Measuring The Natural Course of Antibody Production in Coccidioides Exposed Dogs

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Measuring Dog Antibodies in *Coccidioides* Infections

- Current veterinary measurements of antibodies in *Coccidioides* infection is based on Ouchterlony/Immunodiffusion

- This method is semi-quantitative, labor intensive, and gives varying inter-laboratory results

- Since we are developing a vaccine candidate in dogs, we need to develop a better *Coccidioides* diagnostic and monitor antibody responses in dogs

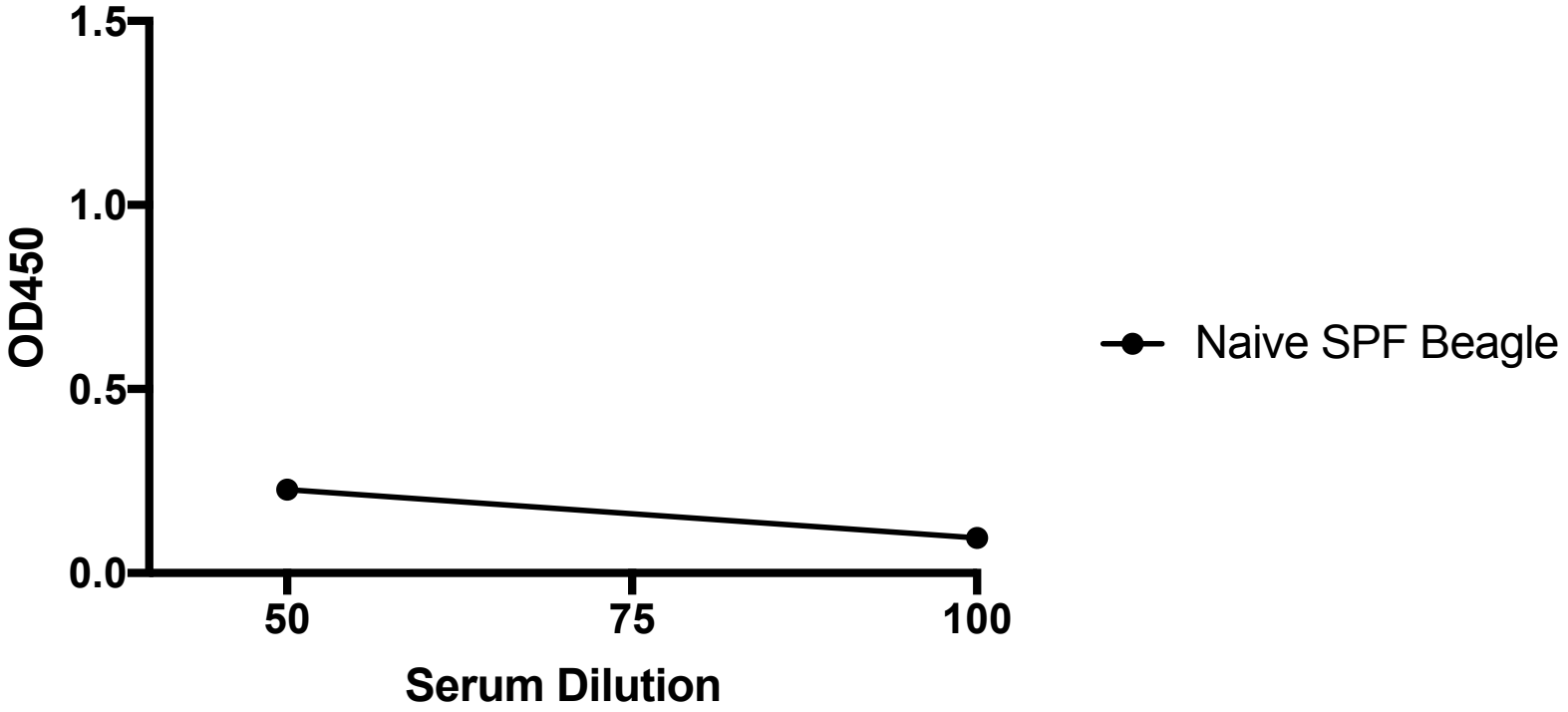
Chitinase-1 Antigen

- Previous work has identified truncations of the Chitinase-1 (CTS1) antigen from *Coccidioides* is recognized by pooled human sera
- Specific truncations reduced/eliminated cross-reactivity with Histoplasmosis patient sera
- rCTS1 is produced in *E.coli* allowing rapid, consistent, and large scale antigen production

Is the CTS1 antigen recognized by immunodiffusion positive dogs?

