GI Parasitology: Utilizing the Keyscreen™ GI Parasite PCR Panel for Enhanced Diagnostic Screening and Improved Standards of Care

Antech Diagnostics

Brad Ryan, MSc, DVM, MPH
Professional Services Veterinarian
Mid-Atlantic and South Atlantic Regions

Stories to make you...

smile!

People

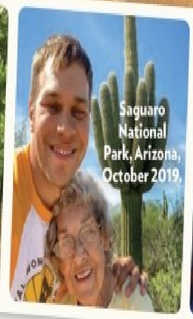
Grandma and grandson set out to visit every U.S. national park together

AMERICA'S NATIONAL PARKS

Six years ago Brad Ryan took his grandmother Joy Ryan on a camping trip to Great Smoky Mountains National Park after she expressed regret about never having seen a mountain in her life. "We had a really life-changing experience that weekend, and it opened the door for me to begin what is now known as Grandma Joy's Road Trip," says Brad, a wildlife veterinarian, 40, who, along with Joy, now 91, set out from their hometown of Duncan Falls, Ohio, to visit all 63 U.S. National Parks. So far they've traveled more than 40,000 miles and visited 61 of them, with parks in West Virginia and American Samoa left to go. Along the way, they've climbed mountains, rafted rapids ("It was like a roller coaster, only with water," says Joy. "It was so much fun!"), ridden a dogsled and more—posting their adventures for their 53,000 followers on Instagram (@grandmajoysroadtrip). With the next leg of their journey starting in October, Joy says, "I plan to keep doing this as long as I hold out. Brad is a wonderful grandson; we've had a lot of fun."

"People tell us they're going to take their grandparents to Yellowstone or the Everglades or wherever. That's a legacy I'm incredibly proud of," says Brad (with Joy at Acadia National Park, Maine, in June 2019).

Mammoth Cave National Park, Kentucky, August 2017.



Saguaro National Park, Arizona, October 2019.

Wrangell-St. Elias National Park, Alaska, August 2021.

CLOCKWISE FROM BOTTOM: COURTESY ZACHARY BATEO; @GRANDMAJOYSROADTRIP(4)

The Columbus Dispatch

THE GOOD LIFE

Grandma Joy's Road Trip helps Ohio State veterinary grad get through mental-health struggles

Megan Henry mhenry@dispatch.com
Published 5:03 a.m. ET Dec. 2, 2019

AVMA | News

Veterinarian, grandma one stop away from completing epic road trip

Duo's quest to see all U.S. national parks spurred by finding passion outside the workplace

By Coco Lederhouse
November 30, 2022

Dr. Brad Ryan and his grandmother Joy Ryan have driven 50,000 miles together. They've also flown to Alaska, Hawaii, and the Virgin Islands—all toward reaching their goal of visiting all 63 U.S. national parks. And now they are closing in on their last stop: the National Park of American Samoa, located in the U.S. territory in the South Pacific. American Samoa is 6,700 miles from their hometown in Duncan Falls, Ohio.

As they prepare to check the final park off their bucket list, Dr. Ryan, who is a professional services veterinarian for Antech Diagnostics, spoke with AVMA News about his memories and lessons learned along the way.

Dr. Brad Ryan and his grandmother Joy Ryan whitewater rafting in Wrangell-St. Elias National Park & Preserve in south-central Alaska (Photos courtesy of Dr. Brad Ryan)

The Washington Post
Democracy Dies in Darkness

INSPIRED LIFE

Grandmother and grandson visit 63 national parks on adventure of a lifetime

Joy Ryan, 92, had never seen a mountain. So her grandson decided to take her to every site that has "national park" in its name.

By Sydney Page
August 30, 2022 at 6:00 a.m. EDT

Brad Ryan with his grandmother, Joy Ryan, during a 2019 visit to Channel Islands National Park in California. (Cheryl Hutchison)

Listen 8 min Comment Gift Article Share

Brad Ryan paused to take in the scene before him: Mountains with perfect peaks, a lush valley and an endless expanse of untouched Alaskan wilderness.

What awed him most, though, was not the Arctic tundra in front of him; it was his grandmother who was hiking through it.

Agenda

What methods are available for diagnosing intestinal parasites?

What is new in intestinal parasite testing?

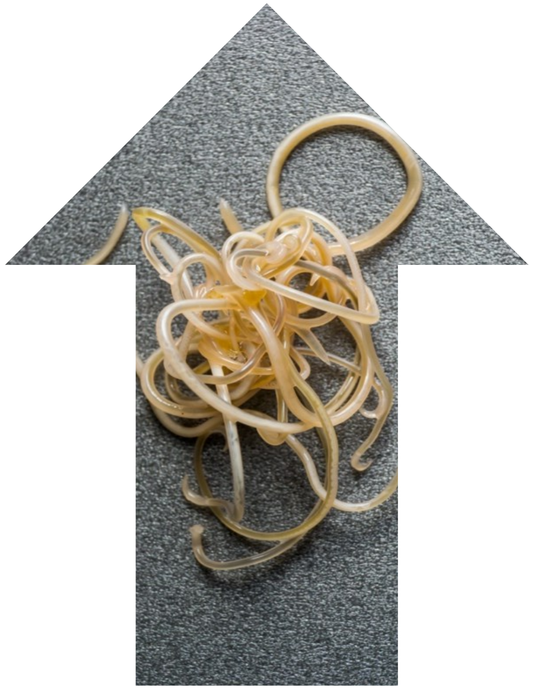
What is the clinical impact of gastrointestinal parasitism?

