Vaccines for preventing cutaneous leishmaniasis (Protocol)

Khanjani N, González U, Leonardi-Bee J, Mohebali M, Saffari M, Khamesipour A

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Vaccines for preventing cutaneous leishmaniasis

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ABSTRACT

This is the protocol for a review and there is no abstract. The objectives are as follows:

To evaluate if vaccination with each of the different available vaccines reduces the occurrence of cutaneous leishmaniasis (CL) in endemic regions.
BACKGROUND

Description of the condition

Leishmaniasis is a disease that 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 (Manson-Bahr 1983).

The disease is geographically and ecologically widespread, occurring in tropical and subtropical regions on all continents except Australia (Roberts 2006). 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). With a prevalence of 12 to 14 million cases (UNDP 1995; Alvar 2006) and a world population of 350 million at risk (Desjeux 2004) leishmaniasis is a health problem in 88 countries, especially in the 72 developing ones (UNDP 1995; Davies 2003). It is globally accepted that these figures are an underestimate since the reporting of the disease is compulsory in only one-third (33 out of 88) of the endemic countries (Alvar 2006).

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 (Ameen 2007);
- 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;
- cutaneous leishmaniasis (CL), which is confined to the skin and which can be disfiguring (Desjeux 2004).

Cutaneous leishmaniasis, the most common form and therefore the main focus of this review, also includes zoonotic cutaneous leishmaniasis (ZCL) where the reservoir is a non-human mammal. Cutaneous leishmaniasis can show at least three clinical forms including acute cutaneous leishmaniasis (ACL), chronic cutaneous leishmaniasis (CCL) and occasionally leishmaniasis recidivans (LR) (Dowlati 1979).

Cutaneous leishmaniasis usually produces skin ulcers on the exposed parts of the body such as the arms, legs and especially the face (WHO 2005). Over 90% of cases of cutaneous leishmaniasis heal spontaneously within 3 - 18 months (Davies 2003).

In ZCL, many patients with acute myeloid leukaemia were reported to have multiple lesions, up to two hundred in number, and one patient had 384 lesions (Dowlati 1979).

In 2 - 5% of cases, ACL becomes chronic or can develop into LR (leishmaniasis recidivans), a persistent presence of parasites that are able to expand and drive a cutaneous lesion around the original lesions during the course of the primary lesions or long after healing (Modabber 2007).

Leishmaniasis recidivans is difficult to treat and leaves extensive scars (Alvar 2006).

Cutaneous leishmaniasis is endemic in more than 70 countries worldwide (Reithinger 2007). The annual incidence of CL is estimated to be 1 to 1.5 million cases with an estimated prevalence of 12 million cases (Ameen 2007). However, 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 not seeking medical or diagnostic facilities (Desjeux 2004). In several areas of the world there is a clear increase in the number of cases; eg Brazil, Kabul (Afghanistan) and Ouagadougou (Burkina Faso) (Desjeux 2004). Many endemic areas have reported a 500% increase over the past seven years (Roberts 2006). 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 (eg, HIV/AIDS), and the emergence of anti-leishmanial drug resistance (Reithinger 2007).

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 (WHO 2005) in the Old World and Lutzomyia in the New World (Markle 2004). Approximately thirty sandfly species are proven vectors (Desjeux 2004) with more than 40 additional species probably involved in transmission (Reithinger 2007). Biting an infected animal or human will infect the sandfly and the organisms are passed on when the sandfly bites its next victim (Desjeux 2004; WHO 2005). Humans are usually accidental hosts of these flies; natural hosts include a variety of rodents, small mammals, and dogs (Roberts 2006).

A striking difference occurs between the so-called Old World (ie, Africa, Europe, and Asia) and New World (ie, 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 (Reithinger 2007).

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 feces, manure, rodent burrows and leaf litter (Markle 2004).
Each species of *Leishmania* favours one or more animal reservoirs, except *Leishmania donovani* (Klaus 2003) and *Leishmania tropica* (Alvar 2006) in which the reservoir is human. The *Leishmania* parasite has different life cycle forms called promastigote (with flagellum) 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 promastigotes enter the skin during each feeding by an infected sandfly. Some of the promastigotes are taken up by the macrophages (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 (Klaus 2003).

The incubation period of CL is usually measured in months, but ranges from a few days to over a year (Bryceson 1998). 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 (Markle 2004). 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.

**Impact**

A total of 11.8% of total worldwide DALYs (Disability Adjusted Life Years) by all leishmaniasis occurs in the eastern Mediterranean countries, where CL is concentrated (Modabber 2007).

