Trial Protocol

Cluster-randomised trial to reduce the incidence of visceral leishmaniasis in NW Iran

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Background

Insecticide-impregnated collars for dogs, the reservoir of *Leishmania infantum*, have been repeatedly shown to affect sandfly feeding success and mortality, and to reduce canine infection incidence (Reithinger et al., 2001, Maroli et al., 2001, David et al., 2001, Killick-Kendrick, 1997, Halbig et al., 2000), in addition to childhood seroconversion incidence when distributed at the community level (Gavgani et al., 2002a). No studies have tested this intervention approach to reduce the incidence of human VL disease.

Objectives

The objectives of the study are (1) to measure the efficacy of the collar intervention against VL case incidence in children <11yrs old (the high-risk group); (2) to evaluate the operational logistics of collar implementation and sustainability by the local Leishmaniasis Control Program; and (3) to assess potential causes for any between village variation in impact on VL incidence, including (i) community coverage with collars; (ii) dog-owner compliance; and (iii) implementation logistics.

Methods

Study location

The study will enroll villages in the rural region of the Kalaybar and Ahar administration districts in East Azerbaijan province, NW Iran (38688131N; 4321696E). In the study region, sandflies are active from late June to the beginning of October indicating an annual transmission season of about 4 months. Two species of sandfly *Phlebotomus (Larroussius) perfeliewi transcaucasicus* and *Ph. (L.) kandelakii* are the likely vectors of *L. infantum* in NW Iran where peridomestic transmission is clearly demonstrated (Gavgani et al., 2002b, Gavgani et al., 2002a).

The study is designed to be conducted in collaboration with the District Ministry of Public Health (DTARH) in Kalaybar and Ahar, which is responsible for the regional VL Control Program. DTARH oversees the District Health Centres (DHCs) that coordinate activities of 12 provincial Rural Health Centres (RHCs), each run by fully trained medical staff and health officers. Each RHC supervises about 10 village health posts where resident Health Promoters are responsible for disease surveillance, control implementation, data collection, and facilitate suspect VL cases attendance at the RHCs. The RHCs provide free VL diagnostic testing and

treatment services, and refer those needing more specialist hospitalization to the DHCs or district hospital.

Study design
The trial is designed by the PI and co-Is ("the scientists") but implemented by DTARH in order to measure the efficacy under operational conditions.

Randomisation and treatment allocation
The trial is designed as a pair matched-cluster randomised trial where study villages are designated as clusters.

Village recruitment
Villages will be recruited in a two-step process:
Step 1. All 417 endemic villages in the two districts were listed with the recorded number of VL cases in the 4.5 years prior to the intervention (January 1998- June 2002) (based on DTARH records). The inclusion criteria for village recruitment were (i) that the village is residential and not nomadic or partially nomadic; (ii) that the human population size is >100; (iii) that at least 1 confirmed clinical VL case was reported between January 1998 and June 2002, indicative of recent transmission; and (iv) that the village was not subject to recent VL interventions (insecticide control against vectors; culling household dogs) during the pre-intervention period. A total 91 villages have been indicated to meet these criteria for potential recruitment. All villages are located >>1km apart this ensuring no treatment contamination between clusters by sandflies that can potentially fly distances up to c. 1km (usually only 300m).

Step 2. Village leaders will be consulted by the RHC supervisors along with the village Health Promotors, to seek informed agreement to be part of the trial. Dog-owners will be informed of the procedures, benefits and risks, regarding collaring their dogs and requested to provide written consent.

Randomisation
Recruited villages will be listed and ranked in descending order of childhood incidence in the pre-intervention period, and the top two villages paired, and then randomly assigned to either collar treatment or no treatment (control) by tossing a coin in the presence of observers. All subsequent pairs in descending order will be randomised in the same way but alternating the first of each subsequent pair to treatment or control group.

Balance of the two arms will be inspected based on data collected by DTARH including: village population size, number of children <11yrs, sex, literacy and employment status, house numbers, dog population sizes, dog occupation (shepherd vs guard or pet). During the trial period, surveys will be conducted by village Health Promotors to inspect these demographics. They will also monitor any other activities associated with VL control (reservoir or vector control, distribution of bednets, etc).

Intervention

Deltamethrin-impregnated collars (Scalibor®ProtectorBand) will be provided by Intervet International free of charge, and fitted following manufacturer's recommendations. With dog-owner consent, collars will be fitted to all eligible dogs before the start of each transmission season over 4 consecutive years (2002-2005) giving 48 months follow-up. The collars have an effective duration of ≥6 months. The exclusion criteria for collaring a dog will follow product label indications (i) dogs <3m old based on owner information; and (ii) dogs showing signs of pregnancy or lactation.

