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CASE REPORT
Myelitis due to reactivated spinal toxoplasmosis in a cat

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- 10 FS Cornish Rex
- 3 week previously – wt bearing lameness of RFL which rapidly progressed to knuckling and non wt bearing lameness
- Bilateral pelvic limb paresis and anisocoria (miotic R pupil)
- Tx with meloxicam , amoxy clav and clindamycin(25mg PO q12hr) and pred (5mg PO q24hr) – no response
- The cat became anorexic non ambulatory and had a left sided head turn
- CEx – dull , non ambulatory with a preference for right lateral recumbency , BP 200mmHg , mental status appropriate, anisocoria was present with the right pupil miotic relative to the left , all other CN normal , left head turn and left torticollis and was unable to stand and ambulate , neck palpation – some pain , postural reactions and proprioceptive paw positioning were absent in right thoracic and pelvic limbs and reduced in the left thoracic and pelvic limbs
- Visual and tactile placing were absent on the right side and normal on the left side
- Reduced muscle tone and atrophy in the right thoracic limb and increased muscle tone in both pelvic limbs
- Segmental spinal reflexes were absent in the right thoracic limb, normal in the left thoracic limb and exaggerated in both pelvic limbs
- A crossed extensor reflex was present in the pelvic limbs
- Neuroanatomical diagnosis was suggestive of primarily a spinal cord lesion affecting the C6-T2 segments, more severe on the right
- Consistent with this localization the miotic pupil was attributed to partial right Horner's syndrome although it could also have been due to intracranial disease
- Ddx neoplasia, inflammation (toxo, dry FIP , cryptococcus, bacterial meningoencephalomyelititis , granulomatous meningoencephalitis , FIV or FeLV related disease
- Toxo titres IgG titre of 1/512 and negative IgM titre measured using the indirect fluorescent antibody technique
- Results consistent with previous exposure to T gondii rather than active toxoplasmosis
- Cat had lack of response to therapy (clindacin 42.5mg PO BID) and deterioration euthanasia
- Post mortem

- Mild reddening of the cervical and thoracic spinal cord meninges
- Remaining CNS normal
- There was marked perivascular cuffing within the white and grey matter, predominately involving small lymphocytes and scattered plasma cells ,
- Marked generalized unilateral cellular infiltration of the spinal cord, mainly involving the grey matter
- This infiltrate was also predominately lymphocytic but contained scattered plasma cells, large mononuclear cells (macrophages) and glial cells
- Multifocal areas of necrosis and malacia were noted
- In the area of marked cellular infiltrate were multiple round, variable sized protozoal cysts
- IHC showed strong positive staining and confirmed the diagnosis of reactivated toxo due to T.gondii
- IHC staining also positively labelled tachyzoites free within the neural parenchyma adjacent to the ruptured cysts
- Inflammatory changes within other regions of the spinal cord were limited to mild to moderate perivascular cuffing with lymphocytes and plasma cells and congestion of vessels
- Examination of the brain sections identified a single toxo cysts within the forebrain that was not associated with an inflammatory response and mild congestion of the subdural vessels of the midbrain region
- Morphological diagnosis of marked segmental non suppurative myelitis and necrosis and marked gliosis was made along with an aetiological diagnosis of CNS toxo

- Encysted form of T gondii can persist for life , is weakly immunogenic and does not stimulate an inflammatory response
- The clinical signs in this cat were assumed to be a consequence of marked CNS inflammation in response to the release of bradyzoites following reactivation of latent T gondii infection
- This is based on the observation of protozoal cysts within the cerebrum and areas of the spinal cord that were not associated with a host inflammatory response the apparent rupture of the cysts and present of tachyzoites within the parenchyma, a lack of addition al systemic signs or organs involvement, T gondii antibody titres suggesting previous exposure rather than active (recent) disease
- This diagnosis concurs with the suggestion that demonstration of neurological (and ocular) signs in the absence of systemic signs is more common with reactivated than acute infection
- While inflammatory CNS disease is typically thought to contribute to demonstration of multifocal or diffuse neurologic signs, a significant number of animals present with clinical signs referrable to focal disease
- Severe necrotising myelitis secondary to reactivation of T gondii or toxoplasma-like organisms has been described in 2 cats of which one was FIV positive
- Reactivation of latent T gondii infection resulting in acute disseminated infection has also been documented secondary to immunosuppression in a cat prescribed prednisolone and azathioprine, in an FIV positive cat treated with repeated depot megestrol acetate injections and in cats administered cyclosporin
- No underlying cause of immunosuppression was found in the cat of this report
- This case demonstrates the difficulty associated with the ante-mortem diagnosis of reactivated toxo o the CNS when there is a lack of serological confirmation for active disease and a poor response to appropriate therapy

- Serology may be used as an adjunct to diagnosing toxoplasmosis but interpretation can be complicated
- A positive IgM titre or a fourfold or greater increase in IgG in paired serum samples taken 2-4 weeks apart are generally considered diagnostic of active toxo
- Reactivation of latent infection to cause disseminated toxo was documented in a cat that was IgM negative and did not demonstrate a rising IgG titre
- Feline toxo associated CNS lesions are similar to those reported in dogs and are characterised by multifocal non suppurative meningoencephalitis and myelitis , the resulting neurological signs may permit localisation of disease but are otherwise non specific
- Analysis of CSF may be normal or demonstrate a mild elevation of protein and cell counts (usually a mixed population of large and small mononuclear cells and neutrophils) , however tachyzoites are rarely detectable in CSF
- Clindamycin is drug of choice effective in crossing the blood brain barrier in CNS toxo , however total resolution of neurological deficits may not occur due tot permanent damage to the CNS as a result of the hosts inflammatory response
- Dose of clindamycin for treatment of feline toxo 12.5mg/kg PO of Im q12hr for 4 weeks in dose range 20-50mg/kg in 2-3 divided doses)

- Obligate intracellular protozoan parasite that can infect virtually all warm blooded animals
- Cats and other Felidae are the definitive hosts while other animals act as intermediate hosts
- Infection can occur congenitally or via ingestion of infected tissue or sporulated oocysts passed by cats
- Following ingestion of sporulated oocysts, sporozoites excyst in the intestinal lumen and penetrate the intestinal cells
- Sporozoites divide into tachyzoites which can multiply in almost any cell of the body eventually encysting to become tissue cysts containing bradyzoites
- Clinical findings in cats are varied , ranging from general signs such as lethargy , fever, inappetence, wt loss , vomiting or diarrhoea to more specific respiratory ocular or neurological signs
- Myocarditis as a result of T gondii has also been reported in a cat
- Polymositis as a result to toxo is recognised in humasn, and dogs and has been described as a potential cause of myopathy in cats and has been identified histologically within skeletal muscle

- Lappin et al reported muscle pain in 3/15 cats diagnosed with toxo although there was no histo confirming a myopathy in those cases
- Case – 6 month history of intermittent bilateral FL lameness , an intermittent bunny hopping Hl gait difficulty using the stairs and jumping and occasional vocalisation suspected to be pain related
- The gait was worse following rest and improved with walking, 18 months prior to presentation there had been a period of right HL lameness followed by a period of l HL lameness that lateral resolved
- Ddx diffuse neuromuscular disease an autoimmune disease such as systemic lupus erythematous or polyarthritis , or an infectious disease such as toxo
- Nerve conduction velocities and EMG findings were consistent with a diffuse muscular disease worse in the proximal thoracic limb and distal pelvic limb muscles
- An axonpathy without secondary myelin disturbances was considered less likely
- Biopsies were taken from the right infraspinatus , right triceps brachii , right extensor carpi radialis right cranial tibialis and right gastrocnemius muscles and submitted for histo and Toxo PCR
- T gondii serology using an IFA returned markedly elevated IgG titre 1:1600 (RI <1:50) and borderline IgM titre of 1:25 (RI <1:25)
- Histo each muscle biopsy revealed a diffuse chronic moderate myopathy with some myofiber resorption
- There was no evidence of toxo DNA on PCR testing of the right triceps brachii muscle
- An improvement was seen within 3 days of starting the clindamycin
- Toxo serology 2 weeks after starting clindamycin revealed a markedly elevated IgG titre of 1:1600 and mildly elevated IgM titre of 1:50
- The cat represented following a 6 week course of clindamycin - occasional FL lameness but otherwise significant improvement
- Ophthalmological examination detected a small chorioretinal scar that could be compatible with a toxo lesion three was no evidence of active lesions and no ophthalmic nerve pathology
- Toxo serology repeated 13 weeks after finishing the clindamycin revealed a positive IgG titre of 1:400 and a normal IgM titre of 1:20
- 20months after cessation of treatment and there was full resolution of clinical signs

Discussion

- Standard diagnostic criteria for an inflammatory myopathy include the presence of compatible clinical signs , an elevated CK , EMG documenting muscle membrane instability and histo evidence of muscle inflammation
- A lack of identifiable cause is required to diagnosis an immune-mediated polymyositis
- There are limited reports identifying infectious causes of myopathies in cats
- Muscle pain has been reported in 3/15 cats that were diagnosed with toxo based on serology clinical signs and response to clindamycin but no histo path performed on those cases to confirm a myopathy
- In general a myopathy does not appear to be typical manifestation of toxo in cats in contrast to dogs
- This case met the diagnostic criteria for an inflammatory myopathy as described by Podell the cat has an abnormal gait with lameness on all 4 limbs , an elevated

creatine kinase , an EMG consistent with a diffuse muscular disease and histopathology consistent with a diffuse and chronic myopathy

- A tentative ante-mortem diagnosis of Toxo can be made cased on serological evidence of recent or active infection consisting of high IgM titres or a 4 fold or greater increase or decrease in IgG titres after treatment ; exclusion of other aetiologies and clinical improvement following an anti-toxo drug
- IgM may appear earlier in the disease but declined more rapidly that IgG antibodies which usually persist for life
- Therefore a positive IgM is not necessary for diagnosis a normal IgM with positive IgG can indicate either previous exposure or a current infection
- Montoya et al have previously discussed the potential for a reduction in toxo PCR sensitivity as a result of the low number of tissue cysts sparsity of parasites and their focal distribution in th tissues
- Evans et al did not identify any toxo organisms on histo in 5 dogs they diagnosed with generalised myopathy as a result of toxo

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Short communication

Comparison of genotypes of *Toxoplasma gondii* in domestic cats from Australia with latent infection or clinical toxoplasmosis

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- Most infections in cats are subclinical and severe toxo is uncommon, as reflected by high seroprevalence among pet cats
- Rarely healthy cats with no detectable immune deficits or comorbidities develop severe clinical toxoplasmosis
- Severity of clinical disease in some infected hosts is influenced by Toxo gondii genotype
- Genotypes of toxo gondii in healthy cats are regionally specific such as non archetypal genotype , Chinese 1
- The amin of this study was to compare the genotype of Toxo gondii in latently infected cats with those from cats with clinical toxoplasmosis
- We applied mulitlocus PCR-RFLP genotyping based on 12 single locus markers and the multicopy *B1* gene
- Materials and methods
    - Tissue samples (skeletal muscle , forebrain , liver and lungs) – formalin and also frozen at -80C from 16 cats seropositive for T gondii specific IgG and archived tissues from one cat previously diagnosed with disseminated toxo were included for analysis