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 barrier to marriage, especially for girls and young women (Modabber 2007). New World CL which is endemic in most countries of Latin America has a dreaded sequel which is mucosal/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 have been recorded due to the stigma associated with ML in Latin America (Modabber 2007).

CL 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 in most cases beyond the means of affected people who are mostly poor (Modabber 2007).

On several occasions, epidemics have significantly delayed the implementation of land development projects (WHO 2005). 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 (Bryceson 1998).

Samples are taken by:

- scraping the affected sore (Markle 2004);
- punch biopsy with tissue impression smears (Markle 2004);
- needle aspiration of tissue fluid from the margin of the lesion (Markle 2004).

The parasite is identified by:

- staining with Giemsa and looking under the microscope;
- cultivation of *Leishmania* species in specific culture media (such as Novy-MacNeal-Nicolle (NNN) medium, etc);
- inoculation of suspected specimens to susceptible lab animals (such as hamsters) (Bryceson 1998, Klaus 2003);
- immunological tests such as antibody detection and a highly sensitive polymerase chain reaction test (Markle 2004).

The leishmanin skin test (LST) is a highly specific test and is of great value in epidemiological studies, although it has little clinical use (Pampiglion 1976). LST can be used for epidemiologic surveys, diagnosis in nonendemic areas particularly in recidivant and mucosal forms of CL and for identification of chronic forms of CL (Dowlati 1979).

In LST a delayed hypersensitivity reaction to intradermal crude *Leishmania* antigen is produced in healing or cured cases of both cutaneous and visceral leishmaniasis. LST could be considered as a simple indicator for Cell Mediated Immunity reaction (Topley & Wilson 2005).

**Description of the intervention**

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 and systemic pentavalent antimonials can be used for problematic sores (Bryceson 1998).
The conventional treatment of cutaneous leishmaniasis with meglumine antimoniate is associated with toxic side effects (WHO 1990) and is contraindicated in pregnant or breastfeeding women, very young children and individuals with certain chronic diseases (Chulay 1985; Berman 1989). Some other drugs are under research. Recently, resistance to many drugs has been reported, requiring the use of more toxic drugs (Davies 2003).

After healing, individuals are normally 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 (Bryceson 1998).

**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) by house spraying or individual protection based on pyrethroid impregnated bed nets (Desjeux 2004). Various repellents, such as dimethyl-phthalate and imidacloprid/permethrin are also used by people to discourage insect bites (Jia 1990; Mencke 2003). Animal reservoir control for CL is based on the use of poisoning baits and environmental management to control rodents (Desjeux 2004).

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 (WHO 1990; WHO 2005) and are short lived (Davies 2003).

Leishmaniasis is considered 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 renders the host resistant to subsequent infection indicate that a successful vaccine is feasible. Extensive evidence from studies in animal models indicates that solid protection can be achieved by immunisation with protein or DNA vaccines (Kedzierski 2004).

Vaccination through artificial inoculation of live parasites (*leishmania*) has been used to induce protection. Though efficacious, this practice was abandoned because a small proportion of people developed severe side effects, including chronic lesions (Nadim 1988). However, this old method has been studied again recently (Khamesipour 2005). Researchers have also recently used deep-freeze L. major promastigotes for immunisation against leishmaniasis according to the World Health Organisation (WHO) protocol in Geneva, 1997 (Mohebali 2003).

Also, attention has been turned to the use of killed (Mayrink 1998; Castes 1994; Dowlati 1996; Bahar 1996; Armijos 1998; Sharifi 1998; Momeni 1999; Velez 2000; Misra 2001; Alimohammadian 2002; Mahmoodi 2003; Mohebali 2004) or attenuated (Daneshvar 2003) parasite vaccines and defined subunit vaccines (Kahl 1989; De Luca 1999; Kenney 1999; Rafati 2001; Follador 2002; Ivory 2004).

*Leishmania* are easily cultured, hence the production of vaccine using the parasite or its components are feasible (Modabber 2000). In addition for the past few decades killed parasites have been used as skin test antigens for diagnosis of leishmaniasis in humans. These killed parasites have also been used with or without adjuvants as vaccines or for immunotherapy in clinical studies (Antunes 1986). Some evidence from experimental, clinical and field studies suggests that anti-*Leishmania* vaccines based on killed whole, fractionated or recombinant parasite promastigotes are safe and capable of inducing immunity to leishmaniasis (Mayrink 1985; Antunes 1986).

