

A live attenuated-vaccine model confers cross-protective immunity against different species of the *Leptospira* genus

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ELS - Webinar on leptospirosis and other rodent borne hemorrhagic fevers
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Yale School of Public Health
Epidemiology of Microbial Diseases



Oswaldo Cruz Foundation (Fiocruz)
Brazilian Ministry of Health
Salvador, Brazil

Whole-leptospire-based vaccines: killed and attenuated

- Routinely administered to livestock and domestic animals for more than 50 years
- Used for immunization of human population in China, Cuba and France
- **Significant limitations:**
 - ✓ High rates of adverse reactions
 - ✓ Confer LPS specific and short-term protection (annual booster)
 - ✓ One vaccine can't represent the diversity of epidemiological settings worldwide
 - ✓ Not universally effective in preventing carriage

THE PROPHYLAXIS OF WEIL'S DISEASE (SPIROCHÆTOSIS ICTEROHÆMORRHAGICA).

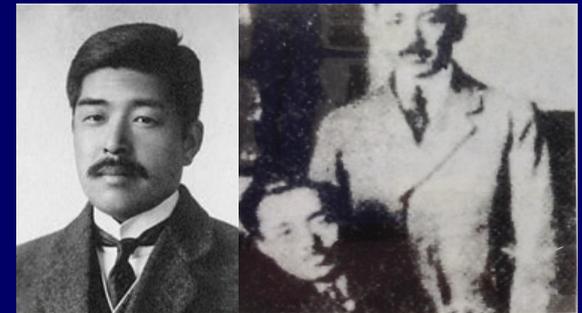
By YUTAKA IDO, M.D., ROKURO HOKI, M.D., HIROSHI ITO, M.D.,
AND H. WANI, M.D.

(From the First Medical Clinic of the Imperial University in Kyushu, Fukuoka.)

(Received for publication, June 27, 1916.)

The prevention of Weil's disease can be undertaken on the basis of the excretion of the pathogenic cause in the urine and feces of patients, and on the routes of invasion of the spirochetes into the human body. But prophylaxis by active immunization seems to offer the surest method.

Ido Y et al. J. Exp. Med. 1916;24:471-483



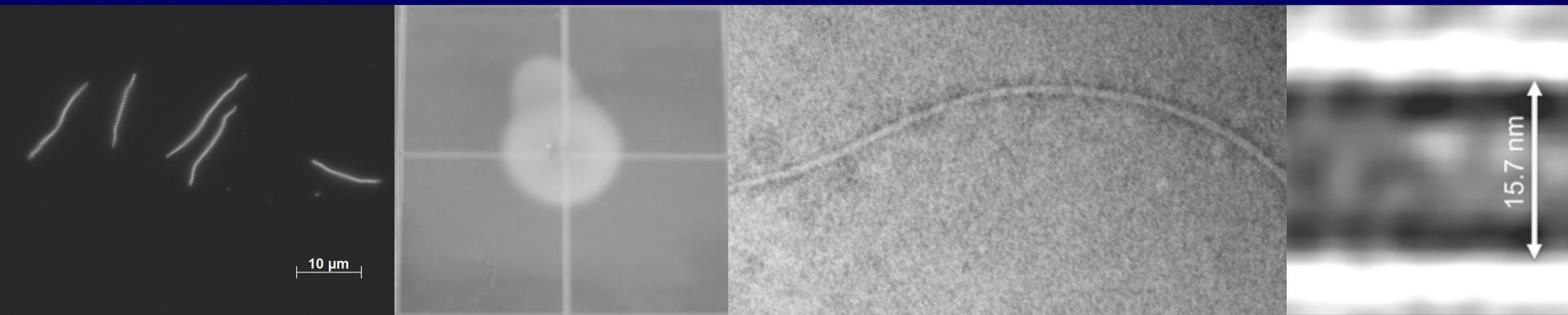
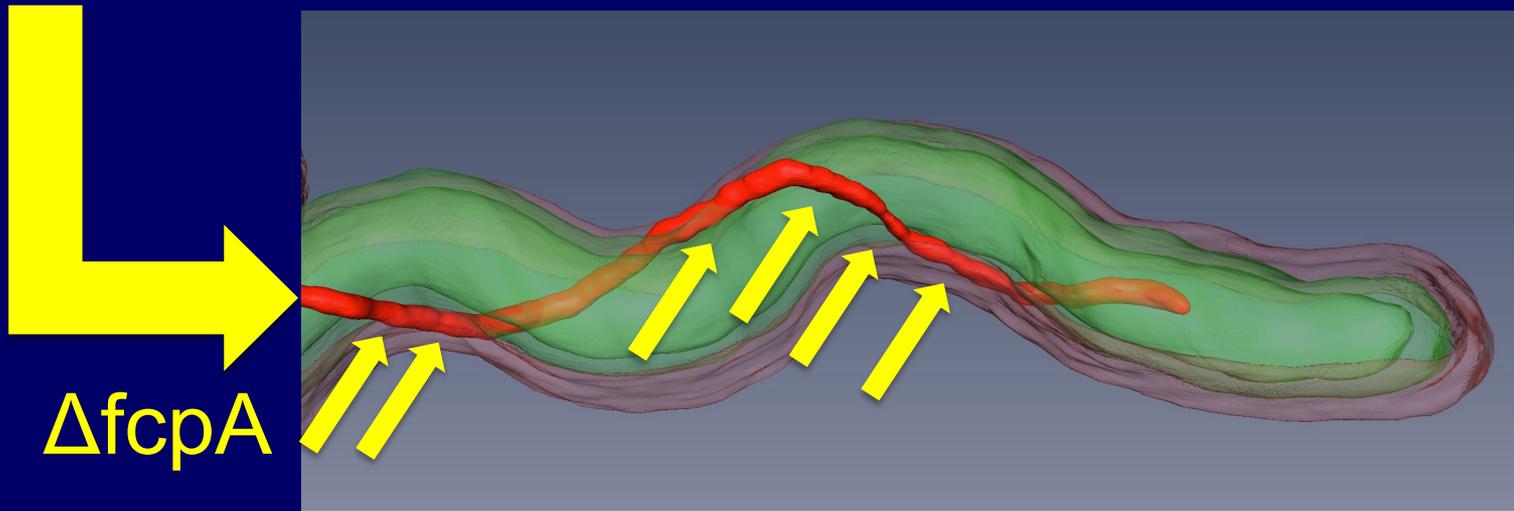
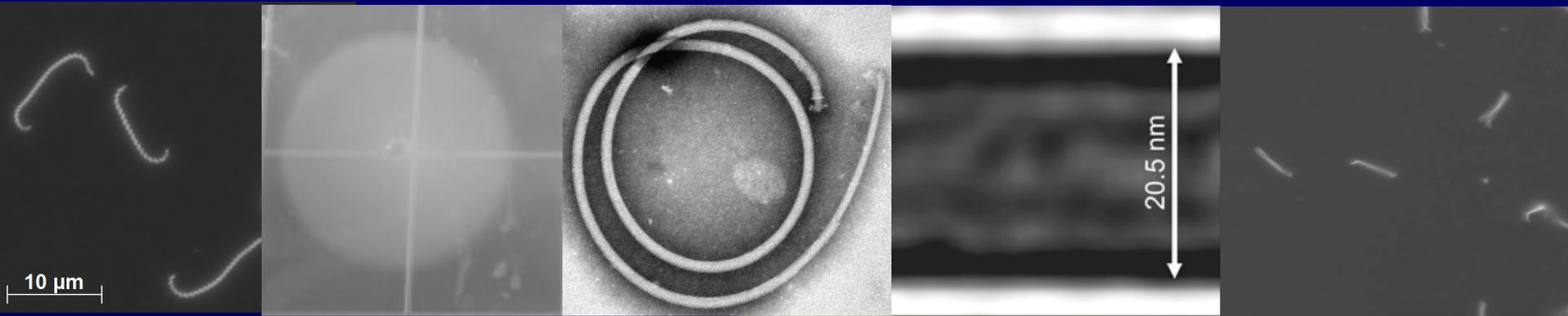
Ryokichi Inada, H. Noguchi and
Yutaka Ido

