Toxoplasma and food

david.buxton@moredun.ac.uk
Toxoplasmosis is arguably…..
“the most widespread and prevalent protozoan parasitic zoonosis”.

(Macpherson 2005)
Toxoplasma and food

1. *Toxoplasma gondii*
   - parasite / animals / environment

2. Zoonosis
   - infection vs disease

3. Control
   - detection / reduction / education
4,000 million years ago  life on earth begins
1,500 mya    Protozoa appear
1,500-500 mya    Apicomplexan protozoa develop

*major clades today*

- piroplasms    *(Theileria, Babesia)*
- haemoporaninids    *(Plasmodium)*
- gregarines
- cryptosporidians
- enteric coccidia    *(Eimeria, Cyclospora)*
- tissue cyst forming coccidia    *(Toxoplasma, Neospora, Sarcocystis)*

LD Sibley Vet Parasitol 2003
tissue cyst forming coccidia
(*Toxoplasma, Neospora, Sarcocystis, Hammondia, Besnoitia*)

common ancestor - 250 mya
mammalian hosts diverge and expand over similar period

LD Sibley Vet Parasitol 2003
gundi (*Ctenodactylus gundi*)
1908 Nicolle Manceaux
Pasteur Institute, Tunis

rabbit (*Oryctolagus cuniculi*)
Splendore 1908 Sao Paulo Brazil
Toxoplasma gondii

- subphyllum: Apicomplexa
- family: Sarcocystidae
- obligate intracellular protozoan
- toxon (arc) plasma (form) Greek
**Toxoplasma gondii**

**Asexual cycle**

- **BRADYZOITES** in tissue cysts
- **TACHYZOITES**

All warm-blooded animals

Likely mode of transmission

Toxoplasma cysts or an “as yet unrecognised” form of the parasite (Hartley 1966)
Toxoplasma gondii

Asexual cycle

all warm-blooded animals

BRADYZOITES in tissue cysts

TACHYZOITES

Hutchison 1965 (+ many others 1970)
Toxoplasma gondii

Asexual cycle
all warm-blooded animals
BRADYZOITES in tissue cysts
TACHYZOITES

Sexual cycle
cats
Gut infection
OOCYSTS

ingestion
Cats and *Toxoplasma* oocyst excretion

<table>
<thead>
<tr>
<th>Ingestion</th>
<th>Day</th>
<th>Oocyst Excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocysts</td>
<td>20</td>
<td>50% thousands</td>
</tr>
<tr>
<td>Tachyzoites</td>
<td>20</td>
<td>50% thousands</td>
</tr>
<tr>
<td>Bradyzoites</td>
<td>4-10</td>
<td>100% millions</td>
</tr>
</tbody>
</table>
Toxoplasma gondii isolates very similar

Molecular strain typing
( Restriction Fragment Length Polymorphisms – RFLP )

>95% discrete clonal groups
Type I  acutely virulent to mice
Type II non-virulent to mice
Type III non-virulent to mice
Toxoplasma gondii
Types I, II and III

Howe and Sibley (1995) J Inf Dis

percent
(n = 106)

4 isolates
mixed lineage

Types I, II and III

Howe and Sibley (1995) J Inf Dis
Toxoplasma gondii
Types I, II and III clinical human cases

Toxoplasma gondii

Type II clinical human toxoplasmosis
persistent animal infections

high prevalence pigs and sheep

Toxoplasma in animals

Clinical toxoplasmosis = *T. gondii* LIVE in environment
BUT tip of the iceberg
Toxoplasma in animals

Clinical toxoplasmosis = *T. gondii* LIVE in environment
BUT tip of the iceberg

Serology infection WIDESPREAD
Toxoplasma in animals
land
water
Ovine toxoplasmosis - temperate sheep rearing countries
New Zealand, Norway, UK, Eire, Spain, Portugal, France
transmission of ovine toxoplasmosis

contam. food/water → primary infection

oocysts

tissue cysts

WILD MICE

abortion
oocysts may contaminate grass
Toxoplasmosis and Risk factors
Seroprevalence of *Toxoplasma* sheep France
(slaughterhouse survey/clin normal) (Dumètre et al 2006)

