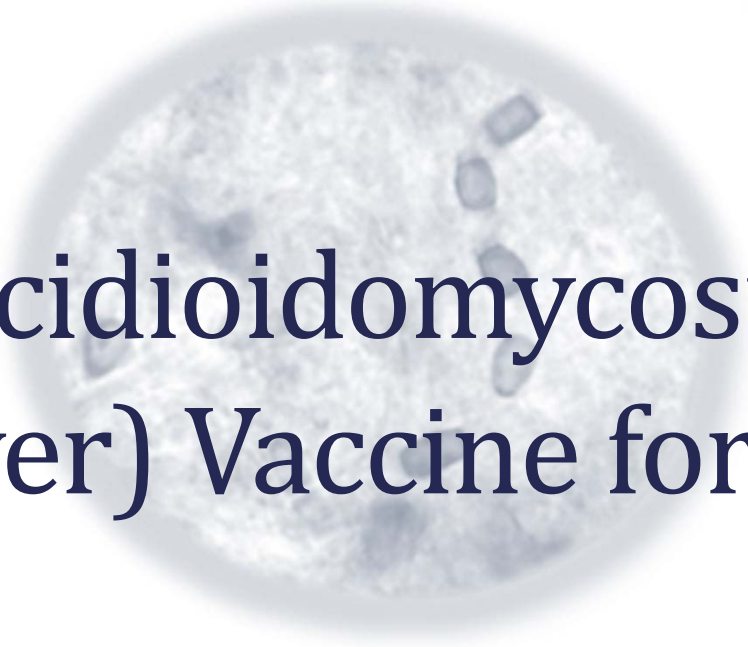

Δcps1 Coccidioidomycosis (Valley Fever) Vaccine for Dogs

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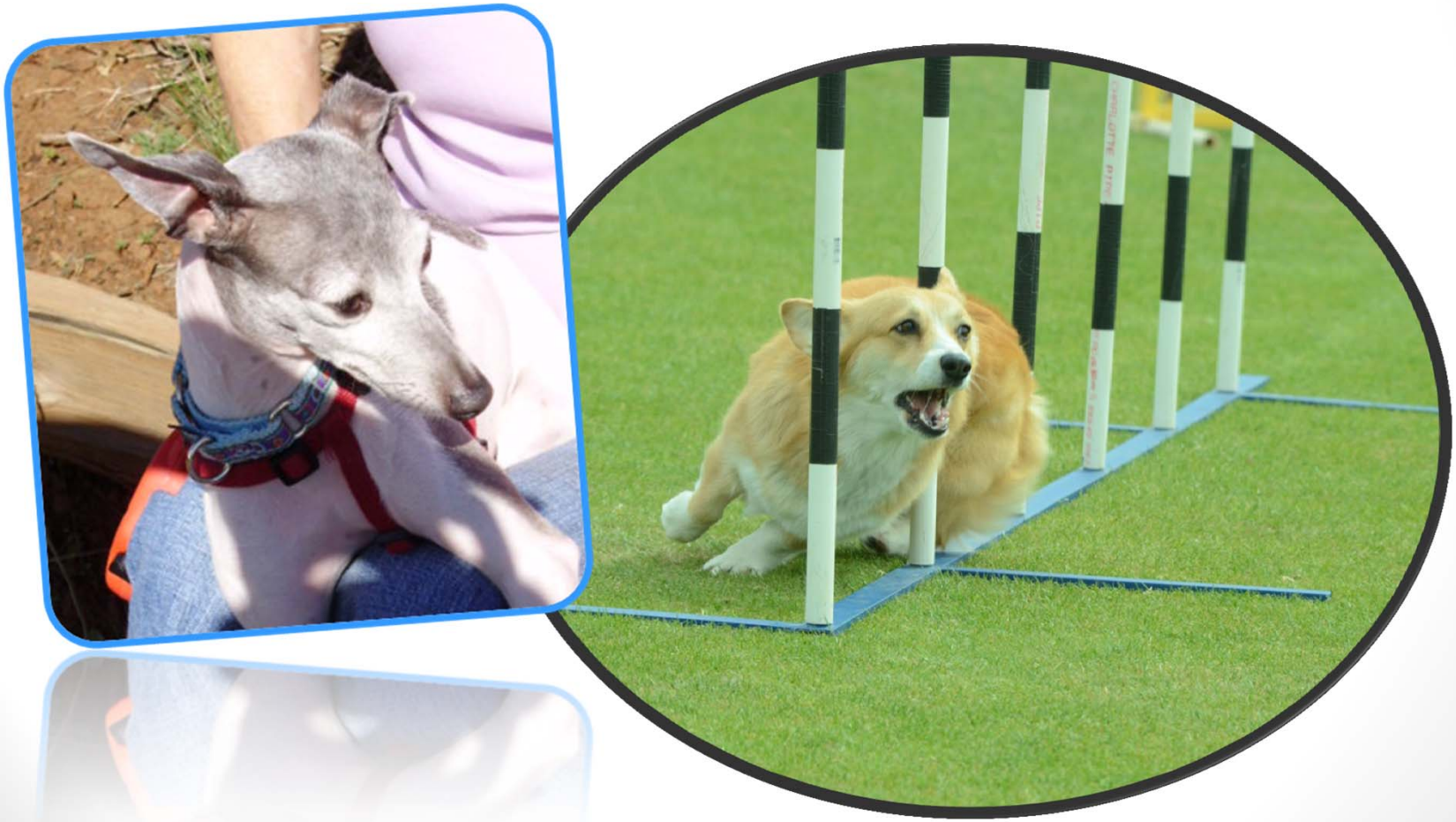
Defining the Problem