- Coat ELISA plates with 50ng Chitinase CTS-1 AA105-310
- Serum samples from 4 immunodiffusion positive dogs
- Negative control from Wisconsin specific pathogen free (SPF) beagles

CTS1 is Recognized by Immunodiffusion Positive Dogs



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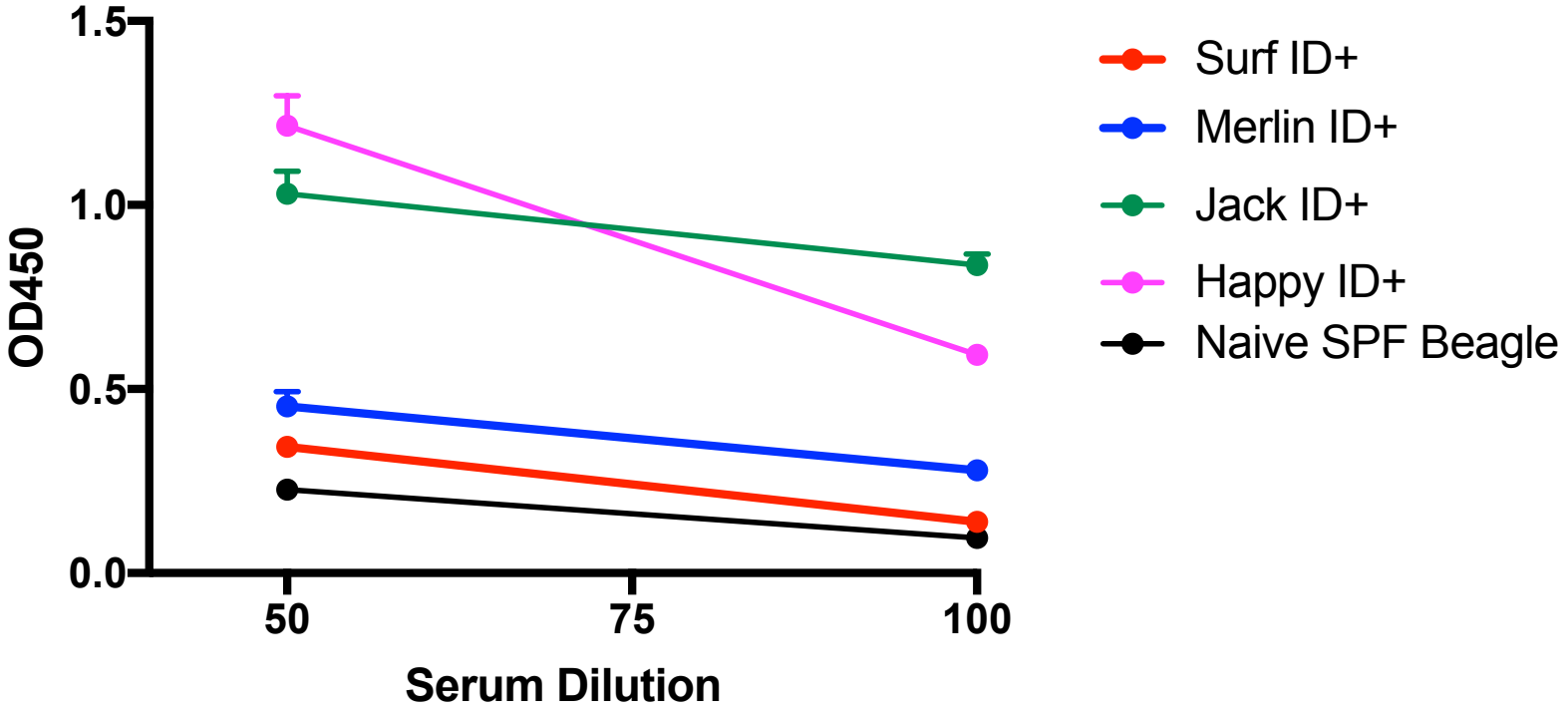


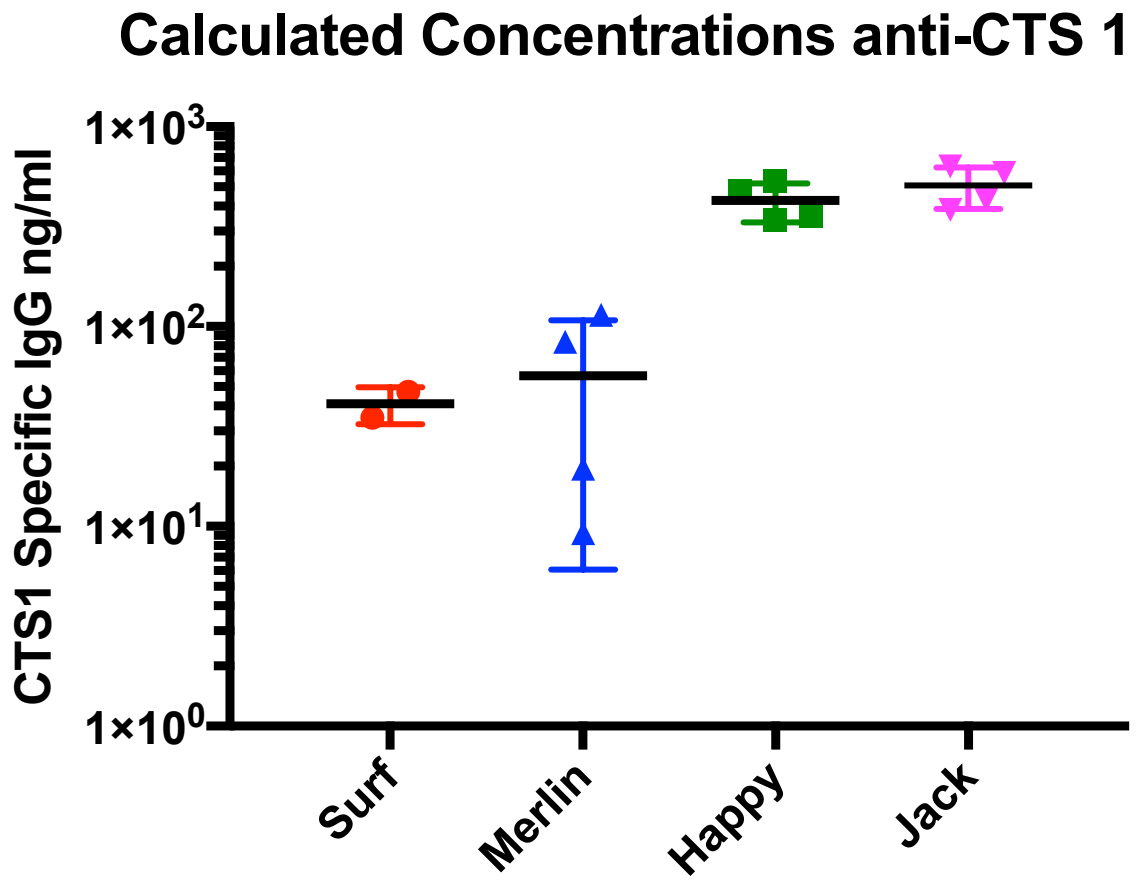
Can we make this assay quantitative to account for test variability?

