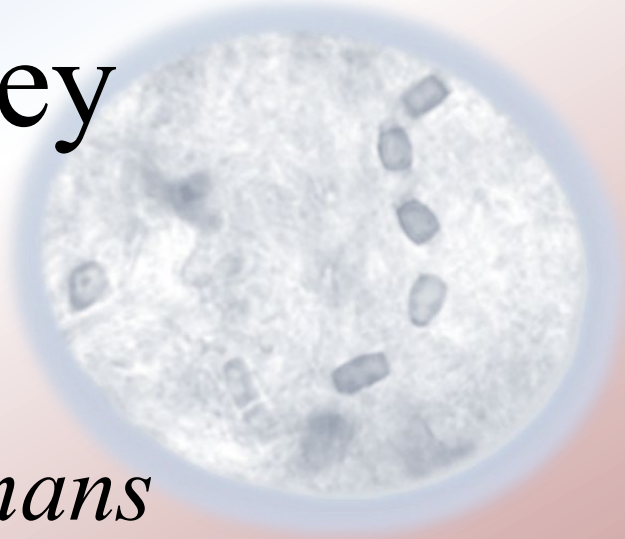
# Δ*cps1* Vaccine to Prevent Valley Fever in Dogs



## *A One Health Journey from Plants to Humans*

Lisa Shubitz, Edward Robb, Marc Orbach, Daniel Powell, Jeffrey Frelinger, John Galgiani

Valley Fever Center for Excellence and the School of Plant Sciences, University of Arizona

Anivive Lifesciences, LLC

THE UNIVERSITY OF ARIZONA
COLLEGE OF MEDICINE TUCSON
Valley Fever Center for Excellence

NAMES, Irvine, CA, November 18, 2022

# Disclosures and Acknowledgements

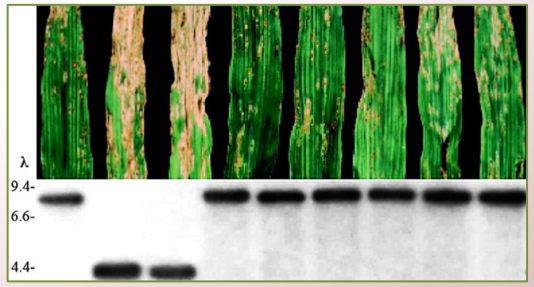
I am listed as an inventor on the patent for Δ*cps1* as a vaccine

Many people and organizations contributed to the studies that resulted in a vaccine ready to license in dogs

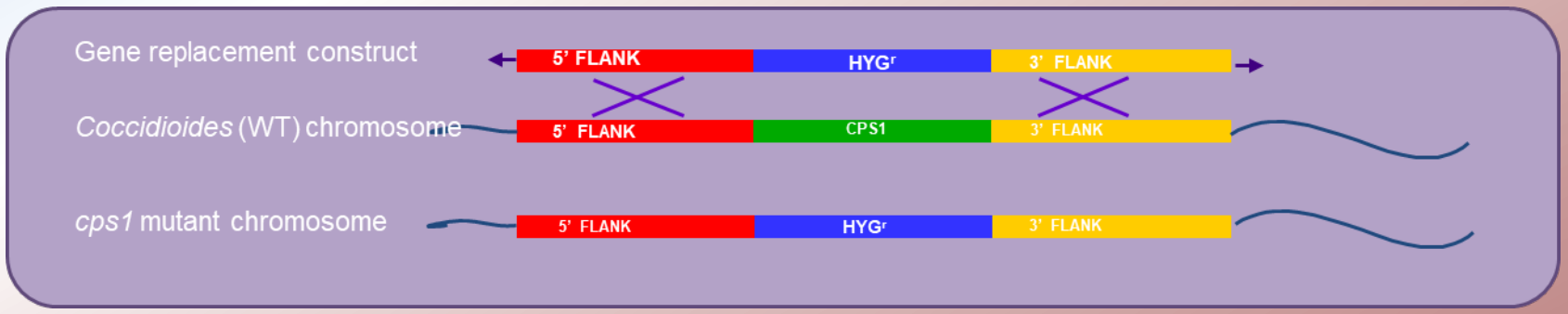
*Dog owners and clubs funded the preliminary data - >$40K*