Valley Fever Basics

- Fungal infection acquired by inhaling spores from environment
  - Dirt, rodent holes, dust storms, generally breathing air
  - Pima, Pinal, Maricopa counties - “Valley Fever Corridor” accounts for ~ 70% of all cases (dog and human!)
- Infects all mammals, occasional reptiles, no birds
- Causes disease in
  - Dogs
  - Cats
  - Horses
  - Non-human primates
  - Llamas and alpacas
  - Other exotic species (zoo animals – Tucson, Phoenix, San Diego)
  - Marine mammals – west coast

Valley Fever in Dogs

- Affects 6-10% of dogs in the "Valley Fever Corridor" annually
  - Estimate 60,000 dogs per year in AZ alone, costing AZ dog owners about $60 million in costs of diagnosis and treatment
  - Dogs in CA, NM, TX, UT, NV affected at lower rates (unknown) and dogs who travel here with winter visitors and for dog shows or performance sports are at risk.
- Dogs are at greater risk of complicated disease than humans
  - Complicated disease includes unresponsive/progressive pneumonia and disseminated disease.
  - 1 publication of a small number of dogs estimates 25%; this is consistent with clinical impression.
  - Treatment of dogs is months to years, depending on severity of disease and whether it has recurred.

Δcps1 Vaccine

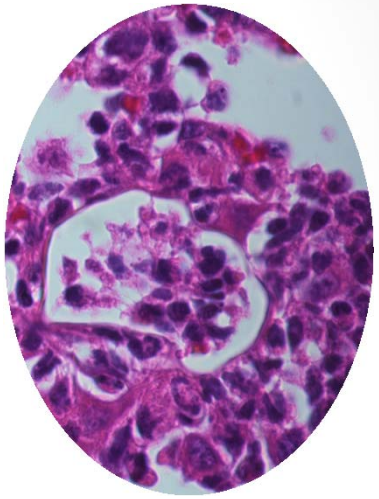
- The vaccine is a live “mutant” strain of *Coccidioides* that doesn’t make animals sick
- Created by removing (“knocking out”) a gene that is required by the valley fever fungus to cause disease in animals
- We don’t know what this gene does in the fungus or why it is essential for causing disease
- The name of the gene is CPS1, so the mutant strain without the gene is called *Δcps1 (Delta-cps1)*
- It would be virtually impossible for this strain to revert back to a disease-causing version