- Purified dog IgG is used to develop a standard curve of IgG concentration

- Optical density readings can be plotted back to this curve and used for quantitation of antigen specific antibody

- Absolute quantitation, with appropriate standards allows for monitoring/correction of inter-test variation

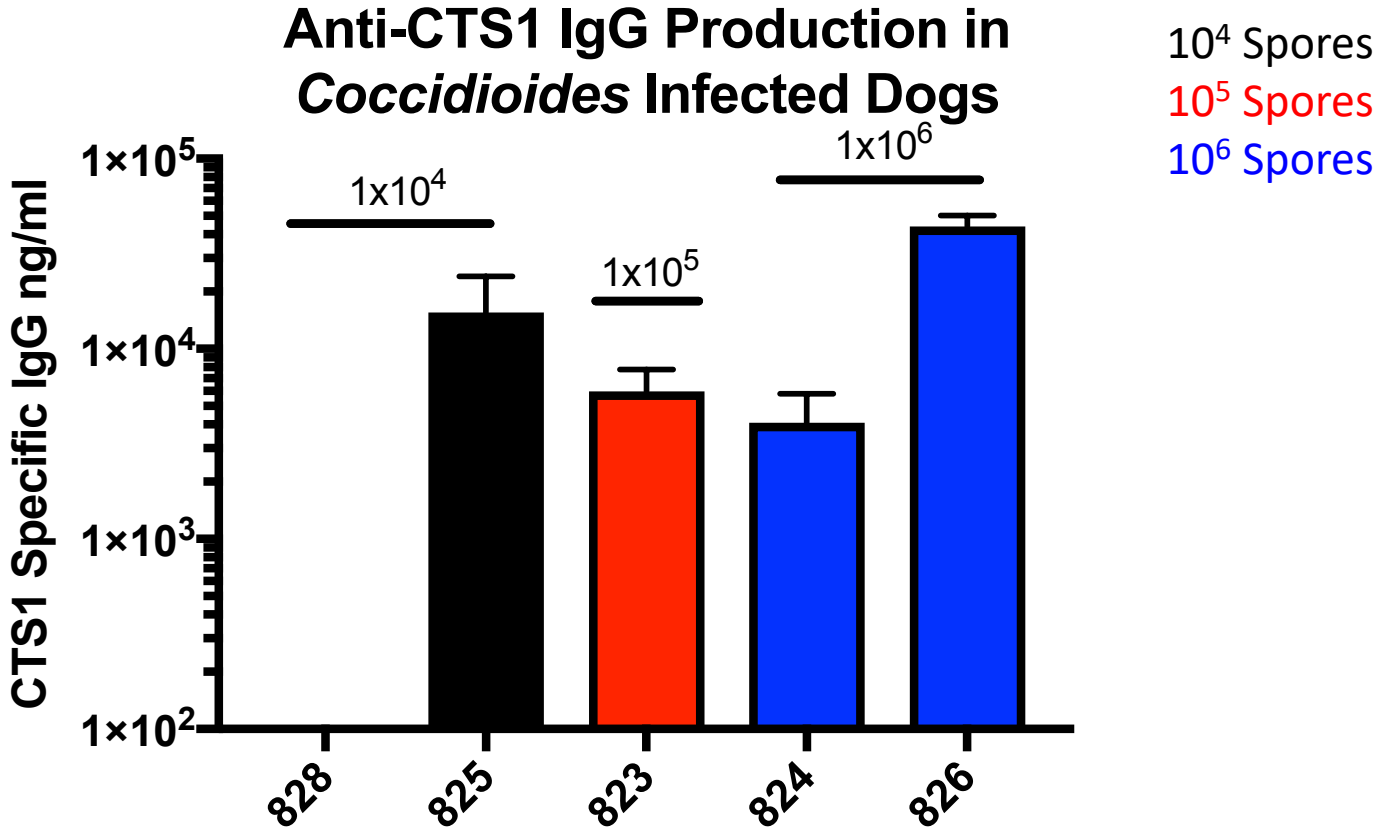
CTS1 is Recognized by Immunodiffusion Positive Dogs



Can we monitor progression of antibody production in experimentally exposed dogs?

