



Cerebral Toxocariasis and Neurodegenerative Diseases

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(范家堃)

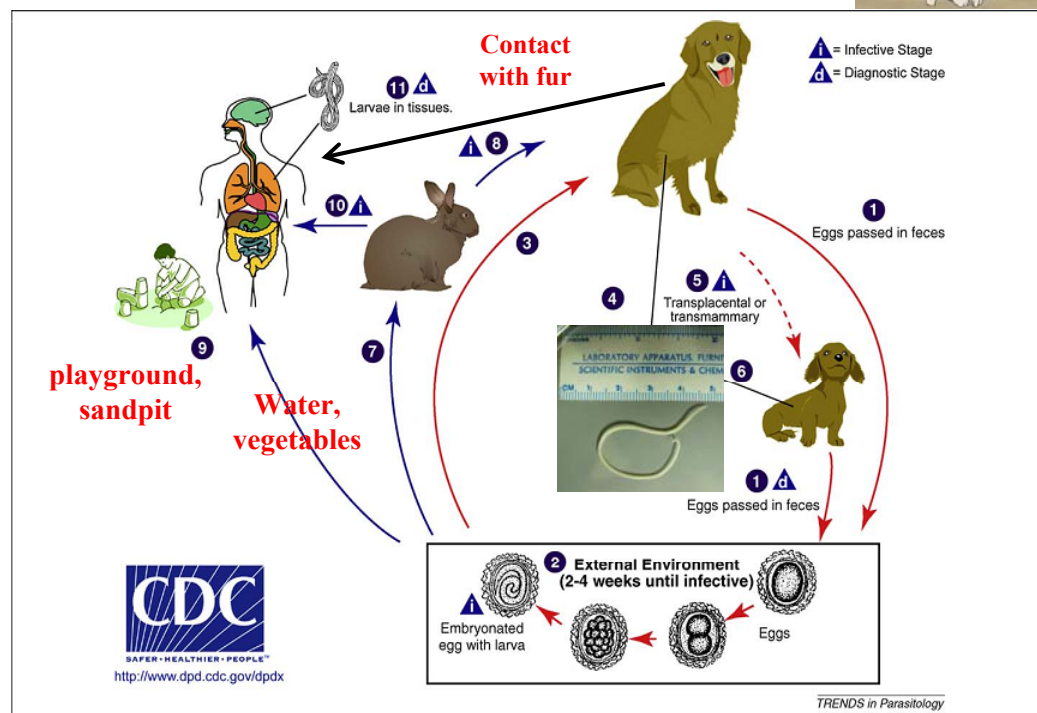
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12/12/2018

I. *Toxocara canis*



- *Toxocara canis* and *T. cati* are intestinal nematodes of dogs and cats, respectively.
- Human toxocariasis is predominantly caused by the accidental ingestion of embryonated *T. canis* (major) or *T. cati* (minor) eggs in contaminated food or water, or by consumption of the paratenic hosts including chicken, lamb or rabbit of the parasite.



