COMPARISON OF TWO LABORATORY PROCEDURES FOR XENODIAGNOSTIC EXAMINATION[1]

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Two xenodiagnostic procedures for detecting the agent of Chagas' disease were compared by duplicate testing of 222 dogs in Venezuela. The results indicate the two methods are about equally sensitive, that one method (Maekelt's procedure) can save considerable laboratory time, but that personnel using Maekelt's procedure need to avoid confusing morphologically similar forms of the Chagas' disease agent (Trypanosoma cruzi) *and the trypanosome* T. rangeli *in areas where* T. rangeli *is prevalent.*

Introduction

Xenodiagnosis—detection of *Trypanosoma (Schizotrypanum) cruzi,* the agent of Chagas' disease, by feeding uninfected triatomid bugs on patients and examining the bugs—is generally considered one of the most sensitive parasitologic methods for detecting acute *T. cruzi* infections. However, the same degree of sensitivity is not attained with chronic infections, only about 60 per cent of which yield positive results.

For the purpose of conducting xenodiagnosis there are well-tested criteria governing selection of the most appropriate triatomid instar, the minimum number of bugs, and the length of time to allow between feeding and examining the insects *(1-4).* In contrast, xenodiagnostic examination techniques have varied greatly, and research directed at determining which are the most practical and reliable is still in progress. Some authors recommend microscopic examination of the intestinal contents of the individual triatomid nymphs *(5, 6),* or else examination of the mixed intestinal contents of all the insects *(4),* while others recommend examination of a centrifuged whole-body homogenate of all the insects. This latter method, as it is generally practiced, is known as Maekelt's procedure *(7).*

The purpose of the study presented here has been to compare the effectiveness of the two basic methods—examination of the individual triatomids' intestinal contents and examination of homogenized triatomids (Maekelt's procedure)—for detecting *T. cruzi* and *T. rangeli* in duplicate xenodiagnoses performed at random on dogs from various rural Venezuelan locales.

Materials and Methods

The Study Areas

The investigation was carried out from February to November 1978 at several rural

[1] Also published in Spanish in the *Boletín de la Oficina Sanitaria Panamericana* 92(1):49-54, 1982.

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localities in the Venezuelan states of Guárico and Trujillo during the course of an epidemiologic survey seeking to determine the prevalence of Chagas' disease.

Xenodiagnoses

Pairs of xenodiagnostic boxes were applied simultaneously for 20 minutes to 222 dogs selected at random. Each box contained 12 fifth-instar nymphs of *Rhodnius prolixus* that had been starved for at least four weeks. Following exposure to a dog, each pair of boxes was stored at room temperature (26-28°C). The boxes were later separated and taken to different laboratories, where the insects they contained were examined in a double-blind study 30 days after the feeding by one of the following procedures:

1) *Examination of intestinal contents:* Two samples of intestinal contents, obtained by gently squeezing each nymph's abdomen, were deposited in saline solution and examined microscopically. This examination was performed at the laboratory of the Research and Reference Center on Vector Biology and Control at Maracay, Venezuela, that is operated jointly by PAHO and Venezuela.

2) *Examination of homogenized insects (Maekelt's procedure):* The procedure described by Maekelt *(7)* was carried out with minimal modification. Specifically, (a) the 12 nymphs in the xenodiagnosis box were homogenized in 10 cc of saline solution by an electric homogenizer[8] for one minute at 9,000 rpm; (b) the homogenized material was first filtered through cotton and then centrifuged in 16 x 150 mm tubes at 1,030 G for 10 minutes; (c) the supernatant was removed and the sediment suspended in the remaining saline solution; and (d) the sediment was examined under the microscope. This examination was performed at the laboratory of Venezuela's Division of Rural Endemic Diseases in Maracay.

[8]The Virtis "23" Homogenizer (the Virtis Company, Gardiner, New York, USA).

Positive preparations obtained by either of the two methods were air-dried, fixed with methanol for three minutes, and stained with Giemsa stain for morphologic identification of trypanosomes. The final results of the two sets of examinations were compared at the end of the study.

Results

As Table 1 shows, the results of the two sets of xenodiagnostic examinations performed were very similar. That is, examination of the intestinal contents of the individual triatomids indicated that 12.2 per cent of the dogs were infected with *T. cruzi* or *T. rangeli,* while Maekelt's homogenization procedure indicated that 11.3 per cent were infected. Likewise, both procedures yielded comparably negative results for dogs in Guárico, an area of low Chagas' disease endemicity, and comparably positive results for dogs in Trujillo, where both *T. cruzi* and *T. rangeli* were prevalent.