- IHC staining for T gondii antigen was performed using T gondii epitope specific rabbit polyclonal antibody at 1:1000 dilution
- Clinical toxo was defined as histological detection of protozoal cysts and/or tachyzoites with positive IHC staining and associated inflammation
- Latent infection was defined as absence of inflammation-associated protozoal cysts or zoites on histopathological and IHC examination of multiple tissues in seropositive cats
- Toxoplasma gondii B1 genePCR amplification and multiclonus genotyping
  - DNA was extracted form 3 25mg aliquots of each tissue (muscle, lung, liver and brain)
  - All DNA extractions included a negative control
  - Presence of amplifiable DNA was confirmed by PCR of feline glyceraldehyde-3-phosphate dehydrogenase
  - A diagnostic PCR amplifying the B1 gene (530 bp) was used to detect T.gondii DNA in triplicate samples of each tissue type
  - T.gondii genotype in cat tissues testing repeatedly positive i=on the B1 PCR was then determined using a multiclonus nested PCR to amplify B1, SAG1, 5'SAG2, 3'SAG2, altSAG2, SAG3, BTUB, GRA6, c22-8,c29-2,L358,PK1,altSAG2 and Apico followed by DNA sequencing and virtual RFLP, PCR products were sequenced bi-directionally using amplifications primers
  - Chromatograms were visually verified using CLC Main workbench
  - Virtual RFLP was performed using NEB-Cutter and compared to reference sequences of clinal t.gondii types 1 and 111.
  - The reference sequence for the B1 gene was AF179871
  - Dinucleotide peaks were detected using the secondary peak calling function on CLC Main Workbench with the cut off of 20% of the maximum peak height (forward and reverse sequence) prior to assembly

Results

- Cats with T.gondii infection
  - T.gondii cysts and tachyzoites were detected in 4 or 17 cays
  - The B1 gene PCR was positive in tissues from 11 to 17 cats tested (65%) including seven with latent infection and 4 with clinical toxo with a varying degree of PCR reproducibility from examined DNA
  - 8 cats returned repeated satisfactory results in the T gondii B1 PCR assay, low conc of T gondii DNA were suspected for cats 9-11, based on PCR results

    - 2 of the 4 cats with clinical toxo were diagnosed antemortem - in 1 of thses , an FIV infected cat , tachyzoites were detected in cytological prepartations of liver from a FNA – despite treatment with clindamycin 12.5mg/kg q12hr IV and pyrimethamine 1mg/kg PO q12hr the cat developed respiratory distress and was euthanised
    - In the other case , a cat receiving pred 1mg/kg PO q24hr and cyclosporin 6mg/kg PO q24 hr for a severe chronic inflammatory polyarthropathy , tachyzoites and fungal hyphae were detected on cytology of pleural fluid
    - In 2 cats clinical toxo was diagnosed post mortem including a cat treated with multiagent chemotherapy for hepatic lymphoma and a cat with chronic progressive neurological signs and multiple CN deficits
- Toxo gondii Type 11 in cats with clinical and latent infections
    - Loci for T.gondii genotyping were amplified in 8 of the 11 cats 73% that tested positive to the B1 gene PCR but not in the other 3 cats despite repeated attempts
    - 7 cats including 4 with clinical toxoplasmosis were genotyped as toxoDB genotype#3 using 12 loci
    - One cat with latent infection could only be typed at 6 single copy loci , the virtual RFLP suggested genotype ToxoxDB#1, ##,#128 or #129
    - Comparison with reference T gondii DNA sequence revealed unique polymorphisms in the 3'AG2 and L358 locus of some strains
    - T.gondiii single copy 3'SAG2 locus from cat 1 had dinucleotide peaks at nucleotide position 1222 and 1275 suggesting co-infection with more than one strain
    - We then typed T.gondii for cats 1-8 at the B1 gene locus because it has been shown to be variable between isolates, in 5 cats T gondii type 11/111 alleles were present at the B1 locus while in 3 cats Tgondii type 11/111 like alleles were detected

Discussion

- First study to characterise the genotypes of T.gondii in Aust cats where disease status was definitely identified
- We did not find an association between genotype and clinical toxo
- While the findings in this study do not rule our the possibility that certain genotypes may be more virulent than others in cats , they suggest that factors that influence host susceptibility are more common determination of clinical disease severity
- Of the 4 cats with clinical toxo , 3 were immunosuppressed
- Although experimental studies in cats have found associations between Tgondii genotype and disease severity differences between Tgondii genotype and disease severity, differences between studies in the inoculum dose, infection parasite life stage (tachyzoites, oocysts or bradyzoites) and route of infection could be confounding variables
- Correlations between virulence and genotypes are likely to be most applicable where the route of inoculation, life stage of the parasite and inoculum dose mimics natural infections
- 8 other reported cases of clinical toxoplasmosis in cats where the infecting strain of T.gondii was genotyped Toxo DB genotype #3 was detected in only 1 cat from Switzerland which is similar to cat 5 in this study did not have concurrent disease
- Other genotypes detected including Type 12 clonal in a cat from YS and clonal type 11 in 6 cats from EU were all endemic to the region where cats were from and had also been detected in cats without clinical lkdisease
- The high prevalence of TOXODB genotype #3 in this study suggests it is endemic in Australia this genotype is one of the dominant genotypes found in EU , Africa and Nth America and is

the second most frequently id genotype of all hosts world wide after the clonal type 11 genotype

- Fe other aust isolates of T.gondii have been genetically characterized using multilocus PCR-RFLP genotyping the most common genotype identified previously was ToxoDB#1 from a seal , dog, goat, wallaby and 2 wombats , toxoDB genotype#3 was identified in 4 hosts including a common wombat and a dolphin
- Similar to cat 5 the only cat for which comorbidities were not detected the wombat and bird both had clinical toxoplasmosis with severe neurological signs
- When data from other studies are combined without study it appears likely that TOXO DB#3 is the most prevalent genotype in Australia
- The addition of the highly polymorphic B1 locus to the other multi-locus DNA markers is important for genotyping of Aust T.gondii isolates since non archetypal and type 11 like alleles have been frequently found at this locus
- Only 2 unique alleles were identified at the B1 locus in cats in this study , whereas diversity in this allele was high in Australian wildlife
- Co-infections with 2 or more strains as detected in cat 1 are also common among Australian wildlife
- In feline hosts with 2 strains there is potential for sexual reproduction to occur between strains
- Cat 4 with latent infection could only be partially genotyped , presumably due to low T gondii DNA conc
- It is possible that the low sensitivity of the technique used for Tgondii DNA extraction in this study created a bias towards detecting genotypes that have a high parasite burden such that the true diversity if T gondii may be underestimated
- Genotyping of the other latently infected cats may have been successful using techniques to concentrate T.gondii DNA such as mice bioassay

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Review article

All about toxoplasmosis in cats: the last decade

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Prevalence

- Serological prevalence
  - World wide
  - Seropositivity increased with the age of the cat, indicating post-natal transmission of T gondii
  - Ab to Toxo gondii have (6-10 weeks) been detected in most cats after weaning
  - Possible low levels of antibodies in some young ats are from maternal ab .

    - Maternally transferred antibodies disappear in the cat by 12 weeks of age
    - Prevalence related to lifestyle of cat – feral cats that hunt for their food
    - Varies among countries and within different areas of a country and within the same city
    - Concurrent infections with certain feline pathogens can affect T.gondii infections in cats – although infections can modify the clinical outcomes of infections there is no evidence that they affect the seropositivity of T.gondii
    - MAT and IFA were the most often tests used to detect antibodies to T.gondii in cat sera and the results are comparable
    - The MAT is simple easy to perfom is not host specific and is available commercially , specific and no cross reactions have been documented with antigens of other microbes
    - The commercially abaliable IHAT and I.AT are less sensitive and less specific compared with the MAT
    - ELISA are easy to perform bu need special equipment and the specificity and sensitivity depend of the antigen used - combination of different recombinant antigens was found useful in the feline toxoplasmosis vs single antigens
- Isolation of viable T.gondii in tissues of cats
    - T.gondii was isolated from tissues of naturally infected cats by bioassay in mice or cell culture
    - Of the 112 cats from Egypt whose tissues were bioassayed in mice individually
    - T.gondii was isolated form heart of 74.1% , tongues 47% and brains 32.1%
    - Thus T.gondii was more prevalent in muscles than in the brain of cats
- Prevalence of T.gondii oocysts in cats
    - Prevalence varied with the method of detection
    - By microscopic examination T.gondii like oocysts wre detected in <1% of cats but cant distinguish from *H.hammondi*
    - T.gondii DNA in feces was detected in as many as 20% of asymptomatic cats
- Clinical infection and oocyst excretion
    - Although any organ may be involved by T.gondii pneumonitis is the most common finding and can be rapidly fatal
    - Of the 31 cases of clinical toxoplasmosis in cats reported concomitant infections/immunosuppressive conditions were present in 11 cats
    - Clinical presentation included jaundice, anorexia, vomiting paresis and dermatitis
    - Ocular lesions were not mentioned in any of these cases
    - In a study of 104 cases of uveitis , antibodies to T.gondii were detected in 19 sick (18.3%) vs 1 (5.3%) of healthy cats – however serological tests should be used only as an aid in diagnosis because antibodies titres may not be different than in healthy population
    - In another of study of 14 seropositive cats that were clinically evaluated , 11 had neurological sings , 5 cats had uveitis and 4 had diarrhoea , T.gondii oocysts were detected in feces of 3 , AST and ALT were elevated in 3 cats
    - In another study from Romania uveitis was diagnosed in 19 (86.4%) of 22 cases of suspected clinical toxo in cats
    - In 3 of the 31 cats dx was made by finding tachyzoites in the biliary contents of 3 cats , in dermal nodule aspirate of 2 cats , lung aspirate of 1 cat , tracheal wash of 1 and CSF of 1 cat