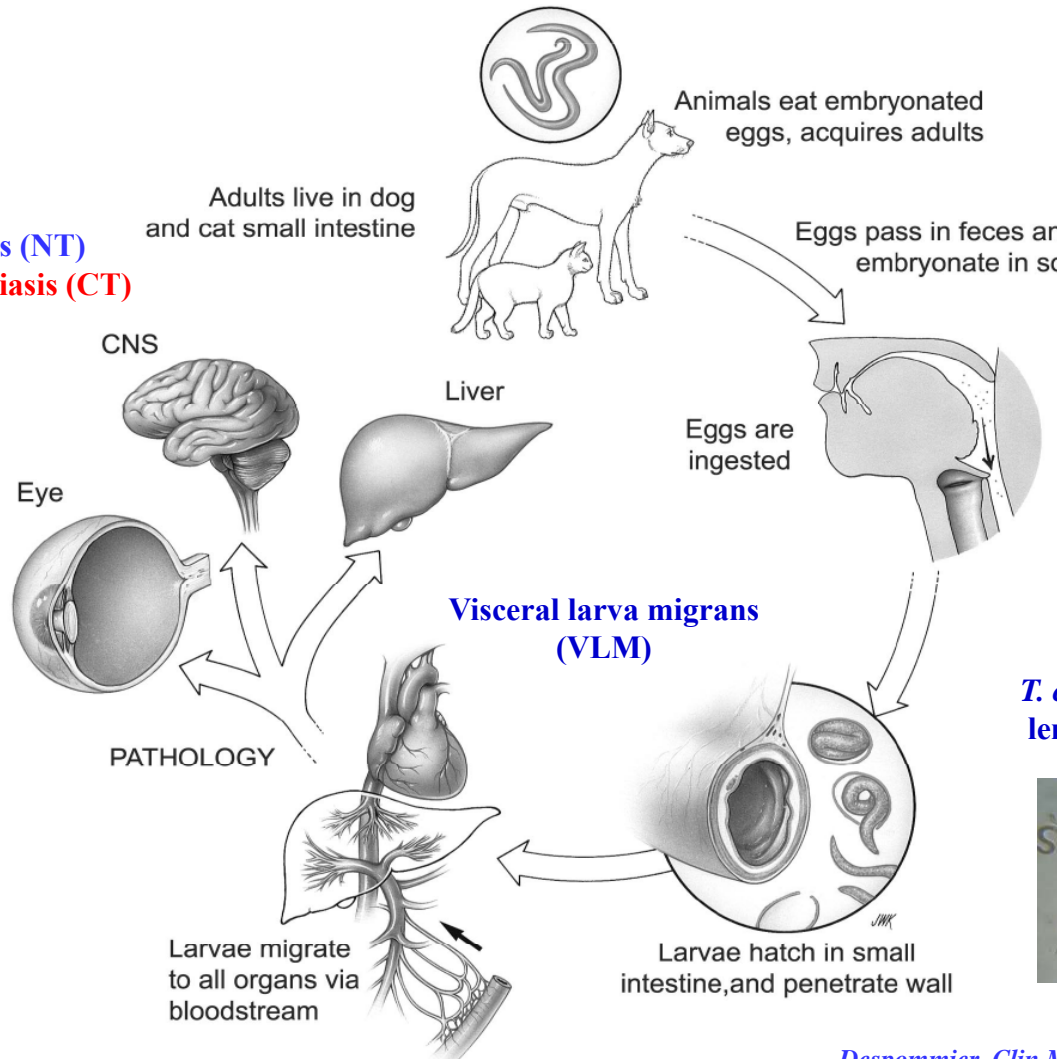
Infective eggs may survive under suitable conditions for at least 1 year (Lloyd, 1993)

Human Toxocariasis



Neurotoxocariasis (NT)
Cerebral toxocariasis (CT)

Ocular larva
migrans (OLM)



* Biological Bullet ?

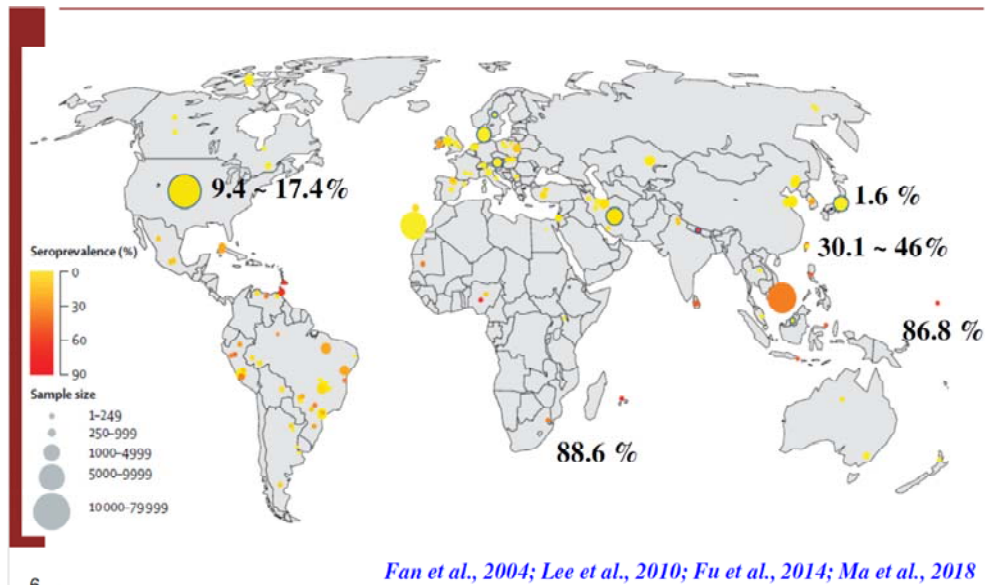
T. canis larva (350~445 μm in length X 20 μm in diameter)



