CHAGAS' CARDIOPATHY AND *TRYPANOSOMA CRUZI* ZYMODEMES IN CHILE[1]

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INTRODUCTION

Chagas' disease, the most widespread parasitic ailment in Latin America, produces clinical pictures with regional variations *(1)*. In Chile, due to the country's geographic and ecologic isolation, specific local conditions influencing Chagas' disease vectors differ from those prevailing in other countries. One such vector, *Triatoma spinolai,* has not been found in other geographic areas. Among other things, this triatomid species is rendered unique by the polymorphism of its wings *(2)*.

Longitudinal studies of clinical disease cases in Chile have shown such cases to possess distinct features in different locales and to differ considerably from clinical cases in other parts of the Americas *(3–11)*. We have reported previously that the observed variations in disease intensity and pathogenicity might be caused by distinct strains of *Trypanosoma cruzi (12)*.

Previous works on *T. cruzi* strains identified by their enzyme patterns (zymodemes) demonstrated the existence of three different strains in Chile *(13, 14)*. One of these, known as Z1, was a wild strain transmitted by *T. spinolai.* The two other strains were transmitted by *Triatoma infestans*. One of these, known as Z2a, was similar to a Brazilian strain (Esmeraldo clone 3); the other, known as Z2b, was similar to the Bolivian strain Z2 *(15)*. Until now, little has been known about the relationships between these strains and the human pathologies involved. The purpose of the work reported here was to shed light on relationships between the strains isolated from infected people (positive by xeno-

[1] This article will also be published in Spanish in the *Boletín de la Oficina Sanitaria Panamericana,* vol. 104, 1988. The work reported here was supported by the WHO/UNDP/World Bank Special Program for Research and Training in Tropical Diseases (Grant No. 820599); by a grant from the Research and Library Department of the University of Chile; and by a grant (No. 801/86) from FONDICYT.

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diagnosis) in endemic areas of Chile and the presence of cardiopathy.

MATERIALS AND METHODS

Isolation of *T. cruzi*

Depending on the stock of bugs available, 10 or 20 third instar laboratory-raised *T. infestans* were fed on each patient. Then, approximately 25 days after feeding, the bugs were washed with a 2% Hibitane (ICI) solution and dried. The hindgut was dissected with sterile instruments in sterile isotonic saline containing 330 μg per ml of 5′ fluorocytosine (Roche) and 330 μg per ml of gentamycin to prevent microbial proliferation *(16, 17)*. Infected feces were dispensed with a sterile syringe into a series of biphasic blood agar cultures previously overlaid with saline containing 5′ fluorocytosine (100 μg per ml) and gentamycin (100 μg per ml) *(13)*. The resulting cultures were examined periodically between 21 and 90 days after inoculation.

Following this isolation on biphasic medium *(13)*, large-volume cultures were prepared using Diamond's liquid culture supplemented with 10% fetal calf serum. Pellets of the isolated *T. cruzi* strains were prepared by centrifugation. The parasites' enzymes were obtained by lysing the organisms through repeated freezing and thawing *(17)*.