Δcps1 Mutant Strain in Mice

- Tested in C57BL/6 and BALB/c mice
  - Both very susceptible strains, and BALB/c are hard to vaccinate effectively
- Studied extreme avirulence in “NSG” mice
  - These mice are missing almost all of their immune system, especially the portions essential to resolve a regular valley fever infection

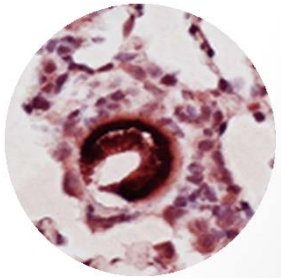
Δcps1 Mutant Strain in Mice

- When we infected BALB/c and C57BL/6 mice with 10-100X lethal doses, they did not get sick
- Microscopic examination showed degradation and clearance of mutant strain from lungs within days.



Δcps1 spore 3 days

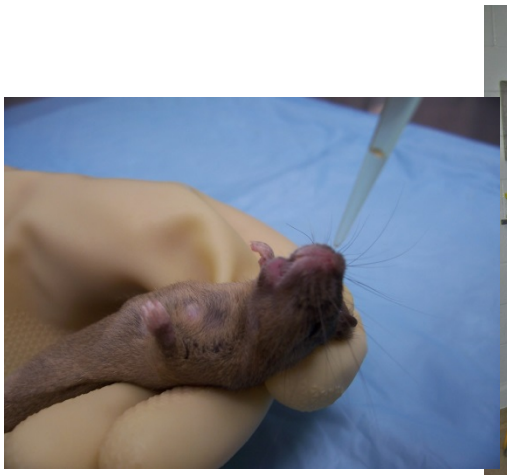
- When we infected NSG mice with a 50-fold lethal dose for the other two mouse strains, they did not get sick
  - NSG mice would die from even one spore of regular valley fever fungus!
  - This has important implications for SAFETY of this strain as a vaccine in dogs or humans