II. Questions?

*Q1 High exposure to *Toxocara* worldwide but human CT cases reported few?*

-Small numbers of larvae involved in human brain that do not present with significant clinical neurological signs. **Underestimated or ignored?**



Ma et al., *Lancet Infectious Diseases*, 18(1) :e14-e24, 2018.

Q2 Although the number of larvae invading the brain is few, may larvae contribute to cerebral pathogenesis owing to longevity?

-*T. canis* larvae may stay in the **primate (10 yrs)** or **mice (67 wks)** brain.

Fan et al., *Vet Parasitol* 113:145-155, 2003.

Clinical Facts (1985-2014)

Fan et al.

* **25 Cerebral** & **61 Myelonic** Toxocariasis reported (1985-2014 PubMed data)

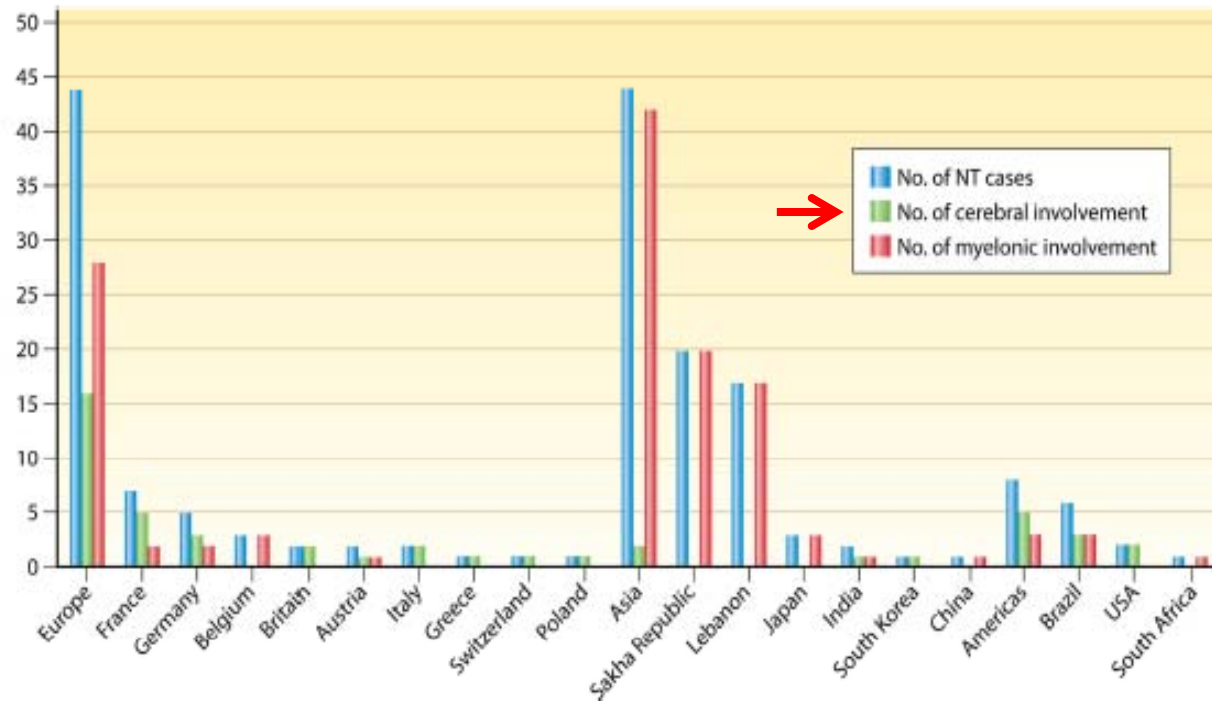


FIG 2 Numbers of neurotoxocariasis (NT) cases—totals and those with cerebral and myelonic involvement—analyzed among 86 clinical NT cases in the PubMed database from 1985 to 2014, by continent and country distributions, ranked from the largest to the smallest number (within each continent distribution). Sakha Republic is one of the 10 autonomous Turkic Republics within the Russian Federation.