- *VFCE and Plant Sciences - U of Arizona*
- *Anivive Lifesciences, LLC*
- *NIH-NIAID [RO1-AI-132140]*
- *Hennessy Research Associates*
- *Colorado State University*
- *University of Kansas*

# *CPS1* is a Virulence Factor in Fungi

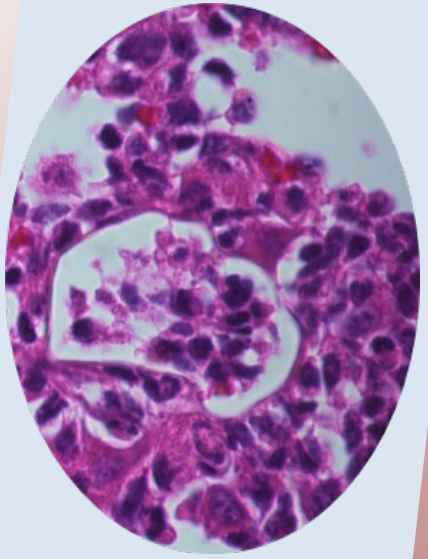



- Disruption of *CPS1* gene in the fungal pathogen *Cochliobolus heterostrophus* reduced virulence on corn plants
- We deleted the homolog in *Coccidioides posadasii*
  - The 6kb gene was removed from Silveira strain and replaced with a hygromycin resistance cassette for selection



# *Δcps1* is Profoundly Avirulent in Mice

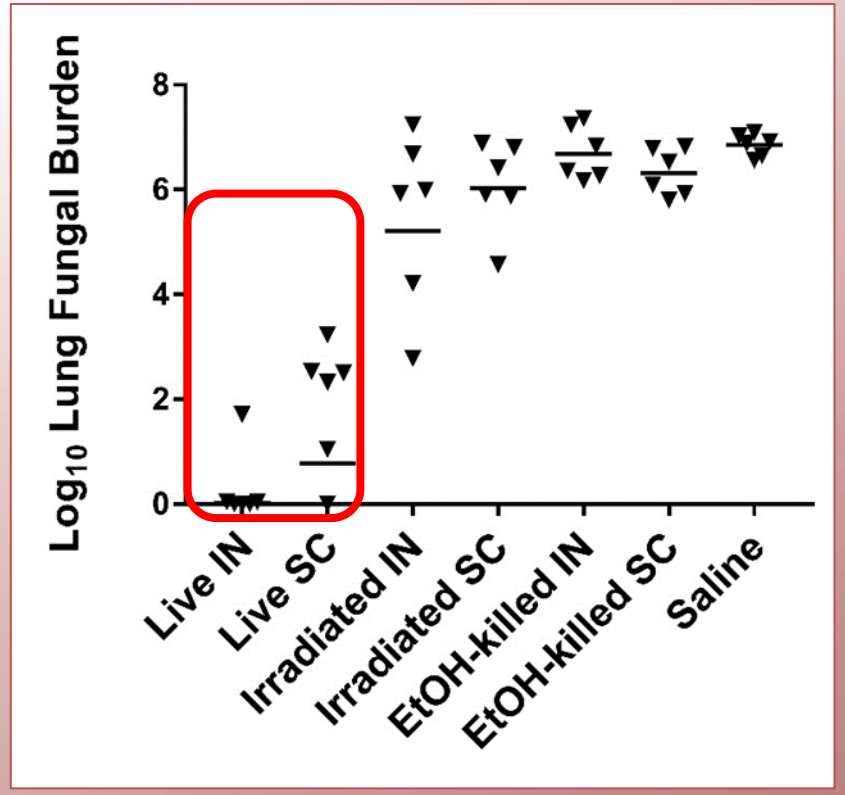
- Not pathogenic in C57BL/6 or BALB/c mice at doses up to 10,000 spores IN
- Immunodeficient NOD-SCID (NSG) given 1000 spores IN remained healthy through 14 days
  - NSG mice lack T-cells, B-cells, NK cells, and other signaling
- Microscopic examination showed degradation and clearance of Δ*cps1* from lungs within days
    - This has important implications for SAFETY of a vaccine in dogs and humans



