CMED 526/EPI 526

B.J. Weigler

Spring 2009

Toxoplasmosis

Agent:	*Toxoplasma gondii*
Taxonomy:	Phylum Apicomplexa (= ~5000 spp.) Sporozoon coccidia
Distribution:	Worldwide
Definitive Host:	Any member of the *Felidae*
Intermed. Hosts:	Any warm blooded animal
Invertebrates:	Coprophagous (“feces-eating”) insects can be transport hosts

Human toxoplasmosis

- Inapparent or mild flu-like illness (most cases)
- Fetal death and mental retardation, blindness, epilepsy…. May not manifest for years
- ~ 400-4000 congenital infections/year (USA)
- Ocular toxoplasmosis
- Severe encephalitis in immunocompromised persons (recrudescent infections)

EMERGING INFECTIOUS DISEASES

Vol. 9, No. 11
November 2003

Synopsis

Toxoplasma gondii and Schizophrenia

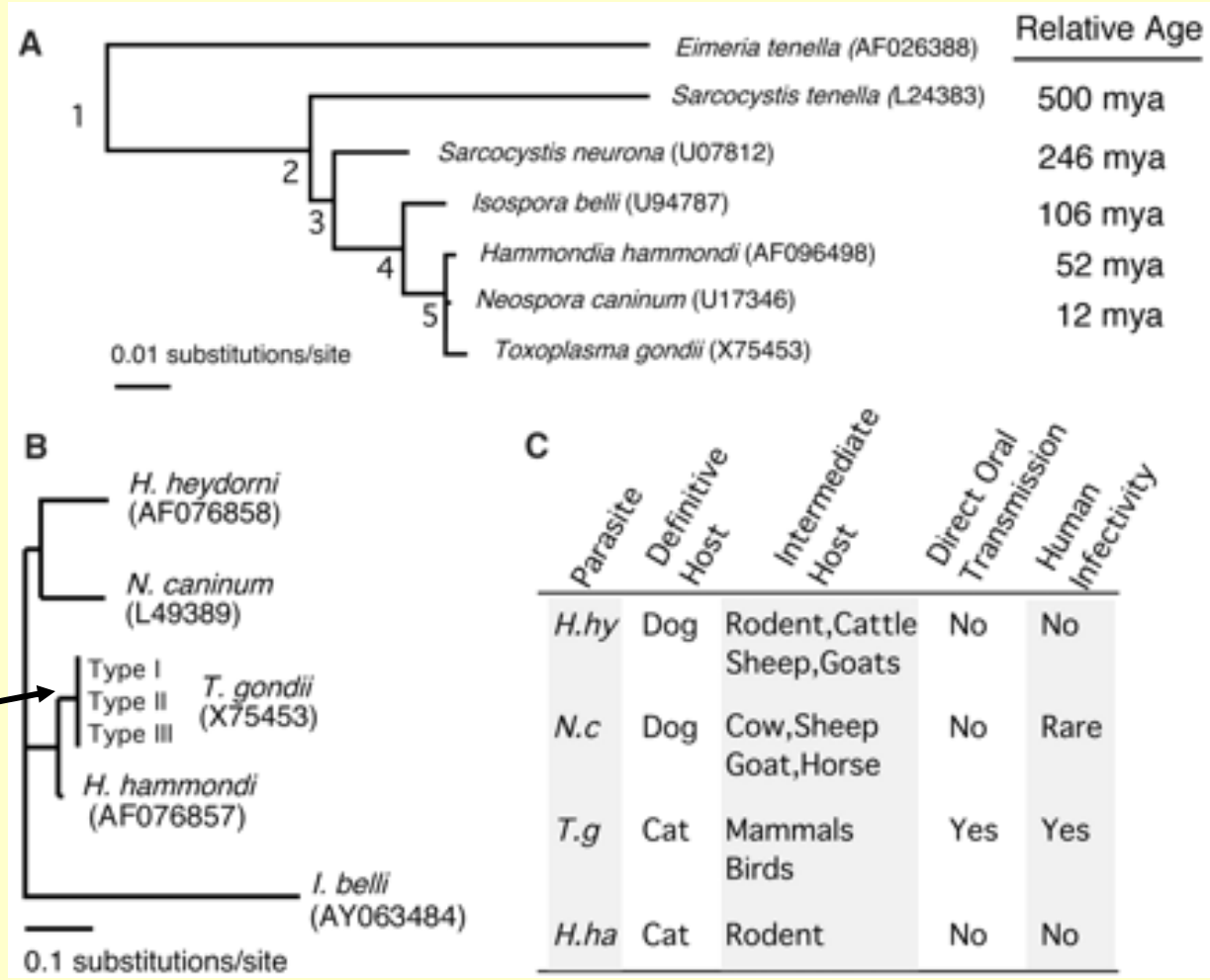
E. Fuller Torrey* and Robert H. Yolken†

*Stanley Medical Research Institute, Bethesda, Maryland, USA; and †Johns Hopkins University Medical Center, Baltimore, Maryland, USA

Recent epidemiologic studies indicate that infectious agents may contribute to some cases of schizophrenia. In animals, infection with *Toxoplasma gondii* can alter behavior and neurotransmitter function. In humans, acute infection with *T. gondii* can produce psychotic symptoms similar to those displayed by persons with schizophrenia. Since 1953, a total of 19 studies of *T. gondii* antibodies in persons with schizophrenia and other severe psychiatric disorders and in controls have been reported; 18 reported a higher percentage of antibodies in the affected persons; in 11 studies the difference was statistically significant. Two other studies found that exposure to cats in childhood was a risk factor for the development of schizophrenia. Some medications used to treat schizophrenia inhibit the replication of *T. gondii* in cell culture. Establishing the role of *T. gondii* in the etiopathogenesis of schizophrenia might lead to new medications for its prevention and treatment.

Phylogeny

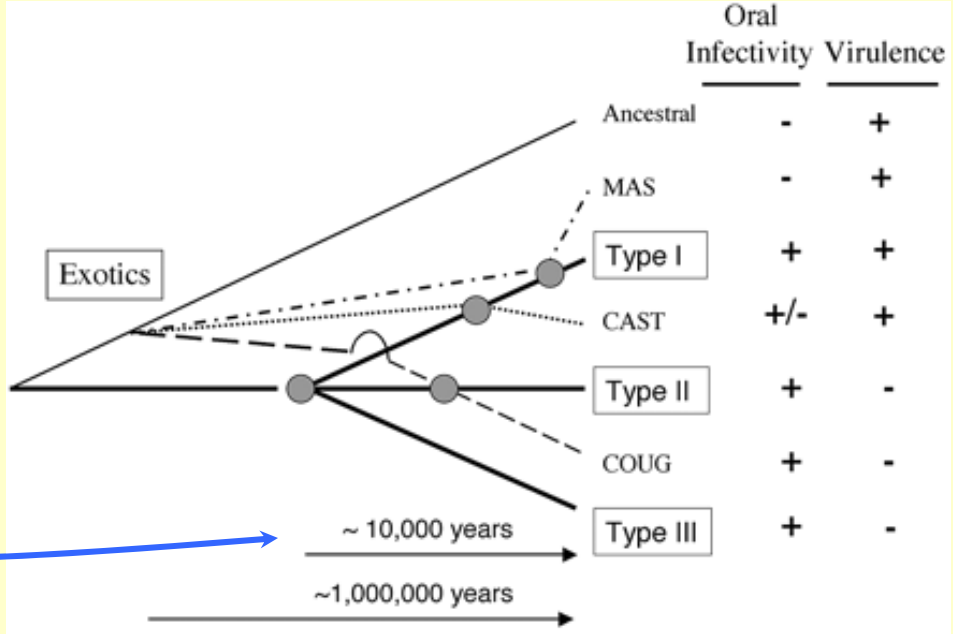
Global predominance of 3 clonal lineages of *T. gondii*



Parasite	Definitive Host	Intermediate Host	Direct Oral Transmission	Human Infectivity
H.hy	Dog	Rodent,Cattle Sheep,Goats	No	No
N.c	Dog	Cow,Sheep Goat,Horse	No	Rare
T.g	Cat	Mammals Birds	Yes	Yes
H.ha	Cat	Rodent	No	No