Electrophoresis

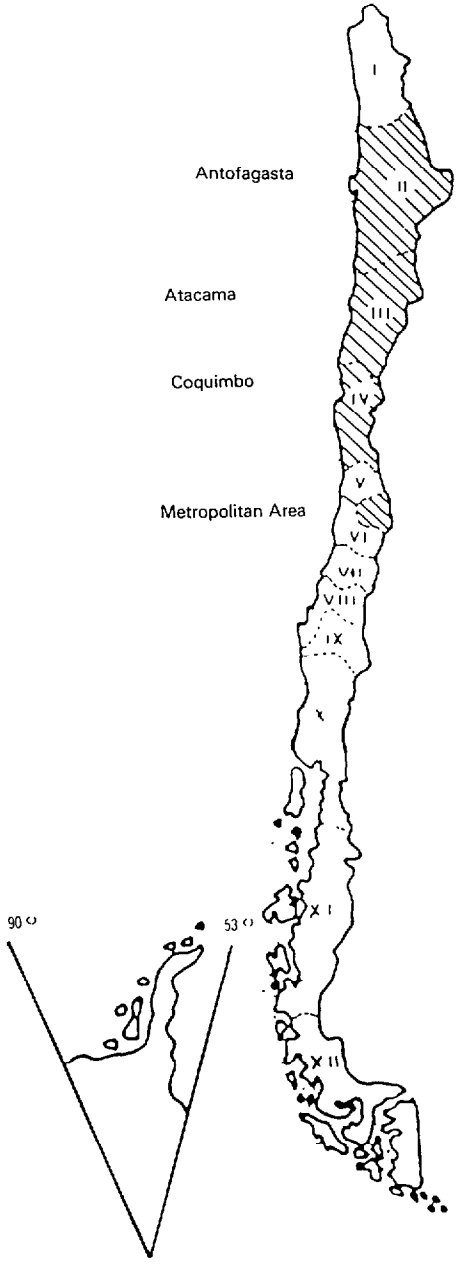
The soluble enzyme extracts produced in this manner were submitted to starch gel and cellulose acetate electrophoresis, a procedure with an appropriate degree of resolution for the selected enzymes *(18, 19)*. Fourteen enzymes were employed in these tests in most cases, although only five were used for identification *(13, 17)*. The electrophoretic migration patterns obtained were compared to those produced by the following zymodeme clones of *T. cruzi:* clone 1 Silvio X 10 (Z1), Esmeraldo clone 3 (Z2), clone 1 SC43 (Z2 Bolivian), and CAN III clone 1 (Z3) *(18, 19)*.

Clinical Study

Patients were selected from those previously found to be seropositive during extensive field studies conducted by Apt and colleagues *(6, 9, 10)*. The number of cases studied was determined by the number of subjects yielding positive xenodiagnostic results from which the parasite was recovered. The overall study sample consisted of 97 people—residents of various villages in Chile's regions II, III, and IV and inhabitants of the Metropolitan Area (Figure 1). These subjects were studied from 1977 through 1980, and were subsequently followed from 1981 through 1986. Each subject was given an initial clinical examination and an EKG in the field, and subsequently received an additional clinical examination and EKG after a lapse of at least four years. The results permitted us to distinguish between patients with and without cardiopathic symptoms.

Each clinical survey obtained data on triatomid bites and infestations, provided information on symptoms of cardiopathy, and included a complete physical examination of each subject. The procedure employed to analyze the EKG tracings has already been described

FIGURE 1. A map of Chile showing the three regions and the Metropolitan Area where the study subjects resided. The area endemic for Chagas' disease extends between regions I and VI.



(10). The statistical significance of observed numerical differences was determined by Student's t test.

All of the subjects tested positive for *T. cruzi* antibodies by the indirect hemagglutination (IHA) and indirect immunofluorescence (IIF) tests *(20–22)*. The *T. cruzi* strains isolated from these subjects were compared in terms of the subjects' geographic areas of residence, clinical pictures, EKG tracings, and clinical cardiopathic symptoms.

RESULTS

T. cruzi Isolation and Identification

T. cruzi cultures free of contamination were obtained from 105 of the 169 persons subjected to xenodiagnosis (62.1%). However, eight of these 105 cultures were discarded because a complete clinical report was not available at the time of evaluation.

The electrophoretic migration patterns (Figure 2) of five of the 14 enzymes studied enabled us to identify three *T. cruzi* zymodemes. The enzymes involved were isocitrate dehydrogenase (ICD), aspartate aminotransferase (ASAT), phosphoglucomutase (PGM), aminopeptidase (PEP), and phosphoglucoisomerase (PGI) *(8)*. Each electrophoretic plate tested had two to four controls and four to six clinical cases; the total number of electrophoretic plates used was around 339.

Table 1 shows the strains isolated and the geographic distribution of the clinical cases studied. Strain Z1 was isolated from two patients (2.1%), strain Z2a was isolated from 16 (16.5%), and strain Z2b was isolated from 79 (81.4%).

Strain Z2b appeared to have the broadest geographic range, extend-

FIGURE 2. Two examples of cellulose acetate electrophoretic zymograms. The enzyme employed in (A) is phosphoglucoisomerase (PGI), while that used in (B) is phosphoglucomutase (PGM). The letters NC, MCC, PAD, JBC, AP, and JCh indicate *T. cruzi* cultures from specific patients; the letters Z2b and Z2a indicate reference zymodemes.