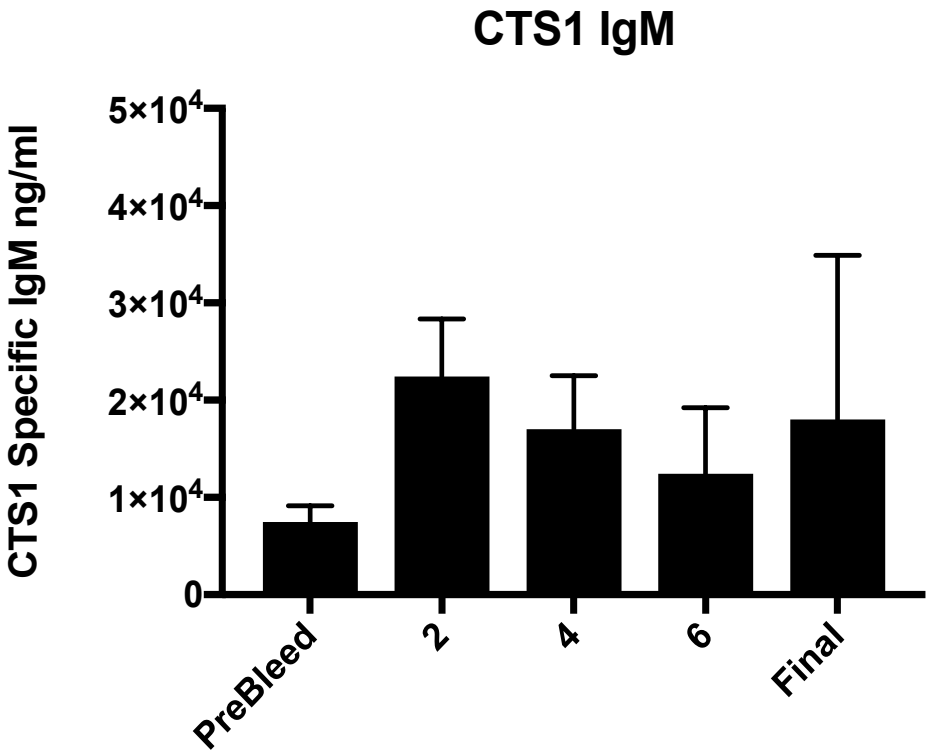
- Federal guidelines for development of vaccines require a working animal challenge model
- Along with Anivive Lifesciences we are working with Dick Bowen at Colorado State to develop a dog model of *Coccidioides* infection to test our vaccine
- 5 beagles were infected with increasing doses of WT *Coccidioides* (10^4, 10^5, 10^6 spores) via nebulizer
- Animals were infected with WT *Coccidioides* for ~8 weeks and monitored for disease outcome; weight loss, cough, respiratory rate, fever, radiography, clinical chemistries, fungal burdens and histology of tissue at necropsy

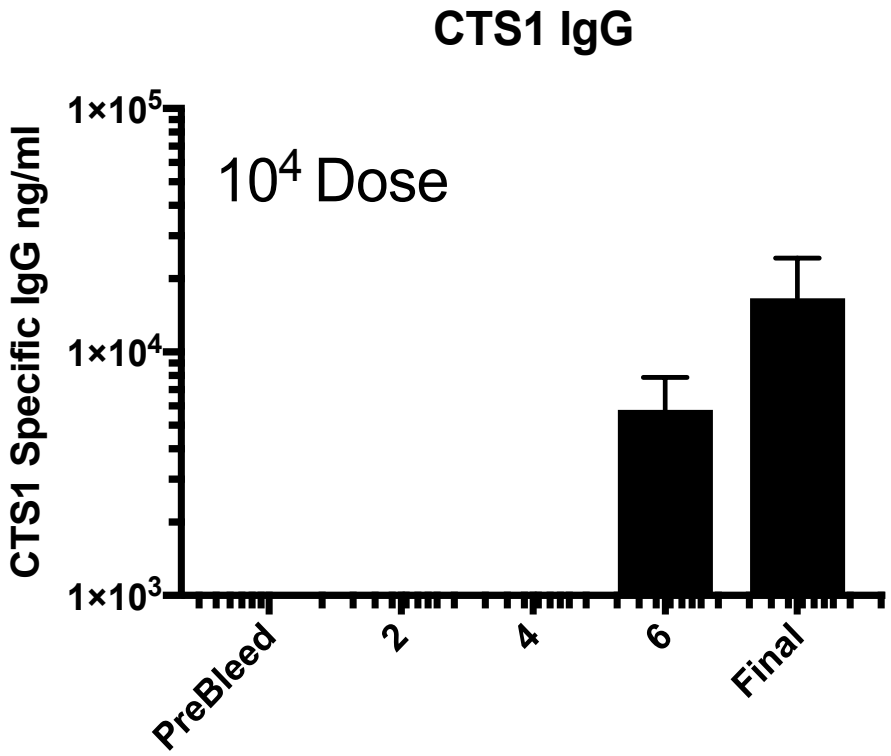
Experimental *Coccidioides* Infection Induces CTS1 specific IgG



Since we know the time of exposure can we explore the kinetics of antibody production in *Coccidioides* infected dogs?