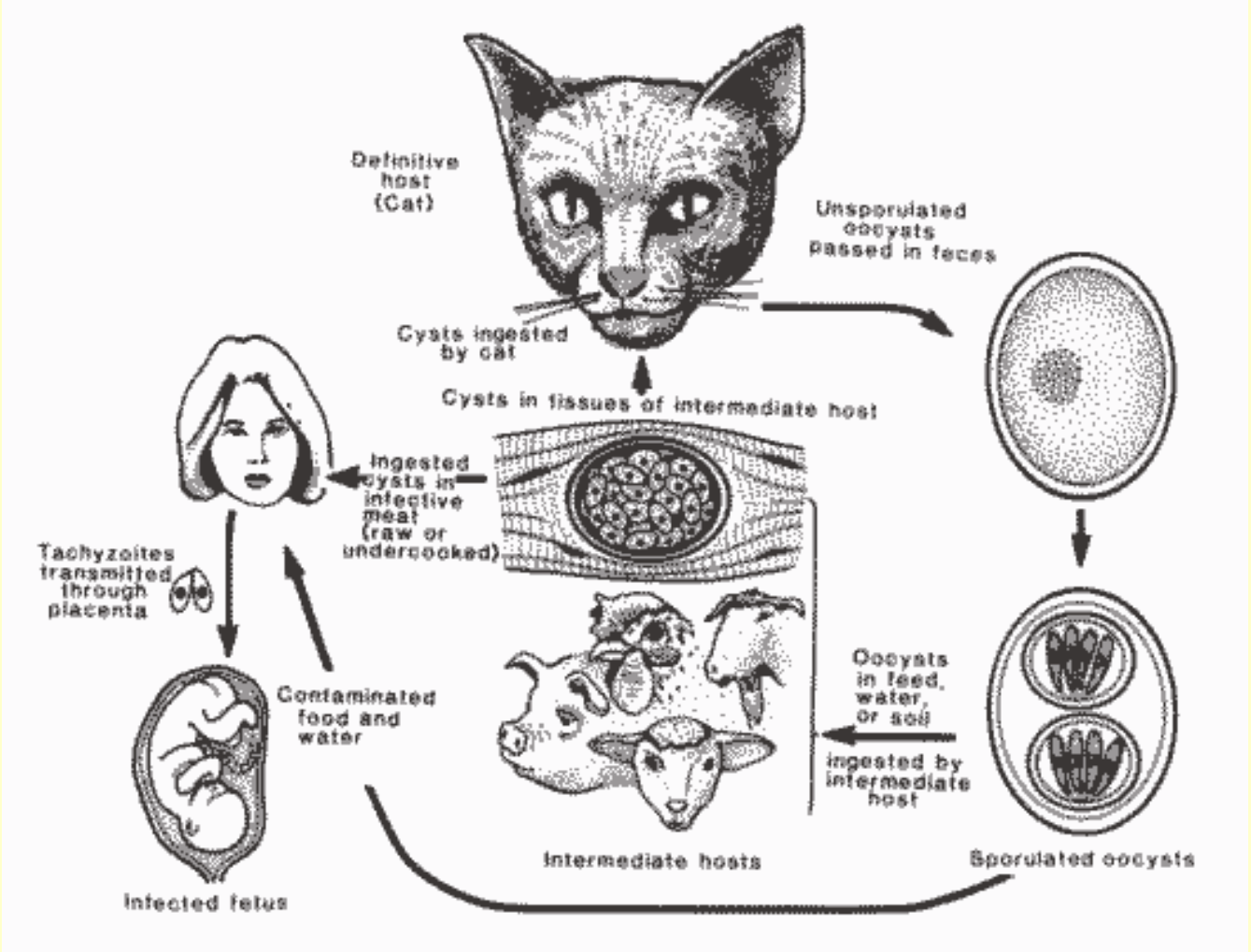
Evolutionary Biology

Time of human agricultural expansion and adaptation of the cat as a pet.



A “recent” genetic cross resulted in the acquisition of oral infectivity, promoting transmission through successive hosts.

Developmental Cycle



Life Stages

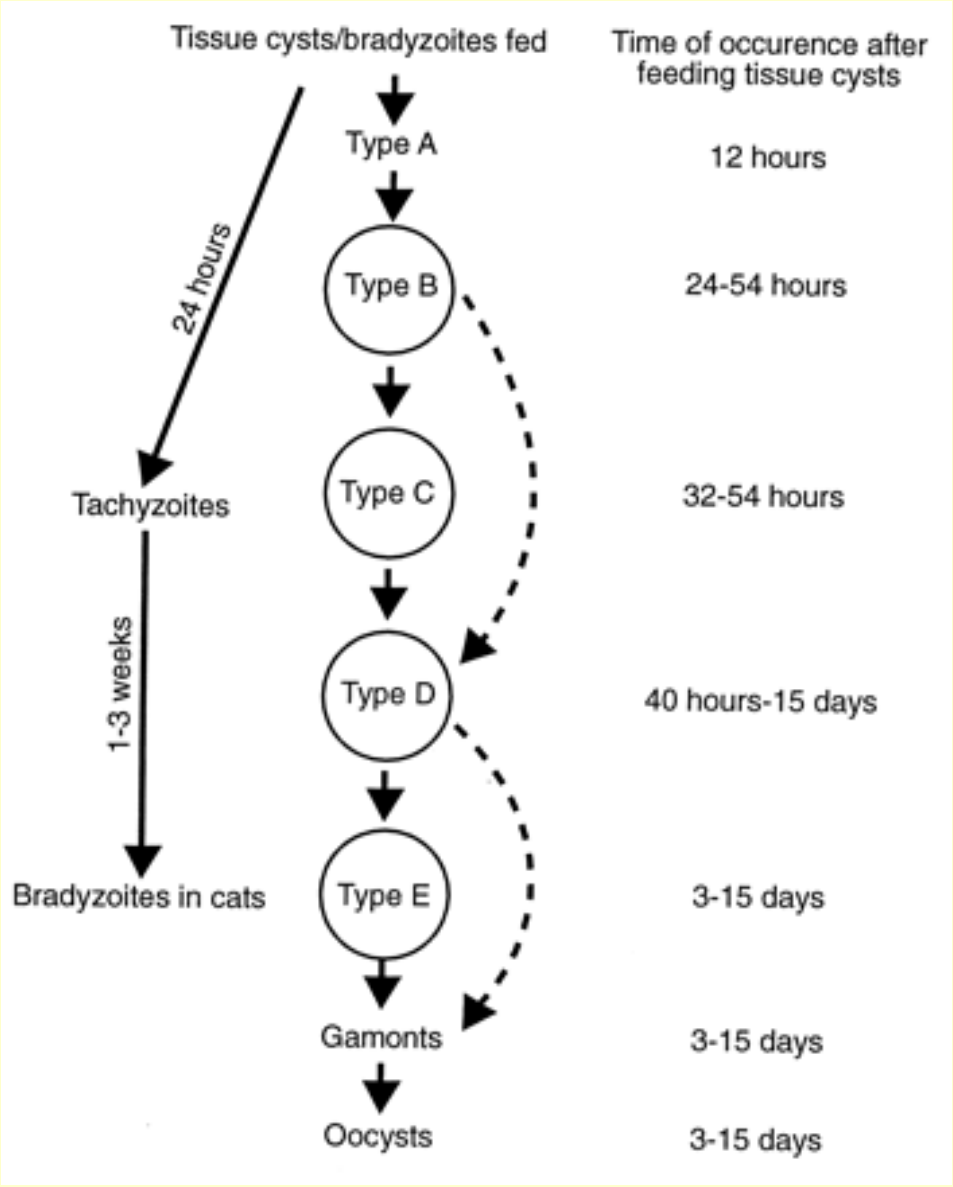
- **Tachyzoites** = proliferative form in blood or CSF, acute or recurrent
- **Bradyzoites** = lifelong “tissue cysts”, any host
- **Oocysts** = (with sporozoites) shed in feces after completion of sexual phase in feline gut epithelium
  - Infectious after 48 hours or more environmental incubation
  - Survive months to years despite freezing, heat, dehydration
  - Thousands-to-millions shed per cat
  - Oocysts are shed from cats for 1-2 weeks only.
  - Only ~1% of cats are shedding oocysts at a given time

Routes of Transmission

- Foodborne (third leading cause of all types)
- Waterborne
- Contaminated soil
- Transplacental
- Organ transplants
- Blood transfusion
- Laboratory accidents