Normal spore 3 days

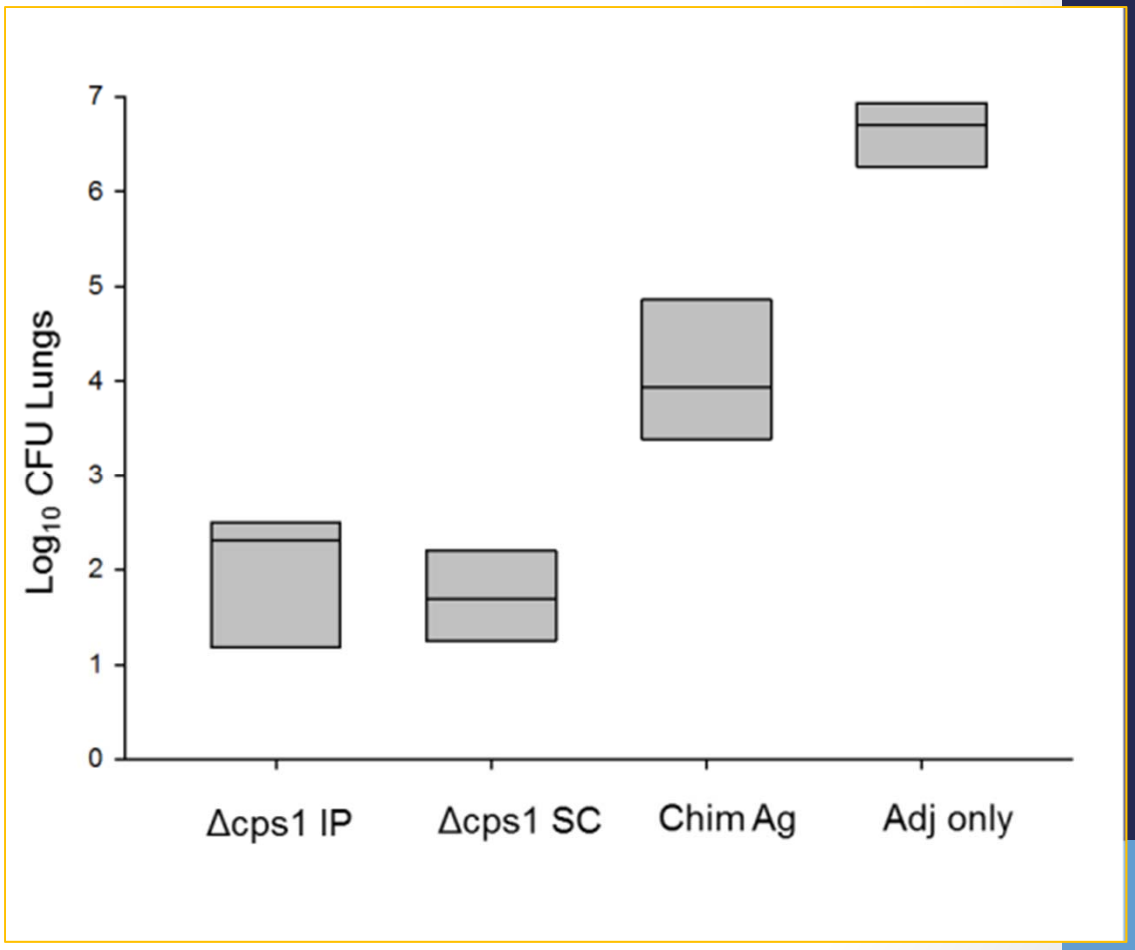
Δcps1 Vaccine in Mice

- Vaccinated C57BL/6 and BALB/c mice with the *Δcps1* and infected them with the regular “virulent” valley fever fungus
- Two types of studies
  - Short term fungal burden (two weeks) and then culture the lungs and count the number of fungal colonies in the lungs
  - Survival over a period of 4 weeks, followed by counting the residual number of colonies in the lungs of the mice that lived



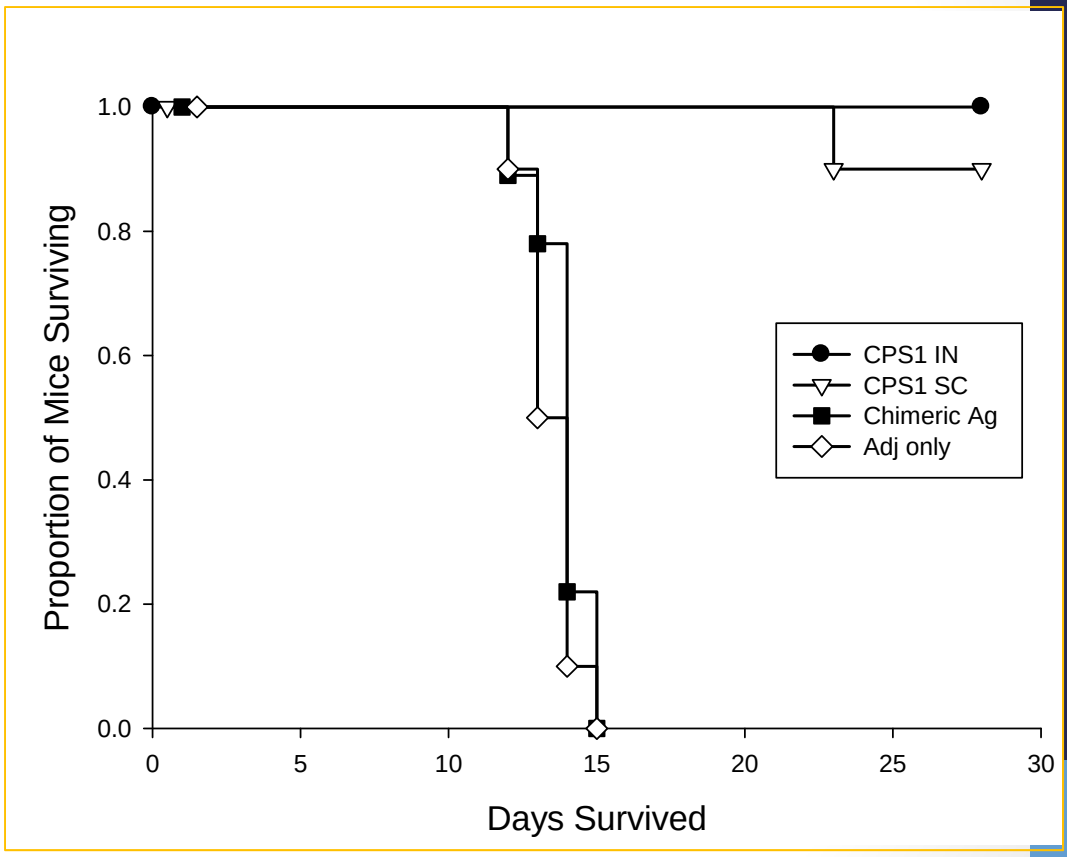
Quantitative lung burden in vaccinated C57BL/6 mice