Gap of Knowledge and Critical Needs - Prevention

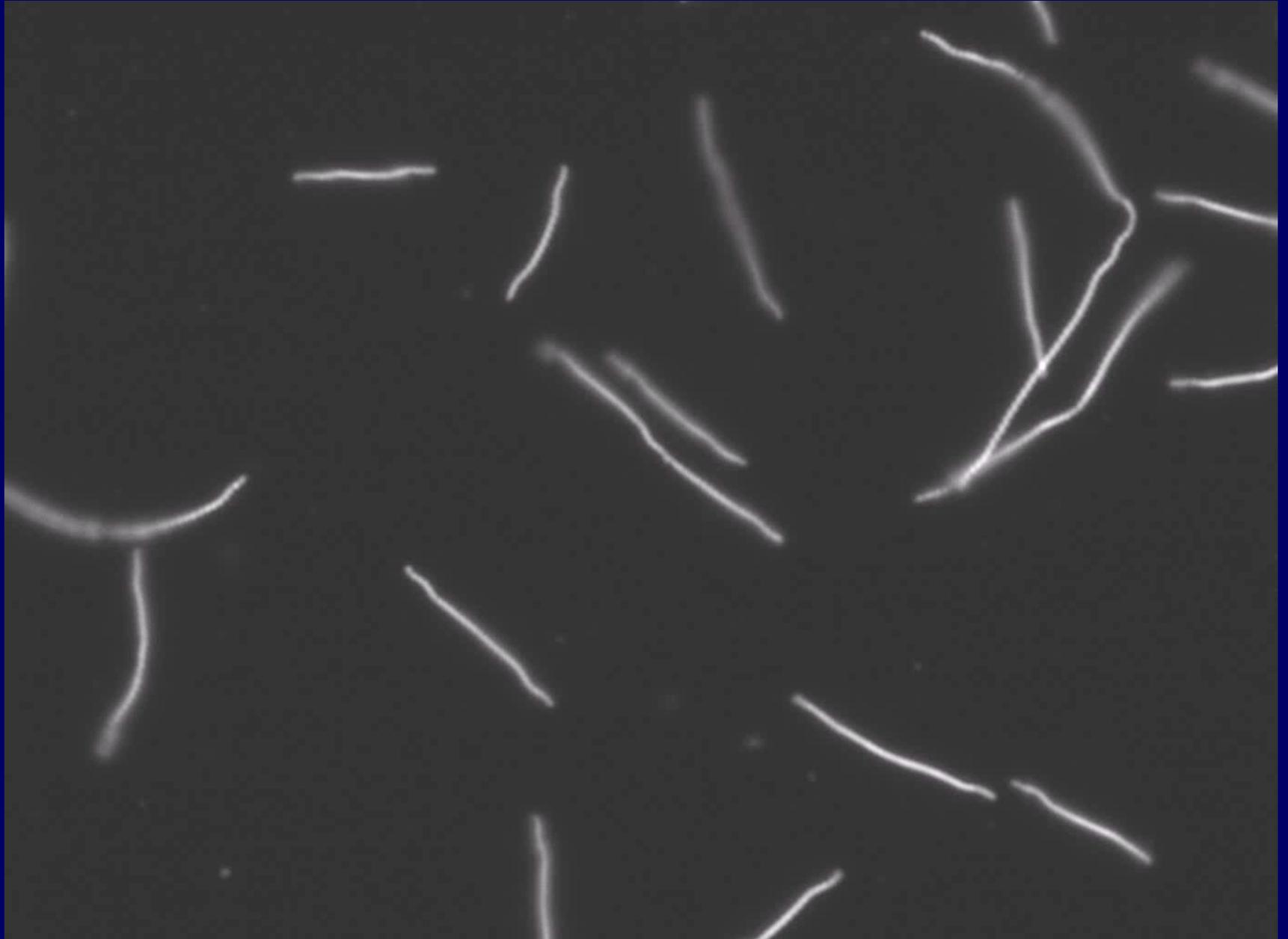
- No effective prevention and control in resource-poor settings
 - ✓ Rural leptospirosis may be difficult to control
- Large number of serovar has been the major challenge to developing a widely-applicable vaccine
 - ✓ Current whole-leptospire-based vaccines that are commercially available confer only serovar-specific immunity
 - ✓ Multivalent vaccines are unable to achieve sufficient coverage against the large spectrum of serovars
- Lack of correlates of naturally-acquired immunity in animals and humans
- Progress is widely believed to be dependent on the identification of effective candidates:
 - ✓ Conserved surface-associated and host-expressed proteins.