Fan et al., Clin Microbiol Rev 28(3):663-686, 2015.

* **Noteworthy, three CT cases showed cognitive impairments, confusion, or dementia syndromes.**

Richartz & Buchkremer, 2002; Maiga et al., 2007; Scheid et al., 2008



III. Hypothesis: Can Cerebral Toxocariasis Progress to Alzheimer's Disease?

Alzheimer's disease (AD)- amyloid cascade hypothesis

Inflammation & NDAFs » » Amyloid precursor protein ↑ APP

NDAFs: Neurodegeneration Associated Factors

- ✚ Alzheimer's disease (AD) is a neurodegenerative dementia.
- ✚ Toxic β -amyloid ($A\beta$) forms amyloid plaques is one of the hallmarks of AD.

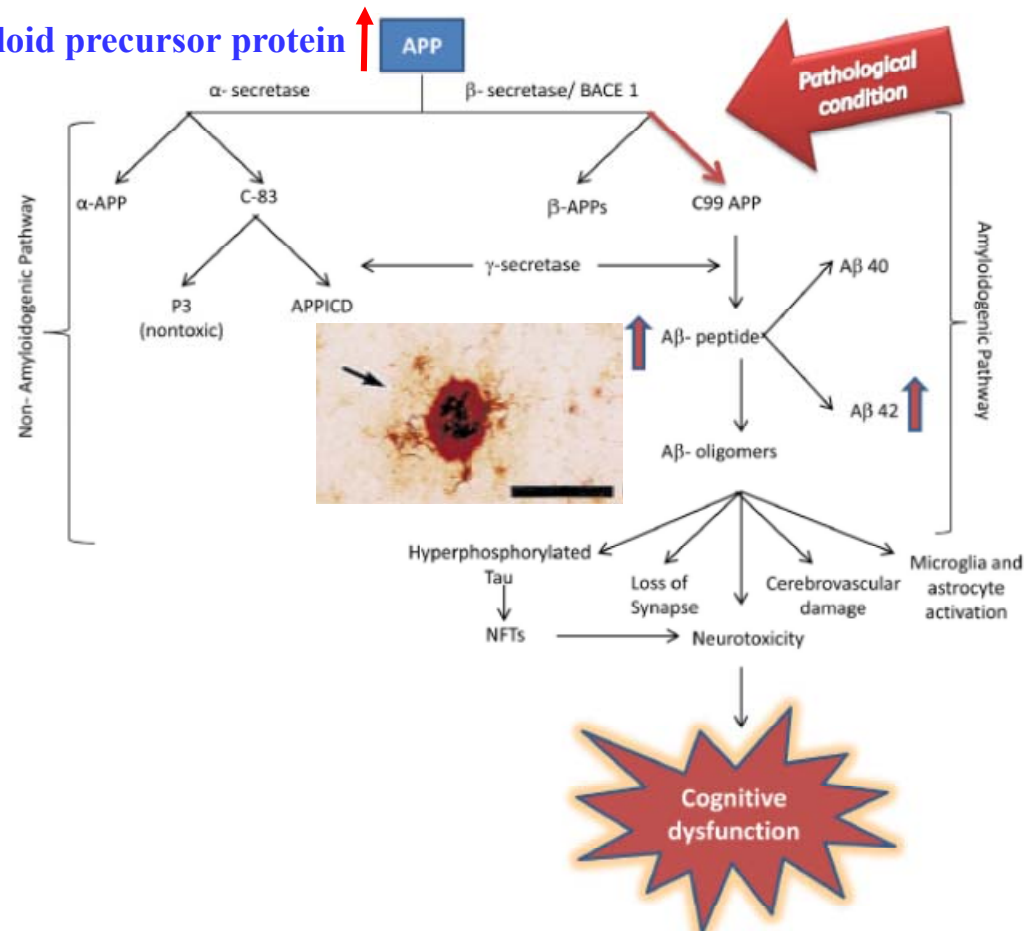


Fig. 1. Diagrammatic presentation of APP processing pathways.