Life Stages of *T. gondii* in Cats

Types A-E are sexual phases, all completed in feline gut epithelium



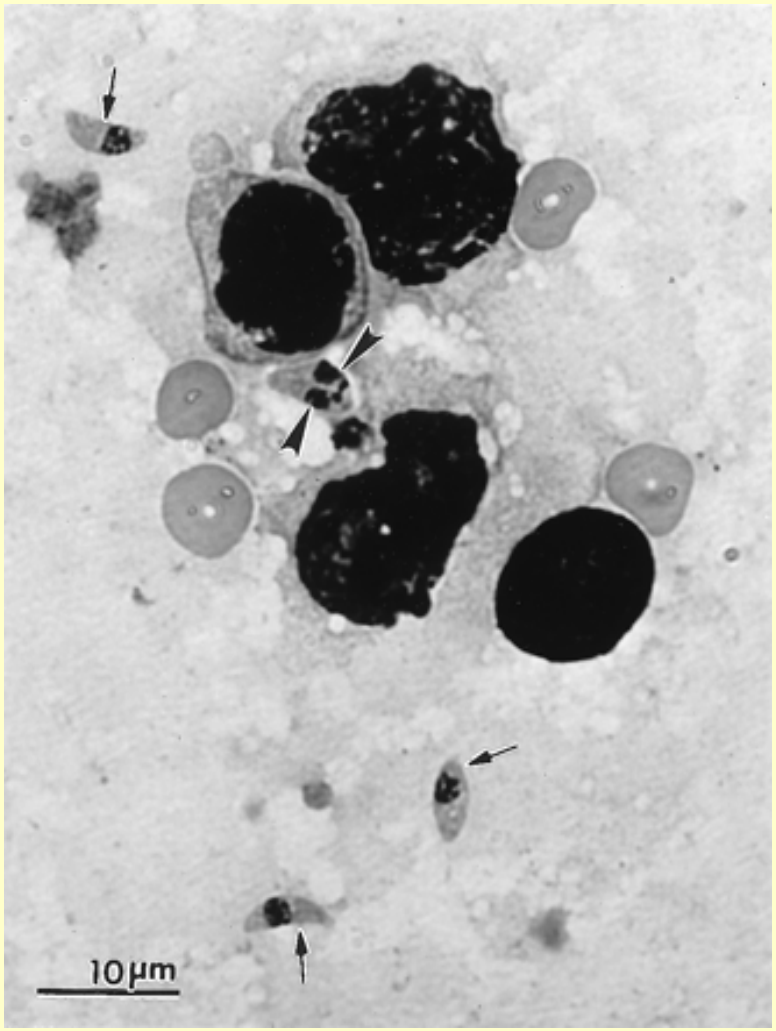
Prepatent periods:

Tissue Cysts: 3-10 days

Oocysts: ≥ 18 days

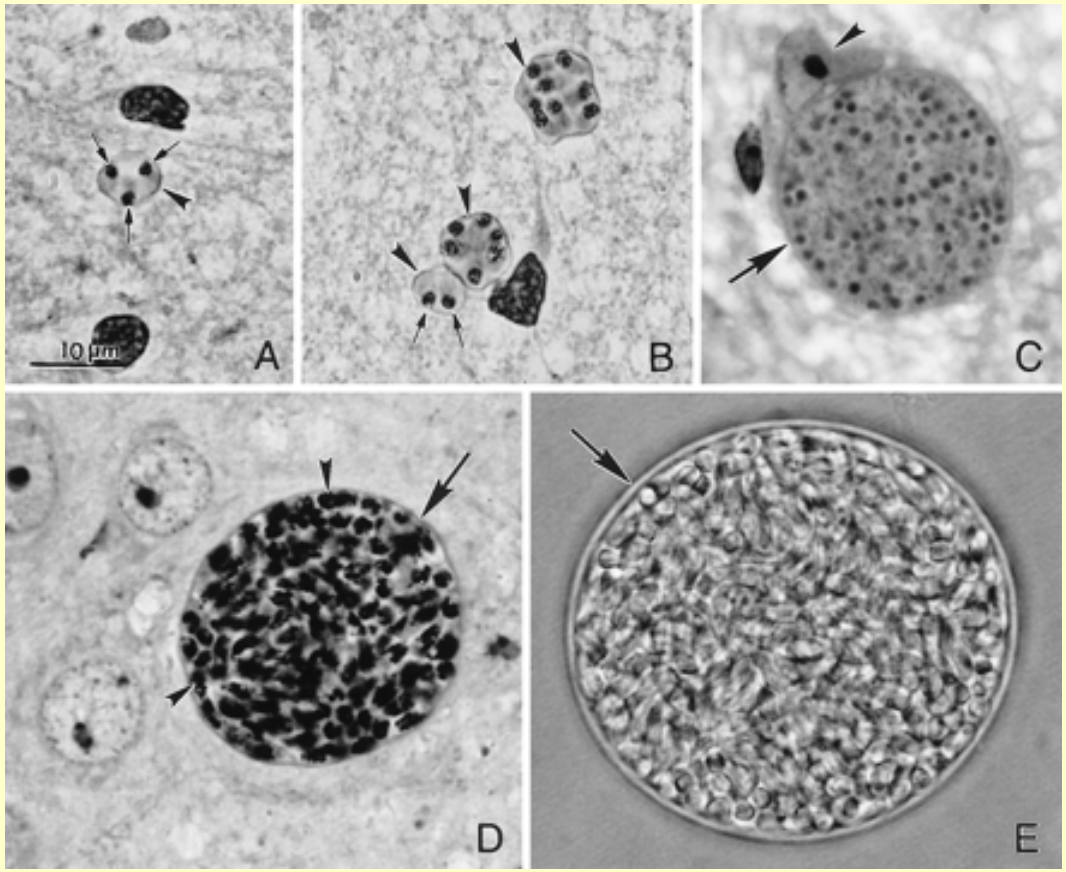
Tachyzoites: ≥ 13 days

Tachyzoites in Mouse Lung



Tissue Cysts in Mouse Brains

3 bradyzoites

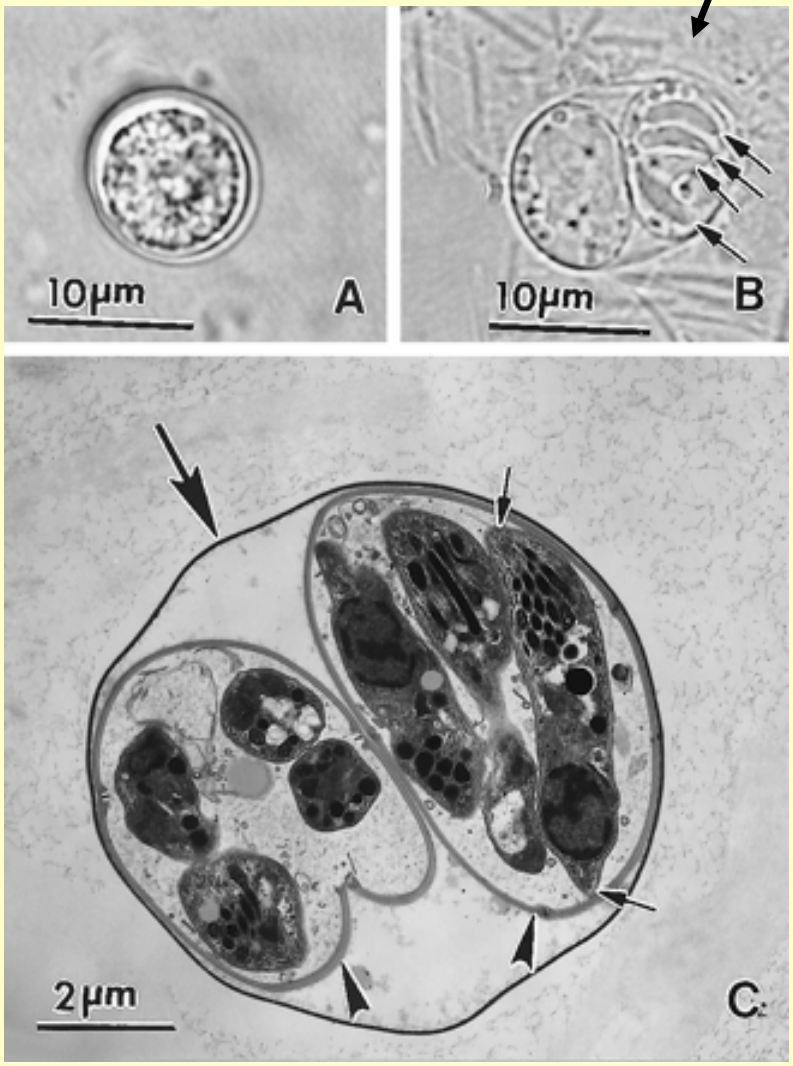


Hundreds of bradyzoites

T. gondii Oocysts

Sporulated with two sporocysts

Unsporulated



Transmission Electron Microscopy - showing sporocysts each containing sporozoites

Sources of *T. gondii* exposure

- Accidental ingestion of contaminated cat feces. For example, accidental touching of hands to mouth after gardening, cleaning a cat's litter box, or touching anything that has come into contact with cat feces.
- Ingestion of raw or partly cooked meat, especially **pork**, **lamb**, or **venison**, or by touching hands to mouth after handling undercooked meat.
- Contamination of knives, utensils, cutting boards and other foods that have had contact with raw meat.
- Drinking water contaminated with *Toxoplasma*.
- Although extremely rare, by receiving an infected organ transplant (solid or hematopoietic) or blood transfusion.