However, those producing a suitable human vaccine have to consider some practical aspects. 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 (Kedzierski 2006). At present there is only one prophylactic live vaccine in population use. This is a mixture of live virulent Leishmania major mixed with killed parasite registered in Uzbekistan (Khamesipour 2006).

**Why it is important to do this review**

Although leishmaniasis has a high incidence, it is a relatively 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 the public health and productivity of many countries. Existing treatments are expensive, associated with adverse effects, and of fairly low efficacy (Sharifi 1998; Desjeux 2004). A separate Cochrane systematic review of treatments for leishmaniasis is currently underway (Gonzalez 2004; Gonzalez 2005).

The main challenge for leishmaniasis control is to translate new knowledge into control tools (Desjeux 2004). Consequently, a safe, efficacious, and affordable vaccine could be the most practical and cost-effective control tool to prevent disease in many situations (Modabber 1996).

This systematic review seeks to evaluate vaccination as a means of preventing leishmaniasis. This review will not only be of interest to people living in endemic regions, but also to other special groups such as travellers, tourists, military personnel on mission, land developers, public health doctors and medical researchers.

**OBJECTIVES**

To evaluate if vaccination with each of the different available vaccines reduces the occurrence of cutaneous leishmaniasis (CL) in endemic regions

**METHODS**

This systematic review seeks to evaluate vaccination as a means of preventing leishmaniasis.
Criteria for considering studies for this review

Types of studies
All randomised 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 will also consider trials not using any form of treatment in the control arm.
We will compare the efficacy of three-dose, two-dose and one-dose regimens. We will also 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.
We will include all types of vaccines, such as:
- live (Leishmanization);
- attenuated;
- killed (by Methiolate, heat, freeze-thaw, etc.);
- fractionated (fractions 5, 9, 6, etc.); purified or
- recombinant parasite antigens (Gp63, , Cystein proteinases, etc.); and other
- molecular or DNA vaccines (IL-12, , LPG, Heat shock protein, etc.).

Exclusion criteria for articles under review:
- the occurrence rate not reported;
- 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.

We will also include papers listing adverse effects even if the occurrence rate (number count) of those adverse effects was not reported, in order to contribute to our qualitative description of adverse effects.

Types of outcome measures

Primary outcomes
(1) Occurrence of cutaneous leishmaniasis at the end of one year, two years and greater than two years.

Secondary outcomes
(1) Leishmanian skin test conversion rate (LST) at the end of one year, two years and greater than two years. Mild skin test reactions (indurations <5mm will be regarded as negative and >5mm as positive).

(2) Side effects of the vaccine, including:
1. local side effects, such as pain, redness, ulcer, lymph node swelling, itch and induration;
2. mortality;
3. quality of life.
We will 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 indirectly to 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

Electronic searches
We shall search the Cochrane Skin Group’s Specialised Register, the Cochrane Central Register of Controlled Trials in the Cochrane Library, MEDLINE (from 2003), EMBASE (from 2005), LILACS (Latin American and Caribbean Health Science Information databases), Persian Databases at www.iranmedex.com, Conference Paper Index at http://md1.csa.com/, Health and Medical Complete (Proquest), and Web of Science.
The UK Cochrane Centre has an ongoing project to systematically search MEDLINE and EMBASE for reports of trials which are then included in the Cochrane Central Register of Controlled Trials. Searching has currently been completed in MEDLINE to 2003 and in EMBASE to 2005. Further searching will be undertaken for this review by the Cochrane Skin Group to cover the years that have not been searched by the UKCC.
We have devised a draft search strategy for RCTs for MEDLINE (OVID) which is displayed in Appendix 1. This is a draft strategy and we will include additional search terms where necessary and also modify it to suit the other databases listed.

Ongoing Trials
We will search the following sources of unpublished and ongoing trials:
- The WHO platform for ongoing trials on www.who.int/trialsearch/
- The Ongoing Skin Trials Register on www.nottingham.ac.uk/ongoingskintrials;
- The US National Institutes of Health on www.clinicaltrials.gov;
- The metaRegister of controlled Trials on www.controlled-trials.com;
- The Australian and New Zealand Clinical Trials Registry on www.anzctr.org.au;
Searching other resources

Reference list of published papers

We will scan the reference list of all retrieved original papers and reviews for possible references to RCTs.

Unpublished literature

We will contact the leading authors of leishmaniasis studies to see if they are aware of any recent or ongoing research or any unpublished data.

Also we will contact 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;
- School of Public Health, Tehran Medical University, Iran.

Adverse Effects

We will look for the adverse effects data reported in papers from our search.