Lambs (n=164) 22% sero+ve

Ewes (n=93) 65.6% sero+ve

Type II Tg (n=50) 100%
oocysts may also contaminate grain
Toxoplasma oocyst contamination of livestock feed

Oocysts in cat faeces 1 million/g
50g cat faeces in 10 tonnes feed
5,000 oocysts /kg feed
100 oocysts will infect one ewe
50 sheep infective doses/kg feed
50 infective doses/kg feed
Pigs become infected when reared outside

Kijlstra et al 2004

Reindeer seroconvert (age related)

Oksanen et al 1997
Antibody to *Toxoplasma gondii*

in red foxes (*Vulpes vulpes*) in Belgium

n = 123

Buxton et al 1997
**T. gondii in game/wildlife (worldwide)**

<table>
<thead>
<tr>
<th>Animal</th>
<th>%</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>bear</td>
<td>15-84</td>
<td>(1,828)</td>
</tr>
<tr>
<td>wild boar</td>
<td>15-37</td>
<td>(1,131)</td>
</tr>
<tr>
<td>deer</td>
<td>7-60</td>
<td>(3,017)</td>
</tr>
<tr>
<td>moose</td>
<td>15</td>
<td>(125)</td>
</tr>
<tr>
<td>reindeer</td>
<td>0-25</td>
<td>(2,577)</td>
</tr>
<tr>
<td>gazelle/antelope</td>
<td>0-37</td>
<td>(694)</td>
</tr>
</tbody>
</table>

(Tenter et al 2000)
Toxoplasma in the environment
land
water
Toxoplasma and aquatic wildlife

Wild otters - *T. gondii*

Sea otters (*Enhydra lutris nereis*) Californian coast
16% mortality *Toxoplasma gondii* infection (n = 35)
Type II (40%) and Type x (60%) isolated
Type x associated with meningoencephalitis

Toxoplasma and aquatic wildlife

Wild otters - serology for *T. gondii*

**River otters** (*Lutra canadensis*) North Carolina
45% +ve (46/103) Tocidlowski *et al* (1997)

**Sea otters** (*Enhydra lutris nereis*) Californian coast
52% +ve (115/223) Miller *et al* (2002)
Toxoplasma and sea mammals (cetaceans)

Beluga whale (*Delphinapterus leucas*)
Toxo (n = 2) 6/22 sero+ve St Lawrence estuary Mikaelian *et al* (2000)

Spinner dolphin (*Stenella longirostris*)
Disseminated Toxo Hawai Migaki *et al* (1990)

Striped dolphin (*Stenella coeruleoalba*)

Risso’s dolphin (*Grampus griseus*)

Minke whale (*Balaenoptera acutorostrata*)
100% sero-ve (n = 202) Nth Atlantic/Arctic Ocean Oksanen *et al* (1998)
Toxoplasma and sea mammals (pinnipeds)

Harbor (Common) seal (*Phoca vitulina*)
Toxo + *S neurona*  California  Miller *et al* (2001)

Harbor (Common) seal (*Phoca vitulina*)
7.6% sero+ve  (29/380) Puget Sound Washington  
Lambourn *et al* (2001)

Harp seal (*Phoca groenlandica*)

Ring seal (*Phoca hispida*)

Hooded seal (*Cystophora cristata*)
100% sero-ve  (n = 443) Nth Atlantic/Arctic Ocean  
Oksanen *et al* (1998)
Water Potential source of zoonotic protozoal infections

*Giardia* spp
*Cryptosporidium* spp
*Toxoplasma gondii*
Toxoplasma in the environment

Land
Infection (+/- disease) widespread
farm livestock and wildlife

Water
Infection (+/- disease)
Associated urban areas
but not always –
Vancouver Island Canada 1994/1995
Toxoplasma and food

1 Toxoplasma gondii
   parasite / animals / environment

2 Zoonosis
   infection vs disease

3 Control
   detection / reduction / education
infection
Human infection
Seroprevalence surveys

North America, Europe 32% (n=17 1956-97)
Central/Sth America, Caribbean 38% (n=17 1956-96)
African Countries 42% (n=18 1977-96)
Middle East, Sth Asia, Pacific 30% (n=27 1973-97)