    - 2 of these 20 cats were excreting T.gondiii oocysts – 1 cat had diarrhoea
    - T.gondii oocysts were detected in the feces of a cat 10 day before onset of clinical signs
- Genetic types of T.gondii in domestic cats
    - Differences in molecular characteristics of T.gondii is a factor in pathogenesis of clinical toxoplasmosis
    - The type of strains prevalent in cats is most important in the epidemiology of the parasite because they are the only hosts that can excrete the environmentally resistant oocyts and can directly transmit the parasite to humans and livestock
    - The frequencies of major genotypes identified in cats agree with global patterns of T.gondii distribution
- Experimental infections in cats
    - Immunization of cats to prevent oocyst excretion
        - Oral inoculation of cats with bradyzoites of a chemically induced mutant of T.gondii can induce protective immunity to oocyst excretion
        - Genetically engineered mutant HAP2KO was produced – oral inoculation with live tissue cysts led to the excretion o f abnormal oocytsts (oocysts that did not sporulate) but cats became immune to oocyst excretion
        - Infections with killed whole T.gondii or recombinant proteins have been unsuccessful to prevent excretion of T.gondii oocysts following challenge with tissue cysts
- Immunity to re-excretion of T.gondii oocysts
    - How often cats excrete oocysts in their lifetime in unknown and it is impossible to stimulate natural conditions for experimental infections in cats
    - Cats that had excreted oocysts did not re-excrete after challenge within 2-3 months after primary infection
    - With respect to oocyst re-excretion there are no data on naturally infected cats
    - Owever naturally exposed cats did excrete oocysts after feeding them tissue cysts in the lab
    - In one study 7/12 adult feral seropostivie cats with dye test titres of 1:4-1:128 and 4 or 6 dye test positive cats excreted T gondii oocysts after feeding tissue cysts
    - The dye test is considered the most specific serological test for T.gondii and even a titre of 1:2 is considered specific – these data indicate that cats might re-excrete oocysts.
    - Most cats usually excrete oocysts for less than 1 week , however studies have shown some cats have a prolonged period of excretion or re-excretion
    - (based on study of cats over 12 days of feces and rectal contents)
    - Malnutrition and concurrent infection with the feline coccidium *Cystoisospora felis* can affect immunity to excretion of oocysts in cats
    - Cystoisospora felis can affect immunity to excretion of oocyst in cats
    - Excretion of millions of T.gondii oocysts by chronically infected cats , in the absence of T.gondii infection from outside is most intriguing because c.felis is usually non pathogenic for cats
    - Immunosuppression by pharmacological doses of immunosuppressive drugs and co-infection with FIV and FeLV do not affect re-excretion of oocysts in cats

    - Prolonged administration of pharmacological doses of cyclosporine 7.5mg/kg/day orally fr 126 days did not lead to re-excretion of T.gondii oocysts
    - Conclusion immunity to re-excretion of T.gondii oocysts in cats is food and cats seldom excrete oocysts after primary infection
    - However there are no guaranties that a cat will not excrete oocysts more than once in life therefore cats should take precautions when handling faeces
- Serological responses of experimentally infected cats
    - Experimentally infected cats SAGI-ELISA for detection of T.gondii Ab – these results obtained with ELISA paralleled IFAT values
- Pathogenesis of congenital infection
    - Uncommon
    - Histological documents of congenital infection in experimentally infected cats is difficult because of the short gestation and because queens cannibalise sick kittens
- Lipid metabolism determines host specificity of T.gondii oocyst excretion in cats
    - Feline are the only mammals that lack delta-6-desaturase activity in their intestines , which is required for linoleic acid metabolism , resulting in systemic excess of linoleic acid
    - It was determined that T.gondii sexual development occurs when culture feline intestinal epithelial cells are supplemented with linoleic acid
    - Inhibition of murine delta-6-desaturase and supplementation of their diet with linoleic acid allowed T.gndii sexual development in mice - this mechanism for species specificity is the first defined for a parasite sexual cycle

Other felids

- Prevalence
    - Serological prevalence
        - Virtually all captive wild felids become exposed to T.gondii
    - Isolation and genetic characterisation of viable T.gondii from wild felids
        - Viable T.gondii has been isolated from several wild felids
        - Genetic typing results showed dominance of TOXODB genotype #5
    - Prevalence of T.gondii oocysts in wild felids
        - Limited data

Conclusions

- the high prevalence of antibodies in cats is indicative of past infection and excretion of T.gondii oocysts in the environment
- compared to high seropositivity T.gondii oocysts were not common
- microscopically oocysts were detected in faeces of around 1% of cats but T.gondii DNA was more prevalent

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Clinical Toxoplasmosis in Dogs and Cats: An Update

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- tachyzoites multiply actively in tissues quickly spread to almost all organs and cause most of the pathology
- once they reach the specific tissue they (CNS, muscle and viscera) they convert into bradyzoites which remain latent in a cyst form leading to a life long chronic infection until a DH (definitive host) ingests it
- then bradyzoites are released and penetrate the SI epithelial cells, giving rise to schizonts that form gamonts and finally oocysts
- oocysts are the environmentally resistant and infective stage
- hosts can be infected horizontally by ingesting tissues containing cysts , consuming water or food contaminated with oocysts or by transfusion or transplantation with parasitized organs
- vertical infection also by congenital infection
- seroprevalence and/or parasite prevalence ranges in dogs and cats from 6-99% worldwide

Clinical presentation and pathology of Toxo in Dogs and Cats

- clinical toxo is more severe in trans placentally infected kittens which frequently develop hepatitis or cholangiohepatitis, pneumonia and encephalitis and show signs of ascites, lethargy and dyspnoea
- adults
    - unspecific clinical signs can be observed ,
    - the occurrence of hepatitis and abdominal involvement hepatic failure and hyperplastic cholangitis ,
    - in disease thickening of the gastric wall due to eosinophilic fibrosing gastritis and regional lymphadenopathy were noted
    - the disease may be rapidly fatal in cats with severe respiratory or neurological disease
    - pneumonia is the main sign of generalized toxo and acute respiratory distress syndrome and septic shock
    - ocular toco has been observed in cats without poly-systemic clinical signs of disease and anterior or posterior uveitis , iritis , iridocyclitis or chorioretinitis , aqueous flare , keratic precipitate , lens luxation , glaucoma, and retinal detachment are common manifestations of uveitis -therefore ocular examination in pyrexic cats
    - less frequent findings such as myocarditis with echocardiographic changes, diarrhoea with oocysts, or pyogranulomatous cystitis after renal transplantation

- primary or reactive neurological disease is frequent in cats – infection of the encephalon, spinal cord and nerves ; general myelitis, marked generalized mononuclear cell inflammation of the grey matter, non-suppurative encephalitis and perivascular cuffing are common findings , cases of intracranial granuloma and panencephalitis have also been reported
- generalized toxoplasmosis in immunocompetent and immunosuppressed cats involving acute interstitial pneumonia, acute and multifocal necrotizing hepatitis , nonsuppurative meningoencephalitis with glial granuloma, moderate lymphadenopathy and splenomegaly
- cutaneous manifestations with nodules that may ulcerate are sometimes related to feline immunosuppression

Congenital infections in dogs and cats

- after primo infection during pregnancy, parasitemia can cause placentitis followed by spread of tachyzoites to the foetus
- kittens born to queens infected with T.gondii during gestation can become infected trans placentally or via suckling
- in general clinical illness is common and severity varies with the stage of gestation at the time of infection
- congenital toxo was diagnosed histologically in nine kittens and one queen from five litters. The queen died of generalized toxoplasmosis and the kittens presented with toxoplasma hepatitis and pneumonia , in addition 3 in 1 month old kittens from another litter were shedding T.gondii like oocysts
- After oral inoculation of 5 full term pregnant queens with T.gondii tissue cysts the clinical signs and lesion were comprehensively described
  - 3 dead kittens were born 16-31 days after inoculation
  - A wide range of histological lesions included :
    - Proliferative interstitial pneumonia, necrotizing hepatitis , myocarditis , skeletal and glossal myositis, non suppurative encephalitis affecting the cerebrum , brain stem and spinal cord , uveitis, necrotizing adrenal adenitis and interstitial nephritis , also placental lesions consisting of grossly visible areas or necrosis and mineralisation
  - Experimental infection of queens in the middle third of pregnancy using 2 different Brazilian isolates showed almost no difference in abortion and premature still bitrth rates
  - Venereal transmission in cats does not appear to occur
  - T.gondii was not detected in semen, testicles or epididymis tissues of primo-infected cats after challenge with T.gondii tissue cysts and tachyzoites
  - Infection thru mild ingestion in cats is supported by results such as the detection of T.gondii by bioassay and PCR in milk of nursing queens after experimental infection
  - Cats of any age may die or develop severe disease following parental inoculation with tachyzoites , bradyzoites or oocysts and large doses of corticosteroids can aggravate clinical toxo
  - Cats infected during pregnancy can develop placentitis and congenitally infected kittens, severe toxoplasmosis including ophthalmitis

Immunosuppression and clinical toxoplasmosis

- Not conclusive that viral infections may predispose to clinical toxo
- A few cases of feline clinical toxo combines with feline FIV and FeLV – in experimental infections challenge with FIV triggered the disease and predisposed cats to acute generalized toxo but later studies on the general population did not identify the same association
- Organ transplantation – cause of acute toxo – 3 cats and 1 dog developed signs 3-6 weeks after renal transplantation – a further case developed pyogranulomatous cystitis was observed in a cat 6 weeks after the same surgery -> all animals received immunosuppressive therapy -> consider the serological status against T.gondii before uses of potent inhibitors of cell mediated immunity
- It has been reported that skin nodules worsened after treatment with CS in 2 cats , and 3 cats of generalized toxo with pneumonia in cats were observed after cyclosporine and pred
- In another case disseminated toxo involved acute resp distress syndrome and septic shock after cyclosporin
- The reshedding of oocysts by cats remains an important aspect in the epidemiology of T.gondii
- It was previously believe that after first infection cats would excrete thousands of oocysts and then elimination would no longer occur during the life of the animal but recent studies have demonstrated reshedding after experimental application of immunosuppressive therapy . In addition it was reported that clinically ill cats can shed oocysts suggesting that special precautions most be taken during clinical care.
- Cats that were experimentally re-infected with T.gondii at 12, 24 and 36 weeks after the primary infection , re-excreted oocysts and then amount was higher the longer the time after the primary infection , especially when a heterologous strain was used

Toxoplasma gondii genotype and clinical toxoplasmosis

- A recent paper in Aust the autors compared the genotypes of T.gondii in latently infected cars with those in cats suffering from clinical toxo by direct genotyping of the DNA isolated from tissue samples
- Toxo DB genotype #3 was commonly found among both sample sets, suggesting that the T.gondii genotype is not a determinant of clinical disease in naturally infected cats in Australia
- Only ToxoDB#3 has been detected in 5 felines with clinical cases from 2 different countries Australia and Switzerland – therefore no specific genotype can be associated with a certain clinical outcome or presentation
- This whether disease spending on immunological status, infection dose , co-infection rates and geographical location (understood as genetic variant distribution), rather than on specific genotype involvement is still under debate
- Three is a wide genetic diversity within the parasites isolated from asymptomatic dogs and cats corresponding to complex epidemiology shown in different geographical areas

