



## Review

## Efficacy of killed whole-parasite vaccines in the prevention of leishmaniasis—A meta-analysis

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## ABSTRACT

Despite decades of investigation in countries on three continents, an efficacious vaccine against *Leishmania* infections has not been developed. Although some indication of protection was observed in some of the controlled trials conducted with “first-generation” whole, inactivated *Leishmania* parasite vaccines, convincing evidence of protection was lacking. After reviewing all previously published or unpublished randomized, controlled field efficacy clinical trials of prophylactic candidate vaccines, a meta-analysis of qualified trials was conducted to evaluate whether there was some evidence of protection revealed by considering the results of all trials together. The findings indicate that the whole-parasite vaccine candidates tested do not confer significant protection against human leishmaniasis.

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### 1. Introduction

Leishmaniasis is endemic in at least 88 countries, some of which are among the poorest in the world [1,2]. The estimated global prevalence of all forms of the disease is 12 million, with

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1.5–2 million cases of cutaneous leishmaniasis (CL) added annually (with duration of lesions typically from few months to a year) and 500,000 cases of visceral form of the disease (with duration of disease from several months to more than a year) [2,3]. Current control measures, including environmental sanitation and drug treatment of cases, are expensive and cannot be sustained effectively by poor countries due to the problems of financing and implementation [4–6]. Moreover, toxicity associated with some of the most widely available drug treatments, including injections of pentavalent antimony compounds, and the resistance developed by the parasite [7–12], underline the need for development of effective methods of prevention, especially vaccines [4,13,14].

### 1.1. Historical perspective

To date, the only effective way of inducing immunity against leishmaniasis in humans is provided by leishmanization (LZ), the practice of injecting live virulent parasites in healthy individuals [15]. LZ has been practiced historically in high incidence endemic foci as a means of controlling the timing and site of the initial lesion, but it is no longer widely used because of rare complications and difficulties in standardization of the injected parasites [15–18].

During the first half of the 20th century researchers in Latin America investigated different antigens as potential vaccines [19,20]. Beginning in the 1970s and 1980s, Mayrink and colleagues in Brazil and Convit and colleagues in Venezuela experimented with the use of whole, killed parasites, both for prophylaxis and therapy. Later studies were conducted with inactivated whole-parasite vaccines in Ecuador (trivalent vaccine composed of three strains of locally obtained parasites), Colombia (Biobras single strain *L. amazonensis* vaccine), Iran and Sudan (autoclaved *L. major* with BCG included as an adjuvant: ALM + BCG) [21–28]. With the exception of the trial by Armijos in Ecuador in which a locally prepared vaccine was used [21], none of the other trials demonstrated significant protection associated with vaccination [26].

Some investigators observed a lower incidence of leishmaniasis in the subset of those in the vaccinated group whose Leishmanin Skin Test (LST) had converted (from an induration of <5 mm to >5 mm) after vaccination [24,25,29]. Also, researchers in Iran observed significant protection in school age boys but not in girls [27]. Evidence of potential clinical value of such vaccines for treatment, rather than the prevention, of disease was demonstrated in trials among leishmaniasis patients in the New World [30,31].

We have re-examined the combined data from all except one published, and unpublished, randomized, controlled clinical trials (RCT) of prophylactic first-generation leishmaniasis vaccine conducted to date to evaluate whether, overall, there is evidence of efficacy, or there is efficacy in some sub-groups of the trial populations.

## 2. Data selected for analysis

### 2.1. Definition of studies

All published and unpublished field efficacy trials of prophylactic candidate vaccines against leishmaniasis conducted to date were considered for inclusion. As a result, publication bias is not a concern in this meta-analysis.