J. Parasitol., 91(5), 2005, pp. 1082–1093

PREVALENCE OF VIABLE *TOXOPLASMA GONDII* IN BEEF, CHICKEN, AND PORK FROM RETAIL MEAT STORES IN THE UNITED STATES: RISK ASSESSMENT TO CONSUMERS

J. P. Dubey, D. E. Hill, J. L. Jones*, A. W. Hightower*, E. Kirkland*, J. M. Roberts*, P. L. Marcet*, T. Lehmann*, M. C. B. Vianna, K. Miska, C. Sreekumar, O. C. H. Kwok, S. K. Shen, and H. R. Gamble

United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, Maryland 20705-2350. *e-mail: jdubey@anri.barc.usda.gov*

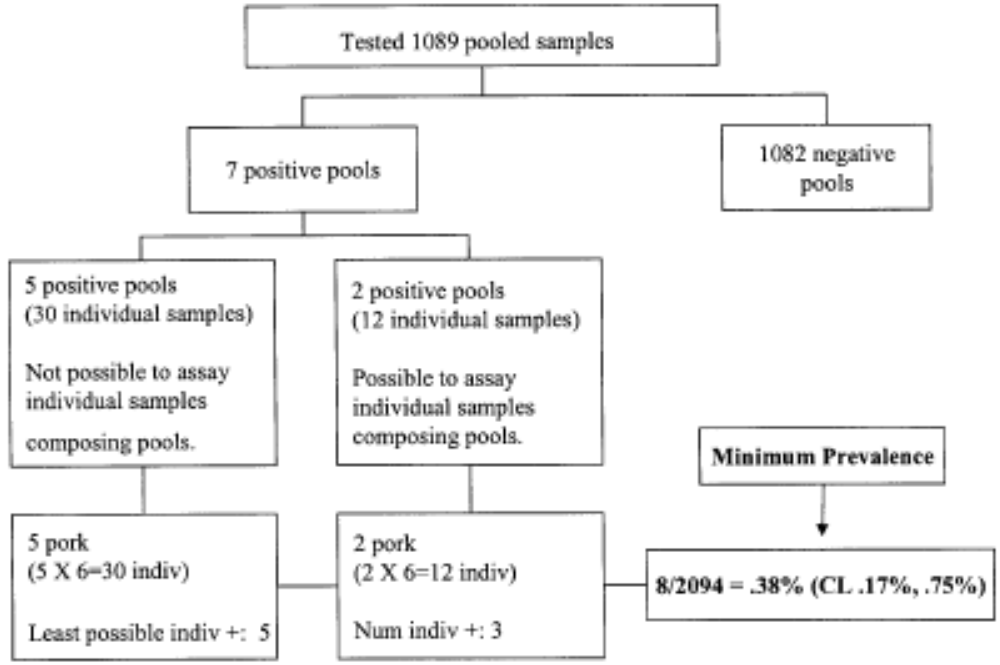


FIGURE 1. Results for viable *Toxoplasma gondii* in 6,282 samples of meat (2,094 each of beef, chicken, and pork) obtained from 698 retail stores in the United States.

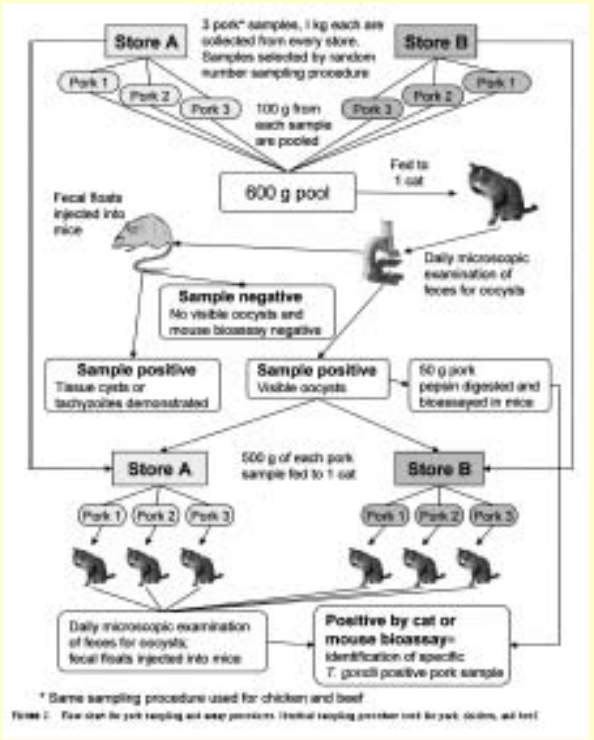


TABLE V. Isolation of *Toxoplasma gondii* from individual pork samples.

Meat survey location, date, and number (enhancement code)*	Store no.	No. days meat stored at 4 C	Bioassay	
			In mice†	In cats (oocysts shed, days)‡
Boston, Massachusetts (November 2002)		12		
388 Pork (54)	BOS17		0/10	AMAS (+) (day 9)§
389 Pork (54)			0/10	ADL5 (−)
390 Pork (54)			0/10	QDM2 (+) (day 9)§
391 Pork (66)	BOS05		0/10	ING4 (−)
392 Pork (66)			0/10	QDY2 (−)
393 Pork (66)			0/10	ADL6 (−)
Columbus, Ohio (April 2003)		8		
781 Pork (74)	COL21		0/10	QLR7 (−)
782 Pork (74)			0/10	879 (−)
783 Pork (74)			0/10	866 (−)
784 Pork (83)	COL24		6/10	IRY4 (+) (days 6–10)‖
785 Pork (83)			0/10	864 (−)
786 Pork (83)			0/10	877 (−)

*See Table III for code.
†Number of mice infected/number of mice inoculated with digest of 50 g of pork.
‡Cats were fed 300–500 g of meat. (+) Indicates oocysts were shed; (−) indicates oocysts were not shed.
§SAG2 Type II, microsatellite code C (see Table IV).
‖SAG2 Type III, microsatellite code B (see Table IV).

TABLE VIII. Risk to consumers of purchasing *Toxoplasma gondii* contaminated meat from U.S. retail stores.

Meat	Annual meat consumption (kg)	Minimum likely prevalence (no. positive/no.) (95% CL)	Probability of purchasing *T. gondii* contaminated meat over time*	
			1 yr	10 yr
Pork	23.5	.0038 (8/2094) (.00165, .0075)	.0626	.4765
Beef	29.5	0 (0/2094) (.0000, .0176)	0	0
Chicken	37.2	0 (0/2094) (.0000, .0176)	0	0

*Binomial probability computations assume a mean sample weight of 1.36 kg.

TABLE IX. Risk to consumers of purchasing *Toxoplasma gondii*-contaminated meat from retail stores in the northeastern United States.

Meat	Annual meat consumption (kg)	Minimum likely prevalence (no. positive/no.) (95% CL)	Probability of purchasing *T. gondii*-contaminated meat over time*	
			1 yr	10 yr
Pork	23.5	.0089 (4/450) (.0024, 0226)	.1409	.7812
Beef	29.5	0 (0/450) (.0000, .0082)	0	0
Chicken	37.2	0 (0/450) (.0000, .0082)	0	0

*Binomial probability computations assume a mean sample weight of 1.36 kg.

US Department of Agriculture

APHIS Animal & Plant Health Inspection Service

2002 Census of Agriculture

Type	# Farms	# Animals
Cattle	1,018,359	95,497,994
Beef	796,436	61,413,259
Dairy	91,989	17,013,361
Hogs	78,895	60,405,103
Sheep	47,464	5,426,904
Poultry	98,315	9,161,425,197

Exposure Frequency

- Of ~750 deaths per year (USA):
- 350 due to eating undercooked meat (<66^{0}F)
- Remainder from ingestion of sporulated oocysts from the soil (e.g., gardening, cat litter, not washing produce), from congenital infections, and from other routes
- Annual estimated economic impact: $7.7B

