Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Randomized, controlled, double-blinded field trial to assess *Leishmania* vaccine effectiveness as immunotherapy for canine leishmaniosis

Angela Toepp [a,b], Mandy Larson [a,b], Geneva Wilson [a,b], Carolyne Bennett [a,b], Adam Leal-Lima [a,b], Bryan Anderson [a], Molly Parrish [a,b], Michael Anderson [a], Hailie Fowler [a], Jessica Hinman [a], Eric Kontowicz [a], Jane Jefferies [c], Marvin Beeman [d], Jesse Buch [e], Jill Saucier [e], Phyllis Tyrrell [e], Radhika Gharpure [f], Caitlin Cotter [f], Christine Petersen [a,b,*]

[a] Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA 52242, USA
[b] Center for Emerging Infectious Diseases, University of Iowa Research Park, Coralville, IA 52241, USA
[c] Noah's Ark Animal Clinic, Kansas City, MO, USA
[d] Littleton Equine Hospital, Littleton, CO, USA
[e] IDEXX Laboratories Inc., Westbrook, ME, USA
[f] Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD 21210, USA

ARTICLE INFO

Article history:
Received 11 May 2018
Received in revised form 3 August 2018
Accepted 4 August 2018
Available online xxxx

Keywords:
Leishmania
Zoonoses
Immunotherapy
Canine

ABSTRACT

Better tools are necessary to eliminate visceral leishmaniasis (VL). Modeling studies for regional *Leishmania* elimination indicate that an effective vaccine is a critical tool. Dogs are the reservoir host of *L. infantum* in Brazil and the Mediterranean basin, and therefore are an important target for public health interventions as well as a relevant disease model for human VL. No vaccine has been efficacious as an immunotherapy to prevent progression of already diagnostically positive individuals to symptomatic leishmaniasis. We performed a double-blinded, block-randomized, placebo-controlled, vaccine immunotherapy trial testing the efficacy of a recombinant *Leishmania* A2 protein, saponin-adjuvanted, vaccine, LeishTec®, in owned hunting dogs infected with *L. infantum*. The primary outcome was reduction of clinical progression, with reduction of mortality as a secondary outcome. Vaccination as an immunotherapy reduced the risk of progression to clinically overt leishmaniasis by 25% in asymptomatic dogs (RR: 1.33 95% C.I. 1.009–1.786 p-value: 0.0450). Receiving vaccine vs. placebo reduced all-cause mortality in younger asymptomatic dogs by 70% (RR: 3.19 95% C.I.: 1.185–8.502 p-value = 0.0245). Vaccination of infected-healthy animals with an anti-*Leishmania* vaccine significantly reduced clinical progression and decreased all-cause mortality. Use of vaccination in infected-healthy dogs can be a tool for *Leishmania* control.

1. Introduction

Use of injection of live *Leishmania major* parasites, known as "leishmanization", has been used in cutaneous leishmaniasis endemic areas to prevent future disfiguring disease [1]. There is no in vivo evidence for use of vaccination to prevent progression of current visceral *Leishmania* spp. infection. Use of vaccination to treat clinical canine leishmaniosis in infected individuals has been used in veterinary settings with no prior investigation regarding the efficacy of such use [2]. Only recently has a growing body of evidence surfaced indicating that immunotherapy can be efficacious as a treatment against progression of an infectious disease [3,4].

Visceral leishmaniasis (VL), as caused by *L. infantum* infection, is a zoonotic disease, causing clinical disease in dogs and humans [5]. VL presents as a chronic immunomodulatory disease affecting the function of the phagocytic immune cells it infects [6]. Dogs are a useful model to understand how immune modulation can impact *Leishmania* infection and its progression in natural infection settings [7,8]. At present, there are no vaccines licensed for human use against leishmaniasis, but there are three veterinary vaccines in clinical use [2]. Epidemiologic studies that modeled control of *Leishmania* transmission have shown that successful canine vaccination would greatly diminish transmission and mortality in both dogs and people [9,10].

Current manufacturers' recommendations for these veterinary vaccines are to vaccinate only seronegative animals. Although

* Corresponding author at: Department of Epidemiology, S429 CPHB, 145 N. Riverside Dr, Iowa City, IA 52241, USA.
E-mail address: christine-petersen@uiowa.edu (C. Petersen).
URL: http://petersen.lab.uiowa.edu/ (C. Petersen).

https://doi.org/10.1016/j.vaccine.2018.08.087
0264-410X/

there are some interesting potential candidate vaccines against VL [11], there are no blinded clinical trials which evaluate the efficacy of anti-*Leishmania* vaccination as an immunotherapy in reducing clinical progression of asymptomatic-infected dogs or humans. There have been two studies evaluating vaccine as immunotherapy, the first using Leishmune in five dogs experimentally infected with *L. donovani* and 21 dogs naturally infected *with L. infantum*, or in a second study where 16 dogs infected with *L. infantum* given an *experimental L. braziliensis* subunit vaccine were followed for several months [12,13]. These were small studies with no, or unmatched, non-blinded, interventions, but provide proof-of-concept that immunotherapy after vaccination is possible. Use of vaccines as immunotherapy is of veterinary importance as approximately 20% of infected dogs likely to be vaccinated are asymptomatic and seronegative [14].