Day 3 Δ*cps1* spherule showing wall degradation, infiltration by neutrophils

# Δ*cps1* Protects Mice as a Vaccine

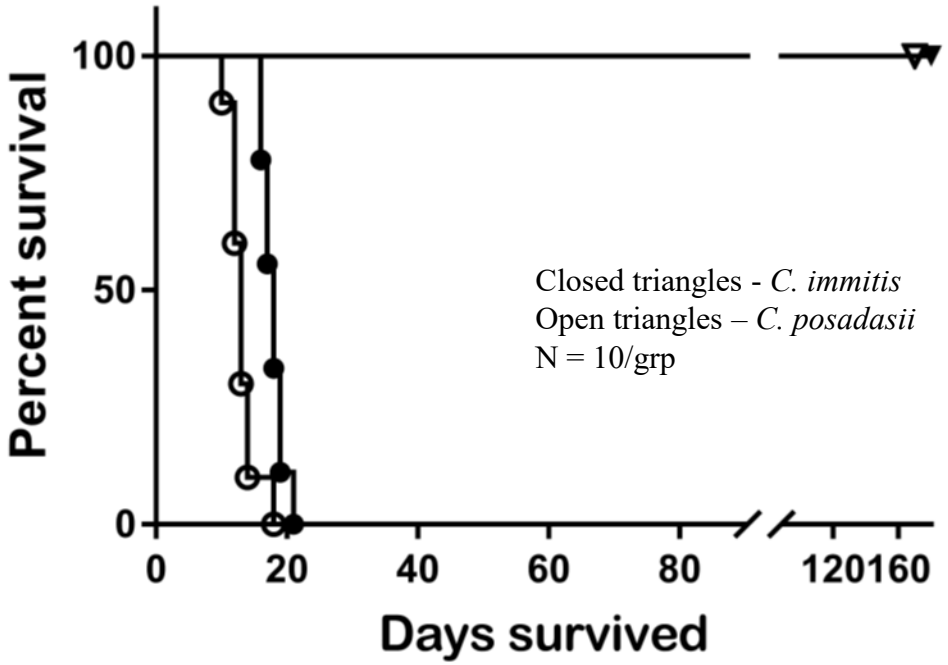
- Balb/c and C57BL/6 mice are highly protected by either IN or SC vaccination
- Only viable spores generate immunity