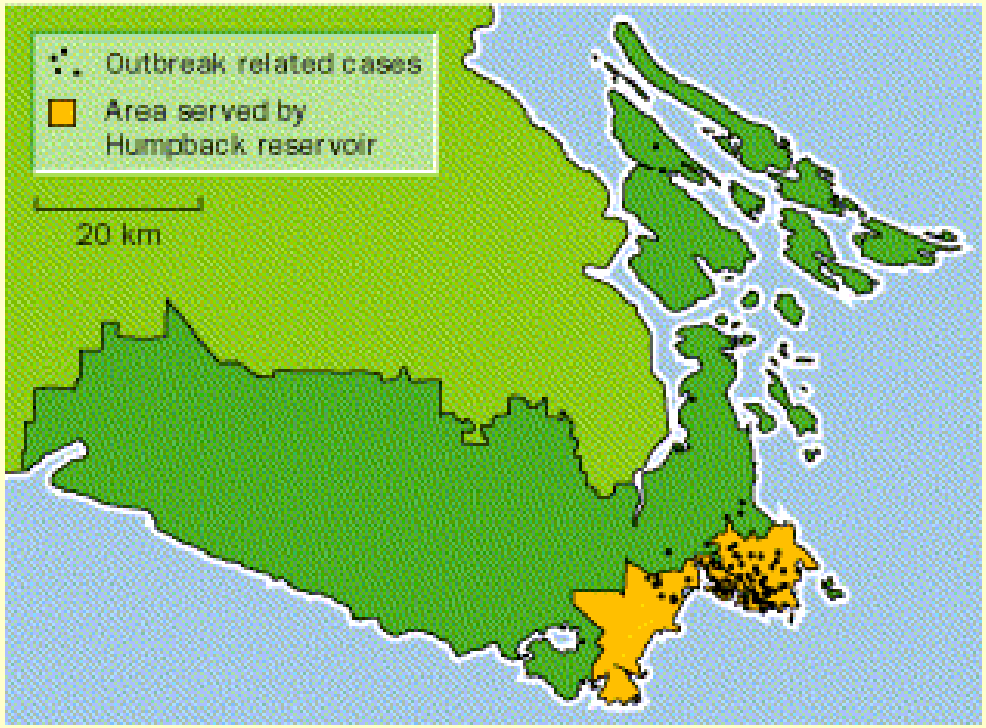
Oocyst Survival in Seawater

J Eukaryot Microbiol. 2003;50 Suppl:687-8

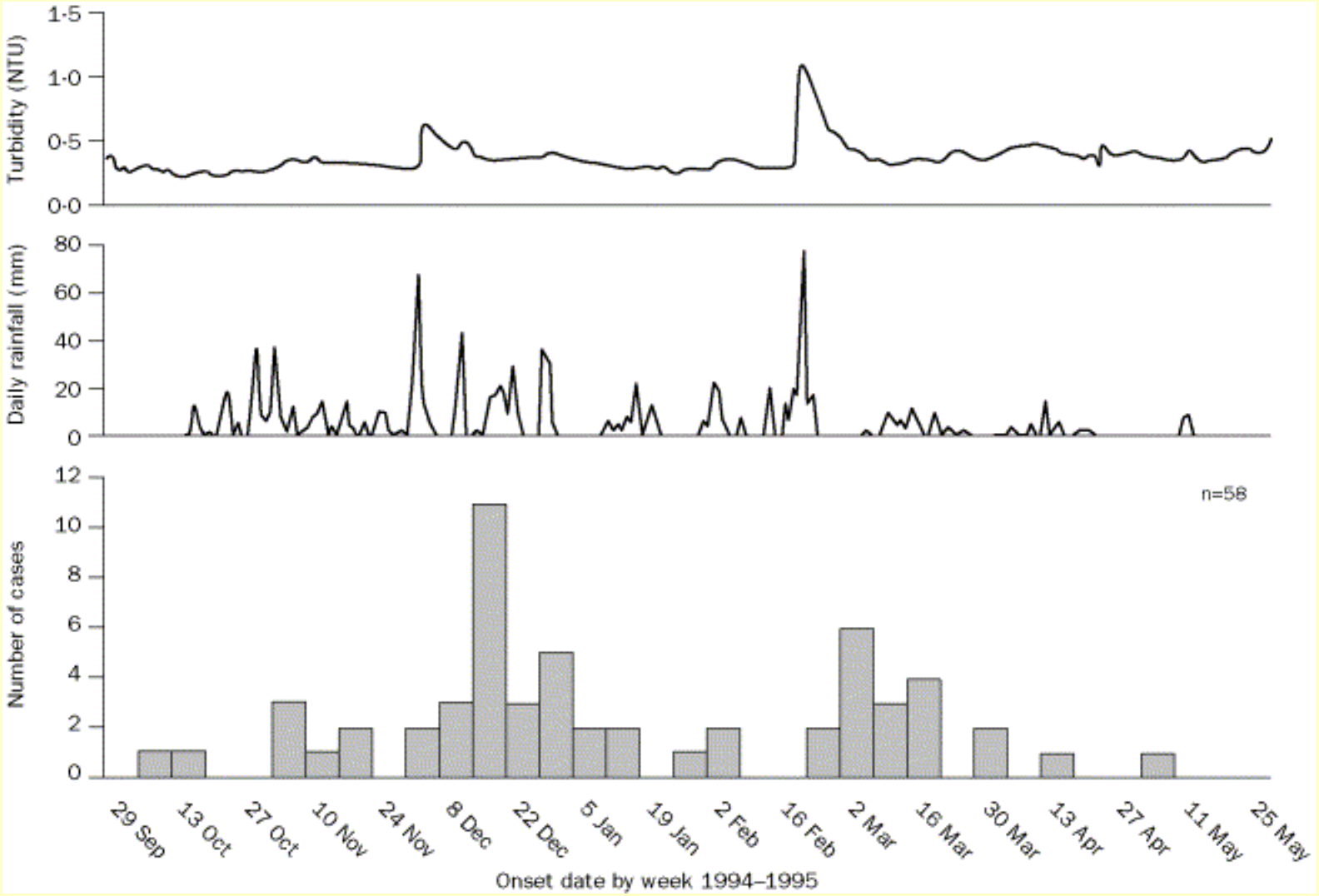
… storm run-off to marine mammals?

- Unsporulated oocysts placed in 15 ppt artificial seawater, 32 ppt artificial seawater or 2% sulfuric acid (positive control) at 24 C.
- From 75 to 80% of the oocysts were sporulated by 3 days post-inoculation under all treatment conditions.
- All mice inoculated with these oocysts developed toxoplasmosis indicating that they were capable of sporulating in seawater.
- Mice fed oocysts that had been stored in seawater for 6 months still became infected.

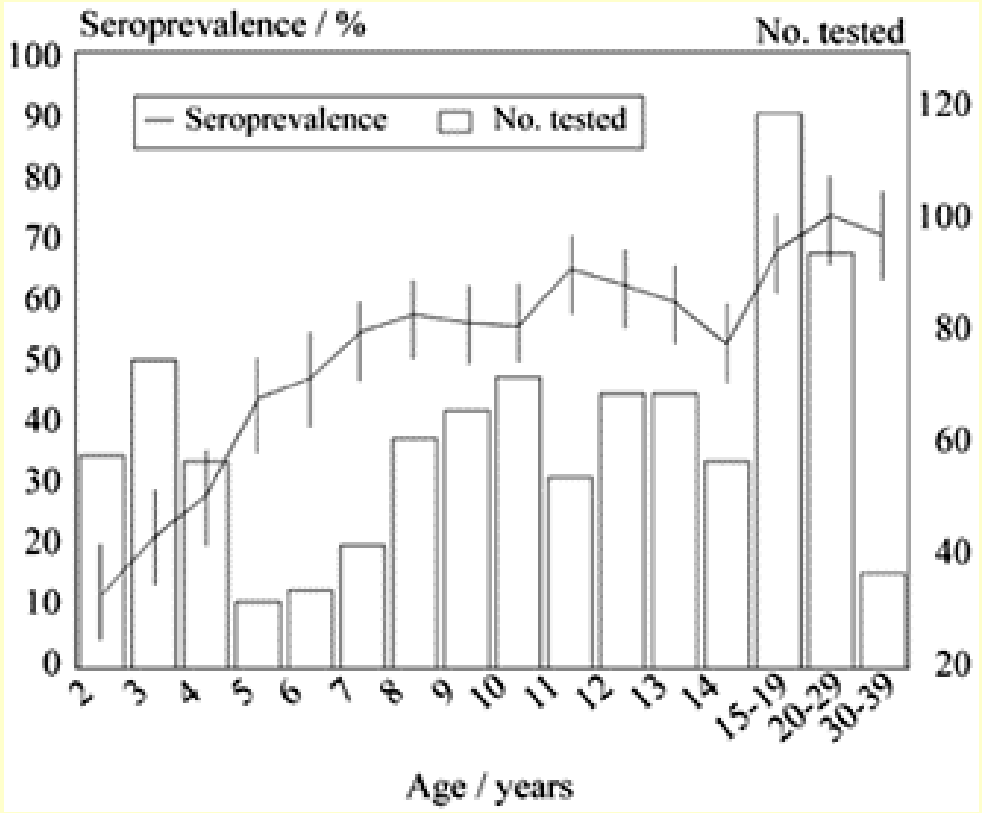
Waterborne *T. gondii* outbreak – Victoria, BC, March 1995



Victoria, BC Outbreak



T. gondii seroprevalence by age, North Rio De Janeiro, Brazil



Prevalence in Wildlife

J Parasitol. 2004 Feb;90(1):67-71.