(Hall Ryan and Buxton 2001)
Toxoplasma antibodies
20 year olds/100
Seroprevalence of toxoplasmosis in the Czech Republic

Seroprevalence of toxoplasmosis in the Czech Republic

Seroprevalence of toxoplasmosis in the Czech Republic

Human toxoplasmosis

Infection vs Disease

Infection common
up to 80%

Disease rare
Confined to risk groups?
Human toxoplasmosis

Risk groups

Immunosuppressed - acute (fatal) illness
AIDS
cancer therapy
certain cancers (lymphoma)
transplant recipients (+ infected transplant)
Pregnancy - congenital infection
Human toxoplasmosis

**Acute illness**
- fever
- headache
- listlessness
- muscle aches
- lymphadenopathy (enlarged tender lymph nodes)
chorioretinitis (pre & post natal)

encephalitis

hepatitis

myositis

pneumonia (interstitial)
Congenital toxoplasmosis

60% mothers no symptoms
40% vague symptoms
fever  headache
listlessness  muscle aches
lymphadenopathy
Congenital Toxoplasma infection per 1000 pregnancies

Stray-Pedersen
Congenital human toxoplasmosis primary infection initiated during pregnancy

1st trimester infection 15% fetuses outcome “severe”

2nd trimester infection 30% fetuses outcome “mod”

3rd trimester infection 60% fetuses outcome “mild”

Desmonts & Couvreur 1984
Toxoplasma and pregnancy

Risk of placental transmission

Risk of fetal damage

gestation

1st trimester
2nd trimester
3rd trimester
Congenital human toxoplasmosis

fetal death
CNS lesions - hydrocephalus spasticity
mild clinically inapparent
longterm 1 in 3 require medical care
mental changes/retardation/learning difficulties
visual handicaps (chorioretinitis)
BUT clinical toxoplasmosis and link to food NOT ONLY in risk groups
Human toxoplasmosis outbreaks linked to food

3 of 4 men (Korea) unilateral chorioretinitis “feasted” on raw pork, liver, spleen of wild pig “in a belief of its special nutritional value”

Patient 1 recovered with treatment (retinal scarring)

Patients 2 and 3 remained blind despite treatment

Human toxoplasmosis outbreaks linked to food

5 of 11 soldiers lymphadenopathy ate raw pork, liver of domestic pig

Sulphonomide/pyrimethamine 1 month
Full recovery

Human toxoplasmosis

Outbreak linked to drinking water
Canada  Vancouver Island 1994/95

Small reservoir supplying unfiltered chlorinated water to 292,000 people

100 cases acute toxoplasmosis
3 times expected incidence

Human toxoplasmosis

Outbreak linked to drinking water
Canada  Vancouver Island 1994/95
100 cases acute toxoplasmosis
51 lymphadenopathy
19 retinitis
11 malais/fever/headache
18 no symptoms
1 no history

Toxoplasma infection in local feral cats
cougars (oocysts in faeces x1)
Toxoplasmosis and Risk factors

most *Toxoplasma*/ocular disease POSTNATAL

retinochoroiditis
### Toxoplasmosis and Risk factors

Most *Toxoplasma*/ocular disease **POSTNATAL**

*(questionnaire - Jones et al 2006)*

<table>
<thead>
<tr>
<th>Increased risk</th>
<th>raw/lightly cooked meat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cured/dried/smoked meat</td>
</tr>
<tr>
<td></td>
<td>eating “frozen lamb”</td>
</tr>
<tr>
<td></td>
<td>garden/yard</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Decreased risk</th>
<th>raw beef/ground beef</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>raw chicken/ground chicken</td>
</tr>
</tbody>
</table>
Toxoplasmosis and Risk factors
Pork and *Toxoplasma* Brazil

Finishing pigs 17% sero+ve (n=286)
*(de A Dos Santos et al 2005)*

Pork sausages (fresh) live *Toxoplasma* 8.7%
*(Dias et al 2005)* (n=149 8 factories)
Human toxoplasmosis

