



Istituto Zooprofilattico Sperimentale  
del Lazio e della Toscana *M. Aleandri*

# ***BAYLISASCARIS PROCYONIS:*** **diagnosi anatomopatologica e parassitologica**

**Arezzo, 12/12/2022**

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DVM

*Istituto Zooprofilattico Sperimentale del Lazio e Toscana «M. Aleandri»*

*Unità Operativa Territoriale Toscana Centro – Sezione di Arezzo*





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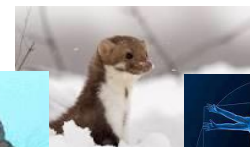
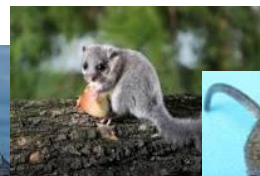
The author with a common raccoon.  
(Photo by Sam Royer)



Prof. Kevin R. Kazacos DVM, Ph.D., DACVM  
Purdue University College of Veterinary Medicine



# Cosa/come cercare



## Negli ospiti definitivi

- 1) Segni clinici (?)
- 2) Parassiti adulti nell'intestino tenue
- 3) Larve/lesioni nella parete intestinale (istologia)
- 4) Uova nelle feci

## Negli ospiti paratenici

- 1) Segni clinici
- 2) Larve/lesioni in organi e tessuti
- 3) Test sierologici





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# Cosa/come cercare negli ospiti definitivi





## 1) Segni clinici

### - Nel procione:

- Solo forma intestinale, per lo più asintomatica
- Segnalate ostruzioni intestinali con morte del soggetto per cachessia nei giovani procioni con infestazioni massive (>400 parassiti adulti)



### - Nel cane:

- Forma intestinale asintomatica o paucisintomatica (enterite) (=ospite definitivo)
- Sindromi da *Larva Migrans* (=paratenici)
- Forme miste
- Carrier meccanico delle uova (infestazione spuria)



# Case Report Rapport de cas

## Neurologic *Baylisascaris procyonis* infection in a young dog

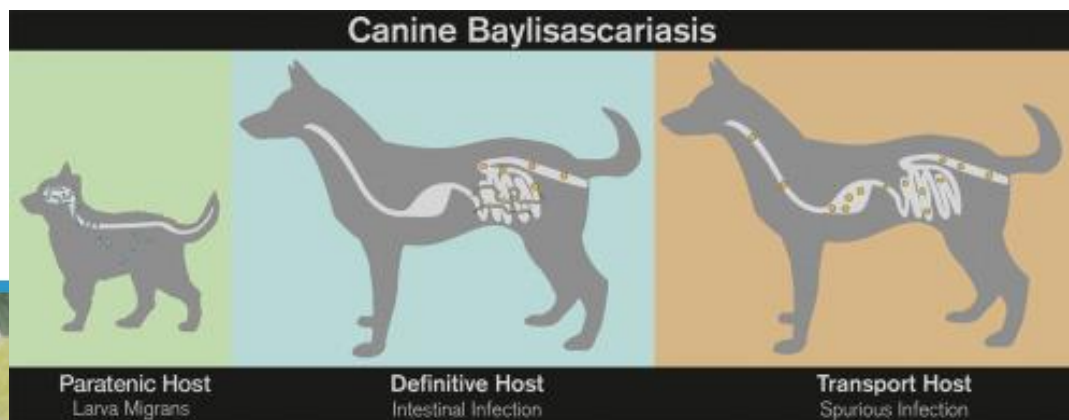
Murray Hazlett, Hugh Y. Cai, Stephanie Sparling, Qiumei You

**Abstract** — A 14-week-old female Boston terrier-cross dog with intermittent gastroenteritis and an eosinophilia developed progressive neurologic disease with ataxia progressing to uncontrolled paddling. Autopsy revealed *Baylisascaris procyonis* larvae in 4 of 7 brain sections, with severe eosinophilic meningoencephalitis. Diagnosis was confirmed with polymerase chain reaction (PCR) and DNA sequencing tests of fresh and paraffin-embedded brain in conjunction with the compatible histologic appearance.

**Résumé** — Infection neurologique à *Baylisascaris procyonis* chez une jeune chienne. Une jeune chienne terrier de Boston de race croisée âgée de 14 semaines a été atteinte de gastroentérite intermittente et d'éosinophilie et a développé une maladie neurologique progressive avec de l'ataxie progressant à des mouvements involontaires. L'autopsie a révélé une larve de *Baylisascaris procyonis* dans 4 des 7 sections du cerveau, avec une méningo-encéphalite éosinophilique grave. Le diagnostic a été confirmé par amplification en chaîne par polymérase (PCR) et des tests de séquençage de l'ADN de tissus du cerveau frais et inclus dans la paraffine conjointement à l'apparence histologique compatible.

(Traduit par Isabelle Vallières)

Can Vet J 2018;59:1325–1328



## 2) Parassiti adulti nell'intestino tenue (*post mortem*)







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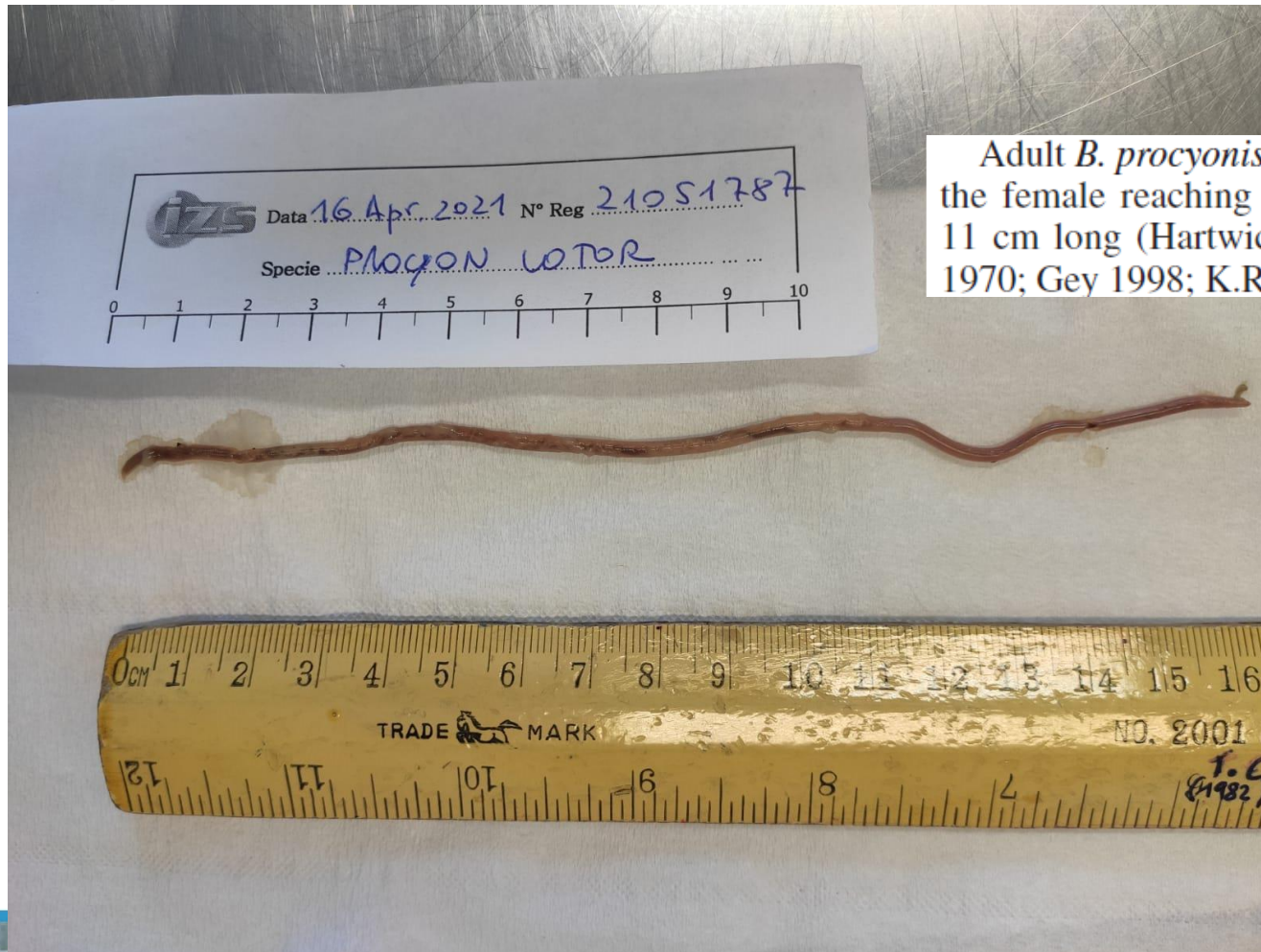
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## 2) Parassiti adulti: identificazione morfometrica



