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A Meta-Analysis of Vaccines for Preventing Cutaneous Leishmaniasis

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Abstract

Background: Cutaneous Leishmaniasis (CL) is a skin infection prevalent in more than 70 countries worldwide. CL can lead to permanent scars and disfigurement. Treatments are costly, hazardous, and with limited effectiveness.

Methods: We searched databases up to October 17, 2017. All randomized controlled clinical trials (RCTs), performed on people living in endemic regions, with no previous history of leishmaniasis of any type, in which Leishmania vaccination was done against placebo or other active preventative means, in order to prevent cutaneous leishmaniasis (CL) were included. Two authors independently screened titles, abstracts, and full texts; extracted data; and assessed risk of bias.

Results: We included 12 trials (nine published, three unpublished) with 28,297 randomised participants. Studies were conducted in endemic populations from Brazil, Colombia, Ecuador and Iran. There was no significant difference between any dose of vaccine versus no Leishmaniasis vaccination in the pooled analysis of trials of Old World and American cutaneous leishmaniasis, after one year follow up (RR=0.87, 95% CI 0.73 to 1.05; 22,566 participants; 11 studies, NNT = 355), or after two years follow up (RR=0.88, 95% CI 0.70 to 1.11; 13,168 participants; 6 studies, NNT = 155). Pooled analysis was not done for 3 studies with more than two years follow up, because the heterogeneity was high. The exact number of these side effects were trivial or not reported. No study measured mortality or quality of life.

Conclusion: The results of this review show that there is no significant difference in the occurrence of cutaneous leishmaniasis after one or two years follow-up between people who were given any dose of the first generation leishmaniasis vaccine and those that were not given the vaccine. More investigation about these vaccines should be done.

Keywords: Cutaneous Leishmaniasis; Vaccination; Preventing; RCT

Highlights:

- The vaccine had no significant effect in preventing CL after one year follow-up.
- The vaccine had no significant effect in preventing CL after two years follow-up either.
- There was no evidence about the impact of different doses in preventing CL.
- Side effects were trivial or not reported.

Background

Description of the condition

Leishmaniasis is caused by a parasite belonging to the genus *Leishmania*, which is spread by the bite of the female sandfly (Phlebotomine). Leishmaniasis is best considered as a spectrum of diseases with distinctive manifestations ranging from infections without symptoms and mild self-healing cutaneous (skin) disease to severe non-healing diffuse cutaneous and lethal visceral leishmaniasis [1].

The disease is geographically and ecologically widespread, occurring in tropical and subtropical regions on all continents except Australia [2]. Cutaneous leishmaniasis (CL) is endemic in more than 70 countries worldwide [3]. There are 1.5 to 2.0 million new cases of leishmaniasis per year worldwide, of which 400,000 to 500,000 are visceral (90% of them in Bangladesh, Brazil, India, Nepal and Sudan) and 1,000,000 to 1,500,000 cutaneous (90% of them in Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Sudan) [4-6]. With a prevalence of 12 to 14 million cases (and a world population of 350 million at risk [7], leishmaniasis is a health problem in 88 countries, especially in lower and middle income economies [4,8]. It is likely that the number of cases occurring

around the world is considerably greater than that officially reported. One reason for this is under-reporting by affected people who are not accessing or seeking medical or diagnostic facilities [7]. In several areas of the world, there is a clear increase in the number of cases, e.g. Brazil, Kabul (Afghanistan) and Ouagadougou (Burkina Faso) [7], and many endemic areas have reported a 5 times increase over a period of seven years [2]. Such increases can be explained in part by improved diagnosis and case notification, but are also a result of inadequate vector or reservoir control, increased detection of cutaneous leishmaniasis associated with opportunistic infections (e.g. HIV/AIDS), and the emergence of anti-leishmanial drug resistance [3]. Also, reporting of the disease is compulsory in only one-third (33 out of 88) of the endemic countries [5].

Clinical Forms: Clinical forms of leishmaniasis are diverse, representing a complex of diseases: visceral leishmaniasis (VL) which affects internal organs and can be fatal; post kala-azar dermal leishmaniasis (PKDL), which arises after visceral infection [6]; muco-cutaneous leishmaniasis (MCL), which is a mutilating disease affecting mucous membranes; diffuse cutaneous leishmaniasis (DCL), which is a long-lasting disease due to a deficient cellular mediated immune response; and cutaneous leishmaniasis (CL), which is confined to the skin and can be disfiguring [7].

Cutaneous leishmaniasis, the most common form and therefore the main focus of this review, includes zoonotic cutaneous leishmaniasis (ZCL) where the reservoir is a non-human mammal and anthroponotic CL where the reservoir is human. Cutaneous leishmaniasis can show at least three clinical forms including acute cutaneous leishmaniasis (ACL), chronic cutaneous leishmaniasis (CCL) and occasionally leishmaniasis recidivans (LR) [9]. In 2-5% of cases, acute cutaneous leishmaniasis becomes chronic or can develop into leishmaniasis recidivans, where the persistent presence of parasites may drive a skin reaction around the original primary lesion long after healing [10]. Leishmaniasis recidivans is difficult to treat and leaves extensive scars [5].

Cutaneous leishmaniasis usually produces skin ulcers on the exposed parts of the body such as the arms, legs and especially the face [11]. Over 90% of cases of cutaneous leishmaniasis heal spontaneously within 3-18 months [8].

Causes and Natural History: The leishmaniases are caused by 20 species of parasites belonging to the genus *Leishmania*, which are pathogenic for humans. The protozoa are transmitted by the bite of a tiny two to three millimetre-long insect vector, the Phlebotomine sandfly in the Old World [11], and Lutzomyia sandfly in the New World [12]. Approximately 30 sandfly species are proven vectors [7] with more than 40 additional species probably involved in transmission [3]. These sandflies are able to pass through the usual netting used for mosquitoes. Sandflies are found around human habitations and breed in specific organic wastes such as faeces, manure, rodent burrows and leaf litter [12]. When a sandfly bites an infected animal or human it becomes infected; the parasitic organisms are then passed on when the sandfly bites its next victim [7,11]. Humans are usually accidental hosts of these flies; natural hosts include a variety of small mammals, and dogs [2].

A striking difference occurs between the so-called Old World (i.e. Africa, Europe, and Asia) and New World (i.e. the Americas) cutaneous leishmaniases in the ecological context of their respective transmission cycles. Old World cutaneous leishmaniasis usually occurs in open semi-arid or even desert conditions, but New World cutaneous leishmaniasis is still mostly associated with forests [3]. Each species of *Leishmania favours* one or more animal reservoirs, except *Leishmania donovani* [13] and *Leishmania tropica* [5] in which the reservoir is human.

The *Leishmania* parasite has two different life cycle forms called promastigote (with flagellum which is the means by which it moves) and amastigote (without flagellum). Parasites, in the form of amastigotes are taken up from the infected tissues or blood of a mammalian host during feeding by female sandflies. Within the midgut of the sandflies, the parasites undergo a change to the promastigote form and multiply. Once the promastigotes are fully developed, they migrate from the gut to the sandfly pharynx and proboscis (the insect's tubular feeding organ), where they remain until they are injected into a new mammalian host during a subsequent blood meal. Between 10 to 200 (or even up to 1000) promastigotes enter the skin during each feeding by an infected sandfly. Some of the promastigotes are taken up by the macrophage cells (cells related to the immune system) in the host skin. Within the macrophages the promastigotes transform into amastigotes. When a macrophage becomes filled with amastigotes the macrophage is disrupted. The amastigotes re-enter the extracellular space and are then taken up again by other macrophages [13]. However, ulceration and tissue destruction in CL has been proposed to be a result of immune activation evoked by the infection rather than a direct effect of the infectious burden of parasites into the skin [14]. The incubation period of CL is usually measured in months, but ranges from a few days (about 15 days) to over a year [15]. The disease begins as a small red swelling (papule) at the site of the sandfly bite. The papule increases in size and becomes a nodule which eventually ulcerates and crusts over. The ulcer is typically painless unless there is secondary bacterial or fungal infection [12]. Sometimes, the ulcer is associated with bleeding and itching. Many people think that covering the ulcer delays its healing, and allow it to be exposed to the air. However, this may facilitate transmission of leishmaniasis to others [10]. Usually, these scars do not disappear. They stay forever [16].