- Mice were vaccinated with *Δcps1* spores either intraperitoneally (IP) or subcutaneously (SC)
- Control groups vaccinated with a known protective recombinant antigen (Chim Ag) or placebo (Adj only)



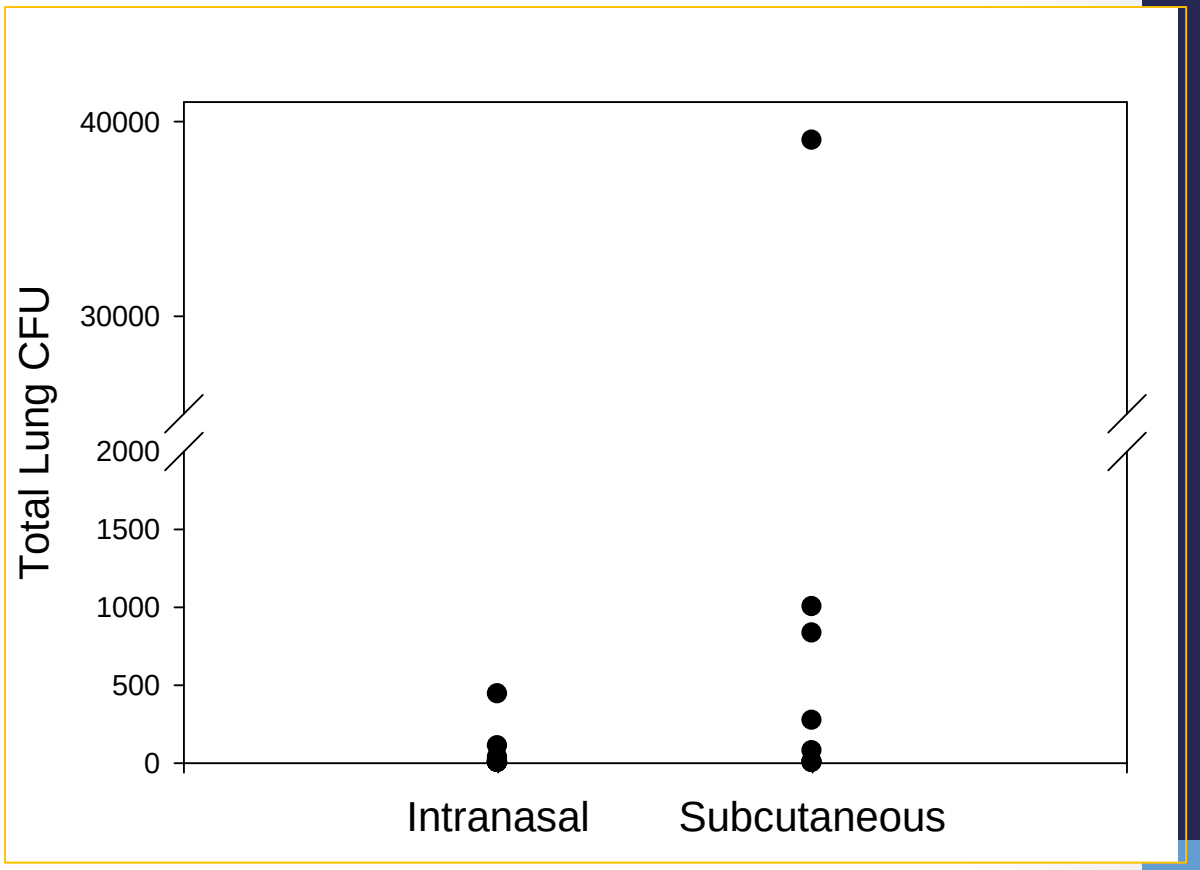
Survival of BALB/c mice following lethal challenge after vaccination