Adult *B. procyonis* are large, tan-colored nematodes, the female reaching 20–22 cm long and the male 9–11 cm long (Hartwich 1962; Sprent 1968; Overstreet 1970; Gey 1998; K.R. Kazacos, unpublished.)

Kazacos, 2011





## 2) Parassiti adulti: Identificazione morfometrica



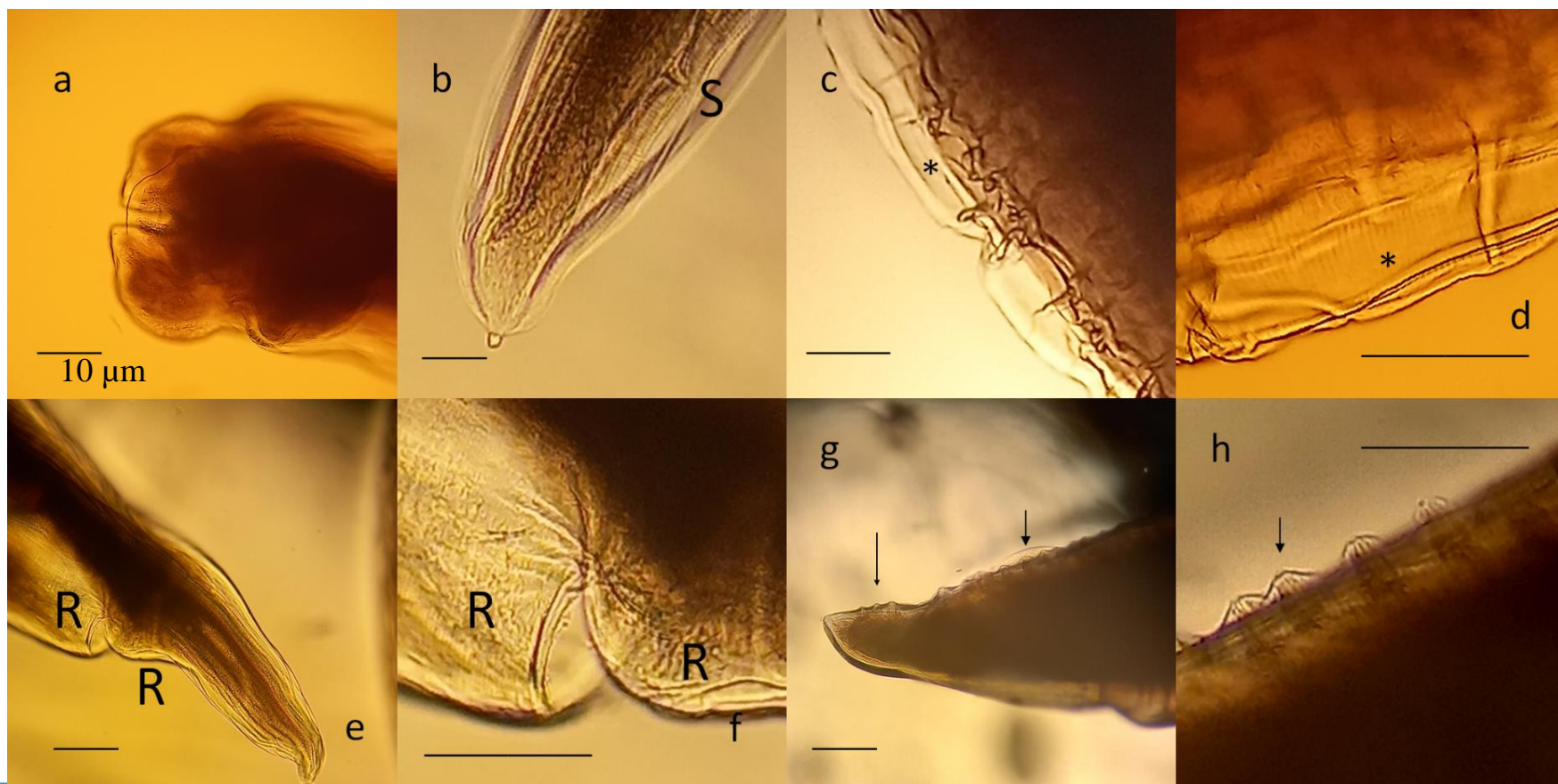
## 2) Parassiti adulti: Identificazione morfometrica





### 3) Parassiti adulti: Identificazione morfometrica

(Foto: dr. Gianluca Fichi)





## 4) Larve intestinali e reperti istologici (intestino tenue)

(Foto: Dr. Claudia Eleni)