### 2.2. Information sources

Trial protocols and progress reports for studies in Iran, Sudan and Colombia were reviewed. For each clinical trial identified, the principal investigator was requested to provide the original trial database. Thus, individual-level data for trials conducted in Iran and Sudan as well as aggregated data for the trial in Colombia were

**Table 1**  
Design characteristics of selected trials.

Study label	Number of injections	Vaccine parasite	Inactivation method	Adjuvant	Control treatment	Causative parasite	Area, Country	Study population origin	Investigators (year published)
A	3	<i>L. major</i>	Autoclaved	BCG	BCG	<i>L. major</i>	Zavareh, Iran	Endemic	Khamesipour, et al. (not published)
B	3	<i>L. major</i>	Autoclaved	BCG	BCG	<i>L. major</i>	Borkhar, Iran	Endemic	Khamesipour, et al. (not published)
C	3	<i>L. major</i>	Autoclaved	BCC	BCG	<i>L. tropica</i>	Bam, Iran	Endemic	Sharifi et al. (not published)
D	1	<i>L. major</i>	Autoclaved	BCG	BCG	<i>L. tropica</i>	Bam, Iran	Endemic	Sharifi et al. (1998)
E	1	<i>L. major</i>	Autoclaved	BCG	BCG	<i>L. major</i>	Esfahan, Iran	Endemic and non-endemic	Momeni et al. (1999)
F	2	<i>L. major</i>	Autoclaved	BCG	BCG	<i>L. donovani</i>	Gedarif, Sudan	Endemic	Khalil et al. (2000)
G	2	5-strain vaccine (species of <i>brasilensis</i> and <i>mexicana</i> complexes, including <i>L. guyanensis</i> and <i>L. amazonensis</i> )	Merthiolate	None	Phosphate buffer + merthiolate	?	Amazonas, Brazil—exposure during military missions	Endemic and non-endemic-army recruits	Antunes et al. (1986)
H	3	<i>L. amazonensis</i>	Merthiolate	None	Saline	<i>L. panamensis</i>	Colombia—exposure during military missions	Endemic and non-endemic-army recruits	Velez et al. (2005)
I	2	3-strain vaccine ( <i>L. guyanensis</i> , <i>L. brasilensis</i> , <i>L. amazonensis</i> )	Merthiolate	BCG	BCG	<i>L. guyanensis</i> , <i>L. brasilensis</i> , and <i>L. amazonensis</i>	Rural rainforest, Ecuador	Endemic	Armijos et al. (1998)

**Table 2**  
Age and gender distribution of participants in trials selected for meta-analysis.

Study	Bam1		Esf1		Bam3		Bor3		Zav3		Sudan2		Brazil 1981-2		Brazil 1983-2		Colombia3		Ecuador2	
	V <sup>a</sup>	C <sup>a</sup>	V	C	V	C	V	C	V	C	V	C	V	C	V	C	V	C	V	C
N	1838	1795	1190	1124	2149	2068	1107	1084	941	1055	1155	1151	667	644	616	1302	1295	438	406	
Age (years)																				
Minimum	6	6	5	5	6	6	6	6	5	5	<3	<3								
Maximum	15	15	67	72	12	12	13	13	59	59										
Mean	9.1	9.1	18.0	18.8	7.4	7.4	8.2	8.7	19.0	19.2	6.5	7.2	18.6	18.6	18.6	19.8	19.8	5.4	5.7	
P-value (Kruskal–Wallis H)	0.822	0.118	0.118	0.132	0.132	0.132	0.000	0.000	0.666	0.666	0.010	0.010						0.271		
SEX																				
% Female	47.1	51.8	52.7	52.6	48.8	49.6	46.3	52.4	50.2	48.9	53.3	55.3	0.0	0.0	0.0	0.0	0.0	57.5	54.3	
P-value (Fisher exact)	0.003	0.496	0.496	0.302	0.302	0.302	0.002	0.002	0.323	0.323	0.599	0.599	N/A	N/A	N/A	N/A	N/A	0.349	0.349	

<sup>a</sup> V = vaccine; C = control.

obtained. These data were used to estimate or verify the effect statistics (relative risk) as well as age and gender composition in Iran studies. For other studies values reported in the published articles were used (see Section 2.5).

2.3. Selection criteria

- (1) Trial objective: Efficacy of a first-generation vaccine for prevention of leishmaniasis in healthy individuals in an endemic area.
- (2) Study design: Randomized, double blind, controlled clinical trial designed to estimate vaccine efficacy.
- (3) Candidate vaccine: Killed, whole *Leishmania* promastigotes.
- (4) Normal field conditions during follow-up: Sample size and power calculations are generally based on previously observed disease rates in the trial area. Unforeseen, major changes in environmental and climatic factors could lead to a significant change in disease incidence, affecting the study power and conclusions. Selected clinical trials were conducted under the usual field conditions that gave rise to the previously observed incidence rates.

