laboratories, Inc.
Attn: Mr. Curt Smith
2 Cessna Court
Gaithersburg, MD 20879
Phone: 301-948-7755 Fax: 301-948-0169

14-12-06	Per anti-bovine IgG (H+L)	100.00
14-20-06	Per anti-cat IgG (H+L)	100.00
14-24-06	Per anti-chicken IgG (H+L)	100.00
14-19-06	Per anti-dog IgG (H+L)	100.00
14-17-06	Per anti-guinea pig IgG (H+L)	100.00
14-21-06	Per anti-horse IgG (H+L)	100.00
14-10-06	Per anti-human IgG (H+L)	100.00
14-18-06	Per anti-mouse IgG (H+L)	105.00
14-15-06	Per anti-rabbit IgG (H+L)	100.00
14-16-06	Per anti-rat IgG (H+L)	100.00
14-23-06	Per anti-sheep IgG (H+L)	100.00
14-14-06	Per anti-swine IgG (H+L)	100.00
50-62-01	ABTS substrate, 2.7 L	212.00
01-12-06	Anti-bovine IgG (H+L), 1 mg	48.40
01-20-06	Anti-cat IgG (H+L), 1 mg	48.40
01-24-06	Anti-chicken IgG (H+L), 1 mg	48.40
01-19-06	Anti-dog IgG (H+L), 1 mg	48.40
01-17-06	Anti-guinea pig IgG (H+L), 1 mg	48.40
01-21-06	Anti-horse IgG (H+L), 1 mg	48.40
01-10-06	Anti-human IgG (H+L), 1 mg	55.90
01-18-06	Anti-mouse IgG (H+L), 1 mg	53.75
01-15-06	Anti-rabbit IgG (H+L), 1 mg	48.40
01-16-06	Anti-rat IgG (H+L), 1 mg	48.40
01-23-06	Anti-sheep IgG (H+L), 1 mg	48.40
01-14-06	Anti-swine IgG (H+L), 1 mg	48.40

Annex C

Source for Marking Paint/Powder and Black Light

BioQuip Products
17803 LaSalle Avenue
Gardena, CA 90248-3602
Phone: 310-324-0620
Fax: 310-324-7931

1.	1166A	Luminous paint kit	$ 7.50
2.	1162A	Luminous powder kit	14.75
3.	1162S	Plastic syringes, dozen	3.00
4.	2827F	Pocket black light	25.50
5.	2827C	Replacement 4 w black light	11.25