- Highly susceptible BALB/c mice were vaccinated with *Δcps1* spores either intranasally (IN) or subcutaneously (SC)
- Control groups vaccinated with a known protective recombinant antigen (Chimeric Ag) or placebo (Adj only)



Residual lung burden in BALB/c mice

- Average fungal burden for both groups was <1000 CFU
- 5 mice vaccinated IN and 3 mice vaccinated SC had undetectable fungal burdens



How Do We Get From Mice to Dogs?

- Development of the vaccine from here requires
  - Additional laboratory studies in mice to look at longer term survival (6 months)
  - Studies to be able to determine if dogs are responding to the vaccine without waiting for them to be infected (underway with funds in the Companion Animal Fund)
    - Draw a blood sample from a vaccinated dog
    - Separate the white blood cells and test them to see if they are producing an “immune response” to the vaccine
  - Formulation of a vaccine – mutant strain plus stabilizers, liquid vs. freeze dried product, shelf life
    - This is what goes in the bottle
    - Estimate 12-18 months of work, unless we are *really* lucky

How Do We Get From Mice to Dogs?

- Manufacture the vaccine in the presumed final formulation in bottles that can be tested for shelf life and effectiveness
- USDA licensing
  - License the manufacturing plant
  - Conditionally license the vaccine
  - Safety study – inject dogs (community) with the vaccine and observe for adverse reactions
  - Efficacy study – vaccinate two groups of dogs (Δcps1 and placebo) and monitor them for a year to determine if the number of dogs in the vaccine group get less valley fever than the placebo dogs
    - This study requires 500 dogs total and half of them are likely to benefit immediately from being in the study by becoming immune

How Much Will It Cost?

- Remainder of mouse studies and vaccine formulation
  - Estimate $100,000-$130,000 (UA campus)
- Manufacturing and licensing
  - ?
  - Depends on whether we have to start a company of our own to manufacture the vaccine or whether a Contract Manufacturing Organization is willing/able to grow the fungus for the vaccine and do the USDA licensing
  - Could cost $2 million if we have to start from scratch
- Safety and Efficacy trials
  - Estimate $100,000-$150,000
  - I hope I am right! These are labor intensive without a lot of materials; cost may depend on ability of local vets to help gratis

In Summary . . .

- I think this vaccine could work and have few adverse effects
- Cost of development is a big obstacle and national or federal sources of funding are a low likelihood
  - Local fundraising
  - Angel investors?
- Will take 3-5 years to licensing once we have identified funds to go forward with the work
- Anticipate it will benefit dogs enrolled in the development studies
- In the long run, could save lives and millions of dollars in veterinary care for dogs in Arizona