Experimentally Infected Dogs Progressively make IgM and IgG





Conclusions

- The CTS1 antigen ELISA can be used similar to immunodiffusion to determine exposure to *Coccidioides* in dogs
- CTS1 ELISA allows for quantitation of both IgG and IgM in experimentally infected dogs over time
- Dogs experimentally infected with *Coccidioides* produce high levels of CTS1 specific IgG
- The CTS1 ELISA may prove useful in monitoring responses to experimental vaccination in dogs

Vaccinations in Mice

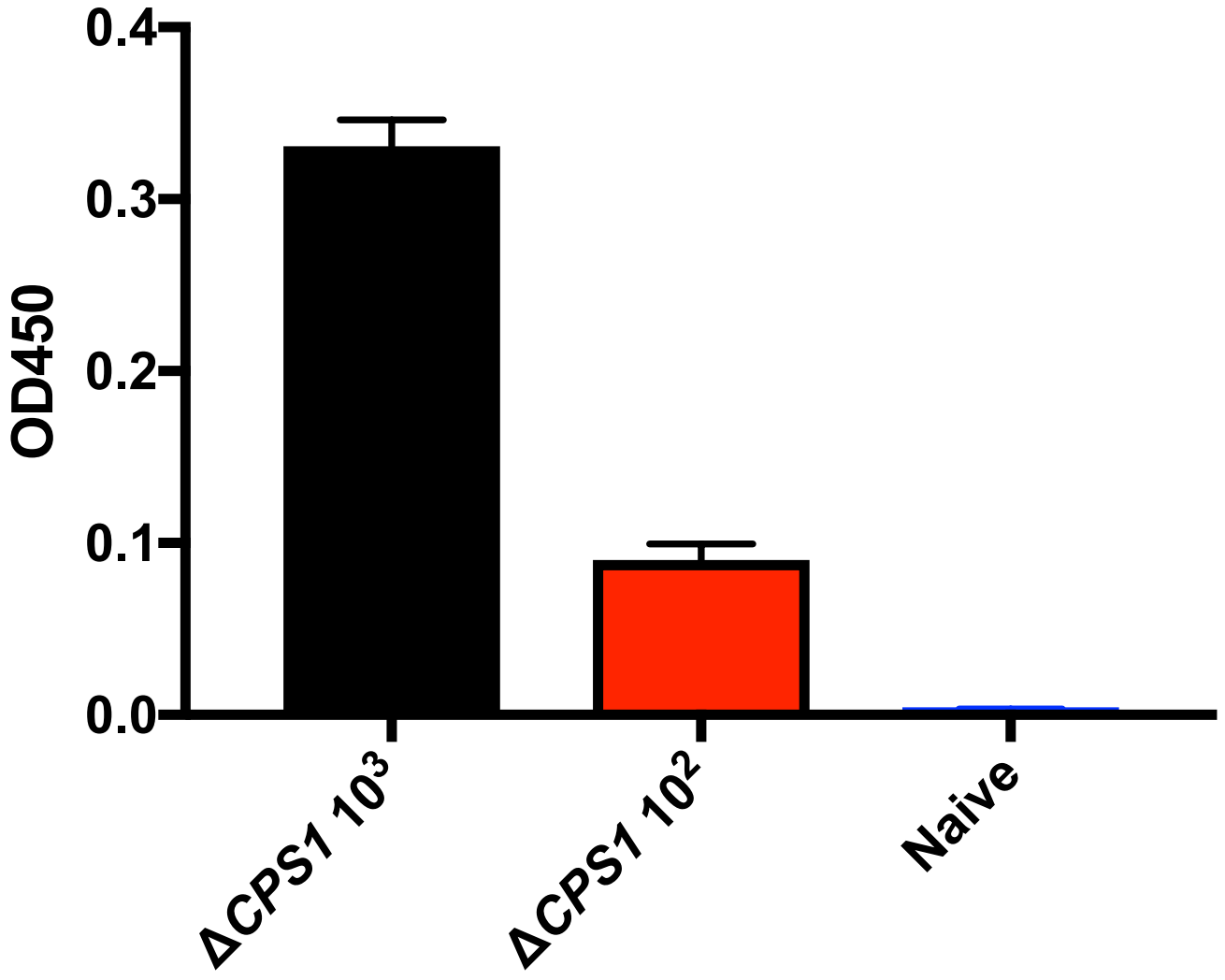
- Vaccination of mice with our live-attenuated Δ*CPS1* induces protective immunity against WT *Coccidioides* challenge

- We can protect multiple mouse strains and the protection is durable and long lasting

- Cell transfers have shown the protection to be mediated by CD4+ T cells

- Can we detect anti-CTS1 antibodies in vaccinated mice?