2.4. Excluded studies

On the basis of the above criteria, one study [32] was excluded due to the unusual climatic changes that were attributed by authors to the El Nino phenomenon during the study follow-up [32]. These changes led to significantly lower disease incidence rate than expected.

2.5. Included studies

Data and reports from the randomized, blinded, controlled efficacy trials listed in Table 1 were used. Trial details are presented in Tables 1–3. Further details are available in Noazin et al. [26].

**Table 3**

LST conversion 42–80 days post-vaccination (among participants with LST = 0 prior to vaccination).

Trial	Trial arm	N	% LST > 5 mm
Bam1	V <sup>a</sup>	1807	16.5
	C <sup>a</sup>	1761	3.3
Esf1	V	1168	36.2
	C	1104	7.9
Bam3	V	1980	18.2
	C	1935	2
Bor3	V	608	29.9
	C	538	6.1
Zav3	V	772	–
	C	901	–
Sudan2	V	1919	30
	C	1005	7
Brazil 1981A-2	V	311	33
	C	–	–
Brazil 1981B-2	V	338	37
	C	–	–
Brazil 1983-2	V	611	68
	C	–	–
Colombia3	V	–	–
	C	–	–
Ecuador2	V	–	–
	C	–	–

– = not measured.

<sup>a</sup> V = vaccine arm; C = control arm.

**Table 4**  
Incidence of leishmaniasis among vaccinated and unvaccinated individuals and estimated vaccine efficacy.

Study	Follow-up (months)	Vaccine Total N	Vaccine Cases	Control Total N	Control Cases	% Case (vaccine)	% Case (control)	Vaccine efficacy
Bam1	24	1838	52	1795	60	2.83	3.34	15%
Esf1	24	1190	214	1124	208	17.98	18.51	3%
Bam3	24	2082	81	2008	93	3.89	4.63	16%
Bor3	36 <sup>a</sup>	604	64	561	63	10.60	11.23	6%
Zav3	24	742	102	868	109	13.75	12.56	–9%
Sudan2	24	1155	133	1151	141	11.52	12.25	6%
Brazil 1981A-2	12	322	28	289	32	8.70	11.07	21%
Brazil 1981B-2	12	345	4	356	5	1.16	1.40	17%
Brazil 1983-2	12	658	4	616	8	0.61	1.30	53%
Colombia3	12	1302	101	1295	88	7.76	6.80	–14%
Ecuador2	12	333	7	316	24	2.10	7.60	72%

<sup>a</sup> Although there were 3 years follow-up in Bor3, only cases from years 2 and 3 are included in this analysis because vaccination was completed (3rd dose) at the end of year 1.

Individual-level data were used for trials A–E, and published information was used for trials F–I (Table 1).

Although most trials excluded individuals with LST > 0 mm, in the Borkhar (Bor3) and Zavareh (Zav3) trials (A and B in Table 1) volunteers with any LST value at screening were enrolled. Since an LST > 0 could indicate previous exposure to leishmaniasis and be associated with immunity, for these two trials we analysed data only on participants with a pre-vaccination LST of zero. This excluded 12% of trial participants in Zavareh and 40% in Borkhar.

The study conducted in Brazil in 1981 was conducted in two separate cohorts [29]. These cohorts were different in several respects, including the risk of disease, duration of exposure and previous vaccination history, and we have treated them as two separate studies (identified as Brazil 1981A and Brazil 1981B).

### 3. Statistical analysis

We used a meta-analysis approach based on relative risk (RR = incidence in the vaccine arm/incidence in the control arm) calculated for each study separately and then pooled across studies. Briefly, in calculating the pooled effect, an average of the trial-specific relative risks was calculated by weighting individual study effects according to their trial size (i.e., weighting by the relative quantity of information provided by the trial). These weights can be calculated using a variety of methods, including the inverse variance (*I-V*) and Mantel–Haenszel (*M-H*) methods. If the relative risks in different studies are not widely different (i.e., studies are homogeneous), a fixed effect model would be appropriate. If the variation is more than would be expected by chance then a random effects model is more appropriate, for which a common method for calculation of the pooled effect is that of DerSimonian–Laird (*D+L*) [33]. To assess heterogeneity, we used a chi-square test of the *Q* statistic ( $Q = \text{sum of squared deviations of weighted RR's from their overall mean}$ ); with degrees of freedom =  $k - 1$ , where  $k$  is the number of studies. In addition, due to limitations of *Q* [34], *I*-squared was also used to assess heterogeneity. *I*-squared measures the percent of variation due to between-studies variability. A value of zero for *I*-squared indicates that all variability in relative risks is due to sampling error [34].