Impact: A total of 11.8% of total worldwide DALYs (Disability Adjusted Life Years) is associated with all the leishmaniases that occur in the eastern Mediterranean countries, where CL is concentrated [10].

There is a social stigma associated with the deformities and disfiguring scars caused by this disease that keeps affected people hidden. Victims are mostly children in endemic areas, and lesions are frequently on the face. The disfigurement caused by CL scars lead to stigma, social isolation, suffering and may be a barrier to marriage, especially for girls and young women [10]. New World CL, which

is endemic in most countries of Latin America, has a serious sequel, which is mucosal or mucocutaneous leishmaniasis (ML). Unless diagnosed and treated early, this disease can progress to destroy tissues in the nose, mouth and throat and in some cases leads to death. Many suicides or attempts at suicide have been recorded due to the stigma associated with ML in Latin America [10].

Cutaneous leishmaniasis also creates a burden on the national economy. Seventy-seven percent of men in Ecuador believe CL diminishes their ability to work. The cost of treatment is high and is in most cases beyond the financial means of affected people who are mostly poor [10]. On several occasions, epidemics have significantly delayed the implementation of land development projects [11]. Leishmaniasis has thus become a disease that impedes socioeconomic development.

Diagnosis: For people in endemic areas, or where travellers return from endemic areas, the clinical diagnosis of typical nodules or sores is not difficult. Deeper sores from beneath the skin, sores arising from lymphatic spread or chronic sores in which scarring predominates may present diagnostic difficulties. Confirmation of diagnosis is through microscopic demonstration of the parasite [15].

Samples are taken by scraping the affected sore [12]; punch biopsy with tissue impression smears [12]; or needle aspiration of tissue fluid from the margin of the lesion [12].

The parasite is identified by staining with Giemsa and looking under the microscope; cultivating *Leishmania* species in specific culture media (such as Novy-MacNeal-Nicolle (NNN) medium, etc); inoculating suspected specimens into susceptible laboratory animals (such as hamsters) [13,15]; or using the highly sensitive polymerase chain reaction test [12].

The leishmanin skin test (LST) is considered as a simple indicator for cell mediated immunity; a delayed hypersensitivity reaction to intradermal crude *Leishmania* antigen is produced in healing or cured cases of both cutaneous and visceral leishmaniasis [17]. It is a highly specific test and is of great value in epidemiological studies, although it has little clinical use [18]. The leishmanin skin test can be used for epidemiologic surveys, diagnosis in nonendemic areas particularly in recidivans and mucosal forms of CL and for identification of chronic forms of CL [9].

Description of the intervention

Treatment: Most leishmaniasis sores will eventually heal spontaneously, but the duration of this process cannot be predicted in an individual case. Topical methods of treatment such as heating, freezing and ointments are used for simple sores. Systemic treatments including antimonials [19,20], amphotericin B, miltefosine and paromomycin can be used for more problematic sores. However, some are expensive, some may be toxic, and, when used as monotherapy, may promote the development of drug resistance. Therefore, the WHO has suggested using drug combinations. Recently authors have suggested immunochemotherapy, whereby a low-dose or short course of an effective drug is prescribed with an injection of a vaccine or immunomodulator to induce an effective immune response [21].

After healing, individuals are usually immune to reinfection from the same species, although secondary sores in old age or due to a parasite of a different kind have been reported [15].

There are reports that show in immunocompromised hosts the disease may return upon stopping treatment, and even in 'cured' or asymptomatically infected individuals, fulminating disease appears after immunosuppression or HIV infection [21].

Prevention and Control: Controlling the sandflies, which transmit the disease (vector control), in and around the home consists of the use of insecticides (usually pyrethroids) being sprayed around the house or individual protection based on pyrethroid-impregnated bed nets [7]. Various repellents, such as dimethyl-phthalate and imidacloprid/permethrin are also used by people to discourage insect bites [22, 23]. Animal reservoir control for CL is based on the use of poisoning baits and environmental management to control rodents [7]. More details about vector and reservoir interventions to prevent CL can be found in references [24].

The complex epidemiological characteristics of the disease and its transmission have limited the success of these disease control efforts. Vector and reservoir control are not always possible or practical in the case of zoonotic diseases, or require infrastructure beyond the means of the affected population. Even if successful, these measures are not maintained because of the cost [11,25] and are short lived [8].

Leishmaniasis is thought to be one of a few parasitic diseases likely to be controllable by vaccination. The relatively uncomplicated *Leishmania* life cycle and the fact that recovery from infection usually renders the host resistant to subsequent infection indicate that a successful vaccine is feasible. Evidence from studies in animal models indicates that protection can be achieved by immunisation with protein or DNA vaccines [26].

The History of the Vaccine: Vaccination through artificial inoculation of live parasites (leishmanisation) has been used to induce protection in the past. Leishmanisation was used in Iran in the 1980s and in Israel in the 1970s as prophylaxis against leishmaniasis, but is not currently practiced in either country [27]. Though reported to be efficacious in some studies, leishmanisation was abandoned for ethical reasons due to its severe side effects such as chronic non-healing lesions [28]. Further problems were the rising incidence of HIV and the use of immunosuppressive agents, parasite persistence in the body and difficulties with the inoculum control [29].

However, this old method has been studied again recently [30]. Researchers have also recently used deep-frozen *Leishmaniamajor* (L. *major*) promastigotes (the parasite or its components) for immunisation against leishmaniasis according to the World Health Organization (WHO) protocol in Geneva, 1997 [31].

Also, attention has been turned to the use of killed [9,32-42] or attenuated [43] parasite vaccines and defined subunit vaccines [44-49].

Leishmania are easily cultured, hence the production of vaccines using the parasite or its components are feasible [50]. In addition, for the past few decades, killed parasites have been used as skin test antigens for diagnosis of leishmaniasis in people. These killed parasites have also been used with or without adjuvants as vaccines or for immunotherapy in clinical studies [51].

Some evidence from experimental, clinical and field studies suggested that anti-*Leishmania* vaccines based on killed whole, fractionated or recombinant parasite promastigotes are safe and capable of inducing immunity to leishmaniasis [51,52].

However, those producing a suitable human vaccine have to consider some practical issues. For example, the vaccine should be delivered as a single, defined molecule to facilitate compliance with regulatory and manufacturing standards and to lower the overall production costs. Ideally, the vaccine should protect against cutaneous as well as visceral leishmaniasis [26].

At present there is only one prophylactic live vaccine for use in human populations [29]. This is a mixture of live virulent *Leishmania major* mixed with killed parasite registered in Uzbekistan [53]. However, there is no registered prophylactic leishmaniasis vaccine against any form of human leishmaniasis [54,55], although three licensed vaccines have been developed for dogs [54].

The Different types of vaccines up to now and their characteristics: Leishmaniasis is a complex disease caused by several different species of parasite that are closely related, so if a single vaccine could be developed there is the possibility that it could protect against several diseases [54]. An ideal Leishmaniasis vaccine should be safe and cost effective, consist of defined components capable of large scale production, contain antigens that are shared among multiple species, induce relevant long-lived T cell responses, protect against infection and disease and be effective for both prophylactic and therapeutic indications [54]. Drug resistance, toxicity and the side effects of expensive chemotherapeutics and difficult reservoir control emphasize the need for a safe and effective vaccine, which is not available yet [56].