Although most canine and human VL occurs in tropical and subtropical climates via vectorborne transmission, there is a surprisingly large prevalence of canine VL among hunting hounds in the U.S. The primary route of transmission in these U.S. dogs is vertical, from dam to pup [15]. This route of transmission occurs globally [16]. The predominant exposure of these animals to *Leishmania* occurs prior to, or possibly at the time of, birth [15]. In settings of vertical transmission of *L. infantum*, a combination of immune control, low parasite burden, and imperfect diagnostics prevents early detection of infection in animals born to an infected mother [16–18]. Dogs may not become diagnostically positive for *L. infantum* infection after vertical transmission for years after birth. In this setting, vaccination prior to exposure is not possible. Instead, vaccination as immunotherapy is best means to prevent progression to clinical disease.

Ex vivo studies, using splenic biopsy or whole blood from Indian VL patients, identified that patients infected with *L. donovani* had a T regulatory response, predominated by CD4+ T cells producing both IFN-γ and predominantly IL-10 [19]. This response also occurred in dogs infected with *L. infantum* progressing to clinical VL [8]. Ex vivo immunomodulation, using antibodies to block IL-10, had limited effectiveness in improving the T cell response [20]. Other means of ex vivo immunomodulation including use of checkpoint inhibitors, particularly anti-programmed death 1, in both canine and human patient cells was thought to have therapeutic potential [8,21,22]. Treatment of canine immune cells with vaccine antigens containing toll-like receptor agonists was shown by our group to recover *Leishmania* antigen-specific T cell responses and robust T helper 1-type immunity [23]. Population-based canine anti-*Leishmania* vaccination has been shown to significantly decrease transmission to people in statistical and mathematical modeling studies [10]. We hypothesized that immunotherapy/vaccination of *L. infantum*-infected/exposed non-clinical animals with LeishTec® will decrease progression of clinical disease.

This block-randomized, double-blinded, placebo-controlled, immunotherapy trial demonstrates the efficacy of a recombinant *Leishmania* A2 protein, saponin-adjuvanted, vaccine, LeishTec®, to prevent progression of *Leishmania* infection, and significantly decrease all-cause mortality in the most relevant animal model for leishmaniasis: the dog. This research provides critical evidence to aid efforts in developing tools, including effective vaccines, to work towards *Leishmania* elimination within the United States and globally.

2. Materials and methods

2.1. Vaccine protocol, study design and participants

We performed a double-blind, block-randomized, placebo-controlled trial in owned hunting dogs from the United States. All dogs were enrolled with signed informed consent and followed the protocol approved by the University of Iowa Institutional Animal Care and Use Committee (IACUC) an AAALAC accredited institution.

LeishTec®, a recombinant A2-targeted, Quil A adjuvanted vaccine (Lot 042/15, Ceva Animal Health, Brazil) [24] was imported into the US from Brazil (permit no: VB-150792BRA). Permission was obtained from the state veterinarian from each participating state for the clinical team to provide this experimental vaccine to animals within their state's borders. 650 hounds from across the continental U.S. were assessed for enrollment into the trial. Physical exam was performed and blood collected to evaluate inclusion criteria. Dogs younger than six months of age, pregnant, or not current for their routine deworming, rabies virus, or core respiratory disease vaccinations were excluded. Blood samples were analyzed for seropositivity to *Borrelia burgdorferi, Ehrlichia* spp., *Anaplasma* spp. and antigen detection of *Dirofilaria immitis* via the SNAP® 4Dx® Plus Test [25] with confirmatory ELISA and/or qPCR. DualPath Platform® (DPP) Canine Visceral Leishmaniasis (CVL) test with detection via Chembios DPP® Micro Reader was employed to detect *L. infantum* seropositive samples [26] and qPCR for *L. infantum* rDNA was used to detect *Leishmania* parasite burden. Dogs that had clinically apparent infection with *Borrelia burgdorferi, Ehrlichia* spp., *Anaplasma* spp., *D. immitis* or *L. infantum*, confirmed diagnostically positive for infection, were excluded from the study.

2.2. Randomization

Based on physical exam and diagnostic testing performed at enrollment, dogs were stratified into two groups: asymptomatic and negative. Dogs were classified as asymptomatic if they had either a positive DPP or qPCR leishmaniasis diagnostic test result and had less than two physical signs of infection at enrollment. Dogs were classified as negative if DPP and qPCR were negative. Equal (1:1) randomization to vaccine or placebo groups was performed using SAS 9.4 (SAS Institute, Cary, NC). Asymptomatic and negative groups were randomized separately with dogs allocated to either vaccine or placebo via block randomization to ensure that there was an equal distribution of age, sex, region, and clinical status in treatment (vaccine and placebo) groups. After block randomization, blinded treatment groups were coded by color and evaluated for overall distribution for age, sex, and geographic location. There was even distribution of these key variables in the two groups.

Owners, veterinarians, laboratory technicians, and data analysts were all blinded to treatment. All veterinarians performing physical exams and research team members performing diagnostics or statistical analyses were blinded and restricted from viewing any patient identifiers to maintain good clinical practice and reduce bias. Vaccine and samples were kept at 4 °C while in the field. Upon arrival at the laboratory samples were immediately processed and stored at −20 °C (sera) or −80 °C (whole blood). All blood and serum samples were obtained and stored with unique barcode identifiers.

3. Procedures

3.1. Intervention

The vaccination period for the trial began in February of 2016. Dogs received either three 1 mL subcutaneous injections in the left flank of either LeishTec® vaccine or sterile water, the vaccine eluent. Dogs were vaccinated three times at fourteen-day intervals with a 22 gauge, 1″, needle (Day 0, 14, and 28, Fig. 1). Venous whole blood and serum samples were taken at each time point,

Please cite this article in press as: Toepp A et al. Randomized, controlled, double-blinded field trial to assess *Leishmania* vaccine effectiveness as immunotherapy for canine leishmaniosis. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2018.08.087