Toxoplasmosis

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Toxoplasma gondii

Toxoplasma gondii is an obligate, intracellular, parasitic protozoan that causes the disease toxoplasmosis. T. gondii is capable of infecting virtually all warm-blooded animals, although felids such as domestic cats are the only known definitive hosts in which the parasite can undergo sexual reproduction.
Major forms of *T. gondii* in different stages

- **Tachyzoite:**
  - Rapid replication
  - Induce host-immunity
  - Related with pathology
  - Acute toxoplasmosis
  - Influenza-like symptom

- **Bradyzoite (cyst):**
  - Slow replication
  - Found in muscle and brain
  - Chronic infection
  - 30–50% of human population
  - Foodborne disease

- **Oocyst:**
  - Highly infectious
  - Found in faeces of cat
  - Infectious source
  - in environment

*T. gondii* infection into host cell

- **Attachment:**
  - Secretion of microneme proteins

- **Invasion:**
  - Secretion of rhoptry proteins

- **Formation of parasitophorous vacuole (PV):**
  - Secretion of dense granule proteins

- **Manipulation of host cell?**

Intracellular parasites

Extracellular parasites
Toxoplasmosis

**Acute toxoplasmosis**
In healthy adults
- asymptomatic
  (influenza-like: swollen lymph nodes, or muscle aches and pains)

In young children and immunocompromised people
  (HIV/AIDS, chemotherapy, organ transplant)
- develop severe toxoplasmosis such as damage of the brain
  (encephalitis) or the eyes (necrotizing retinochoroiditis).

In infants infected via placental transmission
- nasal malformations

**Latent toxoplasmosis**
In most immunocompetent people
- The infection enters a latent phase, during which only bradyzoites are present forming cysts in nervous and muscle tissue.

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**T. gondii infection increased incidence rate of major mental disease**

**Human case:** *T. gondii* infects about one-third of the population.

Risk factor
- Schizophrenia (Alipour et al., 2011)
- Alzheimer’s disease (Kusbeci et al., 2011)
- Personality (Nielsen et al., 2011)

**Rodent models:**
- Host: Decreasing aversion of cat odors (Vyas et al., 2007)

(Urpublis data in Nishikawa lab)
Toxoplasmosis in animals

Toxoplasmosis may cause focal or generalized lymphadenitis, encephalitis, pneumonitis, myocarditis, and retinochoroiditis.

Cats usually do not develop any clinical disease associated with oocyst shedding.

Congenital infection with *T. gondii* can cause neurologic disease, birth defects, stillbirth, and ocular disease in particularly sheep and goats.

Hemorrhage  |  LN dropsy  |  Pneumonia

Diagnosis of toxoplasmosis

The diagnosis of toxoplasmosis is typically made by serologic testing.

A test that measures immunoglobulin G (IgG) and IgM is used to determine.

Diagnosis can be made by direct observation of the parasite in stained tissue sections, cerebrospinal fluid (CSF), or other biopsy material.

Parasites can also be isolated from blood or other body fluids (for example, CSF)

Molecular techniques that can detect the parasite’s DNA in the amniotic fluid can be useful in cases of possible mother-to-child (congenital) transmission.

Ocular disease is diagnosed based on the appearance of the lesions in the eye, symptoms, course of disease, and often serologic testing.

**Detection system:**
- Sabin–Feldman dye test (gold standard)
- Latex agglutination test
- ELISA
- PCR
- Immunohistochemical analysis
- IFAT

Useful for livestock animals
Distribution of *Toxoplasma* in women of childbearing age

Modified from data of www.prevebdawareness.org

Distribution of *Toxoplasma* in cats

Modified from data of Jones and Dubey, 2010
Land and sea contamination by *T. gondii* oocysts

In the USA
There are approximately 78 million domestic cats and 73 million feral cats.

It is probable that nearly every farm in the USA has cats: a mean of 8.5 cats per farm, with a mean of six seropositive cats on each farm (Weigel et al., 1999).

*Toxoplasma gondii* oocysts were detected in cat feces, feed, soil or water samples on six farms (Dubey et al., 1995 and Weigel et al., 1999).