Up to the present, three generations of vaccines have been reported for leishmaniasis. The first generation candidate vaccines against leishmaniasis were prepared using inactivated or killed whole parasites as their main ingredient, and were considered useful due to their relative ease of production and low cost. These vaccines have been the subject of many investigations over several decades and are the only leishmaniasis vaccines which have undergone phase 3 clinical trial evaluation. However, although studies have demonstrated the safety of the vaccines and some studies showed immunogenicity and some indication of protection, they generally have poor efficacy [27,29].

The second generation vaccines have been used since the 1990s, and use live genetically modified parasites, or bacteria or viruses containing Leishmania genes, but their success in field trials has not yet been reported. Authors predict that the second generation of leishmaniasis vaccines with native antigens and effective adjuvants are likely to be licensed and used in control programs in the coming 25 years [29].

The third generation vaccines are the multiple-gene DNA vaccines that are stable and do not require refrigerated transportation. They include genes coded for a protective antigen, cloned into a vector containing a eukaryotic promoter [29]. The DNA vaccine against leishmaniasis was successfully tested for immunogenicity and protective effects, in the rodent models of leishmaniasis infection [57] and might be used for prevention in humans in the future. Some authors have also suggested that the saliva of leishmania-transmitting vectors can be a valuable candidate for developing anti-Leishmania vaccines [58].

In this review, we focus on the first generation of vaccines.

How the intervention might work?: The manufacture of this vaccine like many other vaccines was based on observations that after leishmaniasis lesions heal and leaves scars, the human immune system develops resistance to this micro-organism, and reinfection is less likely. Therefore, The cultivated or killed parasite or parasite parts were used for making the vaccine [16].

Why it is important to do this review

Although leishmaniasis has a high incidence, it is a neglected disease and more research is needed for its control. The disease varies in severity and can lead to severe permanent mutilation in thousands of people every year around the world, with repercussions for public health and has an impact on the productivity of many countries. Existing treatments are expensive, associated with adverse effects, and of fairly low efficacy [7]. Cochrane systematic reviews of treatments for leishmaniasis have been performed [19,20]; however, they showed a lack of evidence for potentially beneficial treatments.

The main challenge for leishmaniasis control is to translate new knowledge into control tools [7]. Consequently, a safe, efficacious, and affordable vaccine could be the most practical and cost-effective control tool to prevent disease in many situations [59].

This systematic review seeks to evaluate vaccination as a means of preventing leishmaniasis.

The plans for this review were published as a protocol 'Vaccines for preventing cutaneous leishmaniasis' [60].

Objectives

To assess the effects of vaccines to prevent cutaneous leishmaniasis.

Methods

Criteria for considering studies for this review

Types of studies

All randomized controlled clinical trials (RCTs).

Types of participants

People living in endemic regions, with no previous history of leishmaniasis of any type.

Types of interventions

Leishmania vaccination against placebo or other active preventative means. We also considered trials not using any form of treatment in the control arm.

We included all types of first generation vaccines, such as: live (Leishmanization); attenuated; killed (by Merthiolate, heat, freeze-thaw, etc.); fractionated (fractions 5, 9, 6, etc.); purified or recombinant parasite antigens (Gp63, cysteine proteinases, etc.); and other molecular or DNA vaccines (IL-12, LPG, heat shock protein, etc).

The predefined exclusion criteria for articles under review were:

- The occurrence rate of cutaneous leishmaniasis not reported; i.e. RCTs which look at immunological response to the leishmaniasis vaccine, but did not report the occurrence of CL were excluded, even if they presented information about side effects, mortality or quality of life.
- articles about human leishmaniasis but not including the cutaneous form;
- articles about using the vaccine for treatment of cutaneous leishmaniasis after its presence and not about prevention
- studies including environmental manipulation and not vaccines;
- Studies on animals not humans.

Types of outcome measures

Primary outcomes

1. Occurrence of cutaneous leishmaniasis at the end of one year, two years and more than two years.

Secondary outcomes

1. Leishmanin skin test conversion rate (LST) at the end of one year, two years and greater than two years. Mild skin test reactions (indurations <5mm were regarded as negative and >5mm as positive).
2. Side effects of the vaccine, including:
   o local side effects, such as pain, redness, ulcer, lymph node swelling, itching and induration;
   o mortality;
   o quality of life.

We did not consider other immunological or physiological predictors of immunity. These predictors include changes in the levels of interferons, interleukins or cytokines and the lymphocyte proliferation rates. These measures try to indirectly predict immunity to disease and may not be as reliable as the actual disease occurrence rate or LST conversion rate.

Search methods for identification of studies

We aimed to identify all relevant randomised controlled trials (RCTs) regardless of language or publication status (published, unpublished, and in progress).

Electronic searches: We searched the following databases up to 17 October 2017. Details are in the appendix. The Cochrane Skin Group Specialized Register, the Cochrane Central Register of Controlled Trials (CENTRAL); 2016, Issue 9, in the Cochrane Library, MEDLINE via Ovid (from 1946), Embase via Ovid (from 1974); and LILACS (Latin American and Caribbean Health Science Information database, from 1982), Persian Databases at SID (Scientific Information Database of Iran, www.sid.ir), Conference Papers Index, Health and Medical Complete via Proquest, Web of Knowledge/ Web of Science; and General search engines and meta-search engines(google, alta vista, excite, search, dogpile, metacrawler), using a search strategy similar to the terms used for searching the Cochrane Skin Group's Specialised Register.

We searched the following trials registers up to 17 October 2017, using a search strategy similar to the terms used for searching the Cochrane Skin Group's Specialised Register:

The World Health Organization International Clinical Trials Registry platform (ICTRP) (www.who.int/trialsearch); the Ongoing Skin Trials Register (www.nottingham.ac.uk/ongoingskintrials); ClinicalTrials.gov (www.clinicaltrials.gov); the ISRCTN registry (www.isrctn.com/); the Australian and New Zealand Clinical Trials Registry (www.anzctr.org.au); and Trials Central (www.trialscentral.org).

We checked the bibliographies of included studies and some reviews for further references to relevant trials.

We contacted the leading authors of leishmaniasis studies to see if they were aware of any recent or ongoing research, or any unpublished data. We also contacted the following Tropical Medicine Centres:

Department of Infectious Diseases and Tropical Medicine at the University of Munich, Germany; Swiss Tropical Institute, Switzerland; Prince Leopold Institute of Tropical Medicine, Belgium; McGill Centre for Tropical Disease, Canada; Tulane University School of Public Health & Tropical Medicine, USA; London School of Hygiene & Tropical Medicine, UK; Tropical Medicine at the Liverpool School of Tropical Medicine, UK; Department of Public Health and Tropical Medicine, James Cook University, Australia; Institut Pasteur, France; Bernhard Nocht Institute, Germany; Trop Ed Europ, Spain; Centro Dermatologico Federico Lleras Acosta, Colombia; Skin disease & Leishmaniasis Research Centre, Kerman & Tehran, Iran; and Center for Research and Training in Skin Diseases and Leprosy, School of Public Health, Tehran Medical University, Iran. We did not perform a separate search for adverse effects of vaccines for preventing cutaneous leishmaniasis. Instead we examined data on adverse effects reported in our included studies.

Data collection and analysis

Selection of studies: Two authors (NK and AK) independently screened all references identified through the search and checked the reference list of the relevant clinical trials and review papers, and also contacted the trial authors of the published papers to retrieve unpublished data. Two authors (UG and AK) contacted trial authors in the field of leishmaniasis in different countries to retrieve any additional unpublished articles. Papers matching the inclusion criteria were chosen by NK and AK. We solved any differences of opinion through discussion with the review team. We did not attempt to blind the review authors to the authorship information of the trials during study selection or data extraction.