Table 2 provides a more detailed breakdown of the positive results, showing the species of parasite identified by both procedures. By and large, the parasites identified were the same. However, three dogs (animals 0291, 0500, and 0602) found negative by Maekelt's procedure were found to contain *T. cruzi, T. rangeli,* or both through direct examination of individual nymphs' intestinal contents. Also, one dog (0501) found negative by direct examination of intestinal contents was found positive for *T. rangeli* by Maekelt's procedure. And finally, one dog (0478) found positive for *T. cruzi* by direct examination of intestinal contents was found positive for *T. rangeli* by Maekelt's procedure. In this latter case, both resulting slides were examined by the reference laboratory, which reconfirmed the identification of the parasite—indicating that both parasite species were present, and that each method failed to detect one species.

In addition, the examination of insects fed on dog 0311 revealed an important qualitative difference between the two procedures. That

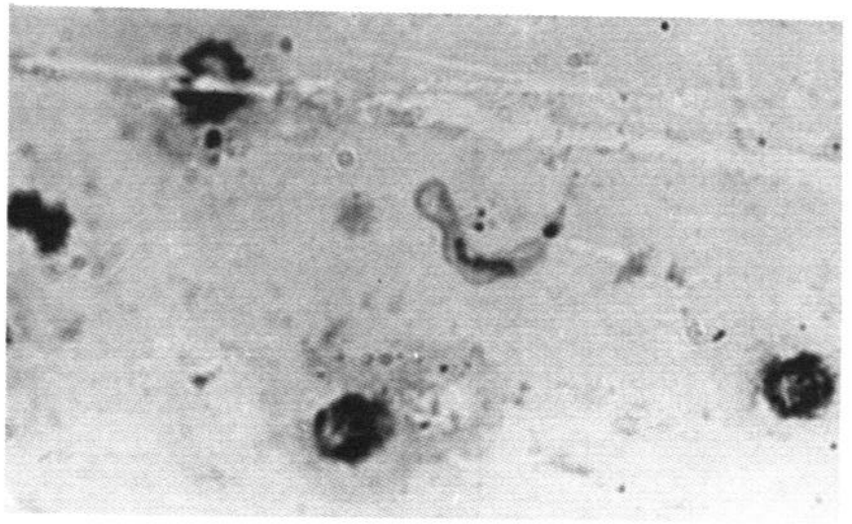
Table 1. Comparative results of 222 duplicate xenodiagnoses performed on dogs using either Maekelt's procedure or direct examination of the exposed bugs' intestinal contents.

Xenodiagnostic procedure used	No. of dogs tested	No. of dogs yielding negative results	No. of dogs found positive for: *T. cruzi*	*T. rangeli*	*T. cruzi* and *T. rangeli*	Total found positive	% positive
Examination of individual bugs' intestinal contents	222	195	4	21	2	27	12.2
Maekelt's procedure	222	197	3	21	1	25	11.3

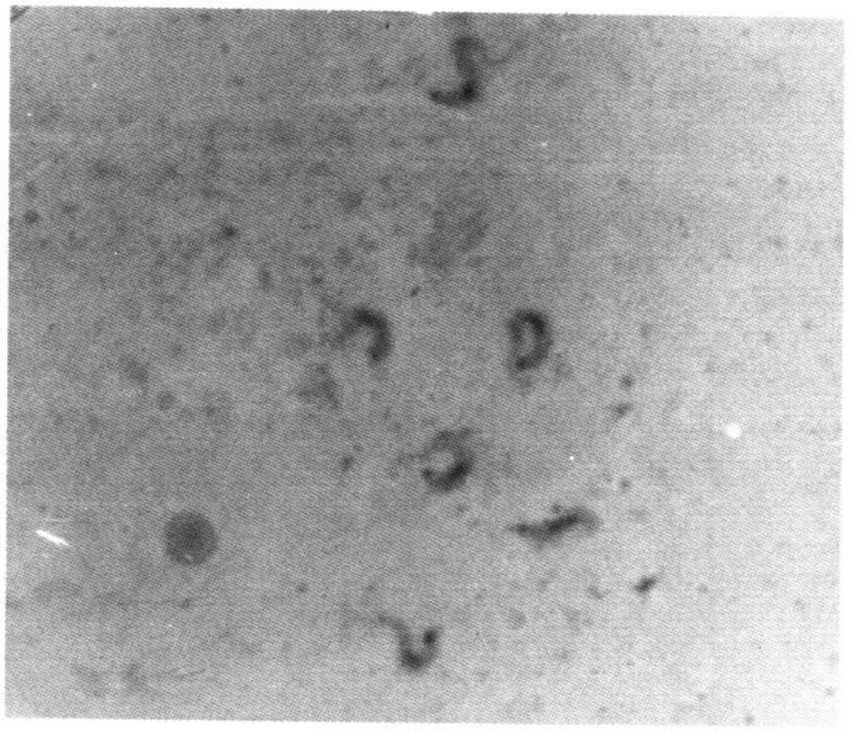
Table 2. A listing of all dogs yielding positive results, showing the trypanosomes identified by both procedures (direct examination of intestinal contents and Maekelt's procedure).