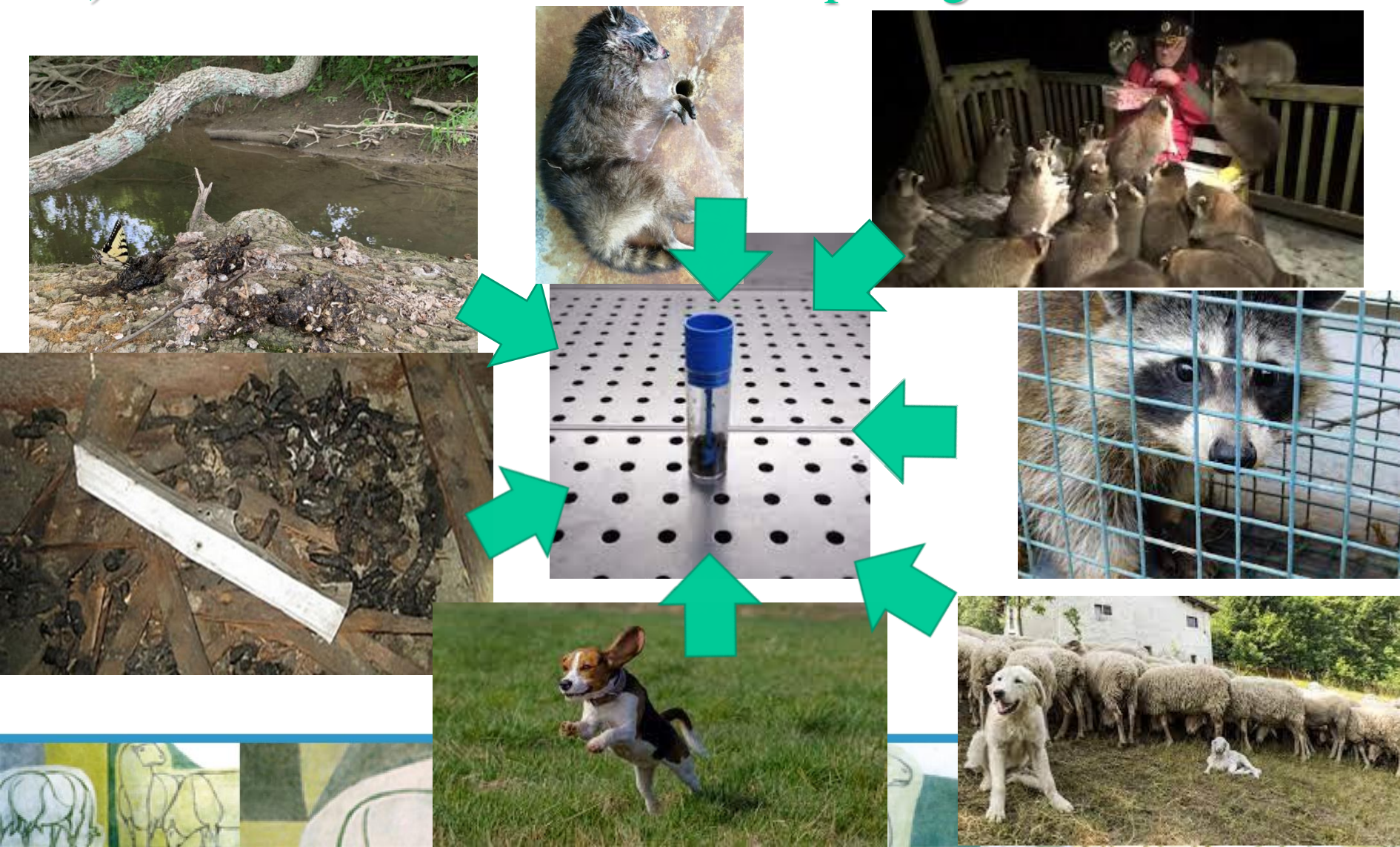
In young raccoons, larvae hatching from eggs enter the mucosa of the small intestine and develop there several weeks before reentering the intestinal lumen to mature, the worms reaching patency in 50–76 days (mean, 63).

Kazacos, 2011

50  $\mu$ m



## 5) Ricerca di uova con tecniche coprologiche



## 5) Ricerca di uova con tecniche coprologiche

- 1) Latrine
- 2) Animali vivi
- 3) Eggs-shedding (anche da cadavere)



- 1) Flottazione
- 2) McMaster
- 3) Tecniche Flotac®







## 5) Ricerca di uova con tecniche coprologiche

### AVVERTENZE (1)

- I campioni «vecchi» o da latrina comportano un rischio biologico elevato per la probabile presenza di uova embrionate «mature»
  - Pretrattamento a bagnomaria a 60°C x 15 min
- Una singola negatività non assicura che l'animale sia *Baylisascaris free*
  - Ripetere il campione a distanza di 60 giorni circa
  - Considerare stagionalità (segnalato in nordamerica calo di incidenza/eggs shedding in inverno, picco in tarda estate-autunno)





## 5) Ricerca di uova con tecniche coprologiche

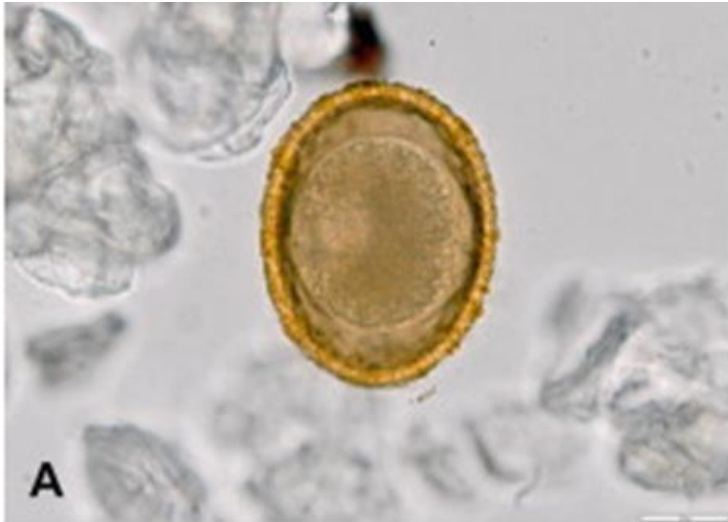
### AVVERTENZE (2)

- Compresenza di uova embrionate, uova non embrionate, uova con morfologia alterata (probabilmente sono tutte *Baylisascaris*)
- *Toxocara Vs Baylisascaris Vs Toxascaris*





## 5) Ricerca di uova con tecniche coprologiche






Handbook of Clinical Neurology  
Volume 114, 2013, Pages 251-262



### Chapter 20 - *Baylisascaris larva migrans*

Kevin R. Kazacos<sup>1</sup>, Linda A. Jelicks<sup>2</sup>, Herbert B. Tanowitz<sup>3,4</sup> 

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<https://doi.org/10.1016/B978-0-444-53490-3.00020-0>

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The eggs of *B. procyonis* are ellipsoidal in shape, brown in color, contain a large single-celled embryo, and have a thick shell with a finely granular surface (Fig. 11.1a); they range in size from 63–88 x 50–70  $\mu\text{m}$ , with most averaging 68–76 x 55–61  $\mu\text{m}$  (Overstreet 1970; Kazacos and Turek 1983; Kazacos and Boyce 1989; Sakla et al. 1989; Miyashita 1993; Averbek et al. 1995; Van Andel et al. 1995; Conboy 1996; Gey 1998). Kazacos, 2011