Mice Produce CTS-1 Specific IgG After Vaccination with Δ*CPS1*



B6 Mice
28 days after
vaccination

Acknowledgements

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  - Angela Bosco-Lauth DVM
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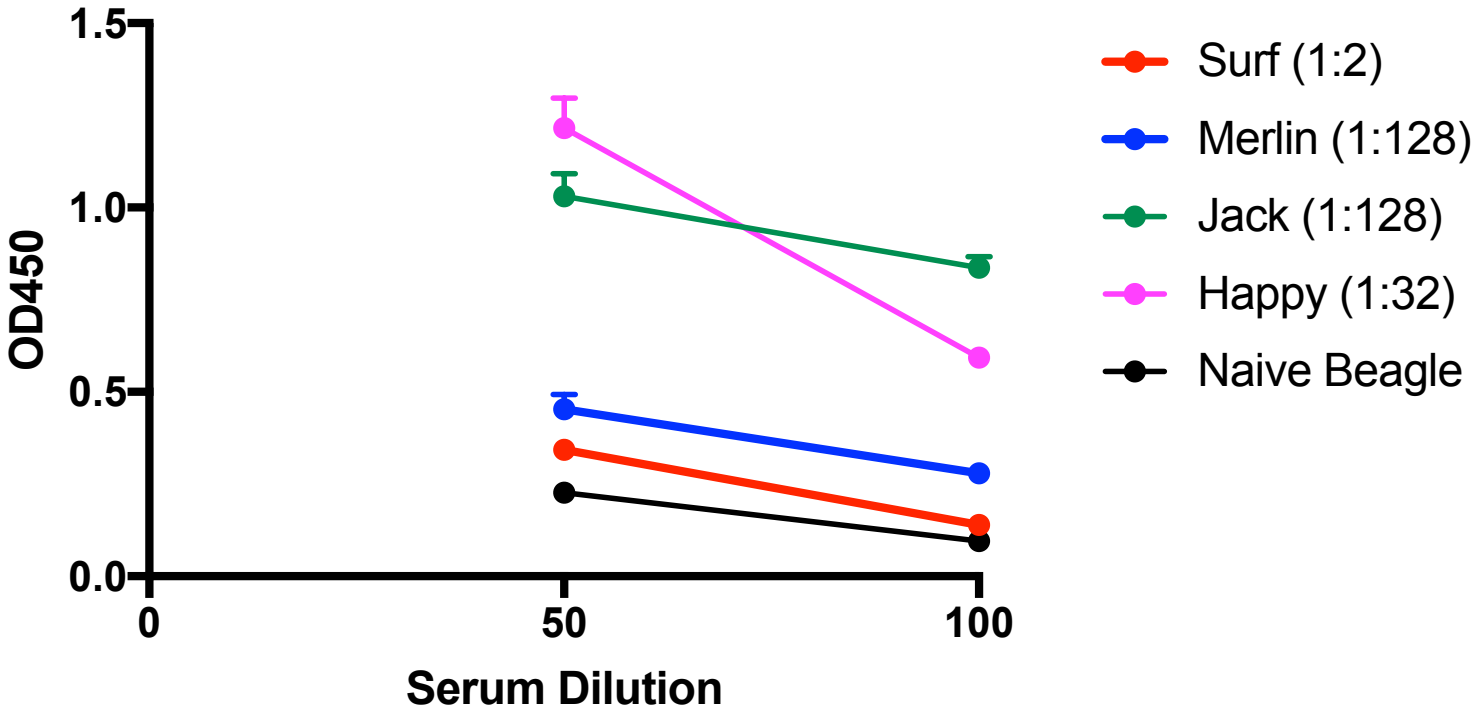
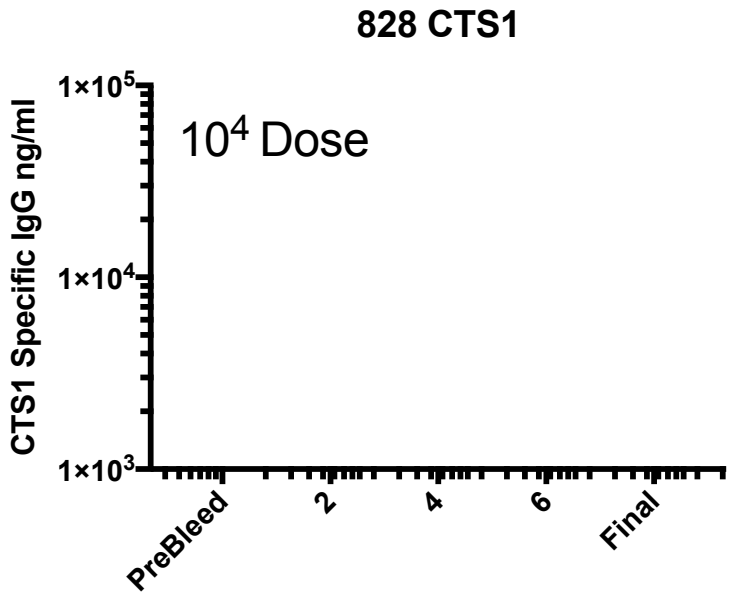
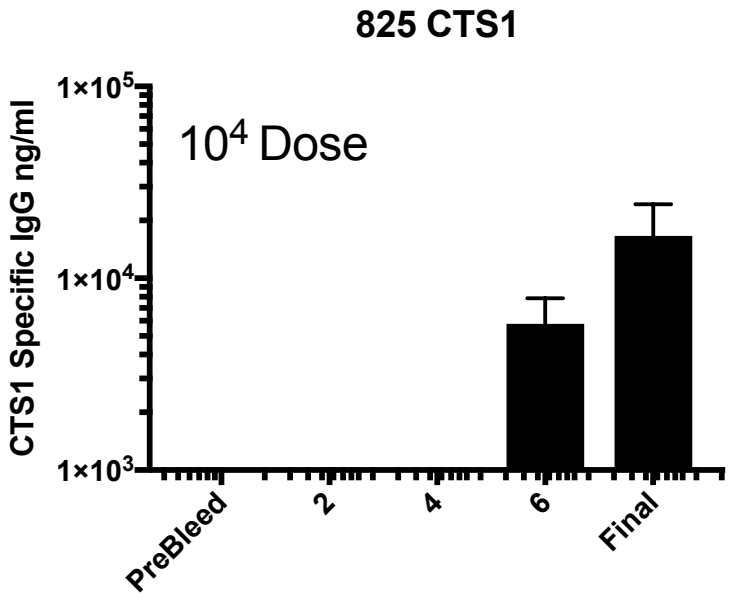
CTS1 is recognized by Immunodiffusion positive Dogs