Identifying no. of dog tested	Results of direct examination of exposed bugs' intestinal contents: No. of *R. prolixus* positive over no. examined	Trypanosome identified	Trypanosomes detected and identified with Maekelt's procedure
0291	8/11	*T. rangeli*	None
0300	8/11	*T. rangeli*	*T. rangeli*
0301	7/11	*T. rangeli*	*T. rangeli*
0302	8/12	*T. rangeli*	*T. rangeli*
0303	7/10	*T. rangeli*	*T. rangeli*
0311	6/12	*T. rangeli*	*T. rangeli* + *T. cruzi*
0317	7/10	*T. rangeli*	*T. rangeli*
0414	11/12	*T. rangeli*	*T. rangeli*
0415	9/12	*T. rangeli*	*T. rangeli*
0416	8/12	*T. rangeli*	*T. rangeli*
0420	12/12	*T. rangeli*	*T. rangeli*
0421	8/11	*T. rangeli*	*T. rangeli*
0422	11/12	*T. rangeli*	*T. rangeli*
0423	9/12	*T. rangeli*	*T. rangeli*
0424	11/11	*T. rangeli*	*T. rangeli*
0430	7/11	*T. rangeli*	*T. rangeli*
0431	8/12	*T. rangeli*	*T. rangeli*
0434	9/12	*T. rangeli*	*T. rangeli*
0440	9/12	*T. rangeli*	*T. rangeli*
0443	6/12	*T. cruzi*	*T. cruzi*
0444	7/12	*T. cruzi* + *T. rangeli*	*T. cruzi*
0451	7/11	*T. rangeli*	*T. rangeli*
0468	9/12	*T. rangeli*	*T. rangeli*
0478	3/12	*T. cruzi*	*T. rangeli*
0500	3/12	*T. cruzi* + *T. rangeli*	None
0501	0/10	None	*T. rangeli*
0542	3/12	*T. cruzi*	*T. cruzi*
0602	9/10	*T. cruzi*	None

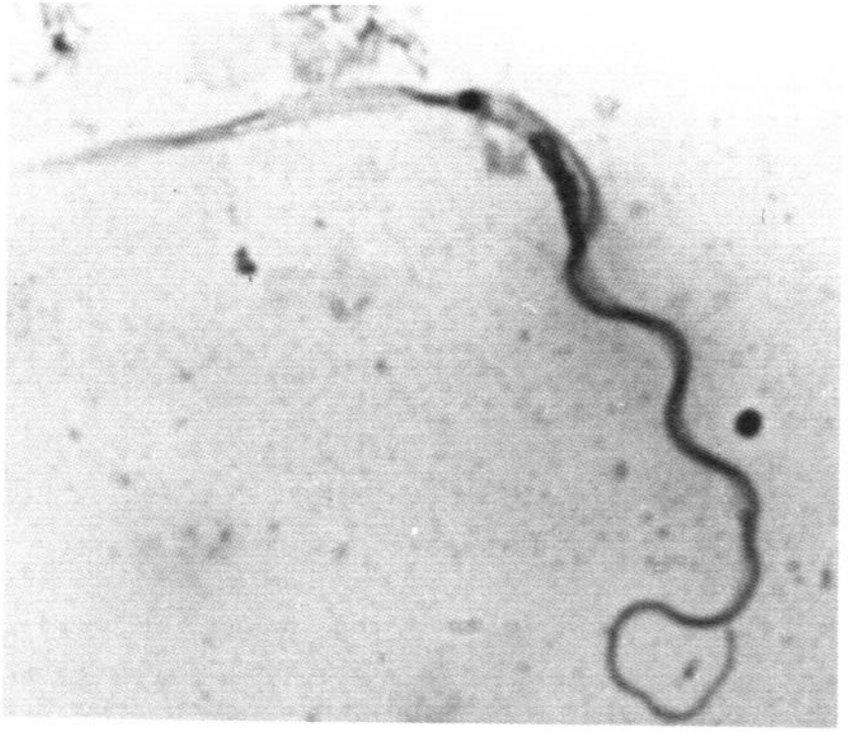
is, direct examination of the intestinal contents of 12 insects fed on this dog revealed the presence of *T. rangeli* but not *T. cruzi*, while Maekelt's procedure indicated that both parasites were present. Subsequent comparison of these parasites demonstrated that those identified as *T. cruzi* were actually metacyclic forms of *T. rangeli* from the homogenized insects' salivary glands. The accompanying photographs show this form of *T. rangeli* in salivary



Metacyclic form of *T. cruzi* found in the intestinal contents of *R. prolixus;* this form differs markedly from the *T. rangeli* seen in the bottom photograph.