EpiInfo 2002, Stata 9 and MS Excel were used in the analyses. The “metan” program in Stata 9 was used to calculate relative risk (RR) estimates and corresponding 95% confidence intervals. The inverse variance (*I-V*) and the Mantel–Haenszel (*M-H*) methods were used separately to fit the fixed effect model and the DerSimonian–Laird (*D+L*) method to fit the random effect model.

### 4. Results

Age and gender breakdown of participants in vaccine trials included in this analysis are provided in Table 2. Some trials were confined to children, whereas other included all ages and the trials among the military in Brazil and Colombia were confined to adult males. In two of the trials there was a significant excess of males in the vaccinated group and in two the vaccinees were significantly younger, on average.

Participants included in the analysis of vaccine immunogenicity were restricted to those with negative pre-vaccination LST. LST measurements 42–80 days post-vaccination (depending on the trial) are displayed in Table 3. LST  $\geq 5$  is generally accepted as an indication of a significant skin test response in volunteers after vaccination. In all trials where immunogenicity was assessed in both vaccinated and unvaccinated participants there was a significantly higher level of skin test conversion among vaccinated individuals. However, the level of skin test conversion varied substantially among trials, ranging from only 16% among those vaccinated in the Bam1 trial to 68% in the Brazil 1983-2 trial. In Table 3 and thereafter, and in our analysis, we have treated the Brazil 1981-2 study as two distinct trials: Brazil 1981A-2 and Brazil 1981B-2. This approach was adopted due to the differences between the two cohorts of volunteers in this study in their duration and timing of exposure as well as the length of time after yellow fever vaccination that the vaccines were given (which could affect their immunological response) [29].

The incidence rates of leishmaniasis in vaccine and control arms in the different trials are summarised in Table 4 (participants in Zav3 and Bor3 with pre-vaccination LST  $\geq 0$  mm were excluded from this analysis). Vaccine efficacy (VE) is calculated as  $(100 \times (1 - RR))$ . The percent of the trial populations who developed disease varied from around 1% to 18%. In only one of the trials (Ecuador2) was the difference in incidences between the vaccinated and unvaccinated group statistically significant.

Table 5 shows the confidence intervals on the relative risk (RR) estimates from the trials and the relative weights derived for each study according to the method of pooling the results.

The weights assigned to the Ecuador trial vary substantially between the three estimation methods. This reflects the tendency of the fixed effect models (*I-V* and *M-H*) to give less weight to smaller trials.

We sought evidence of heterogeneity in the results from the different trials. The heterogeneity statistics estimated by the three methods are very similar, as indicated in Table 6 and in no case was there evidence of significant heterogeneity, providing justification for using fixed effect models. A comparison of the RR's from the Old World and the New World trials indicated such heterogeneity as there is may be attributed to the latter group.

**Table 5**

Relative risks (incidence in vaccinated/incidence in unvaccinated), 95% confidence intervals and relative weights for each study according to the method used for meta-analysis.

Study	N	RR	95% Conf. Int.		Weight (%)		
					I–V	M–H	D+L
Bam1	3633	0.846	0.587	1.22	6.32	7.26	8.33
Esf1	2314	0.972	0.818	1.155	28.37	25.59	19.66
Bam3	4090	0.84	0.628	1.124	9.94	11.32	11.41
Bor3	1165	0.944	0.679	1.31	7.84	7.81	9.72
Zav3	1610	1.095	0.851	1.408	13.34	12.02	13.66
Sudan2	2306	0.94	0.753	1.174	17.08	16.89	15.63
Brazil 1981A-2	611	0.785	0.485	1.271	3.64	4.03	5.39
Brazil 1981B-2	701	0.826	0.224	3.049	0.49	0.59	0.86
Brazil 1983-2	1274	0.468	0.142	1.547	0.59	0.99	1.02
Colombia3	2597	1.142	0.867	1.503	11.15	10.55	12.27
Ecuador2	649	0.277	0.121	0.633	1.23	2.95	2.05
					100	100	100

**Table 6**

Heterogeneity and effect statistics in the three methods of meta-analysis.