Check for updates

Journal of Feline Medicine and Surgery (2018) **20**, 244–255

CLINICAL REVIEW

CICLOSPORIN AND THE CAT
Current understanding and review of clinical use

Silvia Colombo and Roberta Sartori

- Cyclinc undecapeptide metabolite of the fungus *Tolypocladium inflatum*
- Immunosuppressive non cytotoxic activity
- Fat soluble , extremely hydrophobic and poorly absorbed after oral administration
- Currently a microemulsion which enhances oral absorption of the drug
- Recommended dose 7mg/kg PO q24hr

MOA

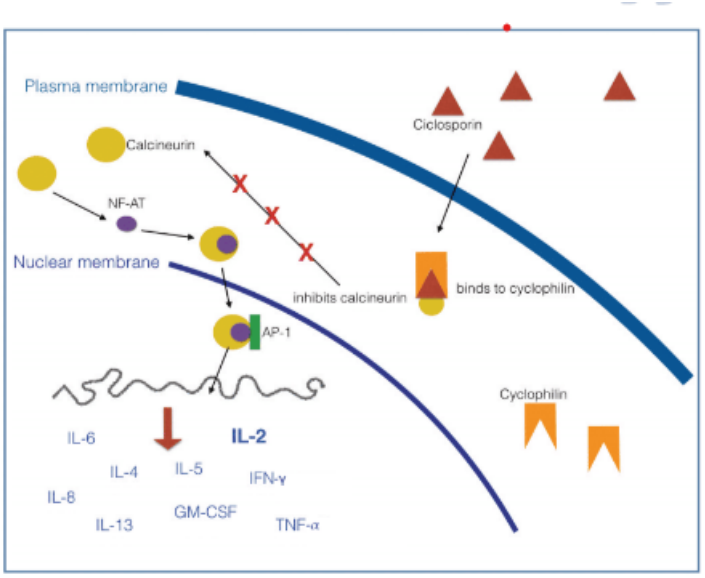


Figure 1 Mechanism of action of CsA. CsA enters the cytoplasm of T cells and binds to the receptor cyclophilin. The drug–receptor complex secondarily binds to calcineurin, inhibiting its action. In the absence of CsA, calcineurin binds to nuclear factor of activated T cells (NF-AT), which in turn enters the nucleus and binds to activator protein 1 (AP-1), a transcription factor. This complex induces the transcription of cytokine genes by the T cell. IL = interleukin; IFN-γ = interferon gamma; TNF-α = tumour necrosis factor alpha; GM-CSF = granulocyte–macrophage colony-stimulating factor

- Exerts its effect mainly on the cell mediated immune system while humoral immunity is less affected
- Suppresses the production of IL-2 receptor on the T lymphocytes , resulting in hinbition of the T cell proliferation and activation
- In also inhibits the production of IL -4, IL-5, IL-6 , IL-8 , IL-13 , granulocyte-macrophage colonly stimulating factor alpha (GM-CSF) , tumour necrosis factor alpha (TNF-alpha) and interferon gamma (IFN-gamma)

- Leads to number of anit-infalmmatory affects including reduction in mast cell degradation , keratinocyte proliferation and cytokine production as wll as decreased tumoouricididal and superoxide activity by macrophages
- Other effects include reduced expression of intracellular adhesion molecule 1 and leucocyte trafficking in endothelial lcells and inhibition of antigen presenting cell function

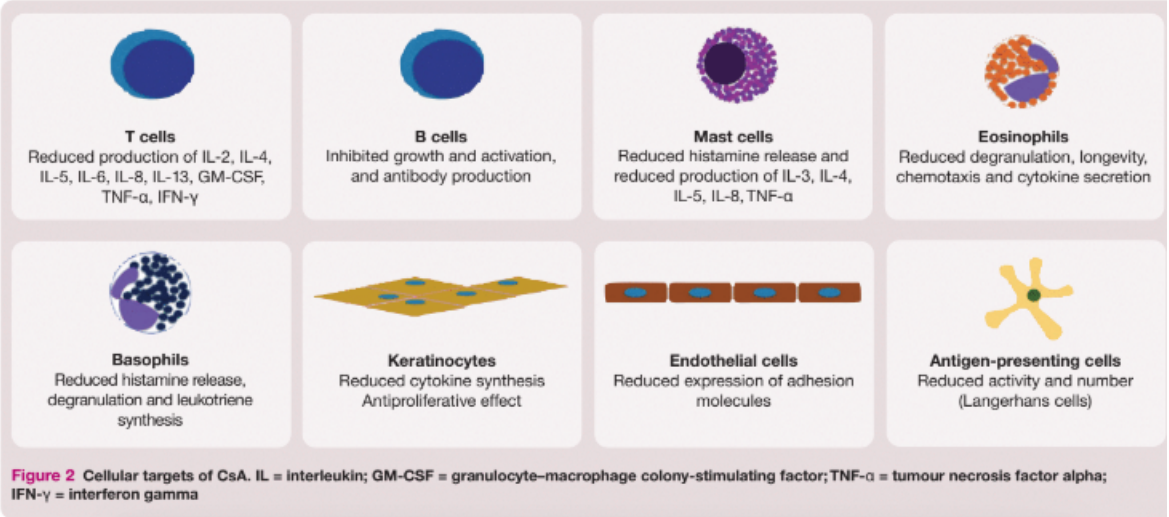


Figure 2 Cellular targets of CsA. IL = interleukin; GM-CSF = granulocyte–macrophage colony-stimulating factor; TNF-α = tumour necrosis factor alpha; IFN-γ = interferon gamma

- In the cat CSA has been shown to supress invitro lymphoblast transformation after stimulation with the mitogens concanavalin A and pokeweed within 7 days of administration with return to normal response within 7 days after withdrawal
- In feline peripheral blood mononuclear cells (PBMCs) , CsA suppresses the expression of mRNA for IL-2, IL-4, IL-10, GM-CSF, INF gamma, TNF alpha and the number of IL-2 secreting lymphocytes , in a dose dependant manner
- In feline intestinal mucosal biopsies CsA is able to reduce neutrophil infiltration

Pharmacokinetics

After oral administration, microemulsified CsA is absorbed across the intestine by passive diffusion. Absorption is limited by P-glycoproteins in the enterocytes, acting as efflux pumps. After absorption, CsA is widely distributed and stored in the skin and adipose tissue, being a very lipophilic molecule.[12,13] In humans and dogs, it has been shown that CsA concentrations are higher in the skin than in whole blood or plasma.[12,14] CsA is metabolised mainly in the liver by enzymes of the cytochrome P450 complex, with minimal metabolism in the kidney and intestine. It is excreted in the bile, with limited excretion in the urine.[6,7] The pharmacokinetics of microemulsified CsA were studied in six healthy cats after intravenous and oral administration. Bioavailability was 29% after 7 days and 25% after 14 days of oral administration at 3 mg/kg q12h. At the same dosage, peak concentration was reached in 1–2 h and elimination half-life was 8.19 h. Trough plasma levels were extremely variable among the tested cats.[15]

In an attempt to overcome problems with oral administration and absorption, topical ocular administration of an oral solution (Sandimmune) and of CsA in olive oil was studied in cats. Both forms were shown to establish whole blood levels of CsA capable of suppressing in vitro lymphocyte stimulation; and, despite noticing great variability in peak concentrations with both formulations, the authors suggested that topical ocular administration of CsA may be an alternative route when oral administration is not tolerated by the cat.[16]

Transdermal CsA is poorly absorbed in cats and therapeutic concentrations are not reached with this route of administration.[17] In 2016, an open trial published as an abstract reported successful use of CsA administered subcutaneously at 2.5–5 mg/kg every 24–48 h in 6/11 cats with non-flea, non-food-induced hypersensitivity dermatitis. Two of the cats showed topical injection site reactions.[18]

CsA is a very lipophilic molecule: concentrations are higher in the skin than in whole blood or plasma.

Therapeutic monitoring

- Because of the extreme variability in absorption and metabolism, monitoring the conc of CsA in the blood has been recommended
- Measure in whole blood rather than plasma because the drug concentrates within blood cells
- Ideally test after 2 weeks of treatment and where available high performance liquid chromatography is better method than immunoassay for evaluating CsA whole blood conc
- The correlation between clinical efficacy of CsA and trough blood levels is usually poor
- Because CsA conc in the skin reaching higher levels compared to blood the current opinion is that when treating , skin disease with CsA , monitoring trough CsA levels is not particulary effective

Drug interactions

- Cytochrome P450 enzymatic system and any drug able to induce or inhibit these enzymes may increase or decrease CsA metabolism thus increase/decrease blood conc
- CsA is also a both a substrate for and an inhibitor of P-glycoprotein, which is an efflux pump able to protect the blood brain barrier from adverse effects of potentially neurotoxic drugs

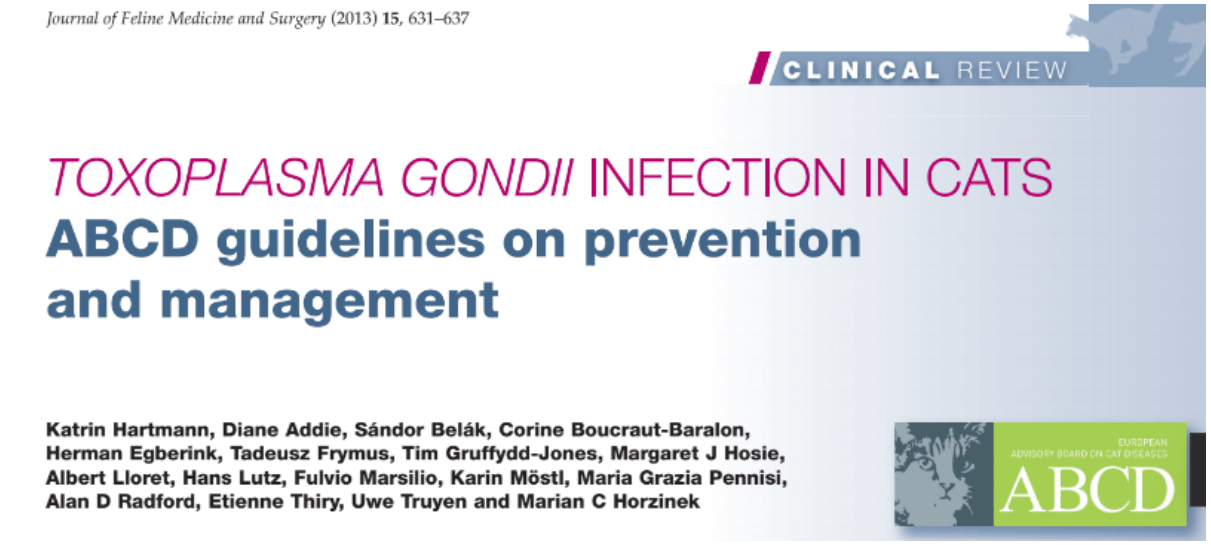
Table 1 Drugs most commonly reported as interacting with CsA in cats and humans