## 5) Ricerca di uova con tecniche coprologiche



RESEARCH

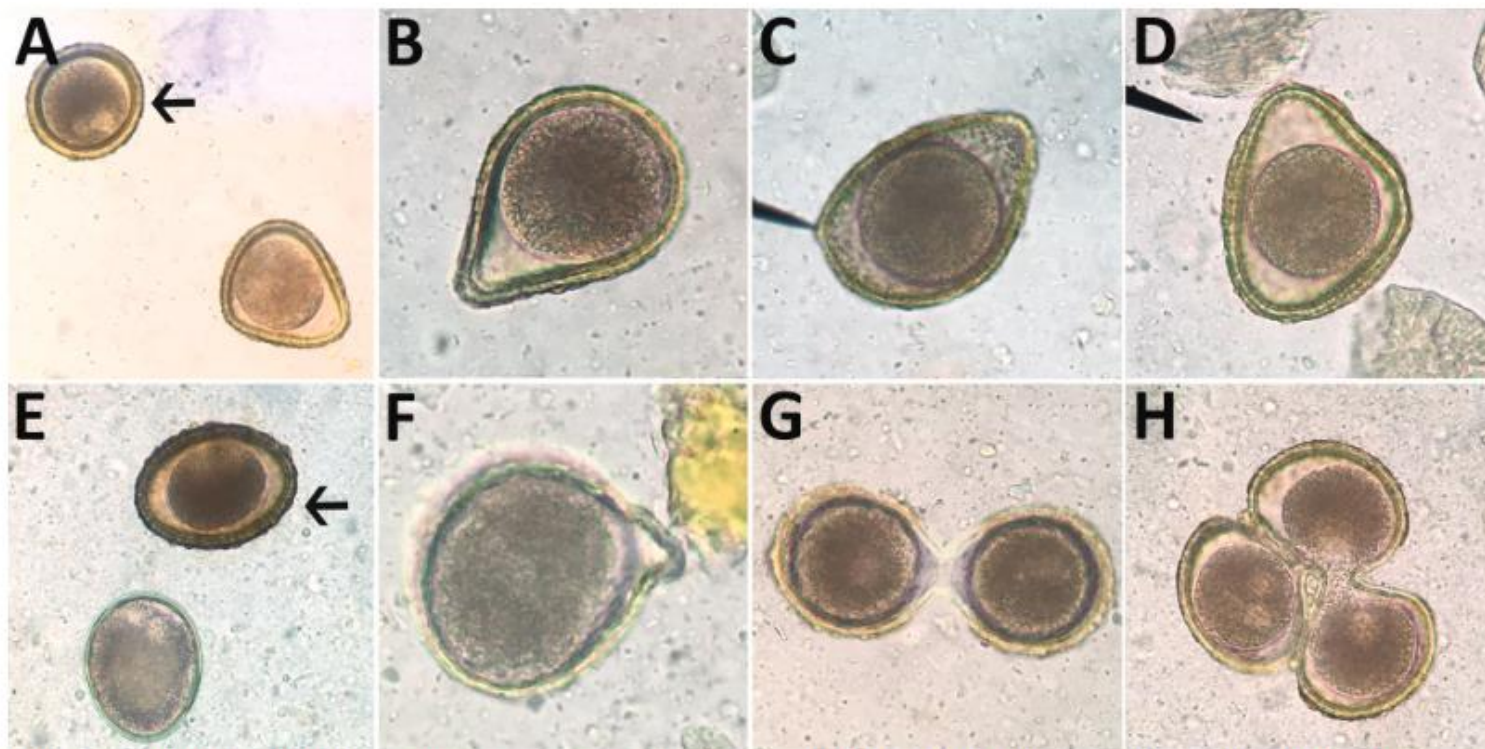
### **Raccoon Roundworm Eggs near Homes and Risk for Larva Migrans Disease, California Communities**

Gabriel P. Roussere,\* William J. Murray,\* Caroline B. Raudenbush,\* Michael J. Kutilek,\*  
Darcy J. Levee,\* and Kevin R. Kazacos†

Emerging Infectious Diseases • [www.cdc.gov/eid](http://www.cdc.gov/eid) • Vol. 9, No. 12,  
December 2003





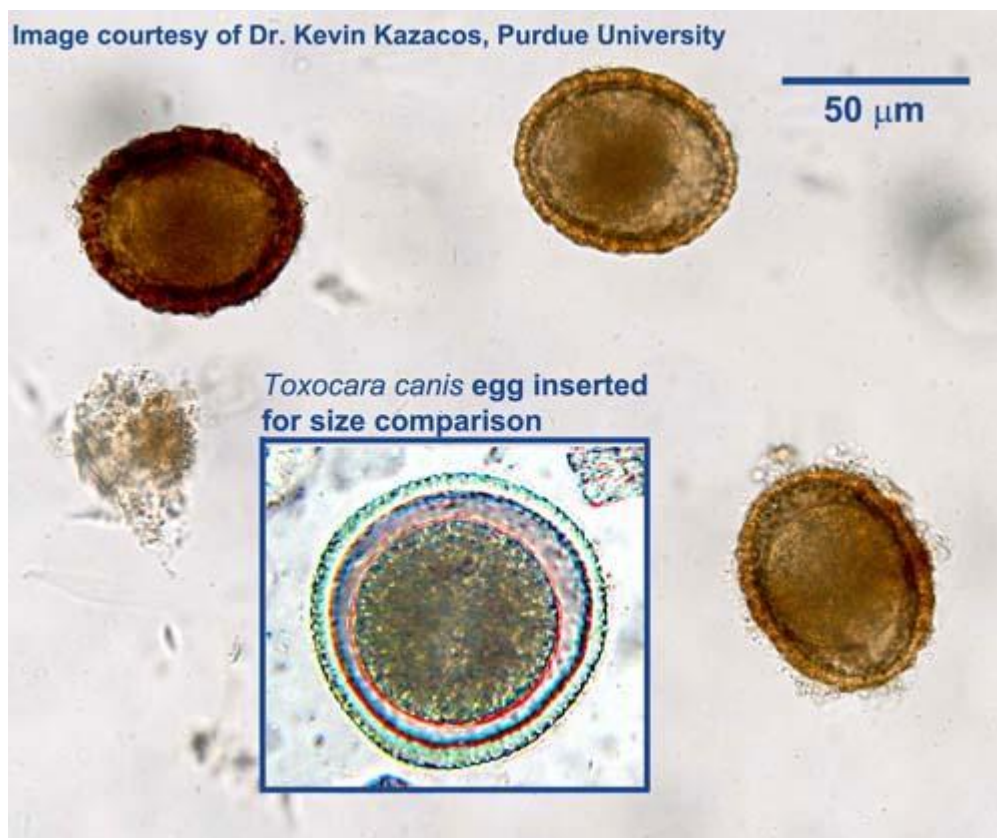


**Figure 3.** Abnormalities of *Baylisascaris procyonis* eggs shed by experimentally inoculated dogs and raccoons, visualized on fecal flotation. A) Triangular egg. B) Pear-shaped egg. C) Almond-shaped egg. D) Triangular egg with indented edge. E) "Immature" egg with underdeveloped morula and no cortex or proteinaceous coat. F) Budded egg. G) Twin conjoined eggs with separate morulae and vitelline membranes. H) Triplet conjoined eggs with distinct morulae; vitelline membrane might be shared between 2 eggs. Arrows indicate eggs of normal morphology (65–75 µm). Original magnification  $\times 400$ .