Conventional approaches have failed to identify a widely-applicable vaccine: the “holy grail” for the field.

Novel Flagellar Protein associated with coiled morphology



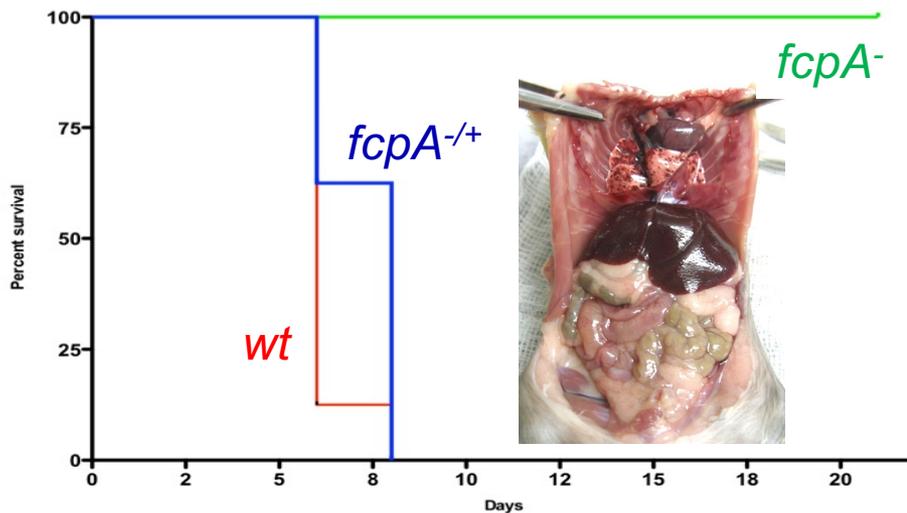
Novel Flagellar Protein associated with coiled morphology



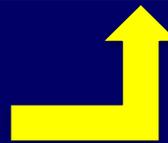
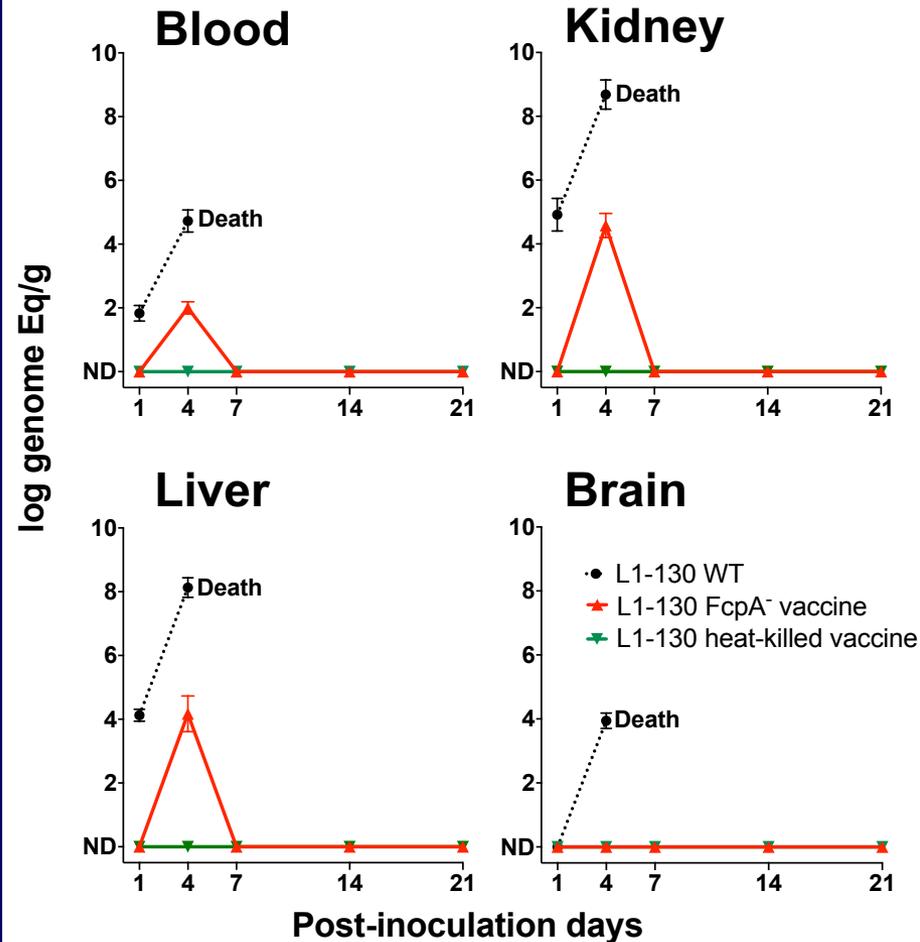
Motility is Essential for Virulence and Dissemination