If one assumes a 30% seropositivity of 151 (78 domestic and 73 feral) million cats and a conservative shedding of 1 million oocysts per cat then there will be enormous numbers of oocysts (50 million × 1 million) in the environment.

An annual burden of 94–4671 oocysts/m² in California (Dabritz et al., 2007 and Dabritz et al., 2007b).

*Toxoplasma* infection is confirmed in sea otters, dolphins, seals and walruses.
Distribution of Toxoplasma in livestock

Modified from data of Dubey, 2009

Show the data of Toxoplasma infection in human and animals in Indonesia
Case study in Indonesia

Seroprevalence of antibody against *Toxoplasma gondii* in livestock animals from the west part of Java, Indonesia

ELISA based on TgGRA7
Sample number: 598 (cattle)
205 (pig)

What is the source of the infection?
The infection source may be from environment (oocyst from feces of cat) or food (Pig meat?).

[City A]
Latex agglutination test
Sample number: 320
Number of positive: 72
% of positive 22.5%

(Unpublished data in Nishikawa lab)
### Table 1

<table>
<thead>
<tr>
<th>Category</th>
<th>District</th>
<th>farm ID</th>
<th>No. tested</th>
<th>No. of positive</th>
<th>Seroprevalence** (%)</th>
<th>95% CI (%)</th>
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<tbody>
<tr>
<td>Cattle</td>
<td>Tangerang</td>
<td>#1</td>
<td>24</td>
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<td>3</td>
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<td>Kuningan</td>
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<td>Pigs</td>
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<td>Bekasi</td>
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<tr>
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<td>Karawang</td>
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<td>#18</td>
<td>36</td>
<td>1</td>
<td>2.8a</td>
<td>0.1-14.5</td>
</tr>
</tbody>
</table>

CI, confidence interval

*Seroprevalence of pigs was statistically higher than that of cattle (P<0.05)

**Within the animal species, prevalence rates with different superscript are statistically different (P<0.05)

(Unpublished data in Nishikawa lab)
What can we say from this result?

The warm and humid weather throughout year across the Indonesia country allows _T. gondii_ oocysts to have longer viability.

Although contact with _T. gondii_ oocysts in the environment is the only infection route for livestock, the transmission by cysts from various resources including meat of other animal species is also possible for human.

The differences detected in seroprevalences of toxoplasmosis in different locations were not due to the climate variation among the locations but due to the management level or the different condition of environmental hygiene in the each locations (farms).

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### Distribution of *Toxoplasma* in Indonesia

<table>
<thead>
<tr>
<th>Location</th>
<th>Seroprevalence Rate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jakarta</td>
<td>70%</td>
<td>(Terazawa et al., 2003)</td>
</tr>
<tr>
<td>Surabaya</td>
<td>58%</td>
<td>(Konishi et al., 2000)</td>
</tr>
<tr>
<td>Sidoarjo, East Java</td>
<td>64%</td>
<td>(Uga et al., 1996)</td>
</tr>
<tr>
<td>Bali</td>
<td>3.1%</td>
<td>(Chomel et al., 1993)</td>
</tr>
<tr>
<td>Irian Jaya</td>
<td>34.6%</td>
<td>(Gandahusada et al., 1980)</td>
</tr>
<tr>
<td>South Sulawesi</td>
<td>62%</td>
<td>(Carney et al., 1978)</td>
</tr>
</tbody>
</table>
Distribution of *Toxoplasma* in Indonesia

**Seroprevalence rate in animal (Gandahusada, 1991. Review)**
- Cats: 35-73%
- Dogs: 75%
- Pigs: 11-36%
- Goats: 11-61%
- Cows: less than 10%

Contact with *T. gondii*-infected animals is a risk factor for the infection in human.

Oocysts from cats may be a risk for the infection from the Environment.

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Prevention and control (General) from CDC

**Reduce Risk from Food**
To prevent risk of toxoplasmosis and other infections from food:

- **Cook food to safe temperatures.**
  USDA recommends:
  - **For Whole Cuts of Meat (excluding poultry)**
    Cook to at least 145°F (63°C) for three minutes (rest time) before carving or consuming.
  - **For Ground Meat (excluding poultry)**
    Cook to at least 160°F (71°C).
  - **For All Poultry (whole cuts and ground)**
    Cook to at least 165°F (74°C) for three minutes (rest time) before carving or consuming.