"…*Toxoplasma gondii* was isolated from the hearts of 21 of 34 seropositive white-tailed deer (Odocoileus virginianus) from Mississippi and from 7 of 29 raccoons (*Procyon lotor*); 5 of 6 bobcats (*Lynx rufus*); and the gray fox (*Urocyon cinereoargenteus*), red fox (*Vulpes vulpes*), and coyote (*Canis latrans*) from Georgia. *Toxoplasma gondii* was also isolated from 7 of 10 seropositive black bears (*Ursus americanus*) from Pennsylvania by bioassay in cats. All 3 genotypes of T. gondii based on the SAG2 locus were circulating among wildlife."

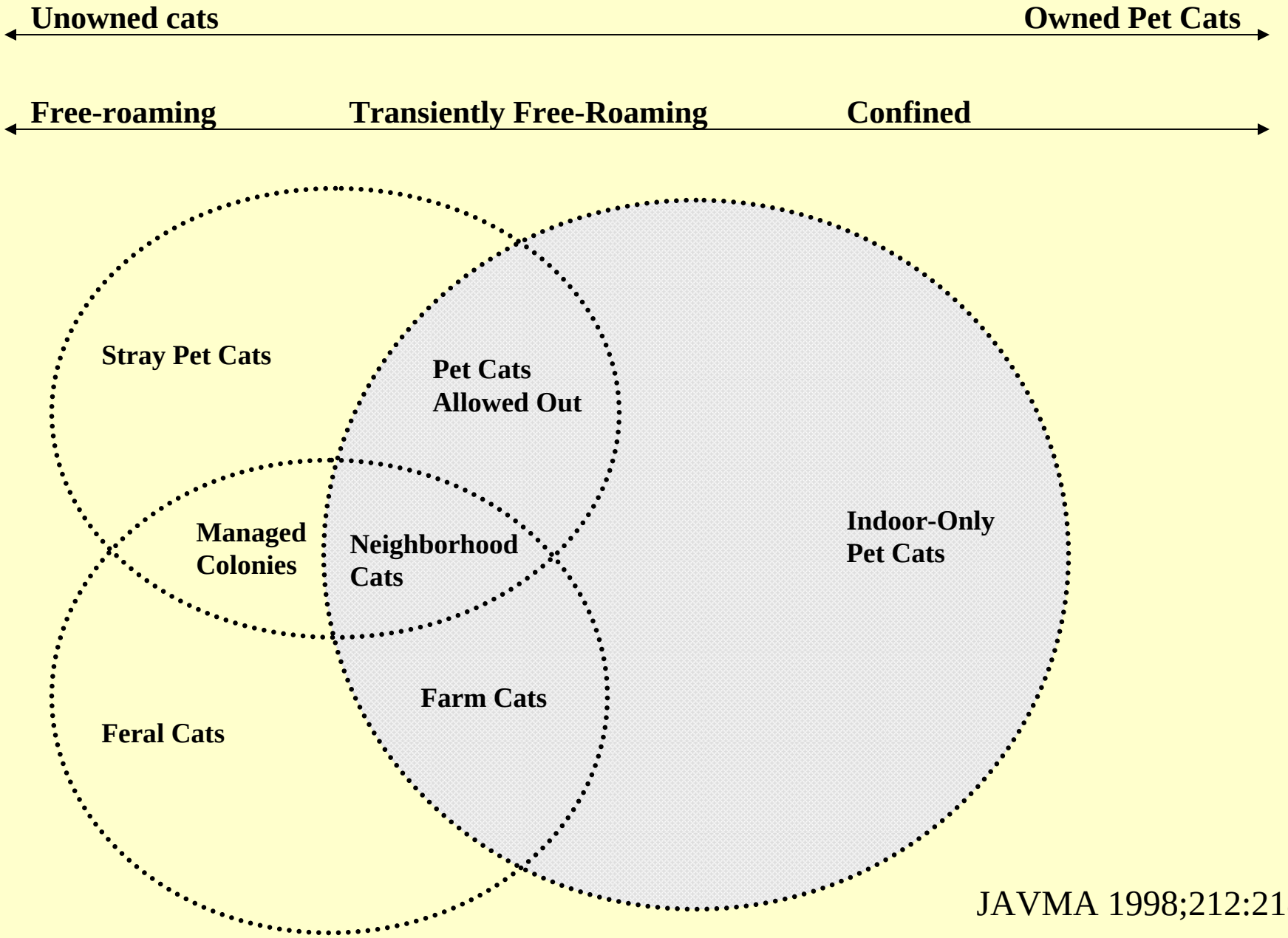
Role of Cats is Pivotal!….

…. BUT indirect!

~ 70 million in USA

"The cat sunning on a pillow in it's owner's apartment today may be considered a neighborhood stray tomorrow, and later may return home. It's likely that very few cats indeed have no dependency on humans for subsistence."

Gary Patronek. Free-roaming and feral cats – their impact on wildlife and human beings. JAVMA 1998;212:218.



JAVMA 1998;212:218

javma News

March 15, 2004

HSUS campaigns to debunk toxoplasmosis myths

The Humane Society of the United States is contacting more than 31,000 obstetricians and gynecologists nationwide with information to help them and their patients understand the risks of toxoplasmosis. The message is that pregnant women need not give up their cats.

"Misinformation about toxoplasmosis is widespread," said Patrick Duff, MD, residency program director of the Department of Obstetrics and Gynecology at the University of Florida.

Human Risks from Cat Contact

- Oocysts sporulate in 48 hours+ at room temperatures.
- Most cats do not leave feces on their fur for two days, so it is unlikely that humans become infected from direct contact with cats themselves.
- Because cats usually exhibit no signs of illness while passing oocysts, it is difficult to determine when a particular cat's feces may be infectious to people or other mammals.
- Most adult cats will not pass oocysts ever again after recovering from an initial exposure to Toxoplasma.

T. gondii seroprevalence in Rhode Island Cats

Am J Vet Res. 2002 Dec;63(12):1714-7.

- Overall, 42% of cats sampled were seropositive
- Seroprevalence was not significantly different between stray versus client-owned cats
- No differences by cat gender
- No differences by type of pet (mostly indoor vs.outdoor)

Feline Toxoplasmosis Disease

- Neurological disease in Feline Immunodeficiency Virus infected cats
- Ophthalmic disease, rarely, in any cat
- Generalized myositis in young cats
- Pneumonia occasionally reported
- Antimicrobial therapy (e.g, pyrimethamine and clindamycin) has been used successfully.

Treatment of Acute Cases in Human Beings

- Generally not indicated for most persons
- Exception: Pregnant women and immunocompromised persons
- Diagnosis typically by IgM plus IgG antibody titers
- Pyrimethamine plus sulfadiazine or clindamycin
- Add folinic acid to overcome thrombocytopenia, leukopenia
- Drugs do not eliminate pre-formed tissue cysts
- Drugs do not completely eliminate infections

Who is at risk for severe toxoplasmosis?

- Infants born to mothers who became infected with *Toxoplasma* for the first time DURING or JUST BEFORE pregnancy.

- Persons with severely weakened immune systems, such as persons with AIDS or transplant recipients. This results from an acute *Toxoplasma* infection or an infection that occurred earlier in life that reactivates and causes damage to the brain, eyes, or other organs. The infection can also be donor-derived (allografts).

Pediatric Blood Cancer
Vol.48, 2 Pages: 222-226

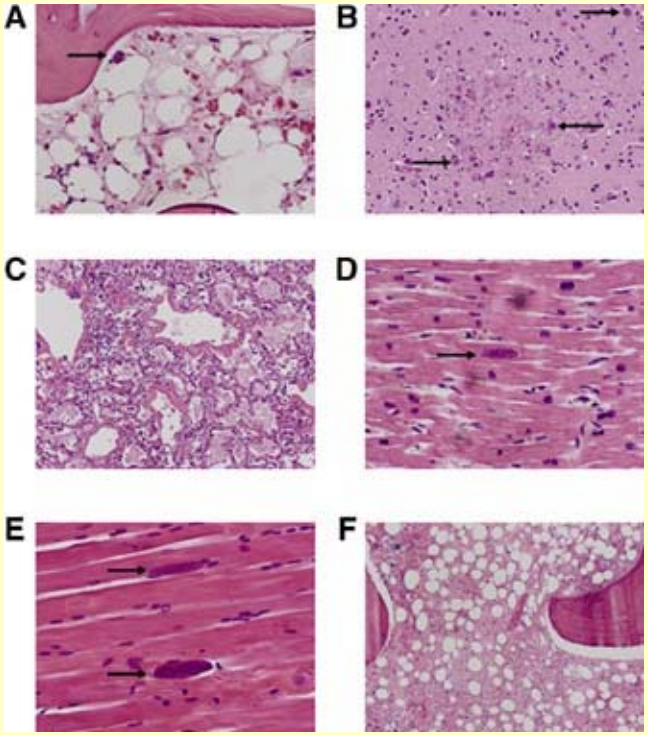


Figure 2. Histologic sections demonstrating the presence of *T. gondii* in various tissues. *T. gondii* cysts and bradyzoites are denoted by arrows. (**A**) Ante-mortem bone marrow biopsy revealing aplasia; this particular section shows a T. gondii organism (400× magnification); (**B**) Section of occipital lobe of brain, which shows necrosis in the center of the slide, and multiple organisms (200×); (**C**) Low-power (100×) section of lung showing diffuse alveolar damage; (**D**) Section of myocardium showing one organism (400×); (**E**) Section of psoas muscle with two organisms (400×); (**F**) Section of vertebral bone marrow demonstrating serous atrophy.