Data extraction and management: We (NK and AK or UG) put the data that was independently extracted onto data summary forms and sent the final extracted data sheets for review to other members of the review team and discussed ambiguous areas through email. If there was uncertainty, we contacted trial authors for clarification. Where included trials were conducted by authors of this review, data extraction was performed by other co-reviewers (NK and UG) who were not involved in the trial.

We described each of the necessary components for each trial and its risk of bias in the Characteristics of included studies table. We also extracted the information mentioned below in our information sheets.

- What type of method was used for the RCT?
- How many, or what percentages of participants, were lost to follow-up in each arm of the study?
- Were the participants analyzed in the groups that they were originally randomized (Intention-to-treat analysis)?
- Was a sample-size calculation mentioned?
- Were the study groups similar at baseline (e.g. for age, sex, location of residence and etc.)?
- Were the inclusion and exclusion criteria of the population specified? How representative was the study population of a real endemic population?
- What was the intervention in the control group? A placebo or nothing?
- Were additional therapeutics used, (such as BCG)? If additional therapeutics were used, were they used identically in both arms?
- Was the outcome (developing cutaneous leishmaniasis) confirmed by pathology or lab results?
- What was the source of funding for the trial? Is it likely to have affected the results of the trial?

Assessment of risk of bias in included studies: We (NK, UG and AK) assessed the risk of bias of the selected studies independently using The Cochrane Collaboration's tool for assessing risk of bias as described in Chapter 8, section 8.5, in the *Cochrane Handbook for Systematic Reviews of Interventions* [61]. With this tool, we assessed the risk of bias as 'low', 'high' or 'unclear' for each of the following domains:

- The method of generation of the random sequence. The satisfactory method of generating the allocation sequence had to be unpredictable (selection bias);
- The method of allocation concealment. It was considered "adequate" if the assignment could not be foreseen (selection bias);
- Blinding of participants and personnel (performance bias);
- Blinding of outcome assessment (detection bias);
- Incomplete outcome data (attrition bias);
- Selective reporting (reporting bias)
- Other bias

Measures of treatment effect: For dichotomous outcomes, we estimated pooled risk ratios (RR) with 95% confidence intervals (CI), and expressed as number needed to treat to benefit from prevention (NNT), where appropriate. For the primary outcomes, data were categorised into one, two and greater than two years. For the latter time point, end points more than two years were used to capture longer term benefits. Side effects were described qualitatively.

In future studies, if we encounter continuous outcomes, we will estimate difference in means (MD) with 95% CI, or as standardised mean differences (SMD) if comparable scales have been used.

Unit of analysis issues: Where there were multiple intervention groups within a trial, pair-wise comparisons were made of similar active interventions versus no treatment, placebo, or another active intervention. Because vaccination is designed to have long term effects, cross-over trials would have been analysed using data from the first phase only and pooled, where possible, with parallel design studies. Internally controlled trials would have been excluded from the analysis as they were not an appropriate method to use for this research question. Cluster randomised trials would have ideally been analysed taking intracluster coefficients (ICC) into account. If this information was not presented in the included study, we could not adjust the standard error to reflect the design.

Dealing with missing data: We conducted an intention-to-treat analysis. If possible, authors of studies would be contacted to provide missing statistics such as standard deviations. We decided to change our methods for dealing with missing data due to the high percentage of drop-outs in some of the included studies. The original statistical analysis plan was to include these missing participants as treatment failures (i.e. they all developed cutaneous leishmaniasis); however, this would have resulted in very high prevalence of disease.

We did not assume that the people with missing data were treatment failures. In other words this meant that for some studies more than 50% of the participants would be assumed to have developed leishmaniasis, which was irrational.

The analysis assumed that the people with missing data did not develop the disease (assuming none of the dropouts had events), which we think is the safest option to use. The other scenarios gave similar non-significant effects for individual study results in most cases.

We additionally compared the effect of allowing for different methods of imputations [62], to find if our assumptions were plausible.

For continuous outcomes, we were unable to allow for missing data in our analyses due to how the data were reported in the original papers, therefore we used data as presented in the original papers.

Assessment of heterogeneity: Statistical heterogeneity was assessed using I^2 statistic. If substantial heterogeneity (I^2>50%) existed between studies for the primary outcomes, reasons for heterogeneity, such as comparing the one-, two-, and three-dose regimens, and between adults and children would have been conducted. Additionally, meta-regression techniques would have been used to explore the relation between the length of the interval between the injections in the two- and three dose regimens and the efficacy of the vaccine. However, in this review there were insufficient numbers of studies included in the comparisons to allow statistical analysis.

Assessment of reporting biases: We had planned to test publication bias by the use of a funnel plot if adequate data had been available for similar types of interventions. But in this review, only a few studies were included in most comparisons, so funnel plots were not used.

Data synthesis: For studies with a similar type of active intervention, a meta-analysis was performed to calculate a weighted preventive effect across trials using a random effects model. Statistical heterogeneity was assessed using I^2. Data was synthesised using meta-analysis techniques, if I^2 was less than 80%. Where it was not possible to perform a meta-analysis, the data were summarised for each trial.

We presented the efficacy of three-dose, two-dose and one-dose regimens in separate meta-analyses.

Subgroup analysis and investigation of heterogeneity: We planned to explore further reasons for heterogeneity, if substantial heterogeneity (I^2 statistic > 50%) existed between studies for the primary outcome. However, due to a lack of sufficient studies, and because generally the level of heterogeneity between the studies was relatively low, we did not conduct any subgroup analyses.

Sensitivity analysis: We planned to conduct sensitivity analyses to examine the effects of excluding poor-quality studies, defined as those with a moderate or high risk of bias as described in the Cochrane Handbook for Systematic Reviews of Interventions [61]; however there were insufficient studies to be able to perform these analyses.

Summary of findings tables were created for all main comparisons, including the primary and key secondary outcomes. The quality of evidence was evaluated with the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) working group grades of evidence.

Results

Description of studies

For a complete description of studies see Appendix.

Results of the search

The electronic database searches identified a total of 408 studies, and three more studies were identified from other sources, 381 articles remained after duplications were removed. We screened 381 references of which 362 were excluded based on titles and abstracts alone. The full text was sought for 19 references. Twelve studies (reported in 10 references) were included and a further nine references were excluded after reading the full text. For a further description of our screening process, see the study flow diagram.

Included studies

Twelve RCTs (reported in 10 papers) were eligible for inclusion in the systematic review [35-37,51,63-67]. A total of 28,297 participants were included in these trials. Details of all the studies are listed in the Appendix tables.

Design: From the 12 trials, nine had been published, and three [64-66] had not been published. All were randomised controlled clinical trials, and one was a cluster randomised trial [65].

The data for Antunes 1986a (1981 I) [51]; Antunes 1986b (1981 II) [51]; Antunes 1986c (1983) [51] are reported in one published paper but have been entered as three separate studies in this review. This is because the paper reports data for two separate trials that were carried out in 1981 and a third trial conducted in 1983. During the 1981 trial, group 1 went into the jungle from March to November for a total of 60 days, whereas group 2 stayed in the jungle for 23 days during the months of February, March and April. Thus, we included them as three individual trials as the populations were different.

In this review, we did not have any cross-over or internally controlled trials, but we did have one cluster randomised trial [65] among our included studies.

Sample sizes: The numbers of participants in the studies were all high and ranged from 611 [51] to 5731 [65] participants.

Setting: The studies were conducted in endemic countries including Iran [36,64-66], Brazil [51,65], Ecuador [35,63] and Colombia [67]; on Old World [36,37,64-66] and American, New World leishmaniasis [35,51,63,65,67]. The study durations were one year, 2 years and more. All participants were healthy individuals living in endemic regions, likely to develop CL. The age range of the participants was wide, including young children, soldiers and adults up to 72 years and both genders.