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Prevention and control (General)

Reduce Risk from Food (continued)
To prevent risk of toxoplasmosis and other infections from food:

Freeze meat for several days at sub-zero (0° F, -18° C) temperatures before cooking to greatly reduce chance of infection.

Peel or wash fruits and vegetables thoroughly before eating.

Wash cutting boards, dishes, counters, utensils, and hands with hot soapy water after contact with raw meat, poultry, seafood, or unwashed fruits or vegetables.

Prevention and control (General)

Reduce Risk from the Environment
To prevent risk of toxoplasmosis from the environment:

Avoid drinking untreated drinking water.

Wear gloves when gardening and during any contact with soil or sand because it might be contaminated with cat feces that contain *Toxoplasma*.

Wash hands with soap and warm water after gardening or contact with soil or sand.

Teach children the importance of washing hands to prevent infection.

Keep outdoor sandboxes covered.

Feed cats only canned or dried commercial food or well-cooked table food, not raw or undercooked meats.
Prevention and control (General)

Reduce Risk from the Environment
To prevent risk of toxoplasmosis from the environment:

Change the litter box daily if you own a cat.
(The Toxoplasma parasite does not become infectious until 1 to 5 days after it is shed in a cat's feces.)

If you are pregnant or immunocompromised:
Avoid changing cat litter if possible. If no one else can perform the task, wear disposable gloves and wash your hands with soap and warm water afterwards.

Keep cats indoors.

Do not adopt or handle stray cats, especially kittens.

Do not get a new cat while you are pregnant.

Treatment

Healthy people (nonpregnant):
Most healthy people recover from toxoplasmosis without treatment. Persons who are ill can be treated with a combination of drugs such as pyrimethamine and sulfadiazine, plus folinic acid.

Pregnant women, newborns, and infants:
Pregnant women, newborns, and infants can be treated, although the parasite is not eliminated completely. The parasites can remain within tissue cells in a less active phase; their location makes it difficult for the medication to completely eliminate them.

Animals:
There is no approved treatment for toxoplasmosis. However, the following medications and regimens have been used successfully in cats or dogs.
- Clindamycin hydrochloride
- Pyrimethamine plus sulfonamide
- Trimethoprim-sulphonamide combination
Current vaccine: S48 strain toxovax® vaccine
Toxovax® is live tachyzoite vaccine for sheep.
<Disadvantages>
Short shelf life, Pathogenicity, Not available for human

Vaccine development is required!

Cytotoxic T cells
Production of specific antibody

Induction of parasite-specific T cells is important for control parasites.

Development of a next-generation vaccine
oligmannose-coated liposome (OML)

oligmannose-coated liposome (OML)
- Uptake of antigen ↑
- Activation of APCs ↑
- No infectious
- No cytotoxicity

Possible induction both humoral and cellular immune responses
Effects of OML vaccine against Toxoplasma

Uptake of OML by macrophage

Uptake of OML FITC-BSA (Green) in lymph node

Increase of mouse survival

Percent survival

PBS

OML

TgPF

TgPF-OML

Days post infection

Ikehara et al., Cancer Res., 2006

Ikehara et al., Cancer Res., 2006

Tanaka et al., Vaccine, 2014

Our goal is practical application of OML vaccine against refractory protozoan diseases

At the end ----

Please consider Neosporosis.

Neospora caninum is an important protozoan pathogen in cattle and dogs.

It is highly transmissible and some herds can have up to a 90% prevalence.

It causes abortions in cattle and up to 33% of pregnancies can result in aborted fetuses on one dairy farm.

Identified in 1988

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It is highly transmissible and some herds can have up to a 90% prevalence.

It causes abortions in cattle and up to 33% of pregnancies can result in aborted fetuses on one dairy farm.
Global economic impact of *Neospora caninum* in cattle

This estimate of global losses due to *N. caninum*, with the identification of clear target markets (countries, as well as cattle industries), should provide an incentive to develop treatment options and/or vaccines.

Thank you very much.

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