## Abnormal Helminth Egg Development, Strange Morphology, and the Identification of Intestinal Helminth Infections

Sarah G.H. Sapp, Michael J. Yabsley, Richard S. Bradbury

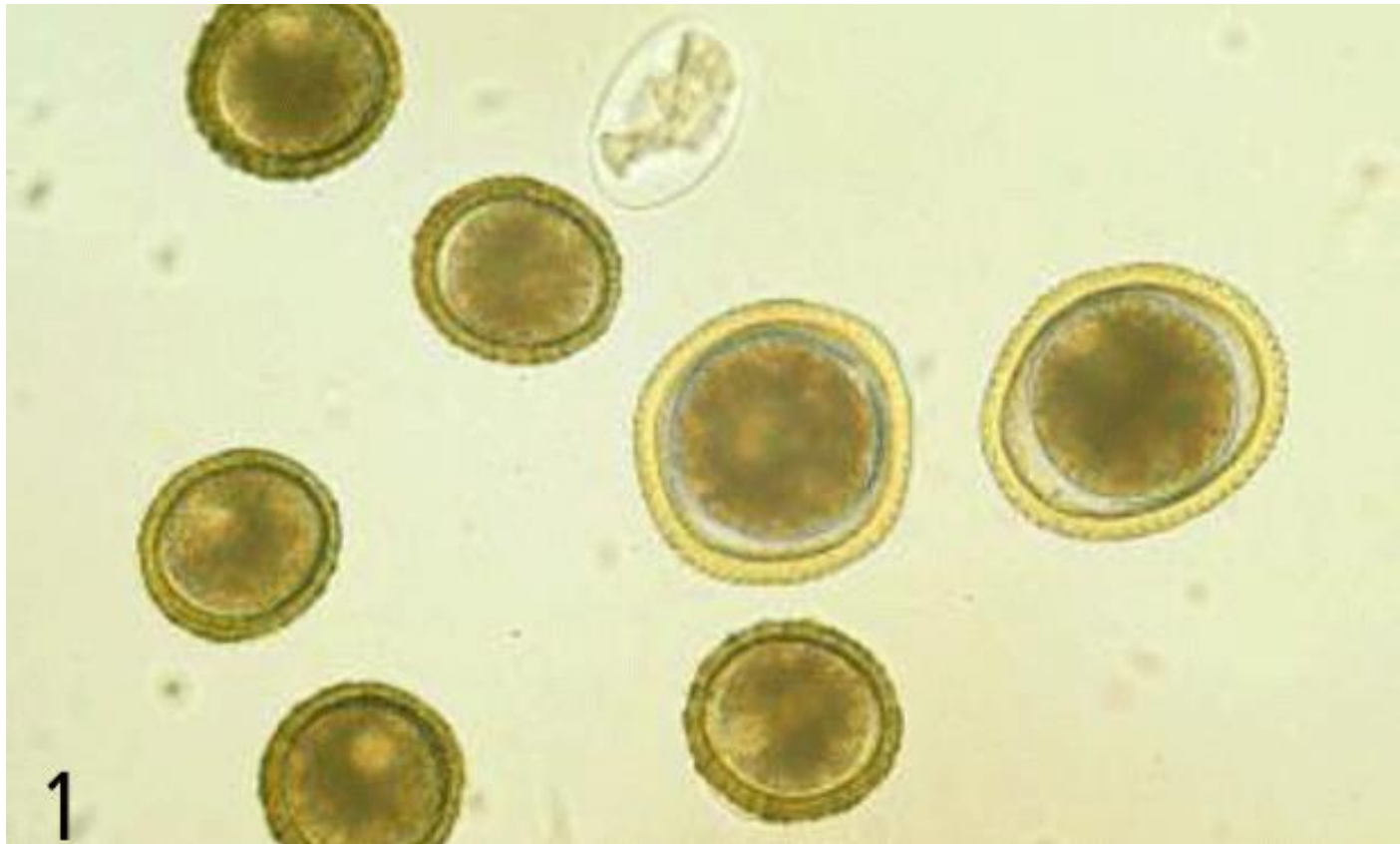
## 5) Ricerca di uova con tecniche coprologiche



Kazacos, 2011

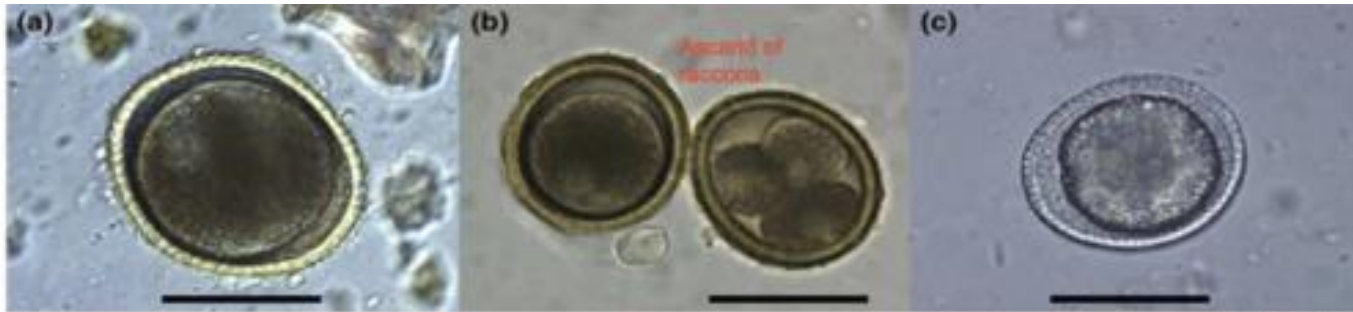






<https://www.cliniciansbrief.com/article/unusual-fecal-parasite-dog>



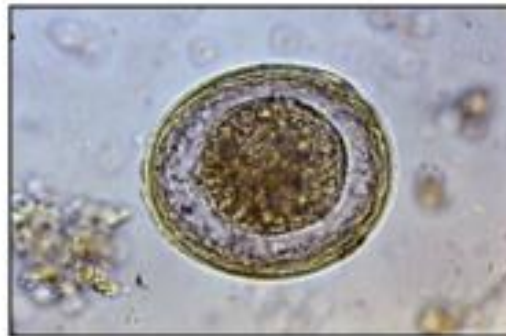


Lee et al., 2010

*Toxocara canis*

*Baylisascaris procyonis*

*Toxocara cati*



*Toxascaris leonina*

<https://quizlet.com/566220504/nematodes-flash-cards/>







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# **Cosa/come cercare negli ospiti paratenici**



## 1) Segni clinici

- Dipendono dal numero di larve migranti e dalla specie ospite
  - Dimensione dell'ospite
  - Numero di uova ingerite
    - Forme asintomatiche
    - Esordio con febbre/ottundimento del sensorio
    - Migrazione larvale (*OLM*, *VLM*, *NLM*)
    - Forma neurologica grave con morte del soggetto



- Per quanto concerne le specie selvatiche, solitamente si assiste a morie o quadri neurologici (+++uccelli e micromammiferi) in animali che si sono alimentati presso una latrina







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# 1) Segni clinici

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Cardinal with twisted neck.  
(Photo by Kevin Kazacos and Sam Royer)

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Bobwhite with twisted neck, ruffled feathers, and rigidity of the feet. (Photo by Kevin Kazacos and Andy Dziubinskyj)

ns



Domestic chicken with twisted neck and rigidity of the legs. (Photo by Kevin Kazacos and Sam Royer)

nd

05);



Domestic duck with twisted neck and inability to stand. (Photo by Kevin Kazacos and Sam Royer)



Blue and gold macaw unable to stand or hold its head up. (Photo by Kevin Kazacos and Sam Royer)



Emu (with *B. columnaris*) unable to stand or use its legs. (Photo by Sam Royer)



# 1) Segni clinici



Woodchuck lying on its side and paddling its forelimbs. (Photo by Kevin Kazacos and Sam Royer)



Gray squirrel with severe head tilt and extension of its forelimbs. (Photo by Kevin Kazacos and Sam Royer)



Chipmunk with head tilt. (Photo by Kevin Kazacos and Sam Royer)



White-footed mouse recumbent on its side with head tilt and rigidity of its forelimbs. (Photo by Claudia Sheppard)



Domestic rabbit with arching of the head and neck. (Photo by Kevin Kazacos and Sam Royer)



Domestic rabbit with arching of the head and neck and extension of its forelimb. (Photo by Kevin Kazacos and Sam Royer)



Ferret with arching of the body and head and extension of its forelimbs. (Photo by Kevin Kazacos and Sam Royer)



Squirrel monkey lying prostrate and semicomatose. (Photo by Kevin Kazacos and Sam Royer)

**Figure 9.** Mammals affected with *Baylisascaris procyonis* neural larva migrans.





## 1) Segni clinici



FIG. 11.4—Gray squirrel with encephalitis due to *Baylisascaris procyonis*, showing arching of the head and neck, and extensor rigidity of forelegs. [Reprinted from Kazacos (1983a) with permission of Purdue Research Foundation.]