Metacyclic form of *T. rangeli* found in the salivary glands of *R. prolixus* that resembles *T. cruzi* found in blood (1,000 x, stained with Giemsa).



Metacyclic form of *T. rangeli* found in the intestinal contents of *R. prolixus* showing classic *T. rangeli* characteristics (1,000 x, stained with Giemsa).

gland tissues (Photo 1), the quite distinct metacyclic form of *T. rangeli* found in bug intestines (Photo 2), and the metacyclic form of *T. cruzi* found in bug intestines (Photo 3).

Discussion

Extensive epidemiologic surveys for Chagas' disease usually require a large number of xenodiagnostic examinations, especially when they involve parasitologic study of domestic and wild animals. The classic xenodiagnostic procedure has consisted of microscopic examination of the intestinal contents of each individual insect, following the recommendations first of Brumpt *(5)* and later of Dias *(6)*. This procedure is highly effective at detecting trypanosomes; but it is also very slow, since it takes about an hour to examine the 10 to 12 nymphs used for each xenodiagnosis. It is therefore significant that another procedure, that of Maekelt, has been proposed as a sensitive and practical method for detecting *T. cruzi* in patients with probable Chagas' disease.

In this regard, it should be noted that Maekelt found his homogenization procedure to yield better results than examination of individual bugs' intestinal contents. Our own findings did not bear this out, but they did indicate that the two procedures were comparably sensitive in detecting trypanosomes *(T. cruzi* or *T. rangeli)* in the 222 dogs tested. The effectiveness of xenodiagnostic examinations involving homogenization and centrifugation of exposed bugs has also been verified in Argentina by Cerisola et al. *(4)*, who used *Triatoma infestans* nymphs to test for *T. cruzi* in patients with acute Chagas' disease.

The advantage of Maekelt's procedure for extensive parasitologic studies is obvious, since it takes an average of only 20 minutes to examine each group of exposed insects.

Moreover, homogenization appears to provide an attractive method for use in "artificial" xenodiagnosis of humans. This procedure—used in our laboratory as an alternative to "natural" xenodiagnosis, which causes allergic reactions of varying intensity in individuals sensitive to the saliva of *R. prolixus*—consists of feeding the patient's blood to between 20 and 40 nymphs through an artificial membrane *(8)*. Obviously, it would take very considerable amounts of laboratory staff time to examine so many insects individually.

Nonetheless, Maekelt's homogenization procedure suffers from a major limitation in areas where *T. rangeli* is prevalent, for the metacyclic forms of this bug in *R. prolixus* salivary glands can be mistaken for forms of *T. cruzi*. As can be seen in Photo 1, the salivary metacyclic trypanosome of *T. rangeli* is small (9.0 to 13.0 μm long) and has a relatively large kinetoplast that makes it appear similar to some forms of *T. cruzi* found in blood. On the other hand, the metacyclic trypanosome of *T. rangeli* that develops in the posterior intestine of the insect (Photo 2) has the classic characteristics of that parasite: a long thin shape (22.0 to 72.0 μm long) with an elongated nucleus and a dot-like kinetoplast at some distance from the posterior extremity. (The metacyclic trypanosome of *T. cruzi* found in the intestinal contents of a triatomid, as shown in Photo 3, is quite distinct from the intestinal metacyclic form of *T. rangeli,* having a length of 17.0 to 22.0 μm, an oval nucleus, and a large subterminal kinetoplast.) Consequently, in areas where *T. rangeli* is prevalent, laboratory personnel should be trained to identify the metacyclic trypanosomes of this parasite that develop in the salivary glands of *R. prolixus*.

SUMMARY

A comparative study was made of two xenodiagnostic procedures for detecting the agent of Chagas' disease, *Trypanosoma cruzi*. One procedure entailed microscopic examination of the intestinal contents of each of the triatomid bugs *(Rhodnius prolixus)* fed on 222 test dogs selected at random; the other

(Maekelt's procedure) entailed microscopic examination of a whole-body homogenate of the exposed bugs.

Both *T. cruzi* and the trypanosome *T. rangeli* were observed. Considering detection of the two taken together, the procedures utilized appeared comparably sensitive, examination of intestinal contents detecting *T. cruzi* or *T. rangeli* in 12.2 per cent of the dogs tested and Maekelt's procedure detecting one or the other trypanosome in 11.3 per cent of the dogs tested. This indicates that Maekelt's procedure should prove useful in cases where microscopic laboratory examination of insect material takes up considerable time, because the time required for such examinations using Maekelt's procedure is relatively short.

However, Maekelt's procedure also permits confusion of *T. rangeli* forms from *R. prolixus* salivary glands with morphologically similar forms of *T. cruzi*. Therefore, in areas where *T. rangeli* is prevalent, laboratory personnel should be trained to distinguish between these similar forms of the two organisms.

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