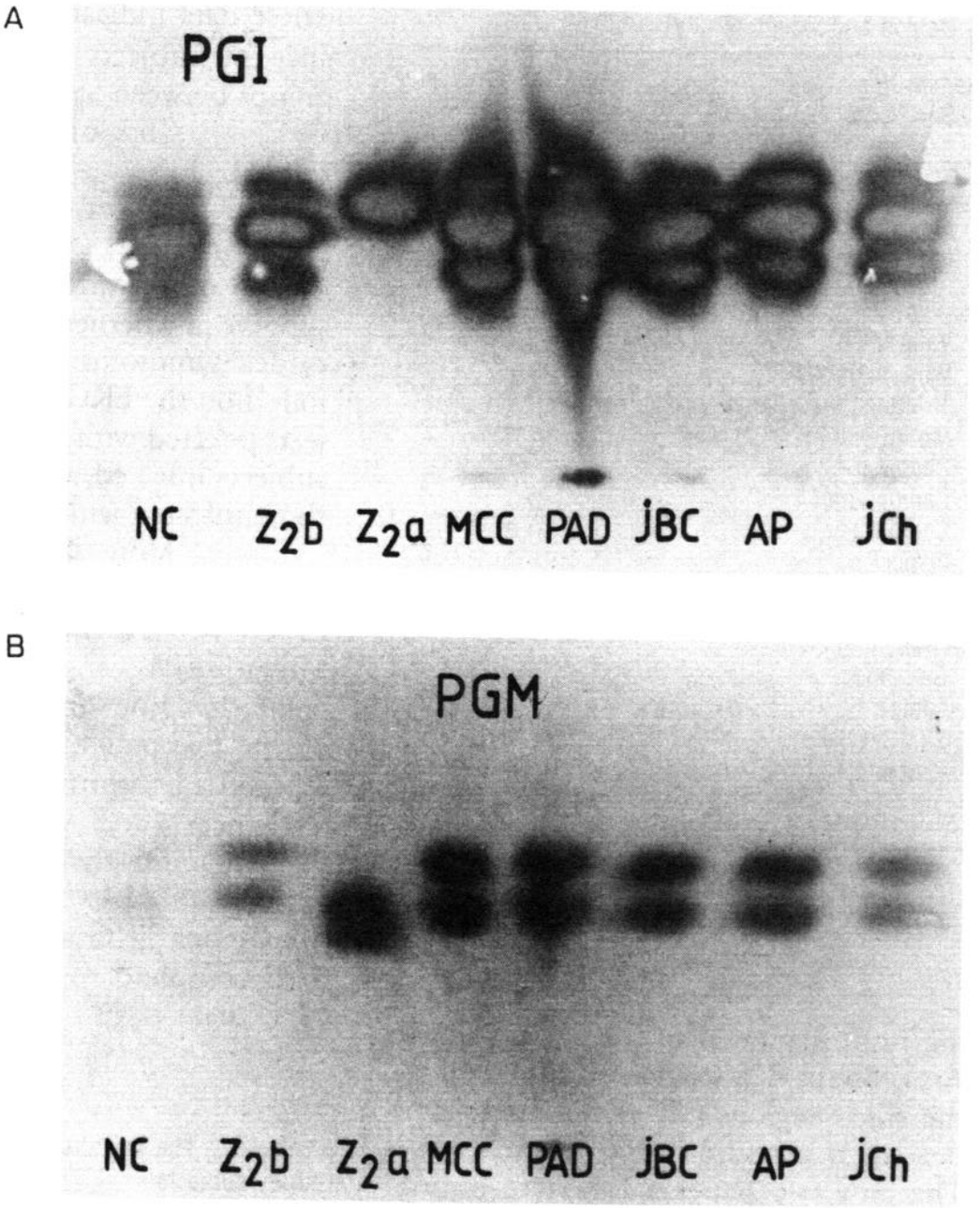


TABLE 1. The three *T. cruzi* strains isolated from 97 infected subjects, by the subjects' residence areas.