Method	Heterogeneity statistics				Effect statistics			Test of RR = 1	
	Chi-square	d.f.	P	I-squared	Pooled RR	95% Conf. Int.		z-Value	P
I–V	14.59	10	0.148	31.5%	0.947	0.864	1.038	1.16	0.246
M–H	14.62	10	0.146	31.6%	0.939	0.857	1.029	1.34	0.179
D+L	14.62	10	0.146	31.6%	0.928	0.821	1.049	1.2	0.231

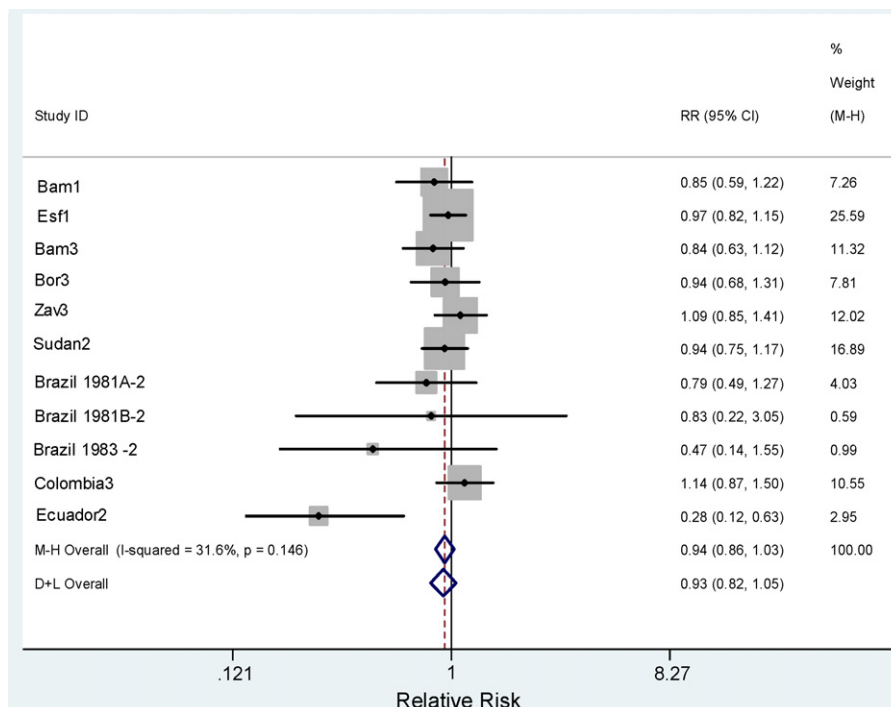
Pooled RR estimates and the 95% confidence interval (CI) estimated by the three methods (Table 6) are very similar, regardless of the model used, providing little evidence to reject the hypothesis of no vaccine effect on leishmaniasis incidence.

Fig. 1 shows the “forest plot” of the findings in the trials. The area of the gray square boxes represent the relative size of each trial with the centre dot and the line in the centre of each square representing the RR and its 95% CI. The overall RR is depicted by two blank diamond boxes, representing the M–H and the D+L estimates.