- *fcpA*⁻ mutant has LD₅₀ of >10⁸ leptospire (wild-type <10)
- Motility deficient clone can disseminate but is eliminated after 7 days of infection and can't cause colonization

Survival curve with IP infection – 10⁸ leptospire



qPCR using SC route - 10⁸ leptospire



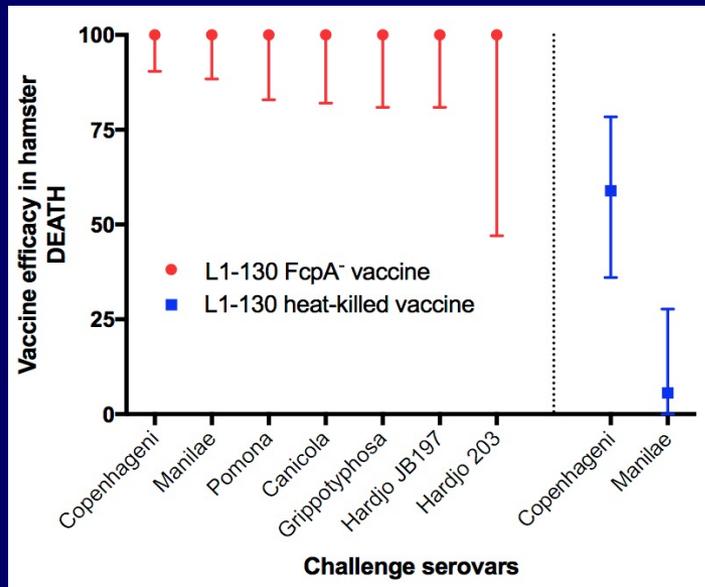
Motility deficient *fcpA*- mutant as a model for attenuated vaccine

- Working hypothesis:
 - ✓ Attenuate strain can disseminate and still express proteins necessary for infection, which will stimulate the immune system;
- Day 1: Subcutaneous vaccination (one dose):
 - ✓ 10^7 leptospire – Motility-deficient mutant
 - ✓ Heat-killed vaccine
 - ✓ PBS – Negative control group
- Day 21: Conjunctival challenge:
 - ✓ 10^8 leptospire;
- Day 42: Outcomes follow-up – death and renal colonization
- Pre and post-vaccination bleeding

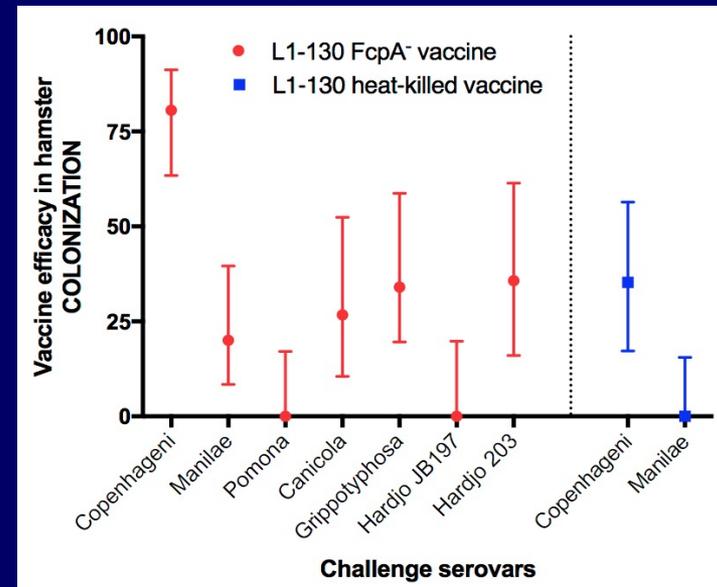
Immunization with *fcpA*⁻ mutant strain confers cross-protective immunity

- Immunization with attenuated *fcpA*⁻ mutant strain protects hamsters against lethal challenge with 10⁸ bacteria via conjunctival route