First Case of Toxoplasmosis Following Small Bowel Transplantation and Systematic Review of Tissue-Invasive Toxoplasmosis Following Noncardiac Solid Organ Transplantation. Campbell, Andrew L.[1,4]; Goldberg, Cindy L.[1]; Magid, Margret S.[2]; Gondolesi, Gabriel[3]; Rumbo, Carolina[1]; Herold, Betsy C.[1]

Volume 81(3), 15 February 2006, pp 408-417

TABLE 2. Demographic features of reported cases of toxoplasmosis

Characteristic	N (%)
Median age, years (range)	38 (6–74)
Sex	
Female	28 (54)
Male	24 (46)
Transplanted organ	
Kidney	34 (66)
Liver	12 (23)
Pancreas	1 (2)
Multiviseral	4 (8)
Small bowel	1 (2)
High risk	16 (31)
Donor positive/recipient negative	16
Standard risk	13 (25)
Donor positive/recipient positive	1
Donor negative/recipient negative	1
Donor negative/recipient unknown	1
Donor negative/recipient positive	2
Donor unknown/recipient positive	8
Unknown risk	23 (44)
Donor positive/recipient unknown	0
Donor unknown/recipient unknown	18
Donor unknown/recipient negative	5
Type of infection	
Primary infection	22 (42)
Allograft definitive source	16
Allograft probable source	5
Nonallograft source	1
Reactivation or reinfection	11 (21)
Indeterminate	19 (37)

Congenital Toxoplasmosis

Manifestations of congenital toxoplasmosis may not become apparent until the second or third decade of life. Serologic tests are used to diagnose acute infection in pregnant women, but false-positive tests occur frequently, therefore, serologic diagnosis must be confirmed at a reference laboratory before treatment with potentially toxic drugs should be considered.

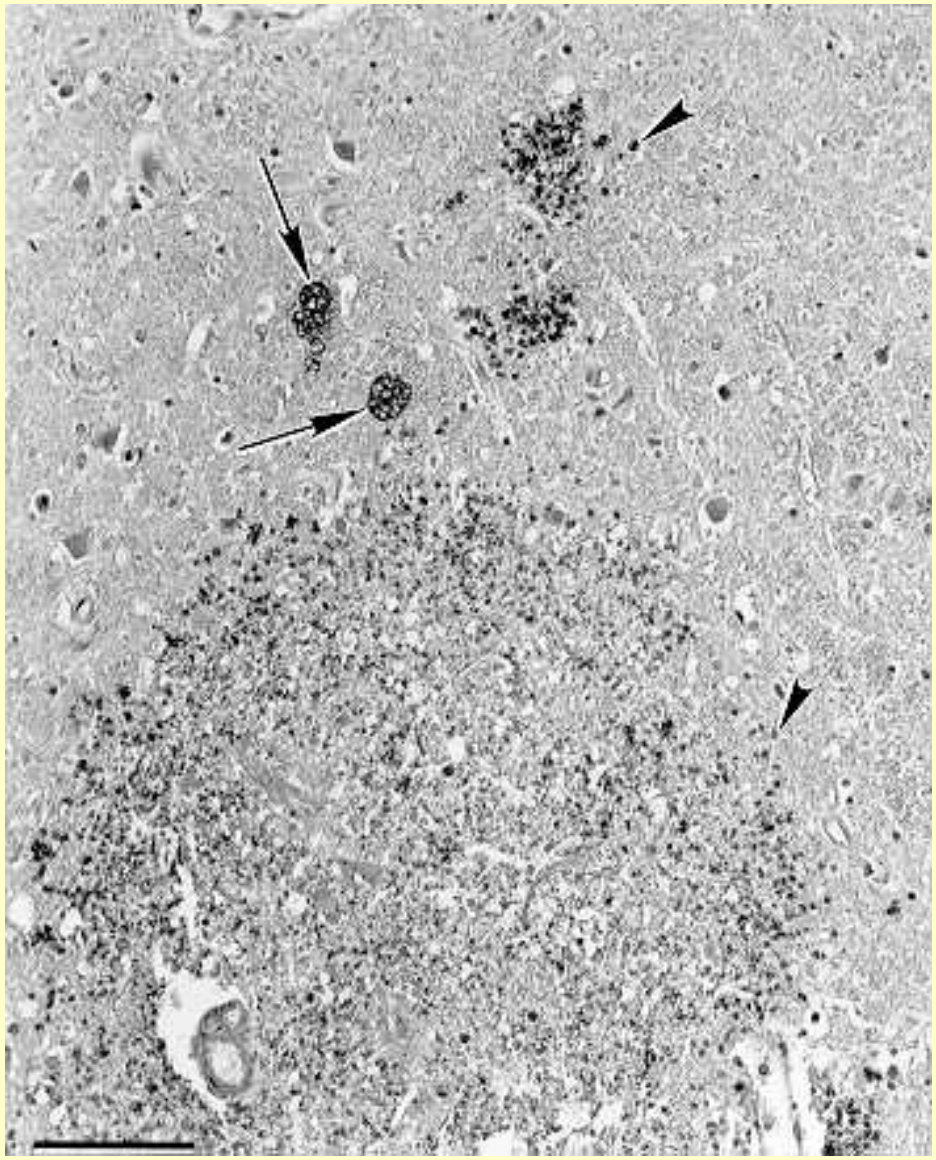
Infant girl with *T. gondii* hydrocephalus

Congenital *T. gondii* Infections

- Many false positives via some kits (8 brands)
- Send to reference lab for confirmation before treatment
- PCR of amniotic fluid useful for test confirmation/exclusion
- Pyrimethamine & sulfonamide for positive PCR-AF tests
- Spiramycin for negative PCR-AF tests