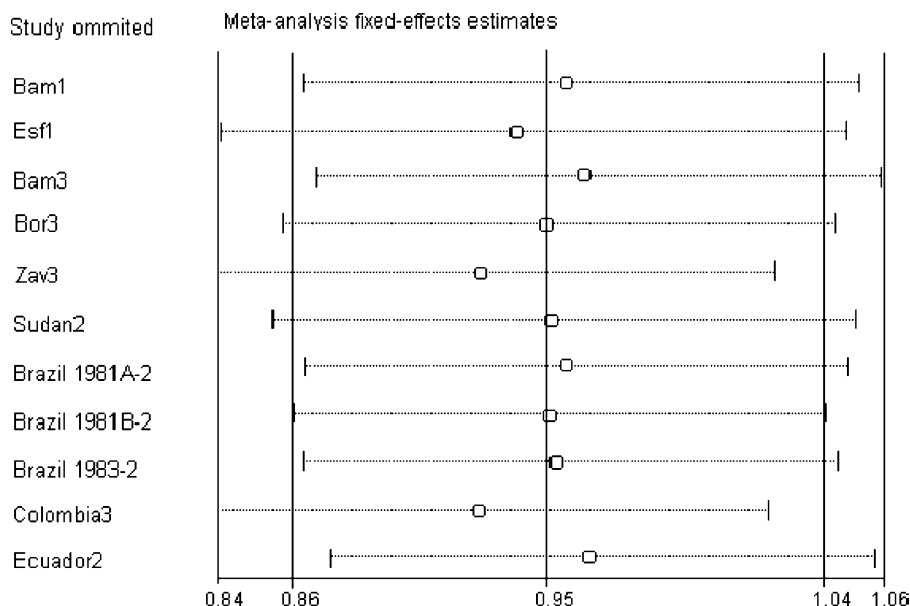
While Old World trials are clustered around the vertical line of RR = 1 (i.e., homogeneous but with minimal efficacy), the results

from the trials conducted in Latin American tend to be scattered on the left of that line, suggesting more heterogeneity but also more efficacious results. The Ecuador trial, the only trial with significant results, is located in the far left of the forest plot. Despite their lower individual RR values, these trials have limited impact on the pooled RR due to their smaller sample sizes (and wide confidence intervals).

A graphical display of the influence of individual trials on the pooled RR is presented in Fig. 2. This graph shows the values of the pooled RR, when studies are omitted one at a time. The reference line is the overall, pooled RR. Thus, the pooled RR is lower when Esf1, Zav3 or Colombia3 trials are omitted and the reverse is the case



**Fig. 1.** Forest plot of vaccine efficacy measures in different leishmaniasis vaccine trials.



**Fig. 2.** Influence of individual trials on pooled RR. The circles indicate the pooled relative risk estimate when each individual trial is omitted. 95% confidence intervals are also shown.

when any one of the Bam trials, Brazil 1981A-2 and the Ecuador2 are omitted.

## 5. Discussion

In some trials, vaccinated participants who skin test converted following vaccination were reported to have a lower incidence of leishmaniasis than other trial participants [24,25,29]. However, our meta-analysis clearly demonstrates the overall inability of first-generation leishmaniasis vaccines evaluated to date in phase 3 clinical trials to protect vaccinated individuals against infection by the *Leishmania* parasite.

The apparent absence of efficacy of these vaccines may be due to a number of potential factors. First, the immune stimulation provided by a single dose, or even multiple doses, of inactivated parasite antigen, even when mixed with BCG as an adjuvant, may be inadequate. Secondly, BCG was used in the control arm in several of the studies and was also used as a vaccine adjuvant. BCG stimulates Th1 response and contributes to the immunogenicity of the vaccine [35]. However, when used as the clinical trial control vaccine (for blinding), and as a vaccine adjuvant, it induces Th1 response in both arms, thus making it potentially more difficult for any potential vaccine effect to be detected. To the extent that BCG alone might protect against leishmaniasis, the difference in incidence between the two study arms would be reduced and the statistical power of the study would be compromised. Thirdly, LST is an imprecise and highly variable indicator of previous exposure to leishmaniasis. This could lead to misclassification of some individuals with previous exposure and immunity as unexposed and allow their inclusion in both arms of the clinical trial. If such persons are still at some risk of leishmaniasis, but the vaccine confers no additional protection in such partially immune individuals, then the protective efficacy of the vaccine in “unexposed” individuals may be underestimated. Fourthly, it is possible that some genetically non-responsive volunteers in endemic areas would show no LST reaction while they could have been exposed and possibly immune. To the extent that this occurs, the resulting misclassification would contribute to a reduced difference between the two arms and contribute to misleading efficacy estimation.

Our use of the meta-analytic approach is subject to some limitations. The different vaccine candidates used in the trials were similar in their dependence on killed parasites, but the composition of the vaccines varied between trials, BCG was used as an adjuvant in some cases and the ecological setting of the different trials varied substantially. Thus, it may be argued that combining the results from the trials should be done with great caution. We would not disagree with this view but have combined the findings to seek evidence that might encourage further work on first-generation vaccines. In this respect, our findings are depressing and suggest that other approaches to leishmaniasis vaccine development should be vigorously pursued.

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