Funding

Studies were funded by

- UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) [36,37,51, 63-65,67],
- Center for Research & Training in Skin Diseases & Leprosy, TUMS [64,65],
- 'Financiadora de Estudos e Projetos' (F1NEP), 'Superintendencia de Campanhas do Ministerio da Satide' (SUCAM), the Pan American Health Organization (PAHO), from 'Conselho Nacional de Desenvolvimento Cientifico e Tecnologico' (CNPq), 'Ministerio do Exercito' (Comando Militar da Amazonia — CMA) [51],
- US Agency for International Development [35],
- fundacao de Amparo a Pesquisa do Estado de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil [CBB-562/02 and CBB-653/06]; Conselho Nacional de Desenvolvimento Cientıfica e Tecnologica, Brasılia, Distrito Federal, Brazil [350200/1998-0], research fellowships from CNPq [65],
- Universidad de Antioquia [67].

Participants

Participants were people from endemic areas with no previous history of leishmaniasis and in different age groups, including school children [36,64,66], army conscripts [51], rural populations [63,65], military base residents [37] and soldiers [67].

Interventions

The interventions were single doses [36,37], double doses [35,51,63,65] or triple doses [64-67] of vaccines made from killed promastigotes, which were accompanied with BCG injections in eight studies [35-37,63-67].

Outcomes

Studies were selected based on the fact that the study reported the outcome of the incidence of cutaneous leishmaniasis in the population after a specified follow-up time. However, some of these studies had also reported LST (or Montenegro Skin Test (MNST)) [35-37,63,64,66,67] and side effects [35-37,63-65,67]. The LST or the Montenegro Skin Test is used to diagnose cutaneous leishmaniasis. The test measures the delayed type hypersensitivity reactions to an intradermal injection of a suspension of killed promastigotes.

Excluded studies

Nine studies were excluded because they did not meet the inclusion criteria and the reasons are provided below. A comprehensive list is available in the appendix. The reasons are listed below:

- Studies were not a randomised controlled trial [32,52]
- The occurrence rate of cutaneous leishmaniasis not reported (inoculation site not counted) [34,38,40,68-70].
- Articles about using the vaccine for treatment of cutaneous leishmaniasis after its presence and not about prevention [72].

There are two ongoing studies about this topic, currently underway in Iran [72,73].

Risk of bias in included studies

Allocation (selection bias): All studies randomized their participants into the intervention and control groups; however, although sequence generation and allocation concealment was satisfactory for eight of the studies, four did not report details so the risk of bias was judged unclear [35,64,65].

Blinding (performance bias and detection bias): The level of detail about blinding of participants and personnel (performance bias) in the reporting varied but all studies except one were double blind and were assessed as low risk of bias. One trial [65] did not provide details about blinding of participants and personnel and was assessed to have an unclear risk.

Blinding of outcome assessors (detection bias) was only reported by three studies [36,37,63] which were considered at low risk of bias and the remaining studies were judged unclear risk of detection bias.

Incomplete outcome data (attrition bias): All clinical trials had a follow-up rate above 80% except for three studies [35,63,64] which were judged to have a high risk of attrition bias. The risk in two studies was unclear [65,66].

Seven studies were rated as low risk of attrition bias. In the study by Sharifi 1998, there appeared to be no missing data. For Momeni 1999, the important point about loss to follow-up is that the rate was similar between the intervention and control groups (30% versus 28%), even though these rates are both high; therefore it is still valid to assume that none of these drop outs had the event. For Armijos 2004, less than 80% completed the 2nd vaccine; however, we assumed that none of the dropouts had events so we did an ITT analysis based on the number randomized before the first vaccine as we think this is the more appropriate denominator to use.

Selective reporting (reporting bias): All of the studies were assessed to have an unclear risk of reporting bias. It did seem likely that there was selective reporting of the side effects as authors mentioned only some of the side effects and there was no identical list for reporting the side effects between studies. Selective reporting did not seem to be an issue in the main outcomes; however, no study protocols were available and so the possibility of reporting bias was unclear.

Other potential sources of bias: All studies were rated as unclear for other sources of bias. Three studies [64-66] were unpublished and were, therefore, not peer reviewed or refereed. We asked the authors about why they did not publish the studies. The author of Sharifi 2001 mentioned lack of time, although he did present the results in a conference. The author of Khamesipour 2000a and Khamesipour 2000b mentioned that the final reports were sent to the WHO and the results of these trials were later mentioned in two review papers [27,73].

Effects of interventions:

Primary Outcome

Occurrence of cutaneous leishmaniasis at the end of one year, two years and more than two years.

Any dose of vaccine versus no vaccine

One year follow-up

The occurrence of Old World and American cutaneous leishmaniasis with any dose of vaccine versus no vaccination showed no significant differences after one year follow-up (RR 0.87, 95% CI 0.73 to 1.05; participants = 22,566; studies = 11, I^2= 32%, NNT=355, Analysis 1.1.1). The 11 studies were: Antunes 1986a (1981 I); Antunes 1986b (1981 II); Antunes 1986c (1983); Armijos 1998; Armijos 2004; Khamesipour 2000a; Khamesipour 2000b; Momeni 1999; Sharifi 1998; Sharifi 2001; and Velez 2005 (Figure 1).

Two years follow-up

Similarly at two years follow-up in Ecuador and Iran (RR 0.88, 95% CI 0.70 to 1.11; participants = 13,168; studies = 6, I^2= 65%, NNT=155, Analysis 1.1.2), there was no significant difference in the occurrence of CL. The six studies were: Armijos 1998, Armijos 2004, Khamesipour 2000a, Khamesipour 2000b, Momeni 1999, and Sharifi 1998.

More two years follow-up

An extremely high level of heterogeneity (I^2= 90%) was detected between the three studies [64,65,66]; therefore, the results from the pooled analysis were likely to be misleading and are not reported. All three studies reported a RR for the occurrence of CL after two years follow-up, which were: RR = 1.02 (95% CI 0.81 to 1.28), RR = 0.20 (95% CI 0.1[64] to 0.39) [65] and RR = 0.84 (95% CI 0.63 to 1.12) [66]; but only one of these was statistically significant [65].

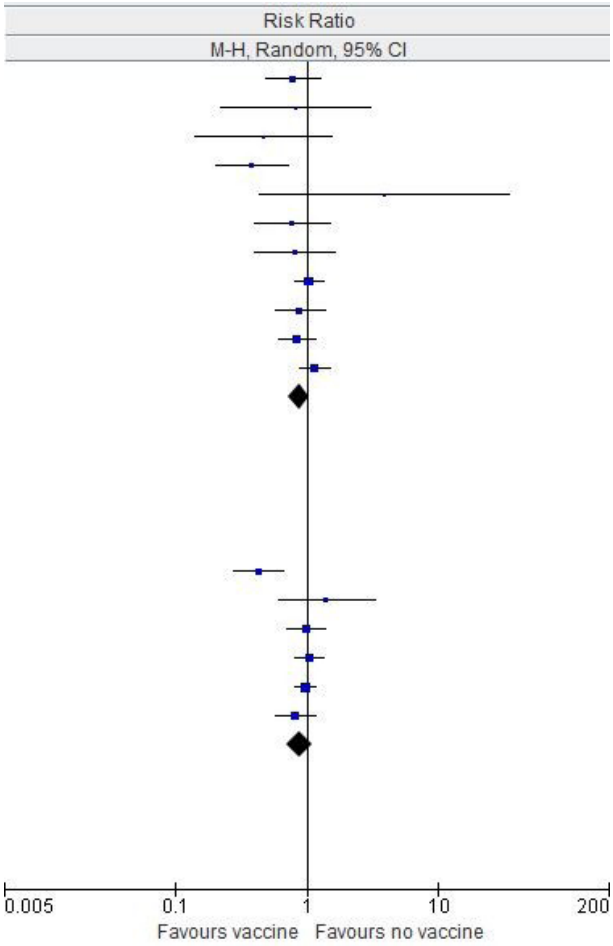

Figure 1: Comparison of using any dose of the vaccine versus no vaccine in occurrence of cutaneous leishmaniasis
Up: at one year follow up
Down: at 2 years follow up

Single doses of vaccine + BCG versus BCG alone

Two studies from Iran [36,37] looked at single doses of vaccine + BCG versus BCG alone (Figure 2).