Survival

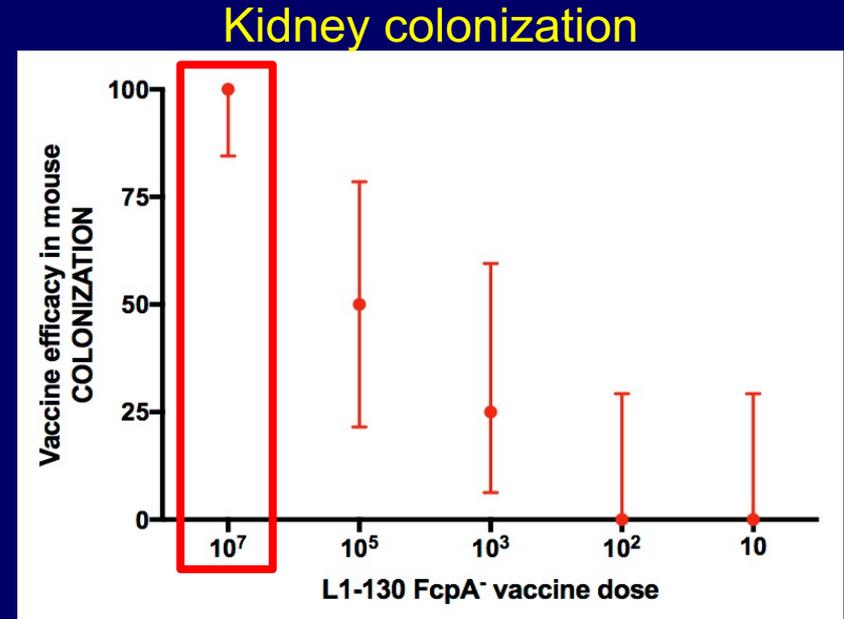
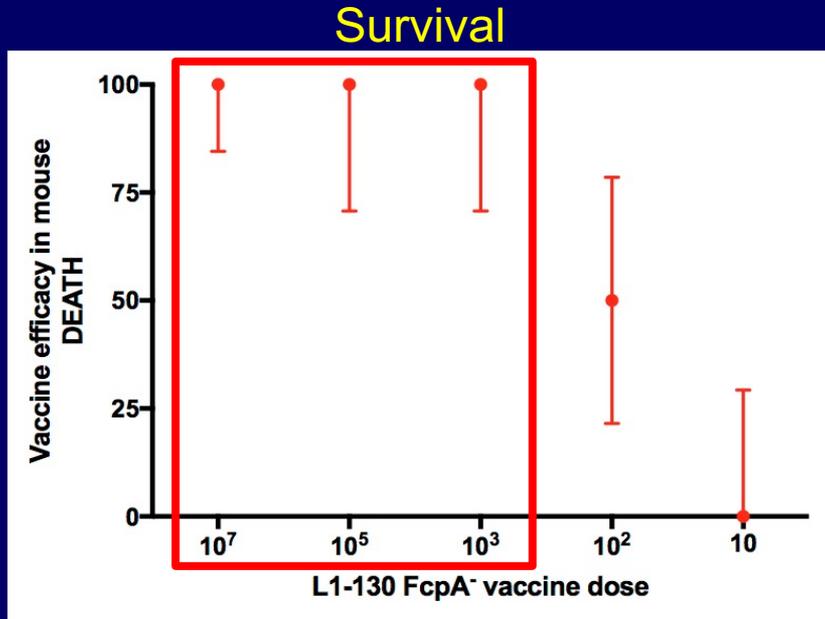


Kidney colonization



- There was only partial protection against colonization after infection with homologous and heterologous serovars
- Immunization with heat-killed leptospires conferred only partial homologous protection and no heterologous protection

Immunization with attenuated *fcpA*- mutant with a dose as low as 10^3 leptospire confers 100% heterologous protection against death in the mice model

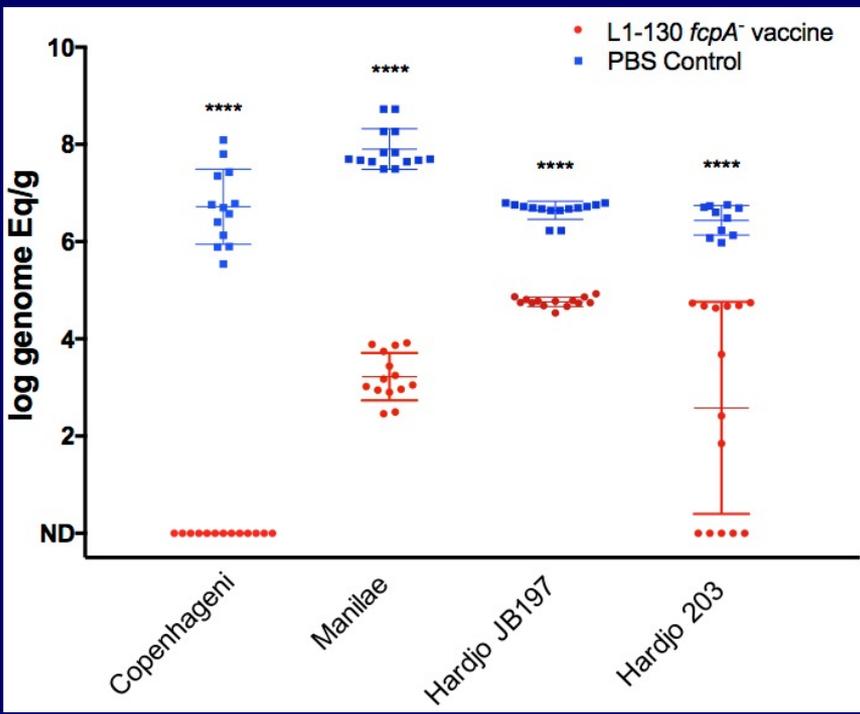


- However, to achieve 100% protection against colonization animals need a dose of at least 10^7 leptospire of *fcpA*⁻ mutant strain

