What has Toxoplasma in Sea Otters Taught Us about the Risks and the One Health Approach to Global Public Health

Clinician Outreach and Communication Activity (COCA) Webinar
Tuesday, February 6, 2018
At the end of this webinar, the participants will be able to:

• Describe the life cycle of *Toxoplasma gondii* and the importance of the oocyst in transmission.

• Explain the different mechanisms for oocyst accumulation in the ocean where sea otters become infected.

• List possible steps to reduce pathogen pollution in coastal habitats.

• Define a keystone species and discuss what we have learned about ecosystem health and human health risks from studying sea otter health.
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Today’s Presenter

Heather Fritz, DVM, PhD
University of California, Davis
What has Toxoplasma in sea otters taught us about risks and the One Health approach to Global Public Health

Heather Fritz DVM, PhD
University of California, Davis
Global Health
Area of study, research and practice with a priority on improving health and achieving health equity for people worldwide.

Transcend national borders
Protect against global threats
One Health

An interdisciplinary approach to solving specific, complex problems that arise at the interface of animals, humans and the environment.
SOUTHERN SEA OTTER
(Enhydra lutris nereis)
The California Southern Sea Otter

- Federally-listed threatened species
- Found only along the central coast of California
- Total population ~3,000 animals
Otters are a Keystone Species
Importance of sea otters to coastal ecosystem

• Kelp forests protect coast from erosion and provide habitat

• Sea urchins destroy kelp

• Sea otters prey on sea urchin
Toxoplasma gondii

How is a terrestrial pathogen causing such profound disease in marine mammals?

What is killing California sea otters?

Most important: Infectious diseases

→ protozoal parasites

Seroprevalence of *T. gondii*:

~38% of 257 live otters

~52% of 305 dead otters

Based on IFAT - Miller et al 2002
California sea otters have been dying in alarming numbers for several years, raising concerns about the future of the species. The deaths have been blamed on pollution, disease, and human interference. A recent study suggests freshwater runoff containing *Toxoplasma gondii* may be partly to blame.

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Could cat waste be killing sea otters?

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A parasite carried by cats is killing off sea otters, a veterinary specialist has told a major US science conference.

The Californian researcher has called for owners to keep their cats indoors.

Cat faeces carrying *Toxoplasma* parasites wash into US waterways and then into the sea where they can infect otters, causing brain disease.

The parasite is familiar to medical researchers, as it can damage human foetuses when expectant mothers become infected while changing cat litter.

The most likely source of infection for sea otters is their cat waste.
Protozoal parasites important cause of death in sea otters

*Toxoplasma gondii*
- Definitive host = cat

*Sarcocystis neurona*
- Definitive host = Opossum

Terrestrial hosts. Terrestrial pathogens.
Toxoplasma gondii

Discovered in 1908 in the North African ‘gundi’ rodent

Toxo = ‘bow’ plasma = ‘form’

- Protozoan
- Apicomplexa
- Obligate intracellular parasite
  - Interacts with the host via secreted proteins
Toxoplasma gondii

Broad host range

Capable of infecting virtually any nucleated cell

Able to cross several anatomical barriers

Only one known definitive host
Three routes of infection

1. Ingestion of sporulated oocysts
2. Ingestion of tissue cysts in undercooked meat
3. Transplacental transmission

Sporozoites  Bradyzoites  Tachyzoites
Lifecycle

Asexual expansion = amazingly efficient

‘Female’ macrogamete

‘Male’ microgamete

Diploid zygote

Unsporulated oocyst = hundreds of millions!
Sporulation occurs in the environment
• 2 sporocysts
• 4 sporozoites in each sporocyst

Intermediate hosts
Oocysts are extremely environmentally-resistant
Oocysts are tough!

- Chlorine
- Ozone
- Ultraviolet Radiation
- Quaternary ammonium compounds
- Ethanol
- 10% formalin
- 2% Sulfuric acid (H$_2$SO$_4$)
One Health Approach:
How is a terrestrial pathogen causing such profound disease in a marine mammal?

1. Develop a model for the transport of oocysts from land to sea.
2. How are oocysts encountered by otters in ocean.
3. What structures/factors are responsible for the remarkable environmental resistance of the oocyst?
4. How can we better identify where oocysts accumulate in the coastal environment to serve as a source of infection to otters?
Modeling the transport of *Toxoplasma* oocysts from land to sea

Liz VanWormer
DVM, PhD
Oocysts per cell = number of cats * infection prevalence * oocysts shed
Differences in infection and shedding
Both domestic and wild felids contribute *Toxoplasma* oocysts to terrestrial coastal environments.
Modeling oocyst transport in freshwater runoff
Figure 2: Spatial distribution of *Toxoplasma gondii* oocysts carried to the ocean via freshwater runoff (light yellow to red shading).
How do coastal development and precipitation influence pathogen flow from terrestrial to aquatic environments?
Land use change has a major impact on increased oocyst delivery to the ocean.
Coastal development and precipitation drive pathogen flow from land to sea: evidence from a *Toxoplasma gondii* and felid host system


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Ecological epidemiology
Infectious diseases
How are oocysts encountered by otters in the ocean

Karen Shapiro
DVM, MPVM, PhD
The puzzle: Toxoplasmosis in California sea otters

It’s a big ocean out there...