Language

We will impose no language restrictions and will seek translations where necessary.

Data collection and analysis

Two authors (NK and AK) will check the reference list of the relevant clinical trials and review papers, and will also contact the trial authors of the published papers for retrieving un-published data.

Two authors (UG and MM) will contact trial authors in the field of leishmaniasis in different countries to retrieve any additional unpublished articles. If the data cannot be handed to the review group we will seek the reason.

Selection of studies

Papers obviously matching the inclusion and exclusion criteria will be chosen by NK and AK. We will list the excluded studies in the Characteristics of Excluded studies. For articles that do not clearly match the inclusion criteria or cannot be clearly ruled out, advice from all members of the group will be sought. We will solve any differences through discussion with the review team. We will not attempt to blind the reviewers to the authorship information of the trials during study selection or data extraction.

Data extraction and management

If necessary the review authors will contact trialists to allow them clarify or verify their published or unpublished missing data.

We will put the data extracted in data summary forms. We will send the final extracted data sheets for review to other reviewers and discuss ambiguous areas through E-mail.

Assessment of risk of bias in included studies

We will not be using summary scores or quality assessment scales for this purpose, because of the low reliability of different scales (Juni 1999). The quality assessment will include the evaluation of the following individual components for each included study, since there is evidence that these are associated with biased estimates of treatment effects (Juni 2001). We will describe each component for each trial in the Characteristics of Included Studies table and the Risk of Bias Table. If the description is not clear enough, we will mention this problem and comment on it.

The components of evaluation are:

(a) The method of generation of the random sequence. The satisfactory method of generating the allocation sequence should be unpredictable;
(b) The method of allocation concealment. It will be considered "adequate" if the assignment cannot be foreseen;
(c) Blinding. Who was blinded and who was not blinded (participants, clinicians and/or outcome assessors)?
(d) How many or what percentages of participants were lost to follow up in each arm of the study?
(e) Were the participants analysed in the groups that they were originally randomised (Intention-to-treat analysis)?
(f) Sample-size calculation mentioned?
(g) Were the study groups similar at baseline (eg for age, sex, location of residence and etc.)?
(h) Were the inclusion and exclusion criteria of the population specified? How representative was the study population of a real endemic population?
(i) What was the intervention in the control group? A placebo or nothing?
(j) Were additional therapeutics used, (such as BCG)? If additional therapeutics were used, were they used identically in both arms?
(k) 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?
We will describe the quality of each study based on these components.

**Unit of analysis issues**
We will do analysis separately for:

- Old World (zoonotic, anthropotic) and New World leishmaniasis;
- Different strains;
- Different adjuvants;
- Different methods of delivering the vaccine; such as natural (the sandfly) or artificial (intra-dermal injection).

For studies which are sufficiently similar, we will perform a meta-analysis using a random effect model. For dichotomous outcomes, we will estimate pooled risk ratios (RR) and the relative risk reduction (RRR) with 95% confidence intervals (CI), and expressed as number needed to treat (NNT) (to prevent), where appropriate. For continuous outcomes, we will estimate difference in means (MD) with 95% CI, or as standardised mean differences (SMD) if comparable scales have been used. Statistical heterogeneity will be assessed using $I^2$. Data will be synthesised using meta-analysis techniques if $I^2$ is less than 80%. Where it is not possible to perform a meta-analysis, the data will be summarised for each trial. Publication bias will be tested by the use of a funnel plot when adequate data are available for similar types of vaccines.

For the primary outcomes, data will be categorised into one-, two- and greater than two years. For the latter time point, end points closest to five years (2 years) will be used capture longer term benefit. The longer term data will be considered the primary endpoint. Systematic side effects and mortality will be considered at the end of trial. Side effects will be described qualitatively and compared between adults and children.

Where there are multiple vaccines within a trial, pair wise comparisons will be made of similar active vaccines versus placebo or another vaccine. Because vaccination is designed to have long-term effects, cross-over trials will be analysed using data from the first phase only and pooled, where possible, with parallel design studies. Internally controlled trials will be excluded from the analysis as they are not an appropriate method to use for the research question.

If participant drop-out leads to missing data we shall conduct an intention-to-treat analysis. Where possible, authors of studies will be contacted to provide missing statistics such as standard deviations. For dichotomous outcomes, participants with missing outcome data will be regarded as treatment failures and included in the analysis. For continuous outcomes, the last recorded value will be carried forward for participants with missing outcome data. If substantial heterogeneity ($I^2 >50\%$) exists between studies for the primary outcomes, reasons for heterogeneity, such as comparing the one-, two-, and three-dose regimens, and between adults and children will be conducted. Additionally, meta-regression techniques will be 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. We plan to conduct sensitivity analyses to examine the effects of excluding poor quality studies from the analysis and comparing the results with the full analysis.