FIG. 11.5—Woodchuck with encephalitis due to *Baylisascaris procyonis*. This animal was submitted as a rabies suspect because of ataxia, circling, and loss of fear of humans.

# 1) Segni clinici



**Figure 10.** The sequence of clinical neural larva migrans in a wild gray squirrel. *A, B, C*, The slow arching of the head and neck with “stargazing” progressing to leaning of the body. *D*, The squirrel with a wide stance, trying to steady itself while eating a sunflower seed. *E, F*, The squirrel has fallen over, lying on its side while continuing to eat the seed. (Photos by Kevin Kazacos)





## 1) Segni clinici



*H. Mice with *Baylisascaris* neural larva migrans, ten days after experimental infection with *B. procyonis* eggs.  
(Photo by Kevin Kazacos and Sam Royer)*



*I. Hamsters with *Baylisascaris* neural larva migrans, ten days after experimental infection with *B. procyonis* eggs.  
(Photo by Kevin Kazacos and Sam Royer)*

# 1) Segni clinici



A. Young child with central nervous system disease and blindness, infected with *B. procyonis* at a university daycare facility in southern California.



B. Young man, severely disabled and confined to a wheelchair as a result of being infected with *B. procyonis* as an infant on a dairy farm in upstate New York.



C. Young man, severely disabled with both NLM and OLM-DUSN after being infected with *B. procyonis* as a child in his yard in the Chicago suburbs.

Human cases of *Baylisascaris* neural larva migrans. (Photos by Kevin Kazacos)

## Clinical signs of neural larva migrans in children.

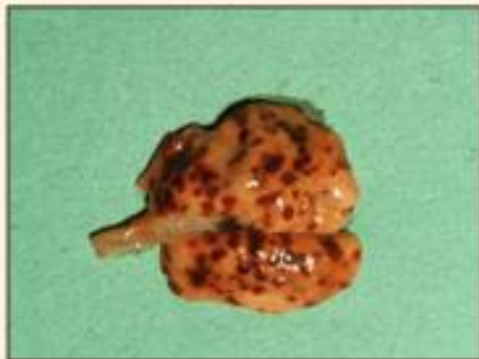
[From Kazacos (2000)]

Lower level infection	Heavy infection
Dullness and somnolence	Sudden onset of lethargy.
Tremors or unsteadiness	Dullness and irritability.
Confusion	Neck rigidity and (or) twisting.
Learning disability	Loss of fine motor skills.
Developmental regression	Decreased head control.
	Inability to sit, stand, or walk without assistance.
	Impaired vision or blindness.
	Eye deviation and (or) rapid involuntary eye movements.
	Speech deterioration.
	Problems with eating.
	Increase or loss of muscle tone.
	Extension of the extremities.
	Arching of the body.
	<b>Stupor.</b>
	Coma.
	Death.





## 2) Lesioni macroscopiche



Early migratory hemorrhages in the lungs of a rat and ferret. (Photos by Kevin Kazacos and Sam Royer)



Early migratory hemorrhages in the brain of a mouse. (Photo by Kevin Kazacos and Sam Royer)

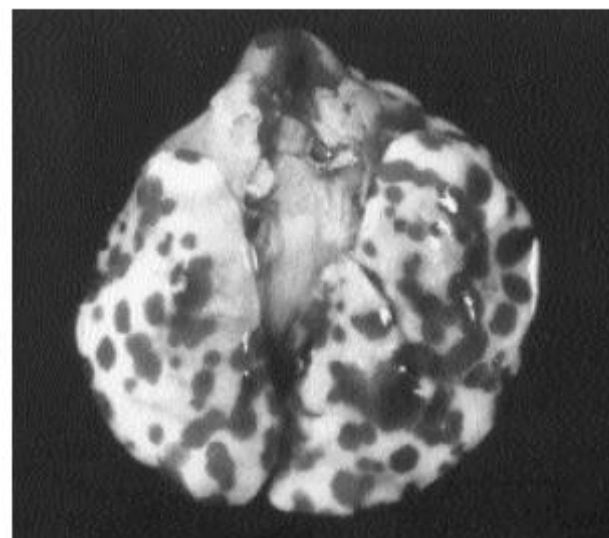


FIG. 11.7—Multifocal, coalescing hemorrhages in the lungs of a lab mouse, due to pulmonary migration of *Baylisascaris columnaris* larvae; 2 days postinfection with eggs.