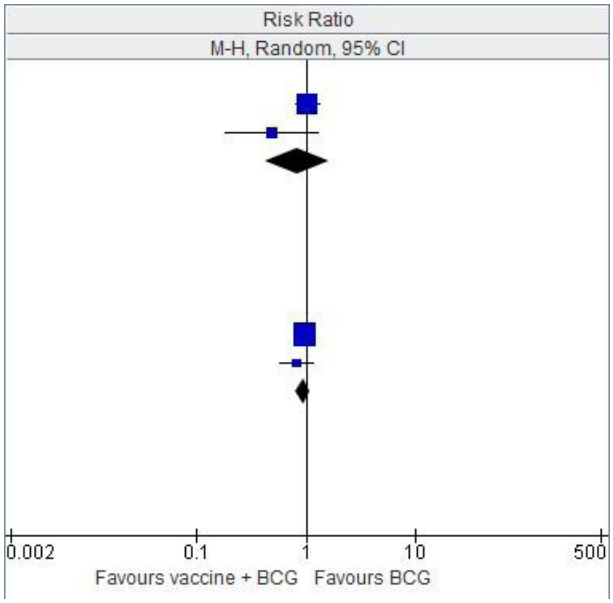

Figure 2: Comparison of using a single dose of the vaccine+BCG versus BCG alone in occurrence of cutaneous leishmaniasis
Up: at one year follow up
Down: at 2 years follow up

One year follow-up

There was no significant differences in the occurrence of CL after one year follow-up (RR=0.83, 95% CI 0.42 to 1.62; participants = 5947; studies = 2, I^2 = 53%, NNT=10535, Analysis 2.1.1).

Two years follow-up

There was no significant differences in the occurrence of CL after 2 years of follow-up (RR=0.94, 95% CI 0.80 to 1.10; participants = 5947; studies = 2, I^2 = 0%, NNT=224, Analysis 2.1.2).

Two doses of vaccine versus no vaccine

A pooled analysis was done for the studies that looked at two doses of the different types of vaccines versus no vaccine (no leishmaniasis vaccine) (Figure 3).

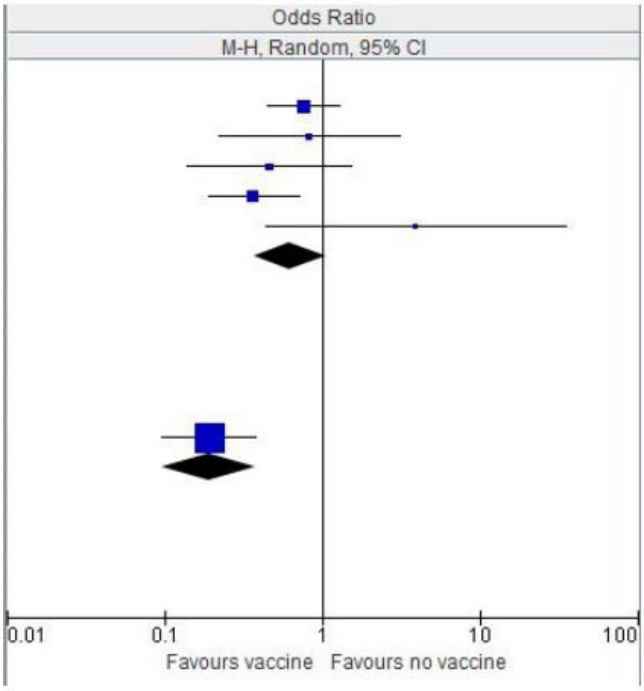

Figure 3: Comparison of using two doses of the vaccine versus no vaccine in occurrence of cutaneous leishmaniasis
Up: at one year follow up
Down: at more than 2 years follow up

One year follow-up

From studies conducted in Brazil and Ecuador after one year follow-up, there may be less CL in the group that was given two doses of the vaccine compared with the group not given any vaccine, but there is uncertainty about the result (RR=0.62, 95% CI 0.37 to 1.05; participants = 5620; studies = 5, I^2= 34%, NNT=106, Analysis 3.1.1). The five studies were [35,51,63].

Two years follow-up

An extremely high level of heterogeneity (I^2 = 83%) was detected between the two studies [35,63]; therefore, the results from the pooled analysis was likely to be misleading and a meta-analysis was not reported. One of the studies found a significant difference in occurrence of CL after two years follow-up in favour of the vaccine (RR=0.43, 95% CI 0.28 to 0.67) [35]; however, the other study found there may be an increase in CL in the group given the vaccine, although the results are very uncertain (RR=1.41, 95% CI 0.61 to 3.29) [63].

More than two years follow-up

One further study [65] assessed the effects at eight years, and found a significant difference in favour of two doses of vaccine versus placebo (phosphate buffer) (RR 0.19, 95% CI 0.10 to 0.38; participants = 5731; studies = 1, NNT=74, Analysis 3.1.2).

Three doses of vaccine + BCG versus BCG alone

Four studies conducted in Iran and Colombia looked at three doses of vaccine plus BCG versus BCG alone (Figure 4).

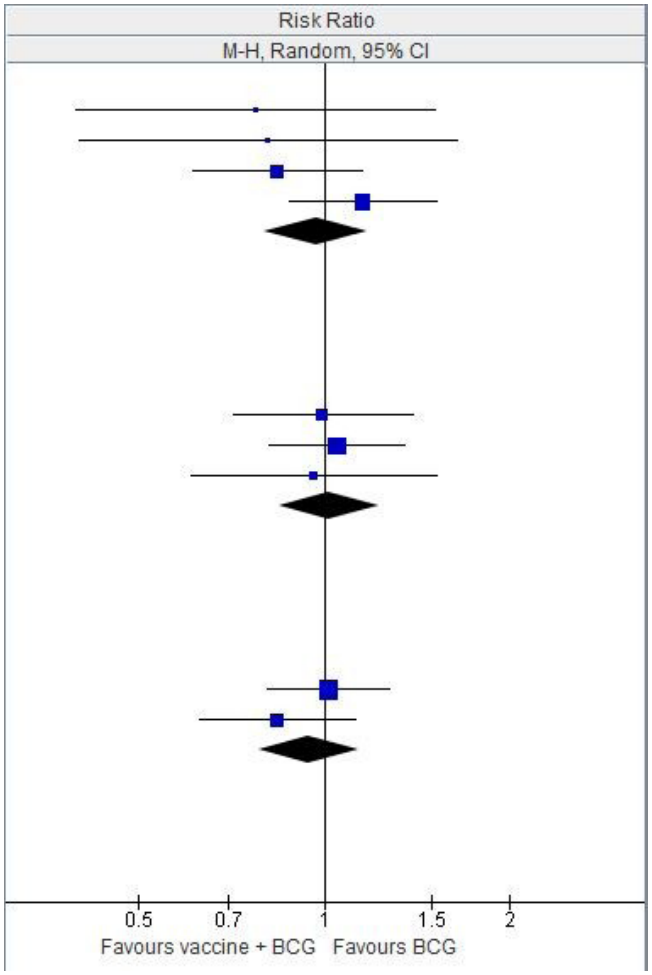

Figure 4: Comparison of using three doses of the vaccine+BCG versus BCG in occurrence of cutaneous leishmaniasis
Up: at one year follow up
Middle: at 2 years follow up
Down: at more than 2 years follow up

One year follow-up

There were no statistically significant differences in occurrence of CL after one year (RR 0.97, 95% CI 0.80 to 1.18; participants = 10999; studies = 4, I^2 = 1%, NNT=1036, Analysis 4.1.1) in Khamesipour 2000a, Khamesipour 2000b, Sharifi 2001, and Velez 2005.