**Other**
MS (our consumer) will comment on the readability and non-technical language of the review. MS will also comment on the questions to be answered and the outcomes of interest to a person living in an endemic region that may be interested in using the vaccine.

We anticipate there may be potential limitations for the review, such as the different dosages of the vaccine, the different preparations of vaccine, geographical settings and the different strains of the *Leishmania* promastigote. We will discuss limitations like these further in the final review.

We will not include trials that are obviously not randomised in the meta-analysis, but will list and comment on them in an additional table if they contain relevant data (outcome or side effect data).

**Acknowledgements**

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The editorial base would like to thank Farrokh Modabber (external content expert) and Chanpen (consumer) for their valuable comments on this protocol.
Additional references

Alimohammadian 2002  

Alvar 2006  

Ameen 2007  

Antunes 1986  

Armijos 1998  

Bahar 1996  

Berman 1989  

Bryceson 1998  

Castes 1994  

Chulay 1985  

Daneshvar 2003  

Davies 2003  

DeLuca 1999  

Desjeux 2004  
Desjeux P. Leishmaniasis: current situation and new perspectives. Comparative Immunology, Microbiology and Infectious Diseases 2004;27:305–18.

Dowlati 1979  

Dowlati 1996  

Follador 2002  

Gonzalez 2004  

Gonzalez 2005  

Ivy 2004  

Jia 1990  

Juni 1999  

Juni 2001  

Kahl 1989  
Kedzierski 2006

Kenney 1999

Khamesipour 2005

Khamesipour 2006

Klaus 2003

Mahmoodi 2003

Manson-Bahr 1983

Markle 2004

Mayrink 1979

Mayrink 1985

Mencne 2006

Misra 2001

Modabber 1996

Modabber 2000

Modabber 2007

Mohabali 2003

Mohabali 2004

Momenni 1999

Nadim 1988

Pampiglione 1976

Rafati 2001

Reithinger 2007

Roberts 2006

Sharifi 1998

UNDP 1995
Velez 2000

WHO 1990

WHO 2005

* Indicates the major publication for the study

Appendix I. MEDLINE search strategy

1. randomized controlled trial.pt.
2. controlled clinical trial.pt.
3. randomized.ab.
4. placebo.ab.
5. clinical trials as topic.sh.
6. randomly.ab.
7. trial.ti.
8. 1 or 2 or 3 or 4 or 5 or 6 or 7
9. humans.sh.
10. 8 and 9
11. exp LEISHMANIASIS, CUTANEOUS/ or exp LEISHMANIASIS/ or exp LEISHMANIASIS, DIFFUSE CUTANEOUS/ or Leishmaniasis.mp.
12. ((aleppo or baghdad or delhi or jericho) and boil$).mp. [mp=title, original title, abstract, name of substance word, subject heading word]
13. ((aden or uta or chiclero or bahia) and ulcer$).mp. [mp=title, original title, abstract, name of substance word, subject heading word]
14. ((balkan or oriental or tropical or bush or pendeh or bay) and sore$).mp. [mp=title, original title, abstract, name of substance word, subject heading word]
15. ((biskra or oriental) and button$).mp. [mp=title, original title, abstract, name of substance word, subject heading word]
16. (saldana or salek or (apeppo and evil) or (forest and yaws) or uta or uto or (dicera and de and baurid) or (pian and bois) or (clou and de and biskra) or ya-te-vi or bejucu).mp. [mp=title, original title, abstract, name of substance word, subject heading word]
17. 11 or 12 or 13 or 14 or 15 or 16
18. vaccines/ or exp protozoan vaccines/ or exp vaccines, attenuated/ or exp vaccines, inactivated/
19. exp VACCINES/
20. sandfly.mp. or exp Psychodidae/
21. promastigote.mp.
22. amastigote.mp.
23. 18 or 19 or 20 or 21 or 22
24. 10 and 17 and 23
HISTORY

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CONTRIBUTIONS OF AUTHORS

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

DECLARATIONS OF INTEREST

None known.

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Internal sources

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• Dept. of Epidemiology & Biostatistics, Kerman Medical University, Kerman, Not specified.
• The Division of Epidemiology & Public Health, Nottingham University, UK.

External sources

• No sources of support supplied