**sources of infection**

**oocysts**
- cat litter trays
- garden – children’s sandpits
- unwashed fruit / vegetables
- contaminated water

**tissue cysts / tachyzoites**
- raw/lightly cooked meat
- aborting sheep (goats, pigs)
Toxoplasma and food

1. *Toxoplasma gondii*
   - parasite / animals / environment

2. Zoonosis
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3. Control
   - detection / reduction / education
DETECTION of *T. gondii*  
clinical signs  
serology  IFAT  ELISA  LAT  
histopathology  
PCR (B1 & P30/SAG genes)  
inoculation (mice  TC)
Killing *Toxoplasma*

*T. gondii* tissue cysts survive up to 3 min 64°C in pork  
(Dubey et al 1990)

Cook red meat until brown
Killing *Toxoplasma*

High Pressure Processing (HPP)
Non thermal treatment
extends shelf-life
retains flavour colour vitamins
Killing *Toxoplasma*

High Pressure Processing (HPP)

Non thermal treatment extends shelf-life retains flavour colour vitamins

*T. gondii* tissue cysts (in ground pork) Survive 0, 100, 200 MPa for 90 seconds

Killed by 300 MPa for 30 seconds

(Lindsay et al 2006)
<table>
<thead>
<tr>
<th>Solution</th>
<th>Concentration</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formalin</td>
<td>10%</td>
<td>24h</td>
</tr>
<tr>
<td>Ammonia</td>
<td>5.5%</td>
<td>1h</td>
</tr>
<tr>
<td>Ethanol</td>
<td>95%</td>
<td>1h</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>7d</td>
</tr>
<tr>
<td>Iodine (tinct)</td>
<td>7%</td>
<td>10m</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>3h</td>
</tr>
<tr>
<td>Water</td>
<td>50°C</td>
<td>30m</td>
</tr>
<tr>
<td>Water</td>
<td>20/22°C</td>
<td>548d</td>
</tr>
<tr>
<td>Water</td>
<td>4°C (2%H$_2$SO$_4$)</td>
<td>548d</td>
</tr>
</tbody>
</table>

*Dubey and Beattie 1988*
Control of toxoplasmosis in animals

management

herbivore - omnivore - carnivore
avoid contamination food/water - oocysts

omnivore - carnivore

treat infected meat (tissue cysts - cooking)

vaccination

ovine toxoplasmosis - live vaccine (Toxovax)

pharmaceuticals
Ovine toxoplasmosis control - vaccination

live toxoplasma vaccine (S48 tachyzoite suspension)
Toxovax (S48 *T. gondii*) and sheep

Will vaccination control clinical disease?

YES
Toxovax (S48 *T. gondii*) and sheep

Will vaccination produce tissue cysts?  
**NO**  S48 *T. gondii* “incomplete” cleared within 3 weeks
Toxovax (S48 *T. gondii*) and sheep

Is meat from vaccinated sheep safe to eat?

**YES** 4 weeks post vacc. no S48 *T. gondii* in meat

**BUT** “wild” strain *T. gondii* may be in meat
Toxovax (S48 *T. gondii*) and sheep

If vaccinated sheep ingest *T. gondii* oocysts will they develop tissue cysts?

S48 suppresses/prevents multiplication of *Toxoplasma*
S48 suppresses/prevents spread in lymph and blood
Toxovax (S48 *T. gondii*) and sheep

Can vaccination control/reduce numbers *T. gondii* tissue cysts in muscle (meat)?

Answer

yes
Toxoplasma and sea mammals

Control
Attempt to reduce toxoplasma oocyst contamination of water (assuming infection is derived from water run-off from the land)

Captive animals possible?
Wild animals difficult
pharmaceuticals

toxoplasmosis

- sulphonamides
- pyrimethamine
- trimethoprim / sulphadiazine
- baquiloprim / sulphadimidine
- clindamycin
Toxoplasma and food

1. *Toxoplasma gondii*
   parasite / animals / environment

2. Zoonosis
   infection vs disease

3. Control
   detection / reduction / education
Toxoplasma IgG in pregnant women

Gilbert 2000