Two years follow-up

There were no statistically significant differences in occurrence of CL after 2 years (RR 1.02, 95% CI 0.84 to 1.23; participants = 8404; studies = 3, I^2= 0%, NNT=762, Analysis 4.1.2) in Khamesipour 2000a, Khamesipour 2000b, and Sharifi 2001.

More than two years follow-up

There were no statistically significant differences in occurrence of CL after more than two years follow-up only in Iran (RR 0.94, 95% CI 0.78 to 1.14; participants = 6408; studies = 2, I^2= 5%, NNT=228, Analysis 4.1.3) in Khamesipour 2000a, and Sharifi 2001.

The reason for the high heterogeneity among the results of different studies maybe due to the differences in the promastigote subtypes, the populations and the settings.

The results using different scenarios to analyze missing data show that even in different approaches for dealing with missing data, the pooled results are not statistically significant.

Secondary Outcomes

Leishmanin skin test conversion rate (LST) at the end of one year, two years and greater than two years. Mild skin test reactions (indurations <5mm were regarded as negative and >5mm as positive).

We completed an analysis of conversion rates (LST); however, in line with our protocol, we excluded papers which did not also report incidence rates of CL and, therefore, caution needs to be used when interpreting the findings for this outcome. Nevertheless, in trials where post-vaccination LST was measured, this evidence of immunogenicity induced by the vaccine was not carried over to a protective effect and this casts doubt on the merit of the vaccine-induced LST response as a correlate of immunity [27].

Three studies (n = 8322) reported results for LST, after one year follow-up [36,37,67], but pooling was not done due to extreme levels of heterogeneity between the studies (I^2 = 98%; Analysis 1.2). Two studies, one from Iran (RR=1.68, 95%CI 1.41 to 2.01; 2310 participants) [36] and the other from Colombia (RR=5.01, 95%CI 4.43 to 5.67; 2597 participants) showed that the vaccine significantly increased the proportion of participants with immunogenicity compared with no vaccine; whilst the third study from Iran [37] showed no significant difference (RR=0.88, 95%CI 0.61-1.25; 3415 participants) in LST rates between the groups.

No studies reported LST data for the second year or longer.

In Armijos 1998 the Montenegro Skin Test (MNST) was used and it was measured at one month after vaccination, and thus these data were not included in the review. We could not add data for Armijos 2004, as although most outcomes were reported at the end of year one, two years, and later; the results for the LST were only reported at two months post vaccine. In Antunes 1986a (1981 I), Antunes 1986b (1981 II) and Antunes 1986c (1983) there was only LST data for the vaccine group and not the control, and thus was not included in the analysis. In Khamesipour 2000b, Khamesipour 2000a and Sharifi 2001 the LST results were not reported. In Mayrink 2013, participants with naturally positive LST were excluded and later the LST test was used in patients presenting lesions to confirm the diagnosis of ACL and it was not reported as a separate outcome.

Side effects of the vaccine

Local side effects, such as pain, redness, ulcer, lymph node swelling, itch and induration

Only seven studies reported side effects for these vaccines [35-37,63-65,67]. The side effects reported in these studies were ulceration, scars, pain, redness (erythema), induration, itching, swelling, lymphadenopathy and secretions at the site of injection (both in the vaccine and placebo group, but usually more severe in the vaccine group) and low grade fever and malaise. However, none of the studies reported side effects associated with the vaccine that was severe enough to stop the trial.

The exact number of these side effects was trivial or not reported in studies. Mortality and quality of life was not reported by any of the studies.

Discussion

Summary of main results

In this review we assessed the effects of vaccines in preventing the occurrence of cutaneous leishmaniasis. Twelve studies from four different countries were included, with a total of 28,297 participants. Studies were mainly well designed RCTs with high sample sizes. We classified the trials and reported them according to the dosage of vaccines and the intervention used in the control groups.

Different regimens were used in the control arm, including BCG [35-37,63-67] and in four studies phosphate buffer 7.4 [51].

When the results for all doses (one, two or three doses) of vaccine were compared with no vaccine, there was very low quality evidence (See SoF table) of no significant difference between the groups with respect to incidence of CL after one year (participants = 22566; studies = 11) or two years (participants = 13168; studies = 6). For more than two years there was significant heterogeneity between the results.

For the secondary outcomes of LST, although there were three studies which reported this outcome, there was significant heterogeneity between the results and we were unable to pool the results. The evidence was low quality. Two studies found the vaccine significantly increased the proportion of participants with immunogenicity as compared to no vaccine and one found no significant difference. However, this evidence of immunogenicity induced by the vaccine (or otherwise) did not appear to be associated with a protective effect against the incidence of cutaneous leishmaniasis in those studies.

Side effects of the vaccine were mentioned by some of the studies, but none were severe enough to stop the trial. Mortality and quality of life were not reported by any of the studies included in this review.

When the results were subgrouped by the number of doses of vaccine given, for a single dose of vaccine, there was low quality evidence (*See SoF table*) of no significant difference in the incidence of CL between the treatment arms at one year (RR 0.83, 95% CI 0.42 to 1.62; participants = 5947; studies = 2) and at two years (RR 0.94, 95% CI 0.80 to 1.10; participants = 5947; studies = 2). All participants included in both treatment groups of all studies also received a BCG. Results for more than two years were not available.

In studies in which two doses of vaccine were given, there was very low quality evidence (*See SoF table*) that there may have been a reduction in CL in the groups receiving the vaccine compared with no vaccine at one year, but the results are uncertain (participants = 5620; studies = 5). The results at two years were too heterogeneous to pool. At more than two years there was one study with unclear risk of bias which showed a significant reduction in the incidence of CL at eight years.

When three doses of vaccine were given, there was *very low quality* evidence (*See SoF table*) of no significant difference in the incidence of CL between the treatment arms at one year (participants = 10999; studies = 4), at two years (participants = 8404; studies = 3) or at more than two years (participants = 6408; studies = 2). All participants included in both treatment groups of all studies also received BCG.

Overall completeness and applicability of evidence

Overall, there was a low rate of participant drop-out from the trials. There were only three studies [35,63,64] with follow-up rates under 80%. However, the authors have reasoned that the remaining population that was analysed in the study had similar characteristics and, therefore, the bias introduced was trivial.

The impact of the bacille Calmette-Guerin vaccine (BCG) alongside the vaccine for leishmaniasis in under question in these articles. It is thought that injection of bacille Calmette-Guerin vaccine (BCG) significantly increases cell mediated immune response and this may reduce the incidence of leishmaniasis infection [27]. Therefore in a clinical trial , BCG may not be a true placebo, and may dilute the true effect of the vaccine [27,73].

Only seven of the included studies reported side effects. The reported side effects were limited and related mainly to BCG. Only in some studies was LST performed, and authors have provided limited information about these outcomes. We also might bear in mind that the vaccines used in different studies were not identical and therefore probably have different side effects anyway. No study investigated quality of life or mortality.

Although both genders and different age groups were evaluated in these studies, but studies were performed in only 4 countries and therefore the results may not be globally generalizable and applicable. The heterogeneity in some outcomes was too high and we were not able to pool the results, which also show that the populations under study and the promastigotes have different characteristics and more studies are needed.

Quality of the evidence

The quality of evidence for the outcomes was generally very low, or low in the results of this review.

The quality of evidence was affected by a number of issues including poor methodological reporting, imprecision and suspicion of publication bias. Although the studies were relatively large with respect to number of participants included, many did not report methods very well and were rated as unclear in a number of different risk of bias domains.

Despite the relatively high participant numbers in the studies, the rate of incidence of CL was relatively low which resulted in increased imprecision. There were wide confidence intervals which increases the uncertainty of findings. In these cases, where imprecision was detected and was judged to be serious, the evidence was downgraded.