How can so many otters become infected with a terrestrial parasite?
Wetlands and Water Quality

- Wetland water effluents have reduced contaminants

- Physical processes
  - Sedimentation
  - Adsorption and straining

- Biological processes
  - Flora and fauna
  - Metabolism and predation

↓ Pesticides
↓ Heavy metals
↓ Pathogens
Effect of Estuarine Wetland Degradation on Transport of *Toxoplasma gondii* Surrogates from Land to Sea

Karen Shapiro, Patricia A. Conrad, Jonna A. K. Mazet, Wesley W. Wallender, Woutrina A. Miller, and John L. Largier
A mechanism for pathogen concentration in the ocean: marine snow

- Clumps of organic and inorganic material
- Snow tends to sink – accumulation zones may determine risk
- Food for invertebrates = entry into marine food webs
Where, when and how does marine snow form?

• Water salinity, currents, particle size and...
  • Transparent Exopolymer Particles (TEP) - Invisible, sticky, gel-like particles – the glue matrix of snow
  • Produced by phytoplankton, cyanobacteria, and...kelp

Hypothesis:
Association of *T. gondii* oocysts with marine snow will increase as a function of TEP
**Aggregation in TEP-spiked seawater**

**Objective:** Test for the association of *T. gondii* with marine snow in seawater spiked with increasing concentrations of alginic acid => TEP produced by kelp

**Findings:** Increased concentrations of TEP typically present in sea otter habitat enhance the association of *T. gondii* oocysts with marine snow
Unraveling the puzzle – Beyond snow

- Many invertebrate species that serve as prey for otters eat snow...
- But only snails identified as a risk factor for sea otter exposure to *T. gondii*
  - 12 X odds of *T. gondii* infection
  - Turban snails are kelp grazers
Association of *T. gondii* with kelp

**Objective:** Can *T. gondii* oocyst surrogates adhere to kelp surfaces?

**Findings:** Up to 30% of *T. gondii* surrogates attach to kelp blades due to TEP coating on kelp.
From kelp to otters – The snail connection

• **Objective:** Can marine snails serve as mechanical hosts for *T. gondii*?

• **Findings:**
  • Retention of oocysts up to 11 days
  • Concentration 2-3 orders of magnitude greater than seawater
**Conclusion**: Snails facilitate *T. gondii* exposure to otters

- Prolong exposure period
- Bio concentrator
Unraveling the puzzle

How can a land parasite infect so many otters?

*T. gondii* oocysts can concentrate in coastal ecosystems through two mechanisms:

1) Enrichment in marine snow

2) Association with kelp surfaces

Snails as mechanical hosts

Snail-specializing otters at greater odds of infection
Terrestrial to Marine System
Oocyst Transport

Shapiro et al 2014
Questions

1. What structures/factors are responsible for the remarkable environmental resistance of the oocyst?

*Hypothesis: Proteins present in one or both layers of the oocyst wall confer environmental resistance.*

2. Where do oocysts accumulate in the environment to serve as a source of infection to humans and animals?

*Hypothesis: Immunomagnetic separation can be used to concentrate Toxoplasma oocysts in water to identify sources of oocyst accumulation.*
Oocyst wall formation

- WFB I – Outer layer
- WFB II – Inner layer

Bleach strips the outer layer of the oocyst wall
Approach

Microarray

Day 0
Unsporulated

Day 4
Mid sporulation

Day 10
Sporulated

Compare same strain (M4):

*in vitro* tachyzoites

*in vivo* bradyzoite cysts

Mass spectrometry

Day 10 oocysts
± bleach treatment

Walls

Sporocysts (sporozoites)
Results: Top 15 oocyst-specific transcripts

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Tyrosine rich protein (TyRP)
Tyrosine-rich proteins are abundantly expressed in oocysts.

### Table: Mass Spectrometry Spectral Counts

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Further investigation of a tyrosine-rich protein

Oocyst walls are autofluorescent

Oocyst walls believed to be composed of tyrosine cross-linked proteins

Dityrosine:
- Autofluorescence
- Sclerotization (hardening)
  - Sea urchin eggs
  - Insect resilin
  - Yeast cell walls
  - Coccidian oocysts
First identification of a tyrosine-rich protein in the oocyst wall

Pre-immune serum

Immune serum
And also in the in macrogamete!

TyRP1 localizes to the macrogametes – role in oocyst wall formation?

David Ferguson
University of Oxford
2. Where do oocysts accumulate in the environment to serve as a source of infection to humans and animals?

*Hypothesis: Immunomagnetic separation can be used to concentrate Toxoplasma oocysts in water to identify sources of oocyst accumulation.*
Oocyst detection in water

EPA-Approved method to test water for Cryptosporidium and Giardia: Immunomagnetic Separation and Immunofluorescence Assay

1. Develop mAb(s) to oocyst wall

1. Couple mAb to paramagnetic beads

2. Co-incubate mAb-coupled beads with concentrated water sample containing oocysts

3. Retain beads + oocysts on magnet

4. Elute oocysts off of beads

5. Detect oocysts by DFA

[Images of DAPI, Ms-α-oocyst, and Oocysts + beads]
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ToxoDB
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Brian Brunk
Brian Gregory
What we’ve learned . . . . .

Studies are more powerful when we integrate information across disciplines

ecosystem-level studies + population health and laboratory studies = Tackle complex problems
Sea otters are sentinels of environmental contamination.

“pathogen pollution”
One Health Approach

Human

Ecosystem

Domestic Animals

Wildlife
Thank You

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