Proc Natl Acad Sci U S A. 92: 3032–3035, 1995.
Pharmacological Reports 67: 195–203, 2015

NDAFs- highly found in AD



* NDAFs: Neurodegeneration Associated Factors Are Commonly Found from AD CSF or Sera

1. **Transforming growth factor β 1 (TGF- β 1):** Induces A β deposition to cause AD development.
2. **Glial fibrillary acidic protein (GFAP):** Implication in CNS response to injury.
3. **S100B:** S100B may increase A β production.
4. **Transglutaminase 2 (TG2):** Dysregulation may contribute to the pathology of AD.
5. **Neurofilament-light chain (NF-L):** marker of axonal injury.
6. **SP:** found in the CNS and is associated with inflammatory processes and pain.

Prog Neurobiol. 61: 439-463, 2000
Acta Neuropathol. 109: 141-150, 2005
J Neurochem 74:143-50, 2000
Glia. 58: 300-14, 2010.
JAMA Neurol. 71:505-8, 2014
Clin Microbiol Rev. 28: 663-86, 2015.

Ubiquitin Proteasome System (UPS)-Normal Function



a

Synthesis of new proteins



Chaperones ensure correct folding



Functional protein

Misfolded proteins tagged with ubiquitin and targeted by the 26S proteasome



Recycling of ubiquitin