	Drug	Interaction	References
Reported in cats	Clarithromycin	Inhibition of P450/CYP3A4 enzymes: decreased CsA metabolism, increased blood CsA concentration	30
	Itraconazole		31
	Ketoconazole		21
Reported in humans	Clarithromycin	Inhibition of P450/CYP3A4 enzymes: decreased CsA metabolism, increased blood CsA concentration	
	Erythromycin		
	Ketoconazole		
	Itraconazole		
	Fluconazole		
	Miconazole		
	Diltiazem		
	Verapamil		
	Nicardipine		
	Methylprednisolone		
	Grapefruit juice		
	Nafcillin	Upregulation of P450/CYP3A4 enzymes: increased CsA metabolism, decreased blood CsA concentration	1,6
	Rifampicin		
	Phenobarbitone		
	Phenytoin		
	Carbamazepine		
	Non-steroidal anti-inflammatory drugs	Nephrotoxicity/renal failure	
	Aminoglycosides		
	Enalapril		
	Captopril		
	Amphotericin B		
	Melphalan		
	Cephalosporins	Other interactions, mechanisms unknown	
	Chloramphenicol		
	Norofloxacin		
	Sulfadiazine		
	Trimethoprim/sulfadimidine		
	Glucocorticoids		
	St John's wort		
	Allopurinol		
	Digoxin		

Contraindications
CsA is contraindicated in feline immunodeficiency virus (FIV)- and/or feline leukaemia virus (FeLV)-positive cats and if there is a past history of malignant neoplasia. It is also not recommended in diabetic cats. It has not been tested in cats less than 6 months of age and 2.3 kg in weight, or in breeding, pregnant or lactating animals.[34]

Adverse effects

- GI – vomiting 12% , soft stools /diarrhoea in 16% - often temporary and many disappear in first few weeks of treatment
- Given maropitant or metoclopramide prior to administration may help decrease GI sigs (metoclopra dose decrease absorption) , freezing capsule also helps decreased GI signs
- Anorexia in 2% -10% of cats
- Weight loss in 5-16% of cats
- Gingival lhyperplasia possibly due to the stimulating effect of CsA on fibroblast proliferation – rae in cats
- CsA has been reported to be a significant risk factor for the development of acute bullous keratopathy



Pathogenesis

<u>Enteroepithelial life cycle</u>

- Ingestion of intermediate host infected with tissue cyst
- Bradyzoites released in stomach and intestine from tissue cysts when digestive enzymes dissolve the cyst wall
- They enter epithelial cells of the small intestine and give rise to schizonts , initiate 5 types of predetermined asexual stages and merozoites released form the schizonts eventually form male and female gamonts
- Fertilization – cyst wall forms around the fertilized macrogamont to form an oocyst – 10-12 um in size and unpsorulated when passed in faeces
- After exposure to air and moisture for 1-5 days they sporulate to contain two sporocysts each with four sporozoites

- This cycle is usually completed within 3-10 days of the ingestion of tissue cysts which is the route of infection in up to 97% of naïve cats
- In the rate event that cats ingest oocysts or tachyzoites , formation of new oocyst is delayed and shedding can occur for up to 18 days (occasionally longer)
- However only 20% of cats fed oocysts will shed

Extraintestinal life cycle

- Same for all hosts including cats, dogs and people irrespective of whether tissue cysts or oocysts are ingested
- Oocysts ingested and sporozoites hatch in the lumen of the small intestine and enter intestinal cells , including those in the lamina propria
- Sporozoites divide into 2 by an asexual process (endodyogeny) and become tachyzoites
- These are lunate (falciform) in shape 6x2um and multiple in almost any cell in the body
- When the cell ruptures, releasing tachyzoites these infect new cells
- Otherwise tachyzoites multiply intracellularly for an undetermined period and eventually encyst
- Tissue cysts vary in size from 15-60um and usually conform to the shape of the parasitized cell
- Tissue cysts are formed mainly in the CNS, muscle and visceral organs and probably persist for the life of the host . thye can be reactivated after immunosuppression which may then lead to clinical signs
- Parasitaemia during preg of the host can cause placentitis and spread of tachyzoites to the foetus
- Many kittens born to queens infected with T.gondii during gestation become infected trans placentally or when suckling – clinical signs are common in these kittens varying with the stage of gestation at the time of infection , some of these newborn kittens shed oocysts

Epidemiology

- Ab prevalence to T.gondii varies geographically – Portugal 24% of cat have Ab in 1 study USA 16-40% AB positive
- Th age of the cat does not play a role in the frequency of T.gondii shedding but season does in Nth Hemisphere more common in July to Dec
- 3 modes of transmission – congenital, ingestion of infected tissue and ingestion of oocyst-contaminated food or water
- Less important blood and organ transfusion
- Lactogenic transmission is suspected because the organism has been detected in queens milk
- toxo blocks the innate aversion of rats for cat urine instead making them attracted by the feline pheromone , which can increase the likelihood of a cat capturing an infected rat. – adaptive behavioural manipulation optimising the chances of completing the parasites life cycle , it reproduces only in the feline intestine
- Behaviour manipulation hypothesis
    - Parasite will specifically manipulate host conduct essential for its transmission
    - Host conduct essential for its transmission
    - However the neural circuits for innate fear , anxiety and acquired fright all overlap raising the possibility that T.gondi can change chemical messages in the CNS that

affect rodent behaviour because the infection can lead to cyst formation in the CNS with production of tyrosine hydroxylase , resulting in a lack of dopamine

- Meat contaminated with T.gondii cysts has been the primary source of infection in people and AB prevalence in humans is relatively high
- Exposure from oocyst contaminated soil or water is common
- Indeed, water borne outbreaks of toxoplasmosis have been reported world wide

Clinical signs

- Caused by inflammation and tissue necrosis resulting from intracellular growth of tachyzoites
- congenital infection tends to be more serious than infection of the adult cat
- clinical toxo develops during dissemination and intracellular replication of tachyzoites
- it usually originates from reactivation of a latent infection rather than after a newly acquired infection
- if a carrier cat is immunosuppressed, bradyzoites in tissue cysts replicate rapidly and disseminate again as tachyzoites
- the most commonly affected tissues are the CNS, muscles, lungs, eyes
- hepatic and pancreatic involvement is less likely cats with toxoplasmosis show neurological signs (eg seizure , ataxia) , muscle hyperaesthesia, dysponea, uveitis, icterus , diarrhoea, fever, depression , anorexia and wt loss
- transplacentally or lactogenically infected kitten develop more severe signs and frequently die of pulmonary or hepatic disease
- immune complex formation and deposition in tissues as well as delayed hypersensitivity reactions can be involved in chronic forms of toxo
- Toxo is not cleared from the body either naturally or through treatment , toxo can recur

Immunity

- Poorly understood
- Mice and human it is highly dependant on cell mediated effector response
- All infected cats develop IgG and IgA antibodies , about 80% also have IgM antibodies d
- IgG can take 4-6 weeks to appear and maximal ab titres are achieved within 2-3 weeks of first appearance

Diagnosis

- Oocyst shedding is diagnosed by microscopy of faecal samples
- Diagnosis of the disease is only confirmed when the organism is found in body fluids or tissues
- IF suitable samples cannot be taken a tentative diagnosis is sometimes based on rising IgM titres , exclusion of other causes for the clinical signs and a favourable clinical response to anti-toxo drugs
- Ooysts 10um in size best demonstrated by centrifugation using Sheather's sugar solution
    - Oocysts are morphologically indistinguishable from those of Hammondia hammondi, Besononita oryctofelisi and Besnoitia darling
- Tachyzoites can be detected in Various tissues and body fluids during acute illness by cytology or PCR
- IFA antibodies of IgM , IgG and IgA isotypes can be detected
    - Helpful in human health

        - AB negative cat will be a greater risk as either currently infected before Ab developed and shedding or at risk of new infection and will shed
        - AB positive cat wil not shed as take 2-3 weeks for AB to develop and thus would have already actively shed oocyst and will not reshed
    - Found in healthy and sick cats thus their presence does not prove clinical toxo

Treatment

- Clindamycin 10-12mg/kg PO q12hr q 4 weeks
- Cats with systemic disease and uveitis should be treated with clindamycin and topical pred drops
- Clinical signs not involving the eyes or the CNS usually begin to resolve within the first 2-3 days of clindamycin administration
- CNS and ocular toxo tend to resolve more slowly
- In cases of pulmonary toxo , radiographic abnormalities might not resolve for several weeks
- The prognosis is usually poor in pulmonary or hepatic disease particularly in immunocompromised patients

Prevention

- Reduce the incidence of infections and the shedding of oocysts into the environment
- No raw meat , freezing or irradiation can kill tissue cysts
- Prevent hunting and eating intermediate hosts or mechanical vectors
- Cats should be prevented from entering areas were food producing animals are sorted or where feed storage areas are located

Box 1 Routes of infection for humans

Common routes

- Ingestion of meat containing tissue cysts is the most common route of infection. Thorough cooking or freezing for several days will kill tissue cysts.[29–32]
- Ingestion of sporulated oocysts, either from the environment (eg, through contact with contaminated soil) or from faeces of shedding cats, is the second most common route. This may also happen when eating unwashed fruit or vegetables. Infection via the environment is more common than direct infection through cat contacts.

Less common routes

- Ingestion of sporulated oocysts through contact with contaminated water.
- Ingestion of raw (unpasteurised) goat's milk.
- Inhalation of sporulated oocysts on dust particles (rare).

Box 2 Recommendations to reduce the risk of parasite transmission from cat to owner

- Litter trays should be emptied daily so that oocysts do not have sufficient time (24 h) to sporulate.
- Gloves should be worn when handling cat litter, and hands should be washed thoroughly after cleaning of litter trays.
- Litter tray liners should be used, if possible, and the tray cleaned regularly with detergent and scalding water.
- Cat litter should be disposed in sealed plastic bags.
- Children's sandpits should be covered when not in use, to prevent cats from using them.
- Only properly cooked food or commercial cat food should be fed.
- Hands should be washed after contact with a cat (especially before eating).

Box 3 Additional advice for households with immunocompromised persons or pregnant women

- Immunosuppressed persons and pregnant women should avoid contact with cat litter.
- Cats should be kept indoors to prevent them hunting and eating intermediate hosts such as voles and mice.
- Cats should not be fed raw or partially cooked meat.
- Cats should be discouraged from eating insects (eg, cockroaches).
- Cats should be tested for *T gondii* antibodies; their presence indicates past infection. These cats will not be a source of infection as they have completed their period of oocyst shedding.
- Cats without antibody will have not been infected previously and, when newly infected, will shed oocysts in their faeces for a short time. They should, therefore, be kept indoors during the phase of immunosuppression or pregnancy of the owner.