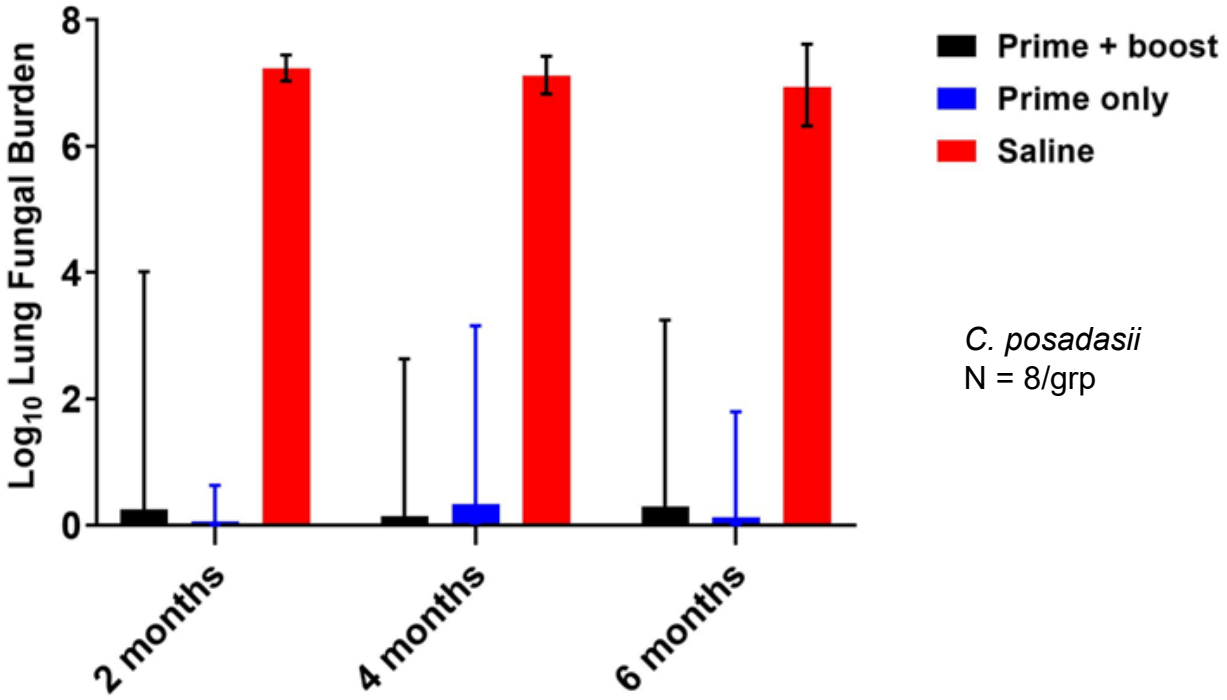


# Immunity is Durable and Survival Extended with Cross-species Protection

100% survival of all vaccinated mice for 6 months after lethal infection; 40% no fungal growth

>7 log suppression of mean lung fungal burden up to 6 months after vaccination; 30% no fungal growth 14 days p.i.




Narra, et.al, 2016, Infect Immun Vol 84:3007-3016

# NIH Awards $4.8M to Valley Fever Center for Work on Dog Vaccine

The UA center will use the funding to accelerate development of a Valley fever vaccine for dogs that one day may lead to a successful human vaccine.

ANIVIVE LIFESCIENCES

Anivive is a biotech company that utilizes advanced technology to optimize the drug discovery and development process of veterinary medicines.

**Objectives: 1)** Produce a canine vaccine candidate ready to progress to USDA licensing

**2)** Proof of concept, safety, and efficacy are stepping stones to developing a vaccine for humans

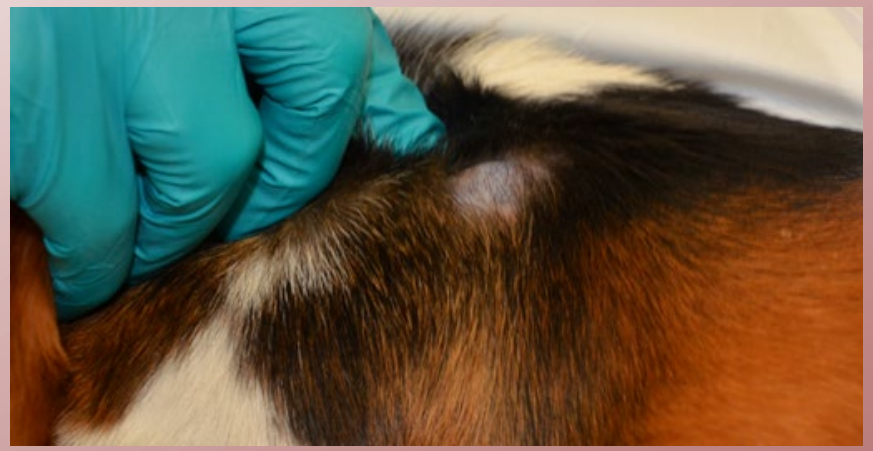
# Canine Vaccine and Challenge Study

**Study Design**

- 34 1-2 YO M and F dogs
- Randomized and vaccinated:
  - 28 days apart with 2 doses of 10K, 50K, or 100K live Δ*cps1* (prime/boost)
  - 1 time only with 100K live Δ*cps1* (prime only)
  - Saline 2x 28 days apart (Controls)

