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)
- 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

Methods

Indirect Fluorescent Antibody Test

- IFAT titer to call a sample positive
- Controls

Histopathology

- Mature and immature S. neurona cysts within a CSL myocyte (Seguel et al. 2010)

Molecular Sequencing

- Chromatogram for S. neurona ITS1 sequence from CSL (Sinnott 2023, unpublished)

Fig 1: Presence of S. neurona IFA performed on horse serum at 25x as an example for CSL IFAT (Pashanth 2023, unpublished)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

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