Public heath issues have come into focus (Box 1), partly because of the increasing number of immunocompromised persons (eg, after infection with human immunodeficiency virus [HIV]). Also, recent research linking psychological and cognitive disorders (eg, reduced IQ, as evidenced by psychomotor and verbal intelligence tests) to *T gondii* infection have contributed. A recent survey among obstetricians and gynaecologists in the USA to determine their knowledge and practices relating to toxoplasmosis prevention and testing found that most overestimated the risk of cat ownership versus environmental risk factors.[27] A systematic review of risk factors for pregnant women is available;[28] it reports a relatively low risk associated with cat ownership [EBM grade I].

Veterinarians commonly get questions from clients as to whether or not to get rid of their cat during pregnancy. If hygiene recommendations are followed (Boxes 2 and 3), the risk of transmission is low (Box 4).

Box 4 Evidence that contact with cats does not increase the risk of *T gondii* infection[33]

- Cats shedding oocysts in faeces are rare.[34] In one study, only about 1/250 cats shed oocysts.[4]
- Contact with cats has no influence on the probability of people developing antibodies to *T gondii*, whereas the consumption of raw meat significantly increases the risk of acquiring the infection.[35]
- Veterinarians working with cats are not more likely to become infected with *T gondii* or to suffer from toxoplasmosis than the general population, including people without cat contacts.[36–38]
- Stroking a cat will not spread the infection. Even when cats are shedding oocysts in their faeces, oocysts cannot be found on their coat.[39] Studies in dogs have shown that oocysts do not sporulate on their fur, and the same is probably true for cats.[40]
- Cat ownership does not increase the risk of toxoplasmosis in people with HIV infection. Although toxoplasmosis is more common in HIV-infected persons, the disease results from reactivation of a previous infection rather than from acquiring a new infection.
- Most people are infected with *T gondii* through ingestion of undercooked meat, especially goat, mutton and pork. The risk of infection from cats is low, except for young children playing in soil contaminated with sporulated oocysts.[41]
- Bites or scratches from an infected cat do not transmit the infection.
- Infected cats under treatment with immunosuppressive drugs at standard doses do not start shedding oocysts in their faeces.[17]
- Infected cats also do not re-shed oocysts in their faeces when they become immunosuppressed due to infection with FIV or FeLV.[42] Cats infected with FIV or FeLV that are subsequently infected with *T gondii* do not shed oocysts for any longer or in any greater numbers than other cats.[2,42]
- Newly identified strains of *T gondii* are highly infectious for species other than cats; thus, cats might actually become less important in the spread of this infection.

VECTOR-BORNE AND ZOONOTIC DISEASES
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Seroprevalence and Risk Factors for *Toxoplasma gondii* Infection in Owned Domestic Cats in Australia

Anthea Brennan,[1] Jennifer Hawley,[2] Navneet Dhand,[1,3] Lara Boland,[1] Julia A. Beatty,[1,3] Michael R. Lappin,[2] and Vanessa R. Barrs[1,3]

- Common risk factors for T.gondii infection in owned cats include increasing age, outdoor access , hunting and a diet containing raw meat

- Previous serological studies T.gondii seroprevalence ranging from 20% to 39% in Victoria ,30% WA , 85-96% TAS,
- The aim of this study was to determine the seroprevalence of T.gondii and risk factors for infection among owned cats in Australia

Materials and Methods

- Cross sectional study design , sera were collected prospectively from owned cats presenting to vet hospitals over a 4 month period
- Owner questionnaire about cats domicile, diet, and lifestyle factors , postcode, environment, diet , hunting , abode

Results

- 417 cats from 18 hosp across 4 states and 1 territory in Australia
- Median age 10 years (range 3months -21 years)
- Data for abode were available for 380 cats of which 83% were from a major city , 9% inner regional 7 % outer regional , 2% in remote location , a high proportion of cats 69% had outdoor access 58% were housed inside at night , whereas the remained had outdoor access during the day and at night
- Litter trays were provided indoors for 87.5% of cats , although 37% of these cats also had outdoor access
- 59% had been fed raw meat by their owners
- 37% raw meat daily , 18% weekly, monthly 6% or occasionally 39%
- The majority of the cats fed raw meat ate meat from more than one species beef most often 62%, chicken 60%, kangaroo 40%
- Overall 39% of cats were seropositive for T.gondii
- The overall prevalence in different regions ranged from 16% in WA to 49% in NSW
- Factors significantly associated with seropositivity were age >1 year , diet containing raw meat , raw kangaroo, raw beef or raw chicken , successful hunting behaviour
- Cats fed raw lamb were significantly more likely to be seronegative

Discussion

- 39% seroprevalence of T.gondi in owned cats in Australia is among the highest reported worldwide
- China 20.3%, US 23% igG
- It is likely that the overall seroprevalence in Australian owned cats is even higher , since active infections may be IgM positive and IgG negative
- Increasing age >12months most consistent risk factor consistent with the major source of transmission of infection in cats being horizontal thru ingestion of encysted bradyzoites in the tissue of intermediate hosts
- Raw meat feeding is a risk factor
- In multivariate analyses did not find association between consumption of specific types of raw meat and T.gondii infection – most common meats fed were beef , chicken and kangaroo
- Given that the infection dose of T.gondii is extremely low , consumption of even a small amount of raw animal products can result in infection and oocysts shedding
- Education of pet owners to freeze meat for several days before feeding it would mitigate this risk since freezing meat for a min of 3 days at -10C for below reliably inactivates T.gondii bradyzoite tissue cysts

- Dataa on T.gondii exposure in animal species used for meat production in Australia are scarce
- However seroprevalence in lambs 16% and sheep 32% is relatively high as it is for kangaroo and other macropods (8-20%)
- The 90% seroprevalence detected among chickens from one free range farm in Australia highlights the increased zoonotic risk of T.gondii infection posed by free range chicken compared with indoor raised poulty
-

Dealing with Toxoplasmosis: Clinical Presentation, Diagnosis, Treatment, and Prevention

Susan Foster

- Obligate , intracellular Apicomplexan parasite
- Apicomplexa is a large, diverse group of eukaryotes most of which possess a unique organelle called an apicoplast (a vestigial plastid that is the site of manufacture and storage of a range of important chemical compounds) and an apical complex structure (involved in penetrating a host's cell)
- DH – felids , IH – felids and non felids that harbour tissue cysts
- 3 infectious stages sporozoites in oocysts , tachyzoites (actively multiplying stage) and bradyzoites enclosed in the tissue cysts (slowly multiplying stage)
- Transmission I s usually by ingestion of infected tissue or oocyst contaminated food or water but congenital infection also occurs
- Nth America and Europe – predominately I,II and III
- Studies of genetic polymorphism revealed that at each gene locus there are only 2 alleles -> common source and since have undergone limited genetic exchange (ie 3 genotypes from 2 alleles)
- In select niches within Europe and Nth America types I , II and III do not predominate some isolates have mixtures of the 2 allele patterns seen in the type of strains , indicating that they are natural recombinants
- Less common are exotic stains which have many unique polymorphisms indicating they have a more ancient origin
- Sth America has the greatest diversity of strains of any region yet examined– suggesting may be birth place of toxo
- Clonal types of toxo show different virulence in mice
    - Type 1 always causes lethal infection
    - Type II and IIIare less virulent
- Less is known about virulence in felines
    - Experimental infection of immunocompetent cats , parental infection with type I (RH strain) resulted in severe to fatal disease

- Parental infection with type II (ME49strain) has usually led to mild transient signs of which chorioretinitis has been the most specific but has also caused death in exp infected cats
- Type II (Apico I) also led to fatal systemic toco in a naturally infected apparently immunocompetent cat
- Natural infection with T.gondii of the type 12 lineage (a forth clonal lineage recognised within Nth American wildlife) has caused disease in a Nth American domestic cat

Clinical Presentation

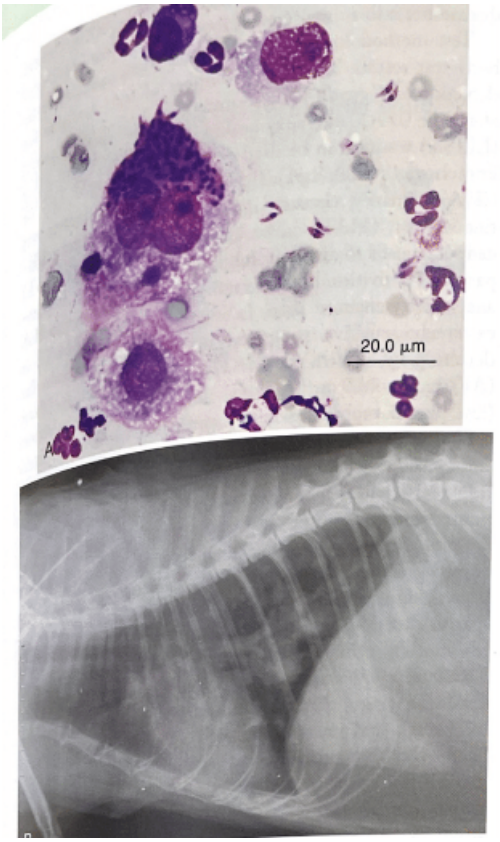
- Acute onset or slow
- Type and severity of clinical illness depends on the strain of T.gondii , host genotype or both
- However for practical purposes type and severity can be related to the degree of tissue injury and location
- Tachyzoites are the invasive asexual forms of the parasite that require intracellular existence for replication and survival
- Cell necrosis is caused by the intracellular growth of T.gondii
- Cats infesting sporulated oocysts or tissue bradyzoites have necrosis of the intestine and associated lymphoid organs and can develop self limiting small bowel diarrhoea lasting up to 10 days – but usually subclinical
- Csx – occur after acute exposure or reactivation of tissue cysts with release of bradyzoites following immunosuppression
- Clinical toxo from systemic spread is most severe in transplacental or locationally infected kittens because tachyzoite replication can be overwhelming
- 100 cats with histologically confirmed systemic toxo lesions in available tissue
    - Lungs 97.7%
    - CNS 96.4%
    - Liver 93.3%
    - Pancreas 84.4%
    - Heart 86.4%
    - Eyes 81.8%
    - The lung appears to be a common target organ in both primary and reactivated toxo in cats
- Kittens with toxo may be still born of diet before weaning – clinical signs pertain to liver, lungs and CNS , lethargy depression , hypothermia and sudden death can occur with some kittens suckling until death , kittens have abdominal distension due to an enlarged liver and ascites , encephalitic kittens may sleep most of the time or cry continuously
- Kittens born to queens with Mozart, Maggie or ME49 strains of T.gondii develop chorioretinitis (sometimes with transient concurrent anterior uveitis), sometimes in the absence of other clinical signs
- Lesion differences were found in kittens from queens infected with Mozart strain of T.gondii compared with kittens from queens infected with ME49 or Maggie strains – but experimental factors may have been responsible