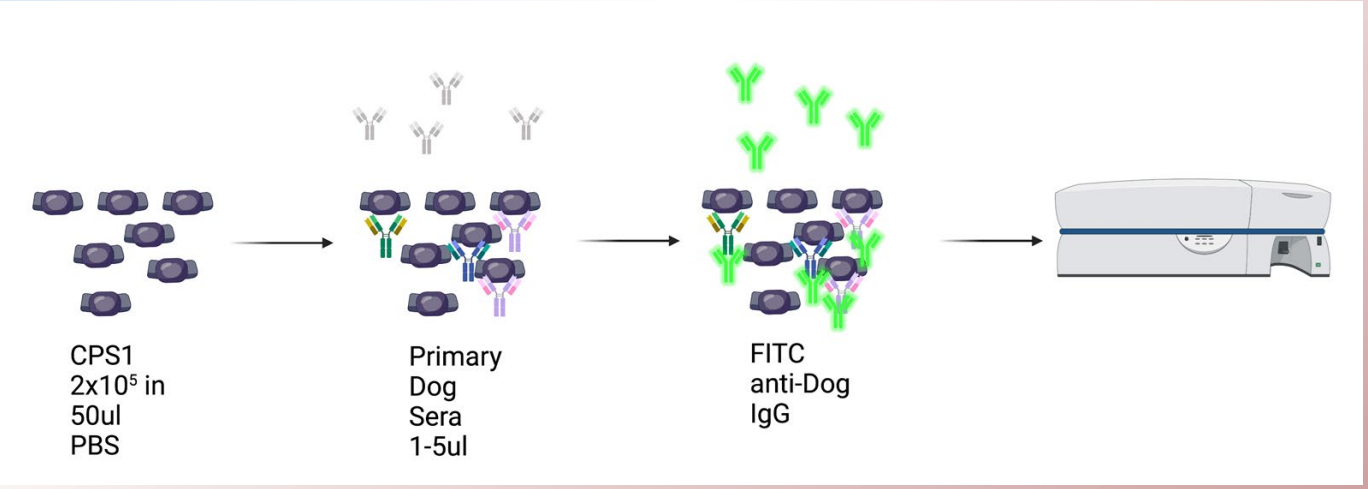
**Vaccine Adverse Effects**

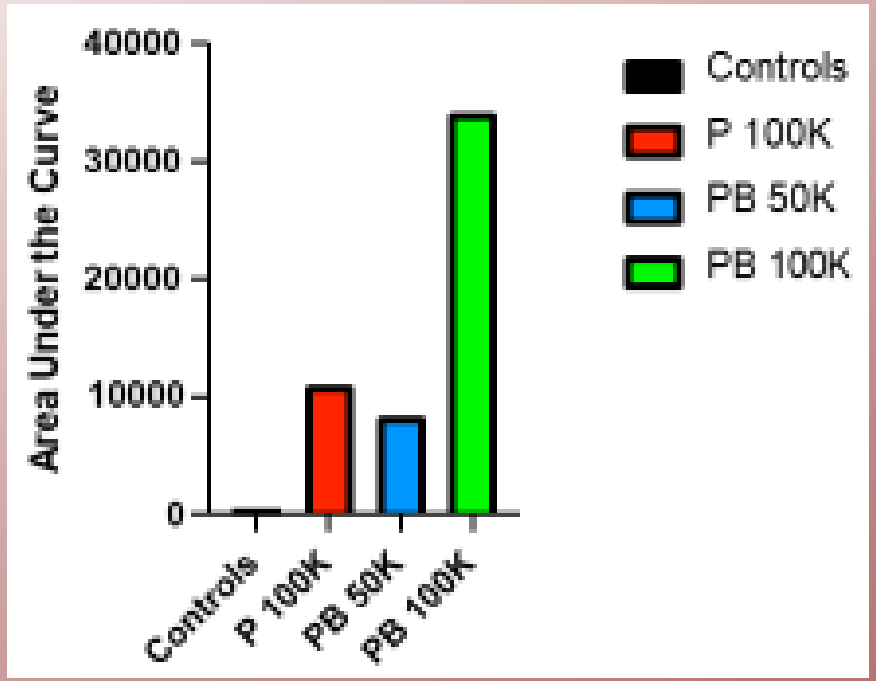
- No systemic effects
- Transient mild/mod injection site swelling that resolved by 2 weeks post-booster



