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Heterologous protection elicited by a live, attenuated, *Leptospira* vaccine

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ABSTRACT

A previously-described live, attenuated vaccine (M1352, serovar Manilae, serogroup Pyrogenes) was tested in the hamster model of infection for cross-protective immunity. The vaccine elicited strong, significant cross-protection against lethal infection by strains representing four serologically distinct leptospiral serovars (Grippotyphosa, Australis, Canicola, and Autumnalis). Combined with our previously reported protection against serovars Pomona and Manilae, this work demonstrates unequivocal proof of concept for cross-protective immunity in leptospirosis.

1. Introduction

Leptospirosis is a zoonosis caused by spirochetes of the genus *Leptospira*, of which there are more than 250 serovars grouped into at least 23 serogroups distinguished by lipopolysaccharide (LPS) antigen (Picardeau, 2017). The greatest burden of disease occurs in resource-poor nations in the tropics and subtropics, and is associated with poor sanitation, deficiency in awareness, and lack of access to health care facilities (Adler and de la Peña Moctezuma, 2010). Vaccination of at-risk communities therefore has the potential to be of great benefit for disease prevention. Notably, with multiple serovars endemic to any one region, a multivalent or cross-protective vaccine is necessary.

To date, progress towards a suitable vaccine has been limited (reviewed in (Adler, 2015)). Currently available vaccines all consist of killed, whole cells. Naturally acquired protective immunity against leptospirosis is predominantly antibody-based, and is directed against the immuno-dominant LPS structure. Hence bacterin (killed, whole cell) vaccines confer protection against a narrow spectrum of serovars, usually within a serogroup (Adler and de la Peña Moctezuma, 2010). A concerted research focus has investigated the immune-protective potential of specific *Leptospira* proteins, with focus on highly abundant, immuno-dominant, or pathogenesis-related proteins such as LipL32, and LigA. However, the majority of studies have not demonstrated a high degree of protection, and only a few have demonstrated a limited level of cross-protection. Furthermore, many studies are prone to spurious claims of vaccine efficacy (Adler, 2015).

L. interrogans strain M1352 is a defined LPS mutant of serovar Manilae (serogroup Pyrogenes) which grows normally *in vitro*, but is avirulent in the hamster model of infection, being unable to colonise the animal beyond a few hours(Murray et al., 2010). We previously demonstrated that M1352 is the first defined, live, attenuated vaccine capable of cross-protecting against leptospirosis in the hamster model of infection; this cross-protection was independent of antibody response to LPS (Srikram et al., 2011). In this study we tested the live vaccine strain against four additional leptospiral serovars, expanding the repertoire of cross-protection conferred by this vaccine.

2. Methods

Leptospiral strains representing different leptospiral serovars were tested for ID_{50} in hamsters using serially diluted cultures. Strains with sufficient virulence were selected for this study and include the following: serovar Australis (serogroup Australis) strain SATT (from Australia); serovar Grippotyphosa (serogroup Grippotypohosa) strain UI8368 (from a patient in Laos); serovar Canicola (serogroup Canicola) strain UI12823 (patient in Laos); serovar Autumnalis (serogroup Autumnalis) strains UI13372 (patient in Laos), RY21 (patient in Thailand), and UT108 (patient in Thailand); and serovar Pomona

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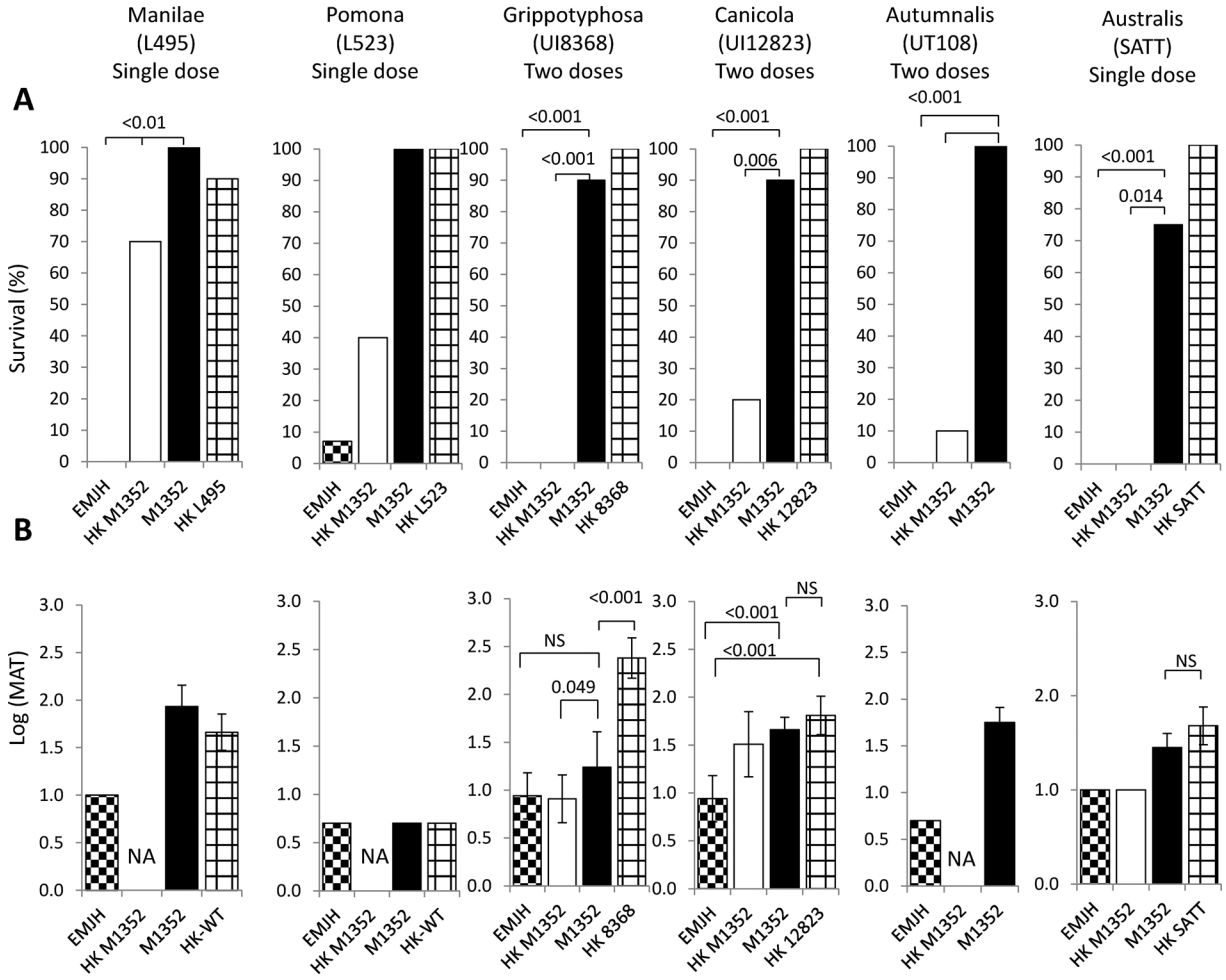