## 2) Lesioni macroscopiche

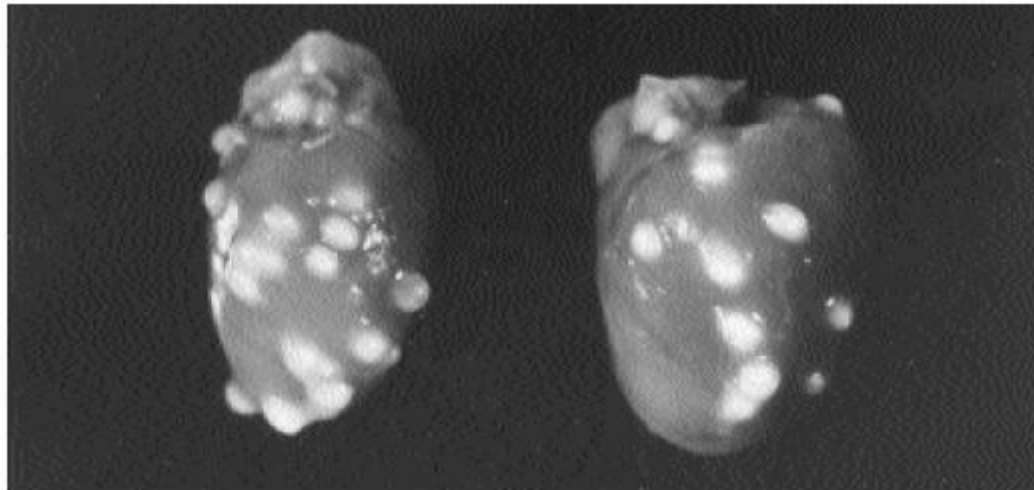


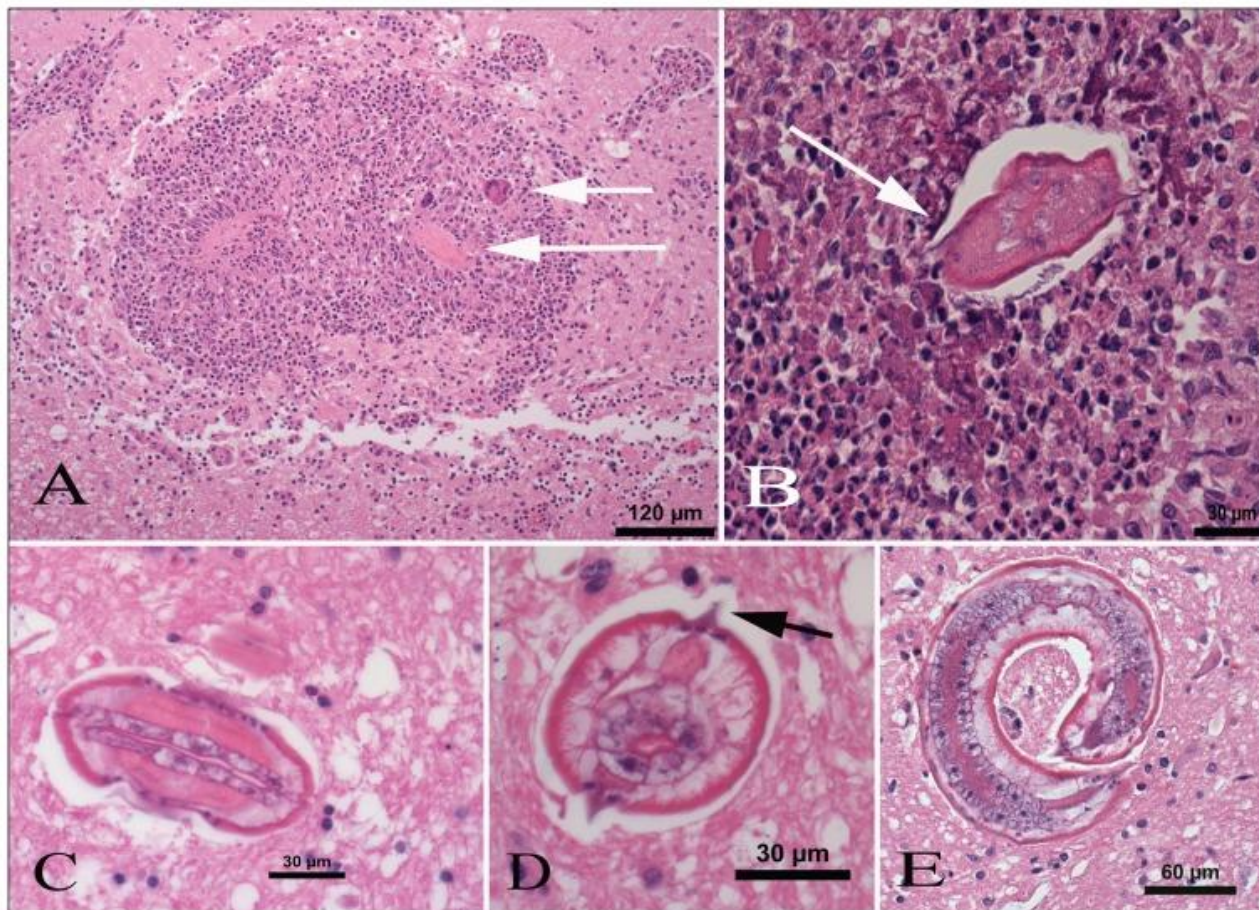
FIG. 11.8—Larval granulomas in the hearts of lab mice infected with *Baylisascaris columnaris*; 15 days postinfection. Similar granulomas develop in *B. procyonis* infections.

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## 2) Lesioni microscopiche (con ricerca larve)



Neurologic *Baylisascaris procyonis* infection in a young dog

Murray Hazlett, Hugh Y. Cai, Stephanie Sparling, Qiumei You



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## 2) Ricerca larve tissutali

- Baermann da organo
- Digestione artificiale
- Brain squash

11

*BAYLISASCARIS PROCYONIS* AND RELATED SPECIES

KEVIN R. KAZACOS

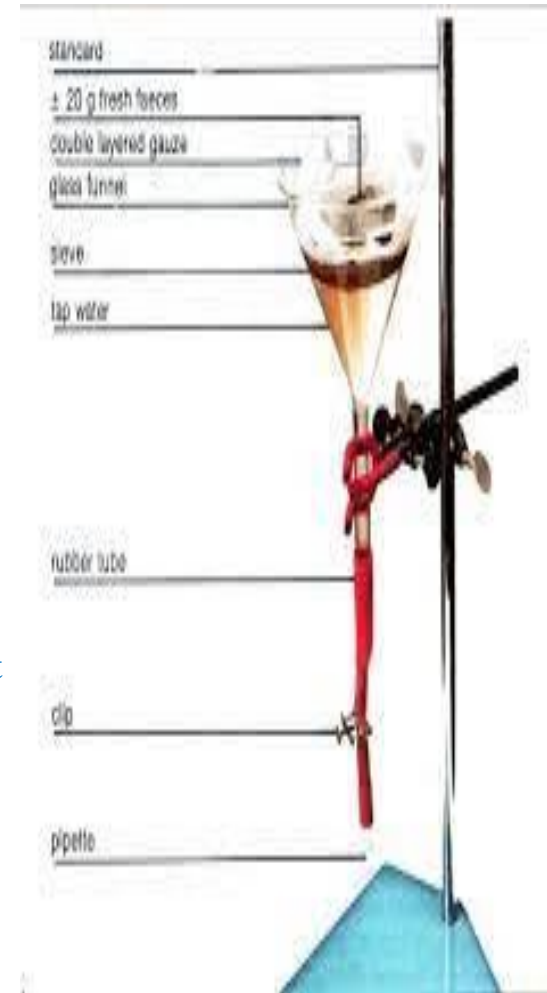




## 2) Ricerca larve tissutali

### Baermann da organo

- Pool di organi target (encefalo, midollo spinale, polmone e fegato) che viene sminuzzato finemente, raccolto in garza e usato in apparato di Baermann come fossero feci
  - Consigliato l'utilizzo di soluzione salina tiepida (o in termostato 37°C)
  - Dopo overnight il fondo viene raccolto in provetta, centrifugato e il pellet risospeso con Lugol e osservato al microscopio
- Metodo rapido, economico e efficace in infestazioni massive
- Sensibilità inferiore agli altri metodi



## 2) Ricerca larve tissutali

### Digestione artificiale

- Pool di organi target (encefalo, midollo spinale, polmone e fegato) che viene sminuzzato finemente
- Ottimo metodo per analisi di più animali in pool
- Digestione artificiale con strumentazione analoga per Test *Trichinella* spp.
  - Soluzione di digestione (20mL di soluzione/1 grammo di tessuto) a 37°C
  - 1% pepsina
  - 1% HCl
  - 1-2 ore di digestione, filtraggio in setaccio e sedimentazione in imbuto separatore 30 min.
  - Osservazione del sedimento in piastra con stereomicroscopio





## 2) Ricerca larve tissutali

### Brain squash

- NCS viene prelevato in toto e diviso in porzioni di 1gr circa
- Ogni porzione viene compressa tra 2 vetrini (squash) e ispezionato al microscopio ottico
- Se il campione è positivo, il materiale viene raschiato dal vetrino e raccolto.
- Molto sensibile
- Utilizzabile solo su piccoli animali, inattuabile su grandi animali



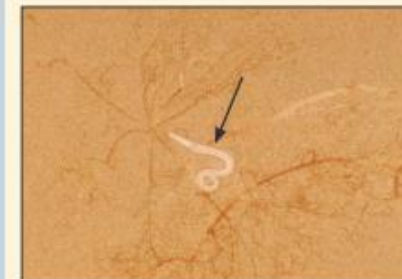
D. Brain squash of a white-footed mouse with two *Baylisascaris* larvae in the brain (circles). (Photo by Kevin Kazacos and Sam Royer)



E. Brain squash of a conure with severe NLM, showing 49 larvae (circles) in about one-third of its brain. (Photo by Kevin Kazacos and Sam Royer)