Residence area (region and village or city)	Strain Z1	Z2a	Z2b
Region II:			
San Pedro de Atacama			1
Region III:			
Diego Almagro		1	3
Salvador			6
Inca de Oro			1
Region IV:			
Valle Elqui			2
Valle Río Limarí			
Tulahuén y Ramadas		4	17
Monte Patria			2
Chañaral Alto		1	
Campanario			8
Combarbalá	1	4	15
Illapel	1	1	6
Salamanca		5	11
Metropolitan area:			
Santiago			1
Lampa			2
San Gabriel			2
Cruceral			2
Total	2	16	79

ing from Region II to the Metropolitan Area. Strain Z2a was restricted to two of the study regions (III and IV) and appeared to produce fewer clinical cases. The only two patients found to be infected with strain Z1 came from two small towns in the northern part of Region IV (see Figure 1).

Zymodemes, Clinical Pictures, and Cardiopathies

Table 2 shows the age distribution and sex of the study subjects. These data indicate a slight preponderance of subjects in the ten-year age groups between ages 30 and 70.

Chagasic cardiopathy detected by EKG was defined as EKG alterations found on at least two tracings taken four years apart, aside from those due to other cardiac pathologies, irrespective of whether the subject exhibited cardiac symptoms. Such alterations were found on the EKG tracings of both subjects infected with strain Z1, 50% of the subjects infected with Z2a, and 33% of those infected with Z2b (Table 3).

More specific data on these EKG alterations are shown in Table 4. (There were 36 subjects with a total of 49 alterations because some subjects had more than one kind of alteration.) Each of the two patients infected with strain Z1 had intraventricular conduction disturbances, and one had myocardial ischemia. Of the 12 patients infected with strain Z2a who were found to have undergone EKG alterations, two exhibited complete right branch block and

TABLE 2. The age distribution and sex of the 97 study subjects.

Age group (in years)	Sex Male	Female	Total
0–10	5	0	5
11–20	7	4	11
21–30	3	6	9
31–40	3	11	14
41–50	7	8	15
51–60	6	11	17
61–70	8	7	15
71–80	5	6	11
Total	44	53	97

TABLE 3. Chagasic cardiopathy detected among the 97 study subjects, grouped according to the *T. cruzi* strain causing the infection.

Chagasic cardiopathy, as indicated by EKG alterations	Patients infected with indicated strain: Z1 No.	Z1 (%)	Z2a No.	Z2a (%)	Z2b No.	Z2b (%)	Total No.	Total (%)
Cardiopathy detected	2	(100)	8	(50)	26	(33)	36	(37)
No cardiopathy detected	0	(0)	8	(50)	55	(67)	61	(63)
Total	2	(100)	16	(100)	79	(100)	97	(100)

TABLE 4. Electrocardiographic alterations detected in 36 of the 97 study subjects, grouped according to the *T. cruzi* strain causing the infection.

EKG alterations	Alterations found in subjects infected with the indicated strain: Z1 No.	Z1 (%)	Z2a No.	Z2a (%)	Z2b No.	Z2b (%)	Total No.	Total (%)
Auricular arrythmias:								
Short PR syndrome					2	(6)	2	(4)
Sick sinus syndrome					1	(3)	1	(2)
Nodal rhythm					2	(6)	2	(4)
Auricular fibrillation			1	(8)	1	(3)	2	(4)
Premature auricular contractions					3	(9)	3	(6)
Ventricular arrythmias:								
Premature ventricular contractions			1	(8)			1	(2)
Unifascicular blocks:								
Left anterior hemiblock			1	(8)	6	(18)	7	(14)
Left posterior hemiblock	1	(33)			1	(3)	2	(4)
Incomplete right bundle block			1	(8)	3	(9)	4	(8)
Complete right bundle block			2	(17)			2	(4)
Bifascicular blocks:								
Left anterior hemiblock plus complete right bundle block	1	(33)	2	(17)	1	(3)	4	(8)
Other alterations:								
A-V block, 3rd degree					2	(6)	2	(4)
Hypertrophy images			2	(17)	4	(12)	6	(12)
Repolarization change			1	(8)	3	(9)	4	(8)
Subepicardial damage			1	(8)			1	(2)
Subendocardial damage					1	(3)	1	(2)
Ischemia image	1	(33)			4	(12)	5	(10)
Total alterations	3	(100)	12	(100)	34	(100)	49	(100)
Total subjects and EKG tracings	2		8		26		36	

two others exhibited bifascicular blocks. Finally, of the 34 patients infected with strain Z2b who showed alterations, nine exhibited auricular arrhythmias—which were generally not seen in patients infected with the other strains. Two other Z2b patients exhibited complete (third degree) A-V block, 11 showed various unifascicular and bifascicular blocks, and four exhibited ischemia images. The latter were two men 53 and 54 years old and two women 33 and 47 years old.