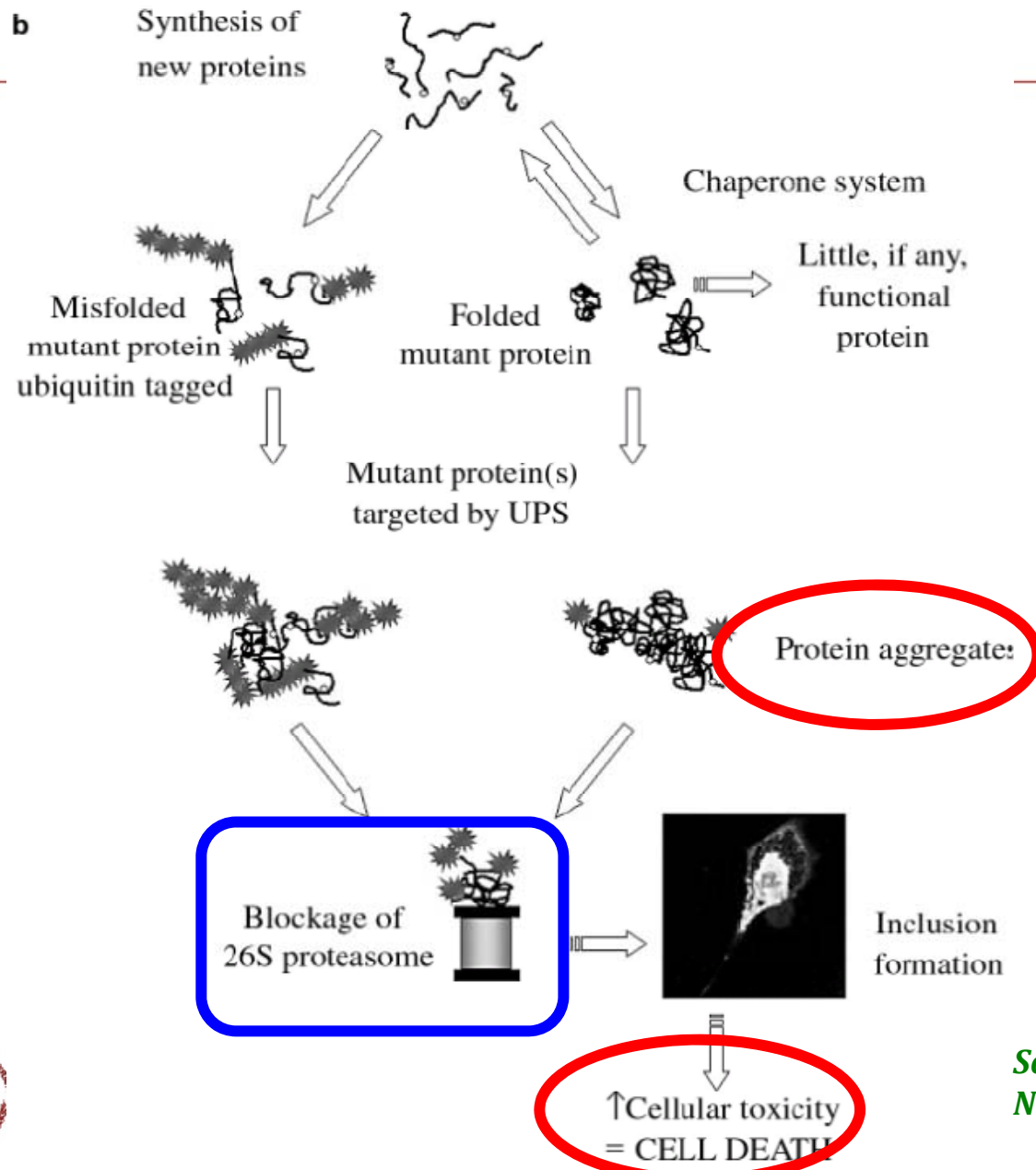


Degradation of misfolded protein to small peptides

Science. 292(5521): 1552-5, 2001;
Neurodegener. Dis. 1:71-87, 2004



Ubiquitin Proteasome System (UPS)-Dysfunction



Science. 292(5521): 1552-5, 2001;
Neurodegener. Dis. 1:71-87, 2004

IV. Our Previous Study-1,2

ICR mice were infected with 250 embryonated *T. canis* ova with an 8-weeks investigation

1

Parasite Immunology, 2008, 30, 525–534



DOI: 10.1111/j.1365-3024.2008.01048.x

Blood–brain barrier impairment with enhanced SP, NK-1R, GFAP and Claudin-5 expressions in experimental cerebral toxocariasis

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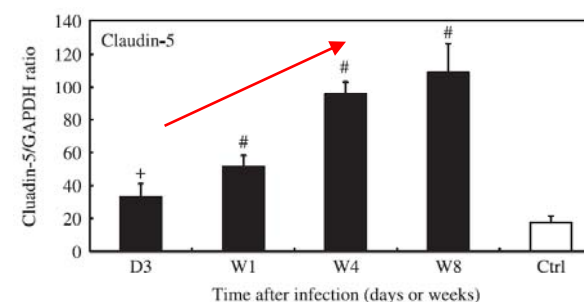
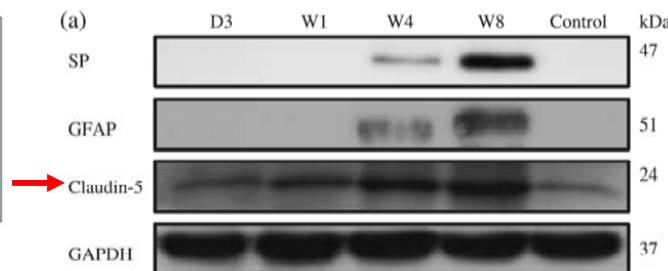
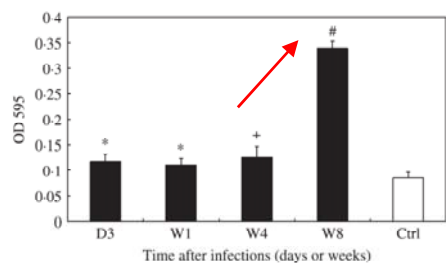


Figure 3 Changes in the amount of Evans blue in the brains of *Toxocara canis*-infected mice. Three to eight mice were in each group. The amount of Evans blue expressed as the optical density measured at 595 nm was significantly higher in infected than uninfected mice (# $P < 0.001$; * $P < 0.01$; + $P < 0.05$).