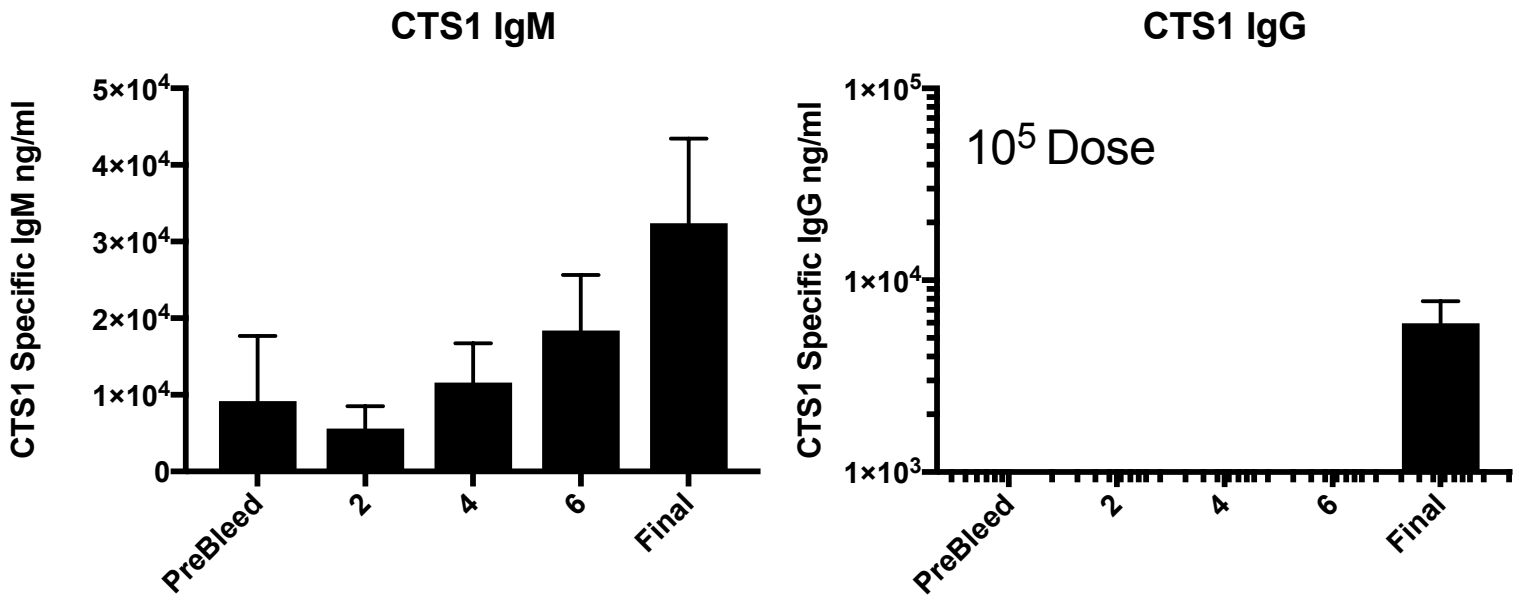


Progression of anti-CTS1 IgG Concentration





Progression of anti-CTS1 Antibodies in Mid Dose Dogs



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