Fig. 1. Vaccine cross-protection experiments using five heterologous challenge strains.
A. Proportions of surviving animals from each vaccine group. Significance is indicated (Fisher's exact test).
B. MAT values for sera taken post immunisation (prior to challenge). Significance is indicated (ANOVA or Student's *t*-test).
Each data set corresponds to one representative experiment. The challenge serovar, strain name and number of vaccine doses are indicated above the figure. Group sizes: n = 10 animals per group apart for the Australis challenge experiment (n = 12 per group, except n = 8 for HK M1352 group).
The results for challenge with *L. interrogans* serovars Manilae and Pomona were published previously (Srikram et al., 2011), and are reproduced here for completeness.

(serogroup Pomona) strain L523 (from Australia) (Srikram et al., 2011). The ID_{50} values for these strains (excluding previously characterised L523) were determined to be approximately 10^2 leptospires. The vaccine strain M1352, and parent strain L495 (serovar Manilae, serogroup Pyrogenes), have been published previously (Murray et al., 2010).

For challenge experiments, four groups of hamsters were vaccinated by intraperitoneal injection of (i) 10^7 live M1352, (ii) 10^7 heat-killed M1352, (iii) 10^7 heat killed challenge strain, or (iv) an equivalent volume of EMJH culture medium (Srikram et al., 2011). A second vaccine dose was administered after two weeks for selected experiments. Prior to challenge (after an additional two weeks), a serum sample was taken, followed by an intraperitoneal injection of enumerated challenge strains (dose of 10^3 leptospires, approximately $10 \times ID_{50}$). Animals were monitored for 21 days, and euthanized if experiencing signs of disease, according to animal ethics requirements. Necropsy was performed on all animals to determine macroscopic pathology and to culture kidneys to determine renal colonisation, as described previously (Bartpho and Murray, 2015). Comparison of groups utilised Fisher's exact test.

Microscopic agglutination tests (MAT) were performed with animal serum, with values log transformed prior to determination of means and comparison of groups (ANOVA or Student's t-test); where agglutination was not detected an arbitrary value was assigned corresponding to the limit of detection (*i.e.* a titre of 5 or 10, depending on the experiment).

Passive immune transfer experiments were conducted as follows. Hamsters (n = 20) were vaccinated twice with the live vaccine (as above), then sera collected, pooled and stored at −20 °C. Two hours prior to challenge with *L. interrogans* serovar Pomona strain L523, hamsters were infused by an intraperitoneal injection with 0.8 mL of serum (passive protection group) or PBS (control group). Additional infusions were performed on days 1, 2, 3 and 9 (experiment 2 only). Animals were monitored to day 21.

Animal experiments were approved by the animal ethics committee, Khon Kaen University, Thailand.

3. Results

Hamsters were vaccinated with a single dose, or two doses of the live attenuated vaccine strain M1352, and then challenged with strains representing four leptospiral serovars (Fig. 1). In terms of animal survival, strong and significant protection was observed against serovars Grippotyphosa (up to 100%, one or two dose, $p < 0.001$ compared to EMJH-vaccinated group), Canicola (62.5–90%, one or two dose, $p < 0.001$ to 0.033) and Australis (75%, one or two dose, $p < 0.001$ to $p = 0.007$). Notably, live M1352 conferred superior protection against these challenge serovars than the killed M1352 bacterin.