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BMC Infectious Diseases

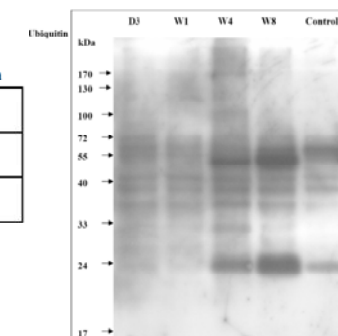
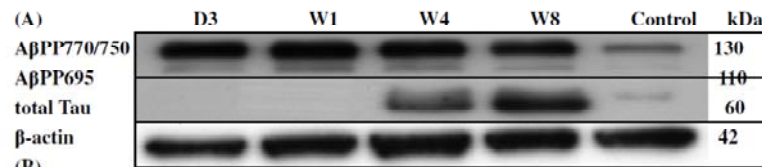
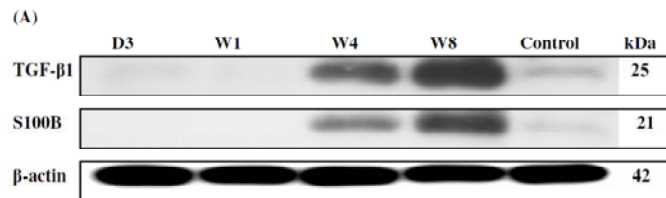


Research article

Open Access

Brain injury-associated biomarkers of TGF-beta1, S100B, GFAP, NF-L, tTG, AbetaPP, and tau were concomitantly enhanced and the UPS was impaired during acute brain injury caused by *Toxocara canis* in mice

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10

Our Previous Study-3

ICR mice were infected with 250 embryonated *T. canis* ova with a 20-weeks investigation

3

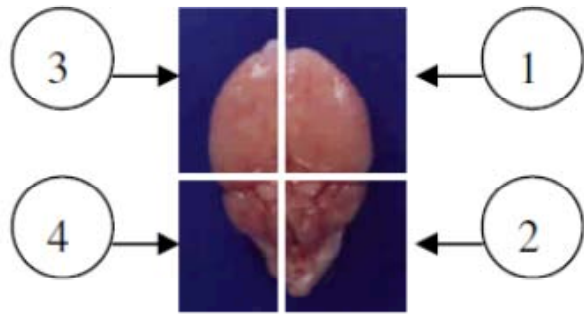
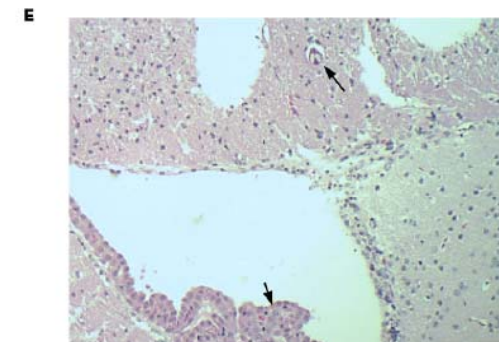
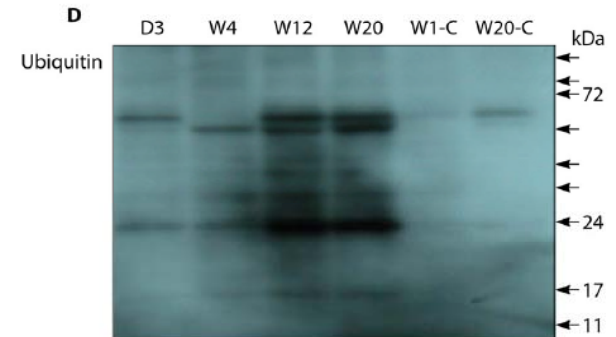
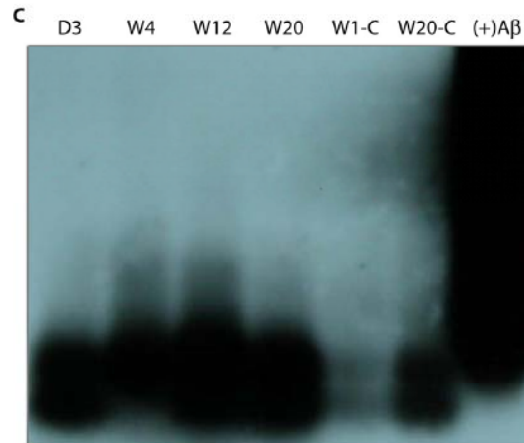
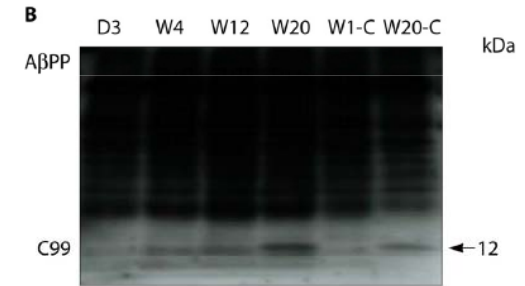
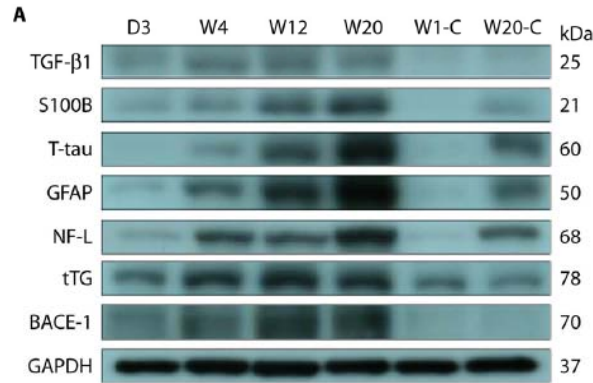