# Measuring Response to Vaccine

- Measuring antibody in blood serum (seroconversion) is a common way to determine an animal made a Vx response
- Detected antibody in only 3 of 34 dogs using conventional tests (ELISA, immunodiffusion)
- New serum antibody test:



# Canine Challenge Study

- Transferred 30 dogs (6 per group) to Colorado State University large animal BSL3 for intratracheal aerosol challenge with virulent *Coccidioides* 63-70 days after first vaccination
  - CSU investigators were blinded to vaccine and control groups
  - Dogs were randomized with new identification numbers
- Infected with 10,000 spores of Silveira and monitored for 8 weeks
  - Infection was non-lethal (mostly subclinical) but produced laboratory, radiographic and histopathologic indicators of Valley Fever infection

# Results

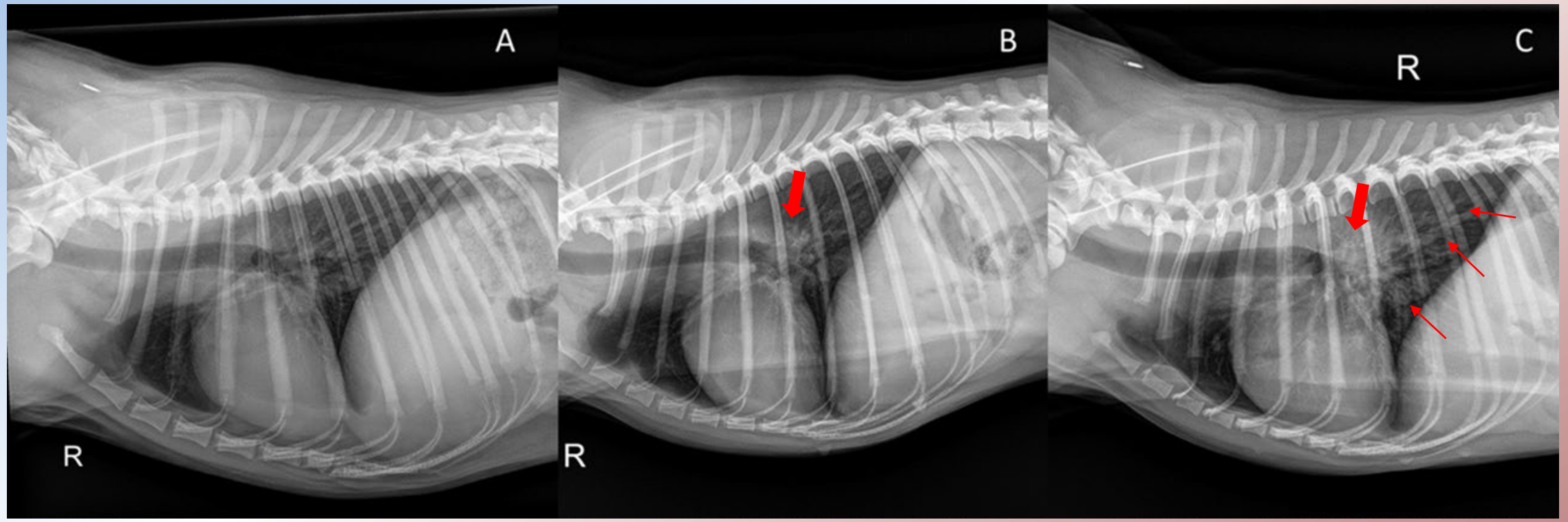
- 1 control group dog had a mild cough, 5% weight loss, fever; 1 dog that received a single dose of vaccine exhibited fever
- Composite total disease score was generated for each dog from 13 scores that measured disease