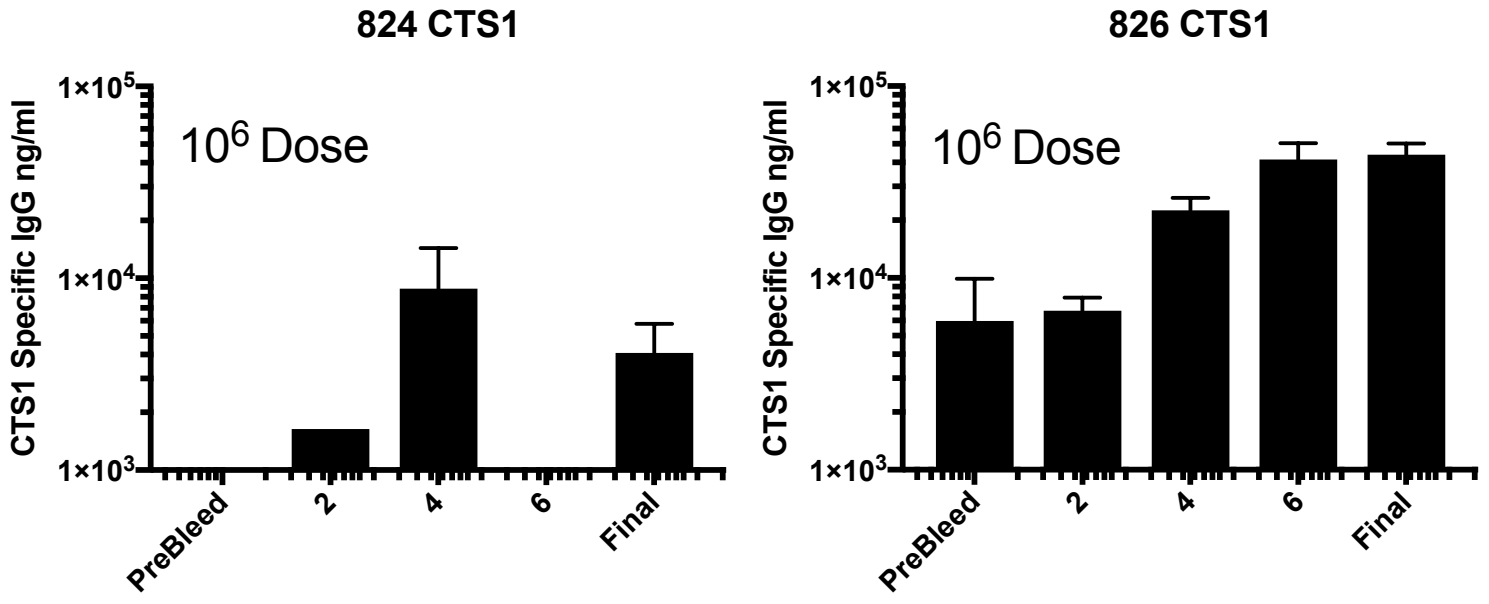
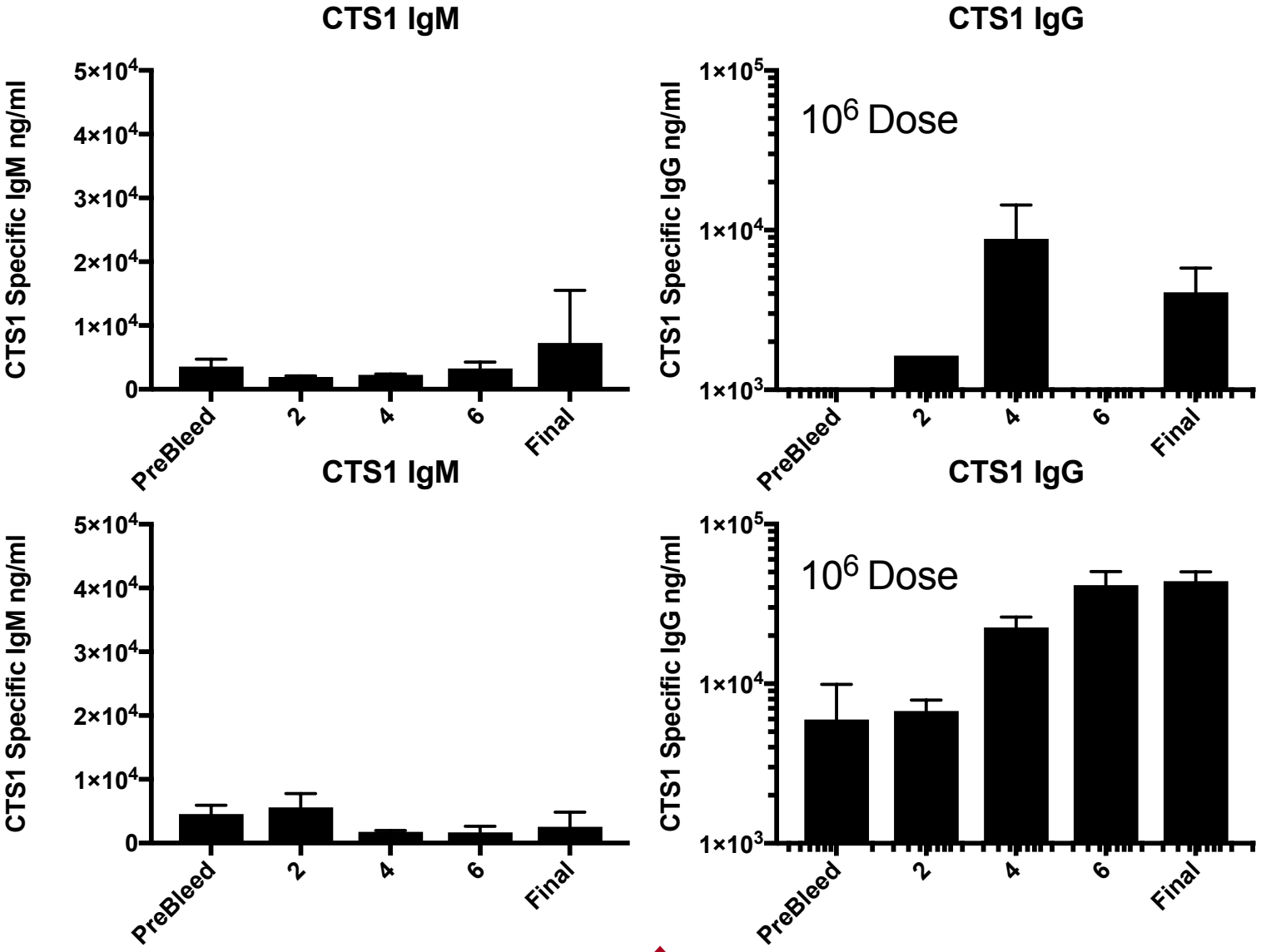


Progression of anti-CTS1 Antibodies in High Dose Dogs



Progression of anti-CTS1 IgG Concentration