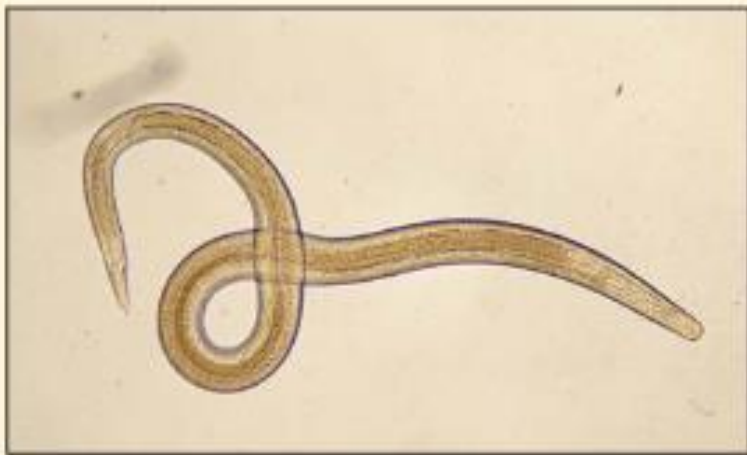
F. Positive brain squash from a conure showing multiple larvae (arrows). (Photo by Kevin Kazacos and Sam Royer)



G. Higher magnification of conure brain squash showing *B. procyonis* larva (arrow). (Photo by Kevin Kazacos and Sam Royer)



## 2) Ricerca larve tissutali



*H. B. procyonis* larva recovered from brain squash of conure with NLM. (Photo by Kevin Kazacos and Sam Royer)

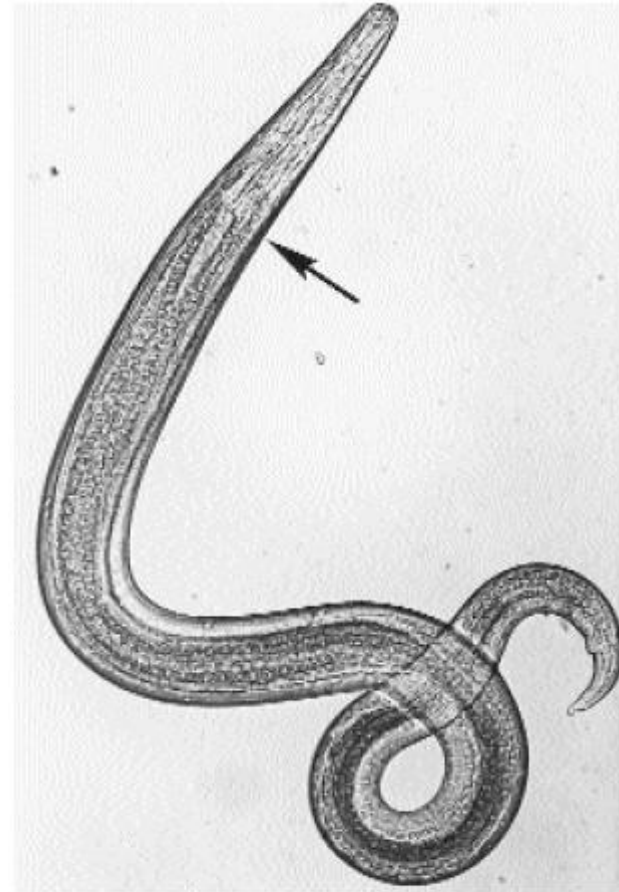
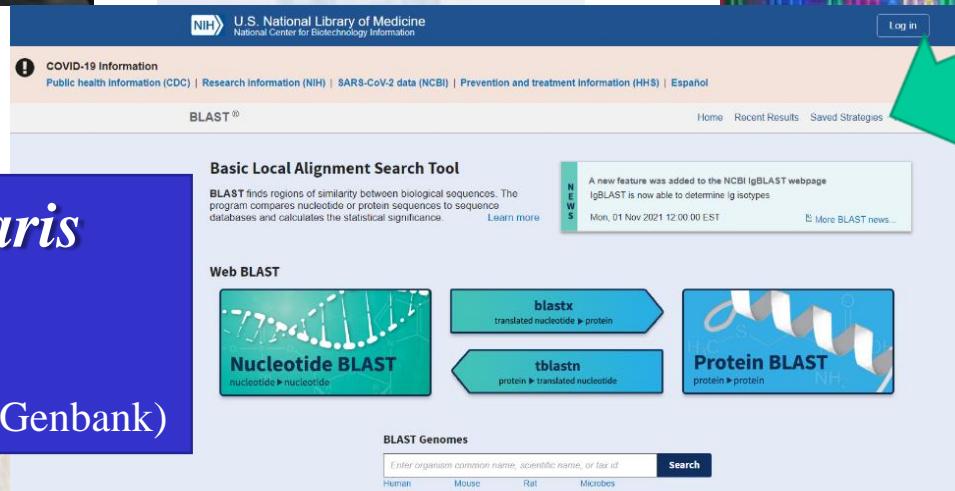
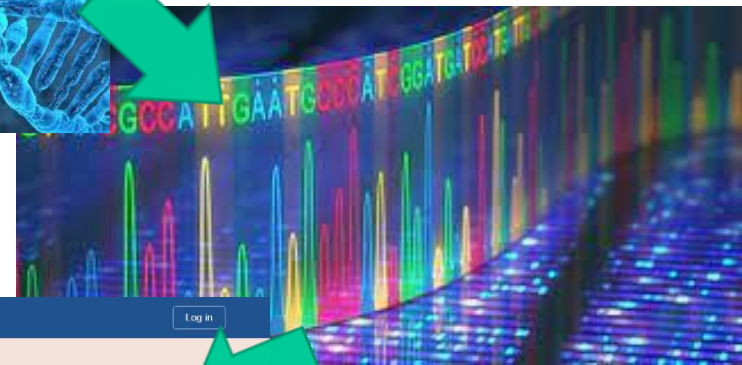


FIG. 11.15—*Baylisascaris procyonis* larva recovered by artificial digestion from the carcass of a conure with encephalitis. Note overall shape and proportions, smoothly rounded anterior end, sharply flexed tail tip, length of esophagus (arrow at esophageal-intestinal junction), prominent intestine, and transverse cuticular striations (seen at bend in mid-body).



**Conferma molecolare tramite sequenziamento NGS o PCR**  
(*Diagnostica Generale, IZSLT Sede di Roma*)



# *Baylisascaris procyonis*

100% identity (Genbank)





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# Test sierologici



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[Clin Microbiol Rev.](#) 2005 Oct; 18(4): 703–718.

doi: [10.1128/CMR.18.4.703-718.2005](https://doi.org/10.1128/CMR.18.4.703-718.2005)

PMCID: PMC1265913

PMID: [16223954](https://pubmed.ncbi.nlm.nih.gov/16223954/)

## Baylisascariasis

[Patrick J. Gavin](#),<sup>1,\*</sup> [Kevin R. Kazacos](#),<sup>2</sup> and [Stanford T. Shulman](#)<sup>3</sup>

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Clin Microbio

## Serology

*Anti-Baylisascaris* antibodies can be demonstrated in CSF and serum by indirect immunofluorescence, enzyme-linked immunosorbent assay, and Western blotting. Enzyme-linked immunosorbent assay is the current test of choice. Serologic testing is currently available only from the Department of Veterinary Pathobiology at Purdue University, West Lafayette, IN (K. Kazacos).





# Grazie per l'attenzione

