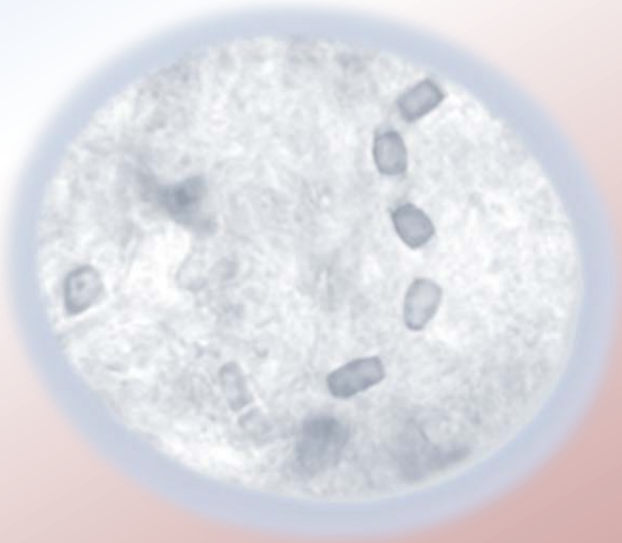
Animal Models of Coccidioidomycosis



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Cocci is Biosafety Level 3 Pathogen

- Must have animal BSL3 (ABSL3) facilities to work with this pathogen
  - Significant aerosol risk to personnel
  - Proper training
  - Class II biosafety cabinet with extra PPE for protection from aerosols or Class III BSC for intranasal infection
    - Guidelines published in the Biosafety in Microbiological and Biomedical Laboratories, 5th Edition (CDC website)

Mice

- Mice constitute the vast majority of animals used to perform preclinical efficacy studies of antifungal candidates to treat *Coccidioides* infection
- Advantages
  - Very well-established coccidioidomycosis models
  - Small and easy to handle in statistically significant numbers in ABSL3 conditions
  - Wide variety of strains, including genetically engineered for metabolic, immune system defects that mimic human disease conditions
- Drawbacks
  - Pharmacokinetics of drugs may differ significantly from humans
  - *Coccidioides* progresses rapidly in mice

Mice – Routes of administration

- Pulmonary High aerosol risk
  - This is the usual way the infection enters the human host
  - Intranasal or intratracheal in saline suspension
  - Aerosol of spores in chamber
- Intravenous – rapid, widespread model of dissemination
- Intraperitoneal – dissemination model w/ LFB common readout
- Intrathecal –meningitis, technically challenging
- Intracerebral - meningitis

Mice – Pulmonary Model

- IN - 50-100 spores in 30-50 µl isotonic saline
  - Under anesthesia
- 4 days to spherule rupture, expansion of infection
    - Some studies treat 48 hrs after pulmonary infection
    - We typically start to treat at 120 hrs, giving time for the infection to establish
      - more similar to human seeking medical care
  - 2-3 weeks to moribundity in untreated
    - Know your model to prevent cage death – thin, hunched posture, tachypneic, weak, dehydrated by skin turgor
    - Estimate won’t survive another 24 hrs

Mice – Disseminated Models

- Intravenous
  - ~50 spores - deaths generally after day 12 (Clemons, et. al., 1984)
    - In published studies, treatment is usually instituted within 48 hrs p.i.
- Intraperitoneal
  - Usually requires more arthroconidia to initiate infection by this route
  - Technically easy to perform at BSL3, reduced aerosol risk vs. IN
  - 2-3 week infection model, similar to IN, IV
  - Granulomas of cranial mesentery, spleen, liver, dissemination to lungs

Mice – Disseminated Models

- Intracerebral, intrathecal – meningitis models
  - Clinical signs 6-8 days p.i., deaths usually start by day 8
  - Paresis, paralysis, ataxia, circling, head tilt, seizures, obtundation
    - Need to be evaluated twice daily for animal welfare reasons once onset of clinical signs
  - Generally institute treatment within 48 hrs p.i.
  - Assess fungal burdens or survival
    - Assess lungs and spleens, not just brain and spinal cord

Assessment of Mouse Models

- Survival (moribundity)
- Organ fungal burdens
  - Common assessment, may be the primary measure
  - Eradication vs. reduction in colony-forming units (cfu)
  - Quantitate cfu by 10-fold serial dilutions of homogenized tissues
    - Usually lung and spleen
    - Can qualitatively assess dissemination by incubating whole organs on plates
- Clinical signs, body weight
  - Body weight is a good indicator of progression of infection even before other signs become visible

Rabbits

- Clemons and colleagues developed a reliable model of coccidioidal meningitis/arteritis in rabbits
  - Cisternal infection
  - Size of animal allows some serial cisternal sampling of CSF
  - Post mortem analysis can include histopathology, fungal burden of spinal cord, brain, CSF parameters
    - Meningitis/arteritis in rabbits appears to be a good model for humans
- Understand drug PK in this species
  - Less utilized than mice in development
- Increased cost of animals, labor, housing
  - Potentially fewer animals, less robust statistics
- Facilities need to be able to manage larger model at ABSL3

Nonhuman Primates

- Experimental - possible but really costly
  - Intratracheal infection with suspension of arthroconidia
    - Recommend a nebulizer after working out a dog model (Soffler, Bosco-Lauth et al. 2012)
  - Current trends seem to support that most drugs at this stage would be implemented in some kind of human trial
- There could be opportunities to treat naturally infected NHPs in primate centers within the endemic area
  - Administration and monitoring offer challenges

Naturally Infected Dogs

- This is an interesting preclinical assessment of drug efficacy
  - In endemic region such as southern and central AZ, very high case load and it is not difficult to enroll cases
- Possible to assess pulmonary disease improvement with 30-60 days of treatment
  - Radiographs, serology, CBC/serum chemistries
  - Dog owners are both grateful and dedicated
    - <10% dropout rate in 2 studies I have done, compliance is very good
- Drawbacks
  - Cost, time to perform, statistically significant numbers, may not drive your development
    - Often get descriptive data from such a study
- Potential advantages
  - Naturally occurring disease in model that is already sick, like the human that presents for medical care
  - Oral administration using forms of drug that would be used in people
  - Might be preclinical PK/toxicology in this species so you know how to dose them

Summary

- The mouse model is the work horse of pre-clinical testing of antifungal drug candidates
  - Small and cost-effective animal for studies that have to be run at BSL3
- The rabbit is a well-developed meningitis/arteritis model that can be used
  - Costs, facilities and technical expertise with the procedures are a drawback
- Larger animal models, both naturally infected and laboratory induced, exist
  - Weigh benefits and costs of these