Regarding the age and sex of the 36 patients with cardiopathy, the two patients infected with strain Z1 were women 22 and 47 years old.

The median age of the Z2a patients with cardiopathy was 51.2 years, within a range of 34 to 71 years. In contrast, the median age of the Z2a patients without cardiopathy was 61.6 years, within a range of 41 to 75 years. The group of eight Z2a patients with cardiopathy included two men and six women, while the group of eight without cardiopathy included five men and three women.

The median age of Z2b patients with cardiopathy was 38.9 years, within a range of 13 to 80 years, while the median age of those without cardiopathy was 39 years within a range of six to 80 years. The group of 26 Z2b patients with cardiopathy included 15 men and 11 women, while the group of 53 without cardiopathy included 22 men and 31 women.

Application of Student's test did not reveal any statistically significant differences between the incidence of EKG alterations in subjects infected with the different strains, perhaps because of the small numbers infected with Z1 and Z2a.

None of the 97 subjects showed acute Chagas' disease symptoms or myocarditis. Nevertheless, symptoms such as precordial pain (of the noncoronary type), palpitations, fainting, and evidence of heart failure were found in different degrees among patients with and without evident EKG alterations. These symptoms, together with the numbers of subjects involved, are shown in Table 5. Regarding those subjects without EKG alterations suggesting chagasic cardiopathy, 25% infected with strain Z2a and 29% infected with Z2b exhibited one or more of the above symptoms. Regarding those with EKG alterations, both of those infected with Z1, 50% of those infected with Z2a, and 57% of those infected with Z2b showed one or more of these symptoms. This last group (Z2b subjects with EKG alterations) included two persons with syncope (fainting) and 10 with symptoms of heart failure. The two patients with syncope were a woman 55 years old and a man of 68; the latter had received a pacemaker to counteract a complete A-V block.

Discussion

These results show that humans can be infected with wild *T. cruzi* strains (in this case the Z1 strain), an occurrence not previously confirmed in Chile *(12)*. It had been thought that such infections were limited almost exclusively to nonhuman creatures. However, our findings indicate that humans can be involved—in this case people who for unknown reasons were bitten by the undomesticated bug *T. spinolai,* which

TABLE 5. Heart-related symptoms found in the 97 study subjects, grouped by presence or absence of EKG alterations indicating cardiopathy and the *T. cruzi* strain causing the infection.

Clinical symptoms	Subjects without cardiopathy infected with indicated strain						Subjects with cardiopathy infected with indicated strain						Total number of subjects[a]
	Z1		Z2a		Z2b		Z1		Z2a		Z2b		
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	
None (asymptomatic patients)			6	(75.0)	38	(71.7)			4	(50.0)	11	(42.3)	59
Precordial pain			1	(12.5)	5	(9.4)	2	(100)	3	(37.5)	3[a]	(11.5)	14
Palpitations			1	(12.5)	6	(11.3)					3[a]		10
Syncope (fainting)											2	(7.7)	2
Heart failure					4	(7.5)			1	(12.5)	10	(38.5)	15
Total			8	(100)	53	(100)	2	(100)	8	(100)	26	(100)	97

[a] The three indicated subjects with precordial pain and with palpitations were the same.

comes into occasional contact with man *(2)*. This suggests that a human population not previously considered endangered does run a risk of acquiring the infection. That population includes people camping in or near the mountains—geologists, engineers, ranch hands, sportsmen, and others.

In this regard, it should be noted that both of the people infected with the Z1 strain showed clear heart compromise. Although the percentage that might be expected to show heart lesions in a larger study population is unknown, the very limited present findings appear important enough to justify more extensive efforts to clarify the situation.