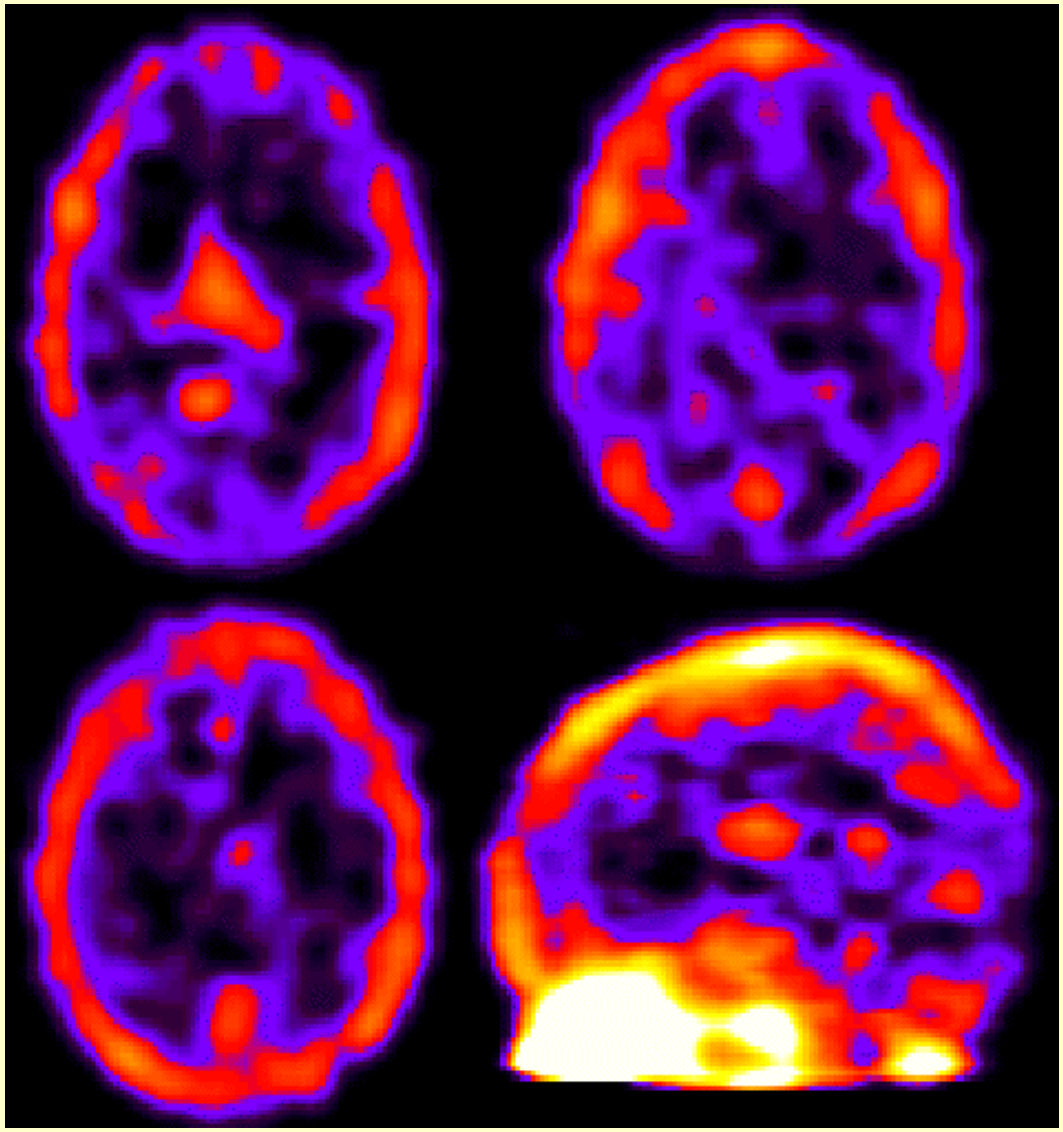
Toxoplasma Encephalitis –

The most frequent cause of focal CNS infections in AIDS patients



T1-201 SPECT Scan: TE Lesions in AIDS Patient

Multiple, bilateral, hypodense, contrast-enhancing focal brain lesions, often with ring-like patterns.



Treatment in AIDS Patients

- Toxoplasma seropositive patients with CD4+ lymphocyte count of < 100/μl:
  - Prophylax against Toxo encephalitis
  - Trimethoprim-sulfamethoxazole or Dapsone
- For TE Patients:
  - Pyrimethamine plus Sulfadiazine or
  - Pyrimethamine plus Clindamycin
  - Alternatively,Atovaquone (1500 mg twice daily)
  - Simultaneous coverage for *Pneumocystis carinii*.

Transplant Patients

• Most toxoplasmosis cases are caused by disease reactivation (tissue cysts), not incident infections… esp. retinochoroiditis

• All SCT recipients should be provided information to reduce their exposure risk

Recommendations for Diagnosis of Toxoplasmosis in SCT Patients

Pre-transplant:

- **Assessment of risk factors- geography/endemicity; exposure to soil, cats, undercooked meats**
- **PCP prophylaxis with trimethoprim-sulfamethoxazole may reduce reactivation**
- **Serologic testing (IgG, IgM, IgA, IgE, and AC/HS) by reference laboratory**

Peri-transplant:

- **Consider trimethoprim-sulfamethoxazole or pyrimethamine-sulfadoxine prophylaxis for seropositive patients, especially in endemic areas**

Post-transplant:

- **High index of suspicion**
- **If CNS symptoms ± characteristic MRI findings, obtain CSF for PCR**
- **PCR and/or tissue biopsy for non-CNS disease**
- **Institute empiric treatment with pyrimethamine-sulfadoxine early**
- **Empiric therapy with pyrimethamine-sulfadiazine for highly suspicious cases while awaiting definitive diagnosis**

Prevention - General Public

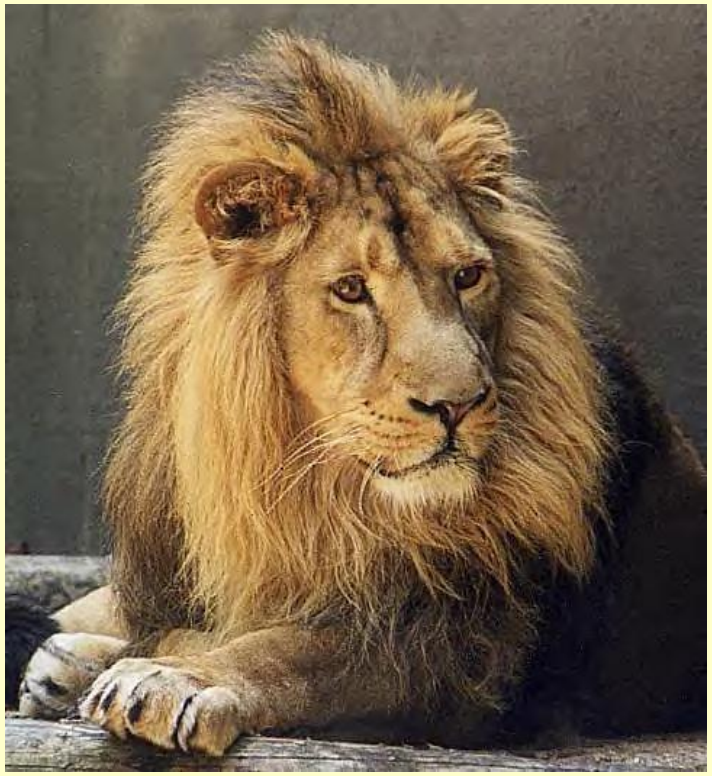
Steps to prevent human exposure to Toxoplasma

- Change litter daily before Toxoplasma oocysts can sporulate to their infectious form. Dispose of used litter safely, preferably in a sealed plastic bag. If pregnant or immune compromised, avoid changing the litter box or use rubber gloves when doing so.
- Wash vegetables thoroughly before eating, especially those grown in backyard gardens. Boil water from ponds and streams when camping/hiking.
- Cover sand boxes when not in use to discourage cats from defecating in them.
- Wash hands with soap and water after working with soil or after handling raw or undercooked meat.
- Cutting boards, knives, sinks and counters should be washed well and disinfected after cutting meats.
- When cooking, avoid tasting meat before it is fully cooked.
- Cook meat thoroughly until the internal temperature reaches 160°F in a conventional oven. Also, be aware that microwaving is not a sure way to kill Toxoplasma in meat.

AVMA Recommendations

Preventing Toxoplasma infection in cats

- Do not allow cats to hunt rodents and birds-keep pets indoors.
- Feed cats only cooked meat or processed food from commercial sources.
- At present there is no vaccine for Toxoplasmosis in cats.
- Efforts are underway to develop a vaccine to prevent oocyst shedding by cats.



American Journal of
EPIDEMIOLOGY

ORIGINAL CONTRIBUTIONS

Toxoplasma gondii Infection in the United States: Seroprevalence and Risk Factors

Jeffrey L. Jones, Deanna Kruszon-Moran, Marianna Wilson, Geraldine McQuillan, Thomas Navin[1] and James B. McAuley

Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

Division of Health Examination Statistics, National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD.

Critique of the AJE Article:

1. What was the main purpose/hypothesis of the study?
2. What was the study design? What are its strengths and weaknesses?
3. What was the study population? Was it representative?
4. What exposures or risk factors were measured? Were there any biases or limitations in their measurement?
5. What was the principal outcome of interest (infection or disease) and how was it measured? Identify advantages and disadvantages with this measure.

Critique of the AJE Article:

6. What were the main findings? Do you agree or disagree? Support your position.What was the study design? What are its strengths and weaknesses?
7. Was there any potential confounding in the data analyses? Was it considered in the data analyses? Explain.
8. Were there shortcomings/limitations to the study? If so, were they of sufficient magnitude to invalidate the results?
9. Write a one-sentence summary of the article that could potentially be used in the context of community health promotion campaigns.
10. Based on this work, what would be the next study you would want to do if you had the necessary resources? Why?

NHANES

National Health and Nutrition Examination Survey

DESCRIPTION: The NHANES target population is the civilian, noninstitutionalized U.S. population. NHANES 1999-2000 includes over-sampling of low-income persons, adolescents 12-19 years, persons 60+ years of age, African Americans and Mexican Americans. The major objectives of the NHANES are: 1) To estimate the number and percent of persons in the U.S. population and designated subgroups with selected disease and risk factors; 2) To monitor trends in the prevalence, awareness, treatment and control of selected diseases; 3) To monitor the trends in risk behaviors and environmental exposures; 4) To analyze risk factors for selected diseases; 5) To study the relationship between diet, nutrition and health; 6) To explore emerging public health issues and new technologies.