Validation of an Indirect Fluorescent Antibody Test for *Sarcocystis neurona* infection in California sea lions

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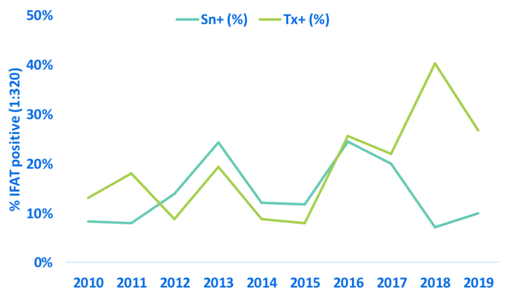
Objective

To evaluate IFAT test performance, identify cases of *Sarcocystis neurona* infections in stranded California sea lions (CSL) and control animals followed by analysis of the correlation between the gold-standard testing approach of histopathology and molecular sequencing against the SarcoFlour IFAT.

Rationale

- Seroprevalence in CSL has increased from 5-6% (1998-2009) to 14% (2010-2019)



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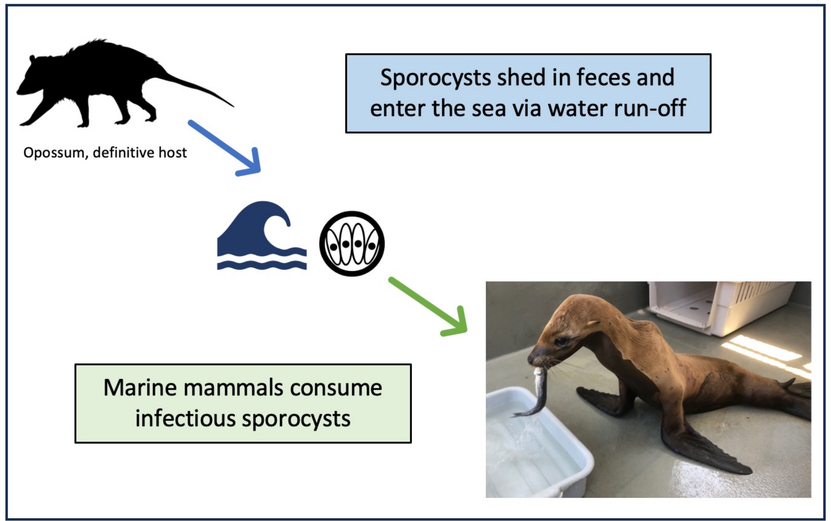
- Infection can result in polyphasic rhabdomyositis and death; prognosis is guarded even with treatment in CSL
- IFAT diagnostic test advantages: low-cost, quick, better informs treatment plans but not yet validated in CSL

Land-to-Sea Transmission



Methods

Indirect Fluorescent Antibody Test

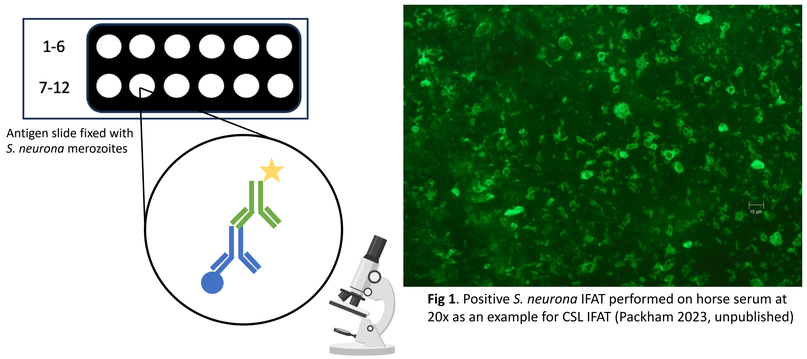


Fig 1. Positive *S. neurona* IFAT performed on horse serum at 20x as an example for CSL IFAT (Packham 2023, unpublished)

Histopathology

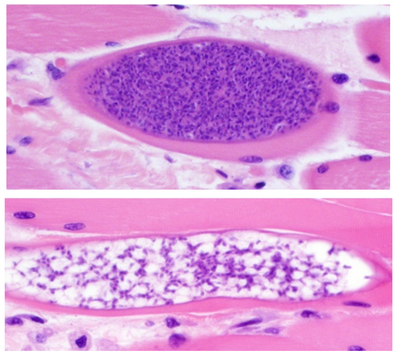


Fig 2. Mature and immature *S. neurona* cyst within a CSL myocyte (Seguel et al. 2019)

Molecular Sequencing

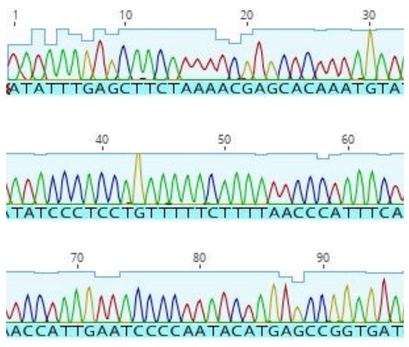


Fig 3. Chromatogram for *S. neurona* ITS1 sequence from CSL (Sinnott 2023, unpublished)

Results

Progress to date

- **20 sea lion cases** and **31 sea lion controls** have been identified. The target sample size is 40 cases and 80 control animals.
- **Cases** are animals with suspected myopathy confirmed by necropsy and evidence of *S. neurona* cysts on histopathology; and will be confirmed with molecular identification.
- **Controls** are animals with a non-protozoal cause of death and no evidence on histopathology of *S. neurona* infection.
- The Kappa statistic on comparing test performance is **0.92**.

IFAT titer to call a sample positive

- **1:320 titer** maximizes sensitivity and specificity at 95 and 96.8%, respectively, with area under the receiver-operating curve of 0.98.

Discussion

Key Findings

- Kappa statistic compares agreement between histopathology and IFAT in classifying a case or control as positive or negative. A value of 0.92 suggests that there is good agreement between the two.
- Preliminary data suggest that an IFAT titer of 1:320 is likely an appropriate threshold for calling a sample positive.

Discussion

- Validation of the low-cost, ante-mortem IFAT diagnostic tool is important due to the increase of sarcocystosis cases in stranded CSL at TMMC and other stranded marine mammal facilities.
- Postulated factors contributing to increased *S. neurona* infections in CSL:
  - Increase in parasite prevalence associated with changing environmental conditions
  - Shift in prey consumption based on altered historic prey availability

Limitations

- Serologic antibody titer does not equal active disease
- Time constraints on TMMC and UCD labs with high-volume case loads
- Financial restraints in studying wildlife diseases

Future Directions

- Risk factors associated with clinical disease and outcome
- Environmental source of infection (e.g. food source, microplastics, etc.) and time until symptom onset in CSL

Acknowledgements

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Miller, M.A. et al. 2002. Evaluation of an Indirect Fluorescent Antibody Test (IFAT) for demonstration of antibodies to *Toxoplasma gondii* in the Sea otter (*Enhydra lutris*). J. of Parasitology, 88(3):549-599.

Seguel et al. 2019. Polyphasic Rhabdomyositis in California Sea Lions (Zalophus Californianus): Pathology and Potential Causes. Veterinary Pathology. 56(4):619-629.

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