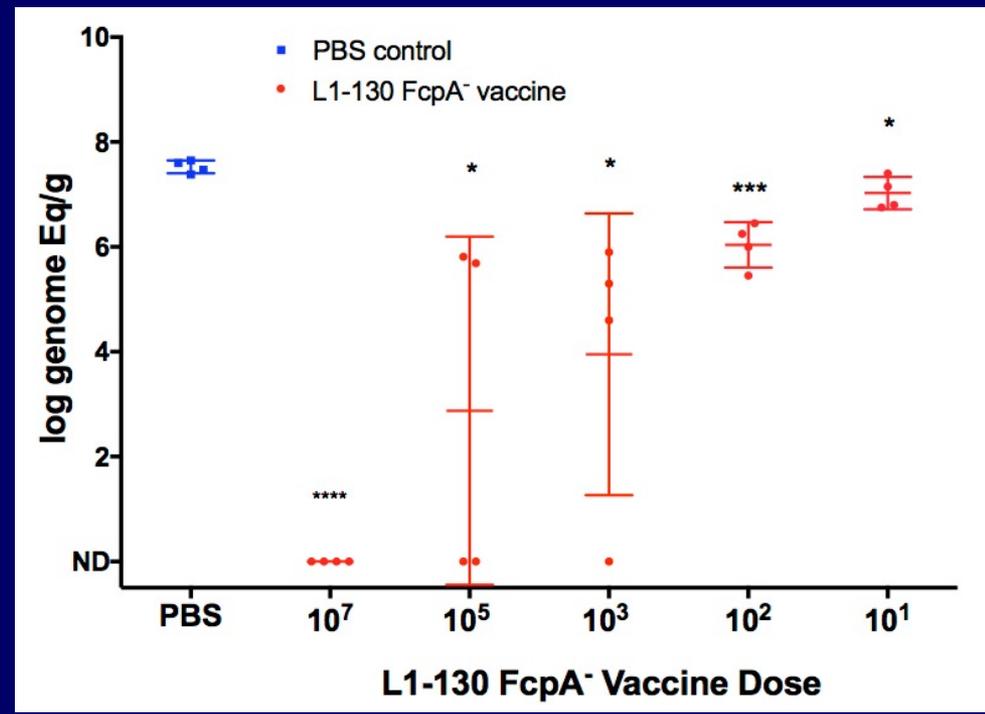
Immunization with *fcpA*⁻ mutant strain reduces the load of bacteria on kidney after heterologous challenge

Kidney colonization (qPCR)

Hamster

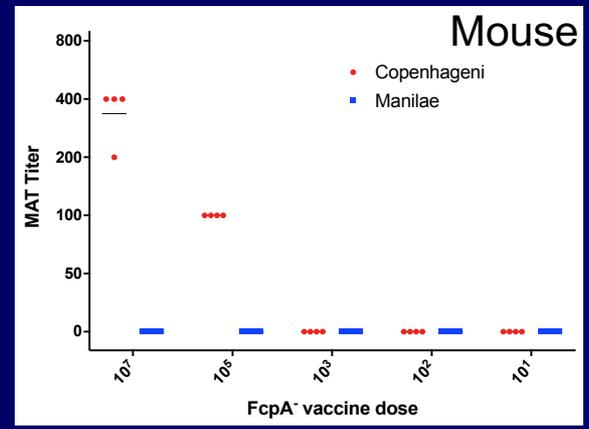
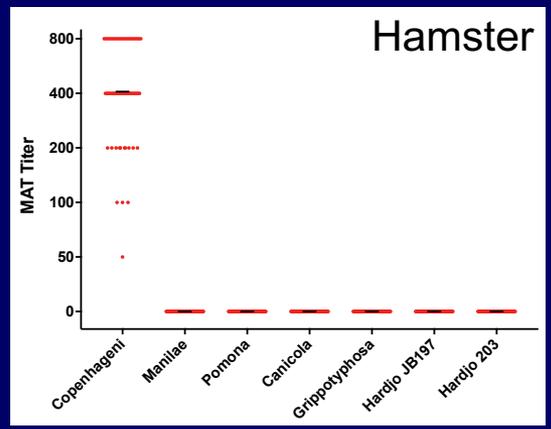