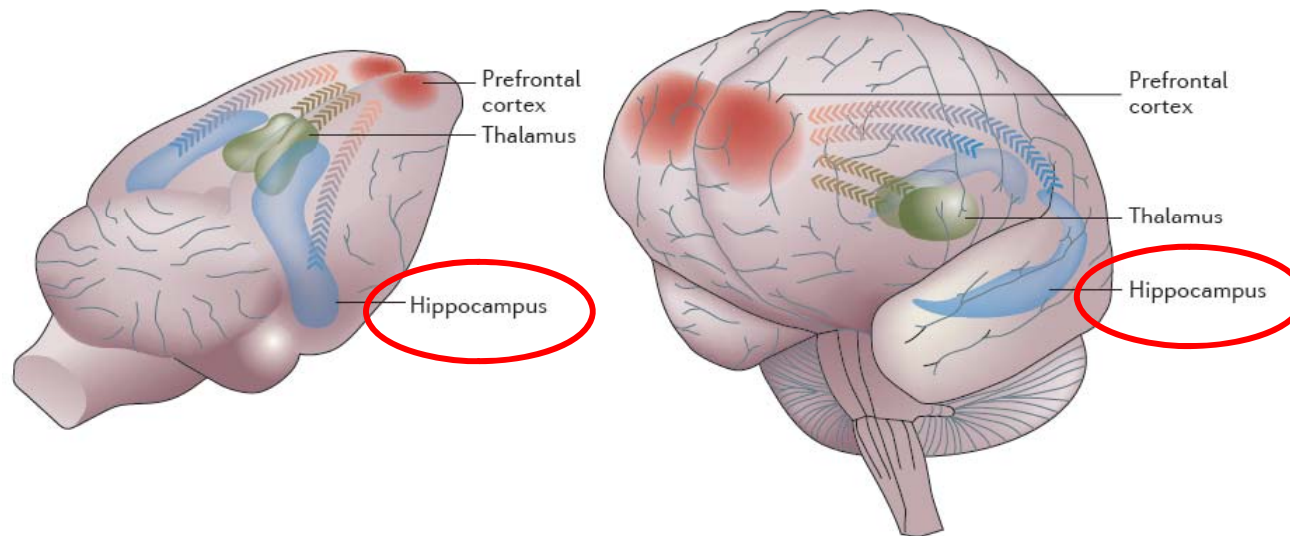
Figure 1
The relative locations of a mouse brain used for various assays in this study. 1, the first part for pathological study; 2, the second for larval recovery study; 3, the third for Western blotting; and 4), the fourth for RT-PCR analysis.

Fan et al., *Clin Microbiol Rev* 28(3):663-686, 2015.



Hippocampus

Critical for learning, spatial and remote memory networks



- **AD**
- Epilepsy
- Schizophrenia

Nat Rev Drug Discov. 2012; 11(7):560-79.

V. Aim

To assess:

1. **Cognitive behavior** in ICR mice orally inoculated with a **low, medium, or high-dose** of *T. canis* ova for a 20-week investigation
2. Pathological changes, neurodegeneration-associated factors (NDAFs) expressions and UPS function in the **hippocampal area**.

VI. Methods & Results

Inoculum size of *T. canis* infective eggs