Disease Scores for Composite

| | |
|---|---|
| **Lung fungal burden** | Log10 total lung CFU |
| **Lymph node burden** | Log10 CFU/g of lymph node |
| **Serology** | Reciprocal of terminal immunodiffusion titer |
| **Lung nodule total** | Total visualized at necropsy |
| **Lymph node score** | Total score at necropsy – thoracic cavity |
| **Histopathology spherule score** | Coccidioides organisms visualized on all tissue sections submitted |
| **Total histopathology score** | Sum of scores for coccidioidal lesions on all tissue sections submitted |
| **Radiology score** | Sum of scores for each radiographic time point |
| **Neutrophilia** | Number of results above the upper limit of normal (11,500) |
| **Monocytosis** | Number of results above the upper limit of normal (1000) |
| **Low albumin** | Number of results below lower limit of normal (3.0) |
| **High globulin** | Number of result above the upper limit of normal (3.2) |
| **A/G ratio** | Reciprocal of A/G ratio |

Shubitz, et.al., *Vaccine* 39:47, 16 Nov 2021, pp. 6894-6901

# Results – Final Lung X-rays




2 doses – normal lungs

1 dose – mild LN swelling

Unvaccinated – severe LN enlargement (thick arrow) + heavy infiltrates (thin arrows)

# Results

- Prime and booster (P/B) generated very good protection
- Not dose dependent between 10,000 and 100,000 spores
- A single vaccine only (P) was intermediate with progression of disease
  - Not statistically reduced vs. unvaccinated (p=0.675)

| Dog Group | Mean Composite Score |
|---|---|
| P/B 10K | 9.5* |
| P/B 50K | 10.7* |
| P/B 100K | 11.7* |
| Prime only 100K | 55.9 |
| Unvaccinated | 123.7 |

P/B = prime with booster at 28 days

P/B groups (*) had significantly reduced disease scores compared to unvaccinated (**p=0.002**) and prime only (**p=0.037**)

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# ANIMAL HEALTH PROGRESSION

## PROGRESSION AS A DEVELOPMENT CANDIDATE

1. **A safe and protective antigen**
   - Live whole spores (arthroconidia) from an avirulent Δ*cps1* mutation of *C posadaslii* with successful testing in mice challenge models
2. **A disease challenge model** to test effectiveness
   - No canine challenge models conducted in dogs since the 1950s. Model is BSL3
3. **A 'potency' assay**
   - Spores can be counted and assayed (cultured) for viability

## HEADWINDS

1. **No known Regulatory Pathway** (no guidance documents, etc.)
2. **Coccidioidies spores have not been grown in bioreactors** (liquid culture)
3. **Lack of Challenge Model Experience (BLS3)**
4. **Formulation requirements and stability for spores unknown**
5. **Live attenuated vaccine with a gene deletion and antibiotic resistance marker (HgB)**

# UPDATE 3

1-Successful Model Development with relevant clinical signs

2- Successful immunogenicity

3- Successful challenge model protection

4- Vaccine well tolerated

5- Low MID and study confirms the need for a Prime and Boost for protection.

6- To date all animal microbiological safety studies are supportive of the safety of the Δ*cps1* mutation of *C. posadaslii*

Spores are easily and rapidly inactivated with common disinfectants. 10 %Bleach/70 %ethanol ( 7 log reduction in<1 min)

7- Master Seed and Testing/Stability, Outline of Production, Yield, Potency and Release Assays and Provisional formulations are available

**8- To date successful engagement with CVB on pathway for regulatory approval**

VALLEY FEVER- ONE HEALTH

## ANIMAL HEALTH PROGRESSION

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**All Development Candidate Requirements Addressed**
**All Headwinds addressed**

# The Valley Fever Vaccine for Humans

- A **key objective** of the VFCE NIH grant was to move Δ*cps1* into development as vaccine to prevent Valley Fever in humans
- Crozet Biopharma (Devens, MA) had a sub-license option for the human vaccine that was dependent on successful funding
  - Unfortunately, funding fell through

# The Valley Fever Vaccine for Humans

This vaccine addresses a clear unmet medical need and requires an infusion of funding and additional human health partners to move forward

The *VFCE, Anivive, and Crozet* are committed to this One Health endeavor to drive the development of Δ*cps1* from dogs to humans… for a health solution on both ends of the leash