- Older cats –
    - anorexia , lethargy dyspnoea coughing less common but has also been reported
    - persistent or intermittent fever has also been reported ,
    - wt loss and wasting are common
    - icterus in 24% of 100 fatal feline cases
    - abdominal pain or discomfort likely attributed to hepatitis , pancreatitis or discomfort from interference with respiration in cats with pneumonia is a frequent finding
    - abdominal effusion
    - eosinophilic fibrosing gastritis has been reported in one cat with confirmed toxo
    - anterior or posterior uveitis in one or both eye
    - iritis iridocyclitis or chorioretinitis can occur alone or concomitantly
    - ocular toxo occurs in some cats without systemic signs of disease
    - both ocular and neurological manifestations in the absence of other systemic signs are reportedly more common with reactivated infection than acute infection
    - signs attributable to CNS involvement have been variable and included hypothermia, blindness, increased affectionate behaviour, stupor, inability to stand , extensor rigidity , HL paralysis and inability to urinated , in-coordination, atypical cry, ear twitch circling , torticollis, head bobbing, anisocoria and seizures
    - other reported findings include vomiting , diarrhoea, cardiac arrhythmias, splenomegaly , lymphadenomagly, muscle hyperesthesia, muscle atrophy, LMN deficits stiffness of gait lameness, joint pain cutaneous nodules and sudden death
    - abdominal mass lesions
- risk increases with immunocompromise caused by infection (eg FIV, FeLV, FIP or hemoplasosis) or drug therapy
- clinical cases of toxoplasmosis in immunosuppressed cats often feature respiratory signs and pulmonary pathology

Diagnosis

- Hematology
    - Non specific
    - Anaemia, leucocytosis or leukopenia
    - In severely affected cats leukopenia can persists until death
    - Exp – leucocytosis has been associated with the recovery phase , although neutrophilic leucocytosis also occurs in the fatal clinical cases
    - A left shift sometimes degenerative has been reported in a number or cases
    - Lymphopenia , likely stress related is often present
    - Eosinophilia is listed as a common finding
- Serum Biochemistry
    - Increased TBIL , ALT AST
    - Increase in AST or ALT activity may reflect liver or muscle necrosis
    - CK can be increased in there is muscle necrosis
    - Hyperproteinemia due to hyperalbuminemia or hyperglobulinemia but also hypo pr and albumin noted
    - When pancreatitis occurs secondary to toxo increase amylase may be seen it is not known whether FPLi is more sensitive marker of pancreatitis in cats with

toxo because the majority of clinical literature on toxoplasmosis preceded the development of this assay
    - Bilirubinuria and proteinuria may be present on UA
- Cytology and Histo
    - Tachyzoites can be detected in tissue and body flids by cytology during acute illness but rarely found in blood or CSF , Most commonly in pleural or peritoneal fluid
    - Tachyzoites rarely reported in BAL or TTA (transtracheal aspirates) j
    - An exp study also demonstrated BAL cytology to be useful in diagnosis of toxo in cats and more sensitive than histo of tissues identification of tachyzoites
    - FNA of lung has dx but can fail to identify (including biopsy) , in addition with organisms not always evident and cellular features sometimes consistent with neoplasia FNA cytology can be misleading



    - Detection of T.gondii by PCR is considered more sensitive and specific than cytology or histo thus do if not seen cytologically
- CSF
    - Protein increased or normal to increased
    - Gunn-Moore and Reed described a mildly increased number of lymphocytes and occasionally neutrophils which would be in agreement with Lappin
    - One report has a mild suppurative inflammation with neuts compromising more than 50% of the nucleated cells
- Radiology
    - Thoracic – esp in acute disease

        - Diffuse interstitial to alveolar pattern with a patchy distribution, mild to marked pleural effusion can be present
        - Variable and symmetrical alveolar coalescence has been noted
        - Asymmetric lung lobe consolidation
        - Discrete mass or nodular pattern
            - Nodules may be ill defined and appear to adjoin bronchi or well defined
            - Combination of nodular pattern of radiographic change and cytology suggestive of neoplasia has resulted in misdiagnosis of pulmonary carcinoma in cats with toxo
    - Abdominal
        - Masses in intestines or mesenteric l.nodes or a homogenous increase in density due to effusion
        - Loss of contrast in the right abdominal quadrant can indicate pancreatitis
- Fecal Oocysts
    - Seroprevalence in Nth, Central and Sth America and Caribbean 9-87%
    - Oocysts prevalence is low <1% in most feline populations because shedding usually occurs of only 1-2 weeks post exposure
    - Some cats experimentally re-infected with different strains of T.gondii from that uses in initial challenge 6 years prior have been shown to reshed oocysts
    - Oocysts are only rarely found in cats ill with systemic toco
    - 100 cats with clinical toxo only has 2 cats with toxo like oocysts
    - Experimental immunosuppression with doses of corticosteroids that would exceed those used clinically has resulted in re-excretion of oocysts but commonly used anti-inflammatory or immunosuppressive doses of glucocorticoids do no appear to predispose to reactivated toxo
    - Not possible to distinguish T.gondii oocysts from oocysts of *Hammondia* or *Besnoitia* sp microscopically thus mouse bioassays is the usual method of differentiation in experimental studies
    - In an individual cat performing T.gondii serology and documenting seroconversion within 3 weeks of oocyst detection is more practical
- Serum Antibody Tests
    - Initial exposure – IgM increases first this is followed by increased IgG
    - Once infected animals have toco tissue cysts for life and this stimulates a long term humoral immune response in infected cats
    - Prevalence of seropositivity increases with age because increased chance of exposure
    - Theoretically a high IgM titre with a negative IgG titre indicates a recent exposure
    - Documentation of an increasing IgG titre 4x or greater can also verify recent infection but not necessarily oocyst shedding or clinical disease

- A high IgG titre with a negative IgM titre indicates chronic infection
- IgM
    - After experimental infection most cats are positive for T.gondii specific IgM within 2 weeks but some cats do not have increased IgM until 4 to 10 weeks post infection despite not having detectable IgG titres after 3-6 weeks depending on the assay used
    - Approximately 20% of cats do not develop IgM titres
    - The antibody class shift from IgM to IgG may not occur in FIV infected cats or cats treated with glucocorticoids
    - IgM is also occasionally detected in serum of cats with chronic or reactivated infection
    - IgM may persist in some cats for months to years after infection
- IgG
    - Most cats there is a narrow window for documentation of a rising titre with maximal titre being reached in 2-3 weeks although some cats may not develop IgG titre for 4-6 weeks and some will die before the IgG titres are increased
    - IgG in chronically affected queens is transferred in colostrum to kittens and these maternal antibodies persist for 8-12 weeks after birth
    - High IgG titres do not prove recent or active infection
    - Chronic persistence of high IgG titres merely reflects continued presence of T.gondii Ag after infection and does not preclude the possibility of reshedding oocysts
- Thus AB class doesn't accurately predict the oocyst shedding period, likelihood of oocysts shedding or stage of infection
- Magnitude of titre is also not helpful , with some healthy cats having extremely high titres and some clinically ill cats having low titres
- AB titres can be used to monitor response to treatment as Ab positive for life
- IFA (indirect fluorescent antibody) and ELISA (enzyme linked immunosorbent assay) can be sued for detection of IgM and IgG or immunoglobulin A (IgA)
- The indirect hemagglutination test (IHA) primarily measures IgG as does the modified agglutination test (MAT)
- Latex agglutination test (LAT) cant be used to distinguish immunoglobulin class
- ELISA = sensitivity as IFA and more sensitive that LAT or IHA
- MAT is extremely sensitive compared to the other methods for detecting IgG
    - Antibodies to acetone fixed (AF) tachyzoites are increased only during acute infection (<3mths) whereas Ab to formalin fixed (FF) tachyzoites can remain high for years

        - This phenomenon has been attributed to the variation in IgG profiles in response to shifting Toxoplasma surface antigens as the infefction progresses form an acute to a more chronic stage
        - The acetone [re[contains stage specific Ag which are recognized by IgG ab formed against t.gondii tachyzoites early in infection
        - These ab have different specificities from those formed later in infection
        - The differential agglutination test which compares AF titres with FF titres has been used in human medicine to help differentiate acute and chronic in human medicine in immunocomproimised individuals and pregnant women
- PCR
    - T.gondii DNA can be detected in the blood of healthy cats so that positive PCR results do not necessarily indicate clinical disease
    - The source of organism in these cats could be bradyzoites from tissue cysts or tachyzoites in sub clinically affected cats
    - Main role is confirmation when tachyzoites are seen cytologically or histologically or not seen but high likely suspicious or diagnosis ocular or CNS toxo when used in conjunction with Ab testing on aqueous humour or CSF
    - Can submit fresh tissue , fluid or aspirates or frozen
    - PCR assays are reportedly less sensitive on FF samples however immunohistochemistry can be performed
- Aqueous Humour and CSF Ab and Ag testing
    - T.gondii specific IgA, IgG and DNA can be detected in both normal and clinically ill aqueous humour and csf
    - When assessing specific ab in aqueous humour or CSF those produced locally must be differentiated from those passively diffusing across a damaged vascular membrane
        - Golmann-Wimer coefficient (GWC)
        - GWC = x/y, where x = T.gondii specific Ab in aqueous or CSF sample divided by total IgG in sample and Y = T.gondii AB in serum divided by the total IgG in serum
        - Alternatively, an antibody co-efficient can be calculated by measuring a specific Ab for an non ocular infection such as FCV for comparison instead of total IgG
        - Co-efficient of 1 to 8 are suggestive of local production of ab and coefficients greater than 8 provide definitive evidence for local production of ab
    - Unpublished data indicate that T.gondii specific IgM has only been detected in the aqueous humour of CSF of clinically ill cats , this may be the best indicator of clinical disease
    - T.gondii DNA in aqueous humor by PCR may correlate to clinical disease in some but not all cats

- Some ophthalmoscopically normal cats have T.gondii detected in aqueous humour by PCR
- Conversely cats with posterior segment disease may have the organism localized in vitreous rather than aqueous humor resulting in false negative tests
- In humans dx on ocular examination but in cats no pathognomonic
- Combination of *T.gondii* specific IgM ab detection in aqueous homour of CSF and organism DNA amplification by PCR is reportedly the most accurate way to diagnose ocular or CNS toxo but sensitive and specificity is lacking

Summary

- Definitive diagnosis of clinical toxo requires demonstration of *T.gondii* tachyzoites in tissues or fluids by cytology, histopathology or immunohistochemistry
- Ocular or CNS toxo may be diagnosed with a combination of PCR and IgM testing on aqueous humor or CSF, respectively
- Because a definitive diagnosis is not always possible antemortem, a provisional diagnosis may be made when there is a combination of serologic evidence of recent or active infection (high IgM titre or 4 x or greater increase in IgG titre), exclusion of other causes of clinical signs and beneficial clinical response to appropriate therapy