Implementation

The intervention will be implemented by the Heads of the DTARH operational DHCs in Kalaybar and Ahar, and their respective village-based Health Promoters. Information on collar fitting and replacement logistics, and dog-owner compliance, will be collected by village Health Promotors following full training in the initial year by the scientific team. The team will monitor the dates and numbers of collars provided to DTARH also ensuring that they receive sufficient collars for replacement.

Case definition and reporting

VL case detection, diagnosis and reporting will follow standard local MoH procedures. Suspect cases are attended at RHCs where free VL diagnostic testing and treatment services are provided; cases needing hospitalization are sent to the DHCs or district hospital. Cases are confirmed by trained physicians, on presentation of clinical signs including prolonged fever, lymphadenopathy, and hepatosplenomegaly, and in the presence of a positive Direct Agglutination Test (DAT) and/or detection of *Leishmania* by microscopic examination of bone marrow aspirate. DATs were performed in the VL laboratory in the DHCs following established MoH protocols, designating DAT titres ≥1/3200 as indicative of *L. infantum* infection. Patients with raised but negative DAT titres in the presence of mild clinical signs are usually considered at risk and thus re-examined at 2-3m intervals to detect ascending titres and clinical progression. All confirmed cases, receive treatment with registered antimonial drugs (Meglumine antimoniate (Glucantime, Sanofi) or sodium stibogluconate (Pentostam, GSK) following WHO guidelines. Amphotericin B is currently used in Iran to treat cases resistant to pentavalent antimonials.

Data management and masking

Following the routine DTARH case reporting processes, confirmed VL case details will be supplied by the RHCs, DHCs and district hospital to DTARH for collation along with data on case age, sex, village of residence, and date when they present at the medical centre. This date will be used to indicate the year of transmission. DTARH will compiled anonymised data relevant to the study villages to provide to the PIs for data analysis.

Village Health Promotors will not be blinded to the intervention as placebo collars are not available. However, the clinicians, lab technicians and DTARH data managers will be fully blinded to the existence of the trial.

Trial outcomes

The primary trial outcome measure will be the cumulative number of confirmed VL incident cases per study village at the end of the 48 months follow-up period. There are no current plans for secondary measures of the intervention efficacy. VL in Iran and elsewhere is almost always in children <11-year-old or younger, being the well documented high-risk group (Mohebali, 2001).

Sample size calculations

The sample size is calculated for a pair-matched randomised control trial (Donner, 2000), and based on the 249 VL cases reported amongst 13,570 children <11 years old recorded in the 91 villages during the 4.5 years pre-intervention period (DTARH unpublished data). The average annual childhood incidence calculated from these records is 0.0041 (4.1 cases per thousand). To detect a 50% reduction in childhood incidence after 48 months follow-up, requires 39 clusters per arm to achieve a power of 90% with α=0.05 to reject the null hypothesis. This assumes that there are an average 120 children per cluster, and a cluster coefficient of variation of $\kappa = 0.124$ following the value estimated from the previous trial of these collars in this region. After the final village (cluster) recruitment, sample size estimation will be repeated using updated values.

Statistical analyses

The intervention effect (incidence risk ratios IRR) on the cumulative childhood incidence over 48 months will be estimated using random-effects regression including variables describing the trial design (the cluster pair-matching structure, baseline village childhood incidence, and the attributed year of transmission), offset (scaled) to the village childhood population size. Interaction terms will be included to explore associations between the intervention effects in each year.

Additional demographic variables will be included as a further potential adjustment to the effect estimates. Changes in nested model deviances will be tested by log-likelihood ratio test. The effect estimates will also by tested by a paired *t*-test of the residuals of the observed /expected VL case ratios per paired clusters (Donner, 2000).

Ethical considerations

The trial protocol has been approved by the Medical Ethics, Tabriz University of Medical Sciences, NW Iran, and the London School of Hygiene and Tropical Medicine. Communities will be informed about the purposes of the trial by the head of the DHCs, and informed consent will be obtained from village leaders and by dog owners for fitting collars. Animal handling procedures has been approved also by the Iranian Department of Environment, East Azerbaijan province division (document #5676). Retrospectively, the University of Warwick's Biomedical and Scientific Research Ethics Committee (BSREC) also confirmed that the trial raised no significant ethical concerns due to its use of secondary anonymous data.

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