Mouse



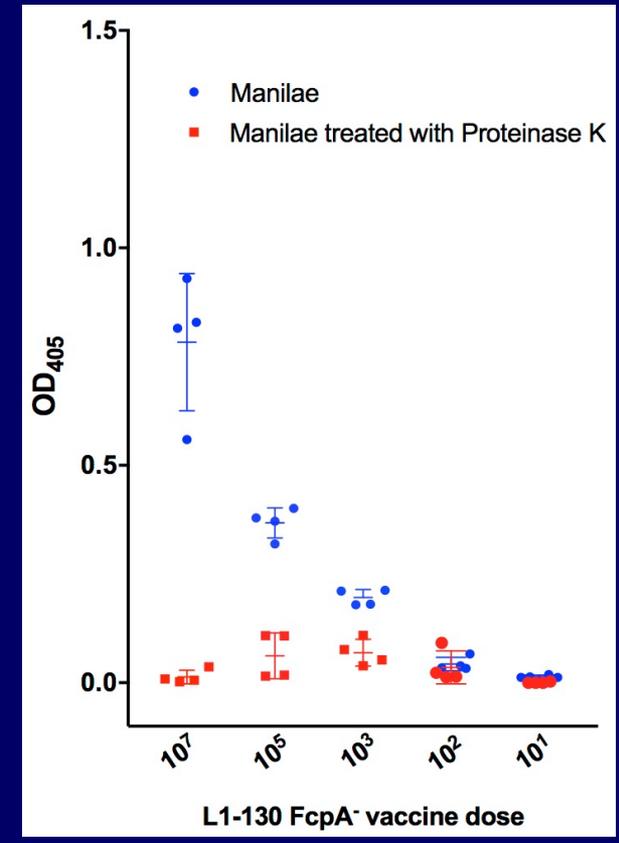
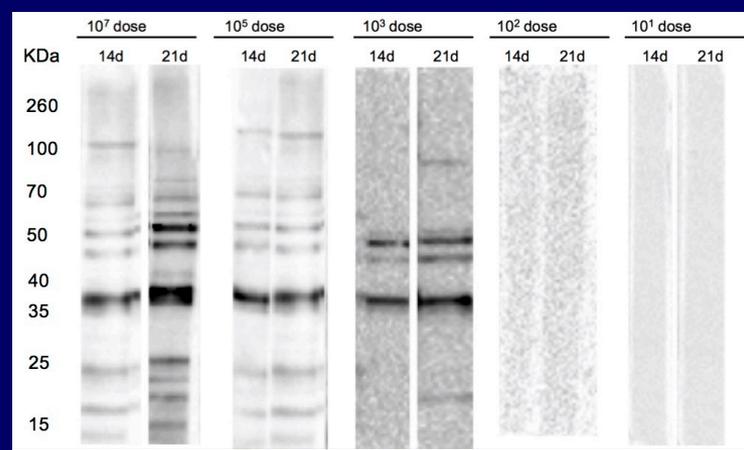
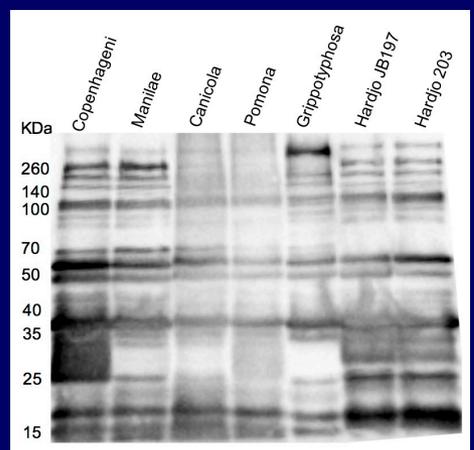
Motility attenuated strain induces immune response against leptospiral proteins

MAT



Protein antibodies are correlates for protection against death

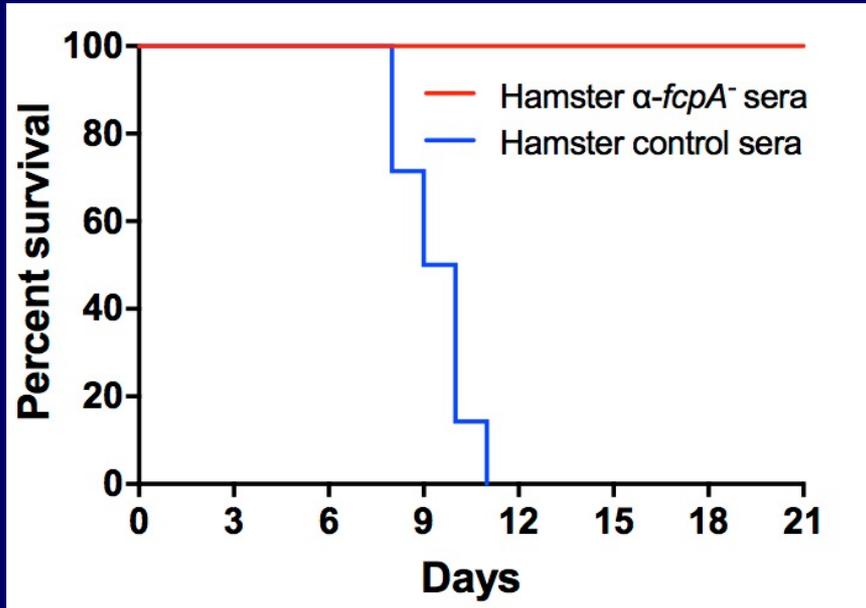
Western blot post-vaccination sera



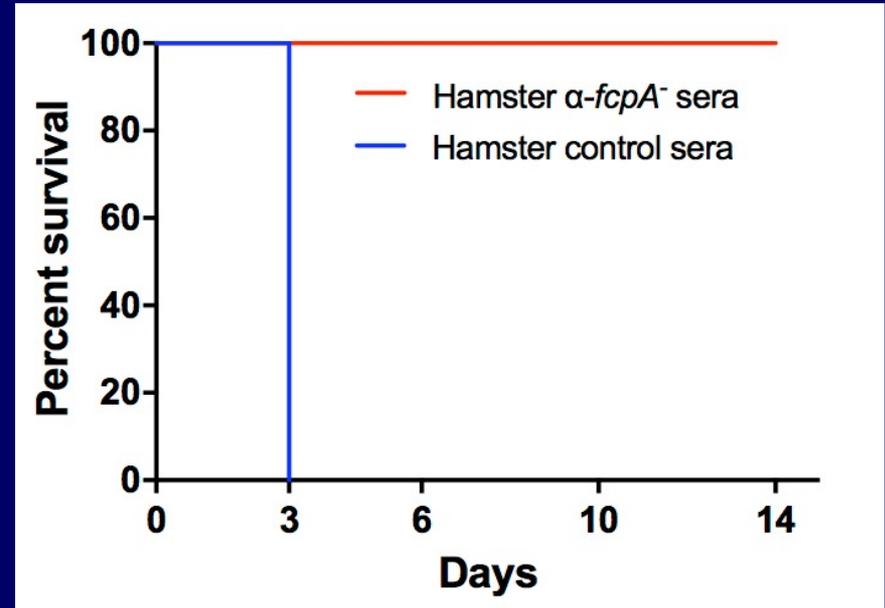
Cross protection induced by motility attenuated strain is antibody mediated