Treatment

- Humans - Inhibitors of parasite nucleotide metabolisms, specifically pyrimethamine (inhibiting dihydrofolate reductase) and sulfa-based compounds (inhibiting dihydropteroate synthase) Clindamycin, an antibiotic that inhibits prokaryotic translation machinery has proven effective as second line drug
- In cats clindamycin is 1st line drug with a concurrent topical, oral or injectable corticosteroid if there is anterior uveitis, TMS has been recommended
- No published work comparing the relative clinical efficacy of drugs used to treat feline toxo
- Little genotyping in clinical cases of feline toco to determine the virulence and drug susceptibility of different strains , it is difficult to assess drug efficacy in reported cases
- Clindamycin
    - Targets ribosomes in the plastid (apicoplast) but the effect of the drug is delayed because of complete loss of plastid DNA is required
    - Given high copy number of DNA in each plastid this could take several parasite divisions to occur
    - Thus the antimicrobial effect of clindamycin is delayed in vitro for 1-3 days
    - Dose 10-12.5mg/kg q12hr PO q4 weeks – followed by food or water
    - Can give SQ at same dose
    - It is reported that clinical sign of systemic illness including inappetence, fever and hyperesthesia usually begin to resolve within 24-48 hours of clindamycin therapy and active chorioretinitis generally subsides within 1 week
    - LMN deficits and muscle atrophy may take longer (weeks) to resolve in animals with polymyositis and neurological deficits may not totally resolve because of permanent damage
    - Anterior uveitis should be treated with clindamycin and concurrent topical, oral or injectable CS to avoid potential adverse sequelae such as lens luxation /glaucoma
    - Although anti-inflammatory doses of GC are likely to exacerbate systemic disease, topical GC therapy is still preferable

- Study of clindamycin efficacy in experimental acute feline too with ME49 strain demonstrated that there was increased morbidity and mortality from hepatitis and interstitial pneumonia in both clindamycin treatment groups (12.5mg/kg q12hr Po of 11mg/kg q24hr PO) compared to both control with placebo (BID or SID)
  - Possible causes were including potential for clindamycin to inhibit phagocyte action , clindamycin is more suppressive than curative , conc found to be inhibitory in vitro may bot be achieved in vivo and the antimicrobial effect of clindamycin is delayed
  - Reported that clindamycin is not effective against extracellular tachyzoites
- Although stated that disease enhancing effects of clindamycin have not been substantiated in naturally infected cats a number of cats in the literature have died despite treatment with clindamycin
- Assessment of the efficacy of clindamycin in clinical cases is thus difficult esp without strain or virulence data
- Clindamycin was reported to be efficacious in one Nth American case study but the selection criteria for that study included resposnse to appropriate treatment or histopathological confirmation and the only case with definitive histo confirmation died without treatment , another cart with possible tachyzoites on histopath responded poorly to clindamycin treatment
- A review of 10 cases of pulmonary toco revealed that only cat survived long term with sole clindamycin therapy and no cats survived with clindamycin and sufonamide tx with or without trimethoprim. The only clindamycin combination therapy that was successful was clindamycin and pyrimethamine
- It may be that cats with pulmonary toxo and critical presentation have a very poor prognosis regardless of which antimicrobials agent was used or that the cats that survived had a less virulent strains or more chronic disease
- It could be said that clindamycin alone is poorly efficacious and possibly even detrimental in these situations as was demonstrated in exp study
- As such based on clinical cases reported, experimetna, and theoretical data it is difficult to recommend clindamycin alone in critical cases of pulmonary toxo or acute disseminated disease

- Pyrimethamine, Trimethoprim, and Sulfonamides
  - Suplonamide 60mg/kg q12hr PO q4 weeks if used as sole therapy or 30mg/kg q12hr PO q4 weeks if used in combo with trimethoprim (15mg/kg q12hr PO) or pyrimethamine (0.25-0.5mg/kg q12hr PO
  - Sulphomides like clindamycin have also been ineffective in clinical cases – Dubey and Carpenter reported that treatment of 17 cats with sulphonamides failed to prevent death , 12 died or were euth within 30hrs and the other 5 lived form 2-13 days , 8 of these cases were treated concurrently with pyrimethamine
  - Pyrimethamine alone has marked invitro activity whereas sulfadiazine (a sulfonamide) has not but pyrimethamine has greter efficacy than trimethoprim when used in combo with sulfonamide but can cause bone marrow suppression
  - Regardless it is probably worth considering pyrimethamine (0.25-0.5mg/kg q12hr PO) in the initial acute phase – if BM suppression occurs can often be corrected with the addition of folinic acid (5mg per cat every 24 hr PO) or breweers yeast (100mg/kg every

24h PO) – not advisable prohalacitally because there will be some decrease in the drug efficacy against the parasite

- Triazines
  - Diclazuril and toltrazuril have reasonable efficacy 60-70% for equine protozoal myelocephalitis
  - Diclazuril esp when combined with pyrimethamine was also efficacious in mice with experimentally induced acute toxo
  - Toltrazuril has been shown to be effective against the intestinal development stages of T.gondii and reasonably effective against extraintestinal stages of T.gondii in cats - dose for treatment of the enteroepithelial cycle is 5-10mg/kg q24hr PO q2weeks
- Fluconazole and Fluconazole Combination Protocols
  - 10day tx with fluconazole had a significant effect of survival in mice exp infected with ME49 strain
  - Th combo of fluconazole, sulfadiazine and pyrimethamine was also very effective resulting in 93% survival in mice infected with highly virulent T.gondii RH strain compared with 36% survival when treated with sulfadiazine and pyrimethamine alone
  - Combination of fluconazole to pyrimethamine was also efficient in reducing mortality compared to treatment without fluconazole
  - Results may be additive , in vivo synergistic effect or pharmacokinetic changes
  - Fluconazole is a potent competitive inhibitor of cytochrome P450 isoform 2C9 which metabolizes sulfadiazine but its effect on pyrimethamine metabolism isn unknown
  - Interestingly itraconazole did not improve survival in experimentally infected mice despite reducing brain cyst burden

Prevention

- Indoors only , no access to rodents or mechanical vecotrs of oocysts and feed them appropriately cooked or commercially processed food
- Raw meat and bones – freeze -12C or lower for at least 3 days before feeding should kill tissue cysts
- Care as cysts can remain viable for more than 11 days at -7C
- Cyclosporin treated cats
  - Weigh up benefits of disease treating (eg atopy) vs risk of inducing a life threatening infection
  - Most toxo cats treated with cyclosporine for atopy is due to acute infection rather than reactivation but there is evidence of reactivation secondary to cyclopsorin
  - No published studies to indicate whether cyclosporin conc plays a role in indiv cats
  - Through cyclopsine conc are variable in cats on similar doses of cyclopsporin and cats with reactivated toxo after cyclosporin have reportedly has extremely high blood conc of the drug – suggested that cats with trough level >1000ng/ml may be at greater risk , also suggested that concurrent pred may increase risk
  - Not known if testing patients of Toxo ab prior to cyclosporin therapy and then treating positive animals with drug against toxo would benefit
  - It would be prudent to reduce chance of acute infection in cyclosporin treated cats indoors and feeding commercial food , measure trough serum levels 2 weeks after starting therapy with aim keeping <1000ng/ml but remember there wil be differences in conc between assays , avoid concurrent pred

- The best advice for for a cat with evidence of toxo exposure prior to cycloporin therapy may be watch carefully for any signs of illness and seek vet
- Experimentally demonstrated that cars dosed at 7.5mg/kg every 24 hour PO prior to toxo infection actually lessened oocyst shedding and did not induce repeat shedding

Public Health

- Zoonotic
- Immunocompetent – relatively innocuous and results in self limiting fever , malaise and lymphadenopathy
- Serious effects on human foetus (still birth, CNS disease and ocular disease) and immunocompromised patients
- Reactivated toxo resulting in encephalitis in patients with AIDS is signif issue
- Possible that infection with Toxo changes the behaviour of its host to increase transmission – more recently rats have been shown to lose their innate fear of cats making them more likely to be prey , people changes in cognition, and suicide(lifestyle that predisposes to infection leads to suicide vs causal link) , schizophrenia (???definitive causal link cant be shown))
- Most horizontal infections are caused by ingestion of tissue cysts in infected meat or by the ingestion of soil , water or food contaminated with sporulated oocysts derived from the environment
- Infection from tissue oocysts occurs with eating or preparing undercooked or raw meat (goat pork or lamb)
- Ingestion of such meat ,failing to wash hands after handling raw meat and contamination of other uncooked foods such as salads, utensils
- Ingestion of raw goats milk, may be another source
- oysters, clam or muscles which presumably filter T.gondii from the sea water
- when cats become infected with toxo they pass unsporulated (non infectious) oocysts in feces for 1-2 weeks after exposure to moisture and air those oocysts mature in 1-5 days to become infectious (sporulated oocysts) and the sporulated oocysts survive for months to years thus frequent gardening and occupations that involve regular soil work are risks
- increased risk of acquired toxo was not associate with cat ownership in studies of vets or AIDS patients and risk of acquiring from litter box are negligible when appropriate care is used because oocysts require at least 24 hours for sporulation and unsporulated oocysts are more susceptible to disinfection and environmental destruction
- touching of cats is probably not a common way to acquire toxo because of the short oocyst shedding period rarity of repeat shedding and cats fastidious grooming habits which result in removal of faeces and thus oocysts
- nb dogs , earthworms, houseflies, cockroaches and snails can act as mechanical vectors
- prevention in humans – avoid exposure to susceptible hosts , cook meat to an internal temp greater than 67C , freeze meat -12 C for at least 24 hours , wash hands after handling raw meat , and care with chopping boards and utensils , gloves for gardening and wash hands, wash hands after touching dogs , care when cleaning litter trays
- sporulated oocysts resist most disinfectants and immersing litter pans in boiling water or scalding water is usually the easiest means of disinfection or change daily
- pregnant women given erroneous advice that seropositive cats should be removed from the household and IgG seropositive cat is not likely to be shedding oocysts if re-exposed or immunocomprimised

- seronegative cats are the greatest public health risk as they will shed oocysts if exposed – thus advise pregnant woman to reduce risk in soil , boil water , cook meat and food hygiene , litter trays , cooked or commercial meat

Environmental contamination

- how to dispose of cat feaes
- clinical toxo in wildlife
- experimental evidence that toxo oocysts can survive chemical and physical inactivation treatments at levels 4-6 x higher than those used to treat raw sewage thus don't dispose of cat litter in toilets – bagged in plastic and disposed in garbage for landfill to prevent waster material leakin gito ground water but most cats shed for no more than 21 days post infection and regardless of if litter is put in landfill or toilets there is still feral cats and dogs that may be shedding

Clinical Infectious Diseases

MAJOR ARTICLE

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Revisiting the Evidence Base for Modern-Day Practice of the Treatment of Toxoplasmic Encephalitis: A Systematic Review and Meta-Analysis

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