Another noteworthy point is that the Z2b strain, similar to Bolivian *T. cruzi* strain Z2, predominated among our study subjects. Previous presentations have postulated that *T. cruzi* infection could have reached Chile from Bolivia by traversing some of the many Andean passes, especially by way of San Pedro de Atacama *(12, 23)*. We feel that further study of parasite structures, perhaps including restriction endonuclease profiles of kinetoplast DNA (schizodemes), might help to determine whether strain Z2b and other strains present in Chile are migratory or native *(24)*.

Compared to the rate of Z2b infection, the rate of Z2a infection in our study subjects was relatively low. Since the number of Z2a subjects exhibiting EKG alterations (50%) was higher than the percentage of Z2b subjects with alterations (33%), it may be argued that infection with the Z2a strain is more dangerous. However, three considerations suggest that the Z2b strain tends to have a greater impact on the heart. These are as follows:

1 In a population at equal risk of heart damage (all of our study subjects had *T. cruzi* in their blood, as demonstrated by xenodiagnosis), the Z2b strain was found among relatively younger subjects (average age 39 years as compared to 56 years for the Z2a subjects).
2 The EKG showed more and wider alterations in each case of Z2b infection. Specifically, there were higher proportions of left

anterior hemiblock, left posterior hemiblock, third degree atrioventricular block, subepicardial and subendocardial damage, and auricular arrhythmias. Whether or not this would still be true if there had been more cases of Z1 infection is uncertain.

3 There were more symptomatic cases among the Z2b subjects and more symptoms of heart failure.

At present there appears to be no information available for determining the relative pathogenicity of the Z2a and Z2b strains. In 1986 Luchetti et al. *(25)* studied 13 chronic cases infected with the Z2 strain (similar to our Z2a). They found two of the patients to be asymptomatic, six to exhibit heart compromise, and 11 to have megasyndromes.

Obviously, it would be useful to know if subjects infected with the Z2a strain had such megasyndromes. Unfortunately, this is not known because our field survey did not have the facilities required to perform X-ray examinations.

A circumstance that we have observed previously is the presence of heart symptoms without EKG alterations *(26, 27)*. In this regard it should be noted that our 97 study subjects were all positive for *T. cruzi* by xenodiagnosis, so their risk of cardiopathy was real, and was also different from that of merely seropositive subjects. It is possible that a portion of the subjects who were asymptomatic had cardiopathy that was passing through an indeterminate phase, or that (as described previously—*26*) their EKGs had at the time reverted to normal. If so, the number of subjects with cardiopathy would have been higher than the number of subjects in whom cardiopathy was detected. For this reason, among others, we feel that all of the cases seen should be closely followed.

Overall, the experience reported here appears to have shed some light on previous questions about whether different *T. cruzi* strains were responsible for different clinical pictures. It has also paved the way for exploring a variety of therapeutic and prognostic possibilities. Hence, there appears ample justification for further studies of a similar nature that include sufficiently large numbers of cases to determine the statistical significance of some of the differences reported here.

Summary

T. cruzi strains isolated from 97 Chilean subjects were cultured, centrifuged, and lysed to yield enzyme extracts. Subsequent electrophoretic testing of these extracts served to identify three strains designated Z1, Z2a, and Z2b. Z1, infecting two subjects, was a wild strain of *T. cruzi* transmitted by the bug *Triatoma spinolai;* Z2a, infecting 16 subjects, was similar to a Brazilian strain (Esmeraldo clone 3, Z2); and Z2b, infecting 79 subjects, was similar to the Bolivian Z2 strain (clone 1 SC43).

Two sets of EKG tracings and clinical examinations spaced at least four years apart provided data relating to cardiopathology. EKG alterations indicating chagasic cardiopathy were found in both of the subjects infected with strain Z1, eight (50%) of those infected with Z2a, and 26 (33%) of those infected with Z2b.

No statistically significant differences were found between the incidence of EKG alterations in subjects infected with different strains, perhaps because of the small numbers involved.

However, identification of the Z1 strain shows that humans can be infected with this wild-type strain transmitted by the undomesticated *T. spinolai,* and that such infection can involve significant cardiopathology. Also, the predominant strain isolated (Z2b) was similar to the Bolivian Z2 strain of *T. cruzi.* This latter finding suggests a need for further structural studies of the parasite in order to determine whether this and other *T. cruzi* types present in Chile are migratory or native strains.

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