Passive transfer experiments with sera from hamster vaccinated with *fcpA*⁻ mutant

Hamster



Mouse



Attenuated vaccine elicits antibodies to a restricted set of *Leptospira* proteins

- Proteome array approach:

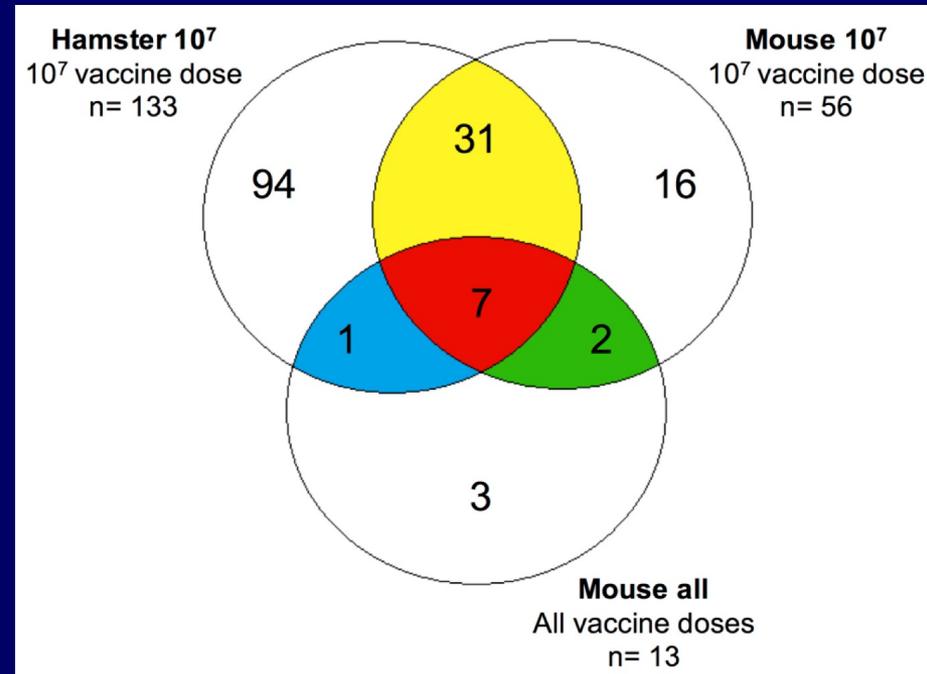
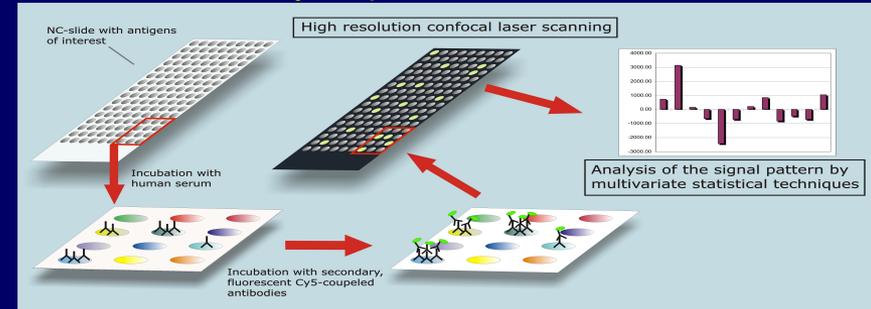
- ✓ *In vitro* translation products
- ✓ Comprehensive analysis of antibody response

- Modeling the dose-response relationship to identify interesting antigens;

- 154 host-expressed proteins identified:

- ✓ Correlates of immunity and protection
- ✓ 23 OMP (15%) ; 52 HP (34%)
- ✓ Potential source for a subunit vaccine or diagnostic marker

Protein Microarray chip



Life-attenuated vaccine as a potential candidate for a whole-leptospire based vaccine

- Potential to formulate a whole-leptospire vaccine with a single strain
 - ✓ Elicit antibodies against *Leptospira* proteins, shared among pathogenic species
- Confers cross-protection immunity against death in hamster and mice model of infection
 - ✓ Same antigens which confer cross-protective immunity in the attenuated vaccine model may play a role in naturally-acquired immunity to reinfection in humans
- Formulate novel approaches for prevention, such as development of targeted vaccines for animal health
 - Different strains for different species
 - Potential to eliminate or reduce the risk of renal carriers

Work-In-Progress on Leptospirosis Prevention

Cross-protective immunity against the spectrum of serovar agents is the *holy grail* for leptospirosis vaccine development.

- Developing an attenuated vaccine formulation:
 - ✓ Research agreement with major Veterinary company;
 - ✓ Knocking out the antibiotic selectable marker;
 - ✓ Veterinary applications – One health benefits
- Evaluate cross-protective antigens as candidates for sub-unit vaccine and diagnostic:
 - ✓ Single protein vs. chimeric approach;
 - ✓ Proteins identified only in infected animals;
- Evaluating correlates of naturally-acquired immunity:
 - ✓ Prospective cohort studies in Brazil and hospital-based studies across the globe;
 - ✓ Better characterize the naturally-acquired immunity against infection caused by different serovars;

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