Although we did not detect any publication bias, but it is possible that there are unpublished studies. Only a few studies were included in most comparisons so funnel plots were not used; this also limits the possibility of detecting publication bias.

Findings were relatively consistent, and heterogeneity ($I^2 > 80\%$) was found in only two analysis. One for the occurrence of cutaneous leishmaniasis at more than two years follow-up (at the any vaccine versus no vaccine group) and at the two dose vaccine versus no vaccine at 2 years follow-up.

Potential biases in the review process

We decided to change our methods for dealing with missing data due to the high percentage of drop-outs in some of the included studies [37,63,66]. The original statistical analysis plan was to include these missing participants as treatment failures (i.e. they all developed cutaneous leishmaniasis); however, this would have resulted in a very high prevalence of disease which was irrational and unrealistic. Therefore we did not do this.

Our units of analysis are different from what was planned in the protocol because after extracting the real data the proposed units of data analysis were not suitable.

We were not able to compare the efficacy of three-dose, two-dose and one-dose regimens, as none were significant except the two-dose regimen after more than 2 years follow-up and this was based on just one study with very low quality.

We did not assess the effect of the length of interval between the doses in the two and three-dose regimen on the preventive effect of the vaccine, because the number of studies was too low to a meta-regression.

We were not able to compare side effects between adults and children because the side effects had not been reported separately for children and adults in studies.

However, we do not think any of these issues has caused a serious bias in the review process.

Agreements and disagreements with other studies or reviews

A previous narrative review concluded that first generation vaccines showed a limited efficacy, and at present there is no vaccine available against leishmaniasis [27]. A meta-analysis also indicated that the whole-parasite vaccines tested until that time, did not induce significant protection against human leishmaniasis [73]. The result of this current review is in line with previous reviews. The studies used in these previous reviews were all included in this currect review.

However, most authors think that although trials with first generation vaccines did not result in identification of an efficacious vaccine, they did show the safety of these vaccines. These trials have made a significant contribution to improving the quality of vaccine investigation in the countries where they were conducted, and where leishmaniasis is endemic. These include training personnel and identifying particular issues related to the development of vaccines against leishmaniasis [27].

Second generation vaccines were evaluated in phase 1 and 2 clinical trials [74], but were not included in this review. It is likely that by the time this review is updated, second generation vaccines will have been assessed in well-designed randomised double blinded studies and we will be able to include them in this review.

These results raise the question about the impact of the Bacille Calmette-Guerin vaccine (BCG) alongside the vaccine for leishmaniasis. In this review eight of the 12 included studies gave participants in both arms of the study some level of BCG vaccine [35-37,63-67]. In Ecuador, Armijos used BCG (about 1/2 the dose normally used for vaccination against tuberculosis) in both his trials [35,63]. In Momeni 1999 and Sharifi 1998, a 1/10 of the normal dose (used in vaccination against tuberculosis) was used in leishmaniasis vaccine trials. In all prophylactic clinical trials of killed parasites plus BCG, BCG alone was used in the control arm and this was to preserve blinding in the trials as BCG may leave a scar similar to leishmaniasis.

It is thought that injection of Bacille Calmette-Guerin vaccine (BCG) significantly increases the cell mediated immune response by an increase in Leishmanin skin test (LST) positivity. Some researchers [27] have observed that the LST conversion due to vaccination is associated with reduced incidence of leishmaniasis infection. Therefore in a clinical trial setting, the use of BCG in the control arm may not constitute a true placebo, since BCG induces LST conversion in individuals in the control arm and may dilute the true effect of the vaccine [27,75].

Our results also showed in some trials where post vaccination LST was measured after a year the responses were larger in the vaccine group. Other reviews have also cast doubt on the merit of the vaccine induced LST response as a predictor of immunity [27]. On the other hand there are reports that there is a strong correlation between LST conversion and protection after recovery from the disease [30]; but, the immunological implication of the LST response also depends on the factors and conditions that gave rise to it [27]. In some trials, the incidence rate was significantly lower in those vaccinated subsets with LST conversion from zero to 5 mm and more [51]. 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 < 5mm to > 5mm) after vaccination [73].

LST is highly variable and not a precise tool of correlation with protection and it is more related to exposure than protection [64,65] (unpublished data). At the time of design of the trials, BCG was the only choice with a high safety profile in people. It was assumed to induce a Th1 response only as an indicator of previous exposure to leishmaniasis. This could have led to misclassification of some individuals with previous exposure and immunity as unexposed (instead of exposed) [73], but this would have allowed their inclusion in both arms of the clinical trial. However, some authors [73] believe that the protective efficacy of the vaccine may have been underestimated because of this misclassification.

Authors' conclusion

Implications for practice

The results of this review, current to October 2017, show that there is generally low or very low quality evidence about the effect of current vaccine on preventing cutaneous lesishmaniasis (CL). Serious vaccine side effects were not reported within the trials. No information about quality of life or mortality is available.

For any dose of vaccine versus no Leishmaniasis vaccination in the pooled analysis, after one year and after two years follow up no significant results in favor of the vaccine were seen. Among 3 studies with more than two years follow up, pooling of the results was not possible and only one of these three studies showed a significant protective effect. All were based on low quality evidence.

Three studies reported results for LST, after one year follow-up, but pooling was not possible. Two studies showed that the vaccine significantly increased the proportion of participants with positive LST compared with no vaccine; whilst the third study showed no significant effect. There was no data about more than one-year follow-up for this outcome and the available evidence was low quality.

There was no significant effect in favor of a single dose of vaccine plus the bacille Calmette-Guerin vaccine (BCG), versus BCG alone after one year follow up or after 2 years of follow up based on some low quality evidence.

In using two doses of vaccine versus no Leishmaniasis vaccine, no significant effect was seen in favor of the vaccine after one year follow up. Pooled analysis was not done for the two studies with two years of follow up. One of the studies found a significant effect in favour of the vaccine; however, the other study found no effect. There was a statistically significant effect, in favor of the vaccine after more than 2 years follow up, but this was based on only one study. The quality of evidence in these studies was all very low.

Three doses of vaccine plus BCG in comparison to BCG alone did not show a significant effect after one year, two years or more than two years either, but the quality of evidence was very low.

There was no conclusive evidence that the number of doses of leishmaniasis vaccine had an impact on the occurrence of CL, compared with no vaccine.

Implications for research

Future studies should be conducted with stronger methodology and less bias. Studies have to be clear in regard to random sequence generation and allocation concealment. Blinding of all patients and personnel and outcome assessors should be done adequately. Attrition bias should be kept to a minimum and researchers should plan to follow all individuals. Researchers should refrain from selective reporting. Side effects should be reported thoroughly and by numbers. Quality of life related to the vaccine should also be evaluated in future studies.

We also ask future researchers to plan appropriate studies to answer these questions.

- How effective is the BCG vaccine in preventing cutaneous leishmaniasis? It would be interesting if it was possible to compare the incidence in BCG vaccinated and not vaccinated individuals.
- Is the two dose vaccine the most effective among the first generation of vaccines?
- How effective are the second and third generation vaccines?
- How much will an effective vaccine cost consumers?
- What are the side effects of an effective vaccine?
- Is it worthwhile vaccinating everyone in endemic areas?

There is hope that the second or third generation of vaccines which are currently being studied might be effective in preventing CL.

Contributions of authors

Co-ordinator of the review (NK)
Searching for studies (NK, with help from UG and AK)
Study Selection (NK, UG, AK)
Assessment of studies (NK, UG, AK)
Data Extraction (NK, AK)
Analysis of data (NK, JLB)
Interpreting the results (NK, UG, JLB, AK)
Drafting the final review (NK, UG, JLB, AK)

Declarations of interest

AK is an author for four of the studies included in this review.

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