How can we improve the standard of preventive care for cats and dogs?

Intestinal parasites

An evaluation of gastrointestinal nematode prevalence data from over 39 million fecal samples was conducted and examined for seven years.

The evaluation revealed a subtle yet significant **increase in prevalence for roundworms, an increasing prevalence for hookworms, and a slightly decreasing prevalence for whipworms.**



Roundworms

Hookworms

Whipworms

OA16.04 Seasonality and Prevalence of Common Canine Gastrointestinal Nematodes in the U.S.A. Jason Drake[1], Thomas Carey[1]
[1]Elanco Animal Health, Greenfield, United States

Intestinal parasite infections are common in cats and dogs despite…

- **Screening:**
  - Juvenile patients
  - Adult patients
  - At-risk patients
  - Patients with appropriate clinical signs
- **Routine de-worming programs:**
  - Puppies and kittens
  - Patients with appropriate clinical signs
- **Antiparasitic preventatives**:
  - Despite routine use, prevalence of many GI parasites remains high

Why current practices are not enough.

- Test limitations
- Re-infection/environmental contamination
- Pet owner compliance
- New medications (Proheart™)
- **Unrecognized resistance**

Environmental contamination by canine geohelminths

- Roundworms, hookworms, and whipworms have a relevant health-risk impact on animals and humans in many cases.
- **High rate of soil and grass contamination with infective parasitic elements** has been demonstrated worldwide in leisure, recreational, public, and urban areas:

  - Parks
  - Green areas
  - Bicycle paths
  - City squares
  - Playgrounds
  - Sandpits
  - Beaches

Donato Traversa, Antonio Frangipane di Regalbono, Angela Di Cesare, Francesco La Torre, Jason Drake & Mario Pietrobelli
Parasites & Vectors ***volume 7****, Article number: 67 (2014)*

Intestinal parasites are present throughout common areas

Results of Portland Oregon Dog Park fecal collection study 2014

Second Study in 2016 found

49%

of all samples were positive for *Giardia*.

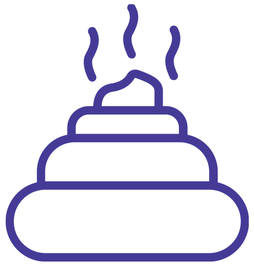
Parasite	Percent Positive
Cryptosporidium	22%
Giardia Trophozoite	9%
Giardia Cysts	21%
Giardia FA	26%
Toxocara	11%
Uncinaria	1%
Spirocerca	1%
Isospora	2%

Parasite	Percent Positive
Ancylostoma	2%
Aelurostrongylus	6%
Larvae	16%
Taenia	1%
Coccidia	1%
Alaria	1%
Trichuris	2%
Strongyloides	2%

Emilio DeBess, DVM, MPH
Oregon State Public Health Veterinarian
Immediate past President of CAPC

Park risk

2019 national dog park study[1]

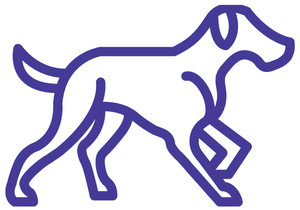


3,006 fecal samples

- 288 dog parks
- 30 cities

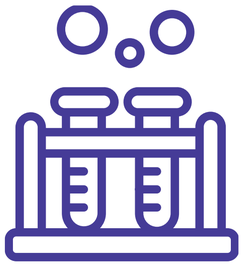
85% of parks positive for nematodes and/or *Giardia*

50% of parks positive for nematodes



622 dogs positive for nematodes and *Giardia*

- 1 in 5 dogs
- 263 positive hookworm + - whipworm + - roundworm



Combined testing

- Detection of infections was most effective using both CF and coproantigen

***CF: Centrifugal flotation**

[1] Elanco. Data on File.

Screening Tests to Detect GI Parasites

O&P

O&P with ELISA

PCR

Improving accuracy

W Telemann, 1908: Eine Methode zur Erleichterung der Auffindung von Parasiteneiern in den Faeces.

Y Carlier, 1982: The use of an excretory-secretory antigen for an ELISA specific sero-diagnosis of visceral larva migrans.

DD Bowman,1987: *Toxocara canis* monoclonal antibodies to larval excretory-secretory antigens that bind …

A Naidich, 2006: Patent and pre-patent detection of *Echinococcus granulosus* in the definitive host.

K Gavina, 2017: A sensitive reverse transcription real-time PCR method for detection of *P. falciparum*.

Paul McVeigh, 2020: Post-genomic progress in helminth parasitology.

Which option works best for you?

O&P

2 gm feces

72 hr stability

O&P with ELISA

2.5 gm feces

72 hr stability

PCR

0.15 gm feces (minimum)

10-day stability (refrigerated)

Improving accuracy

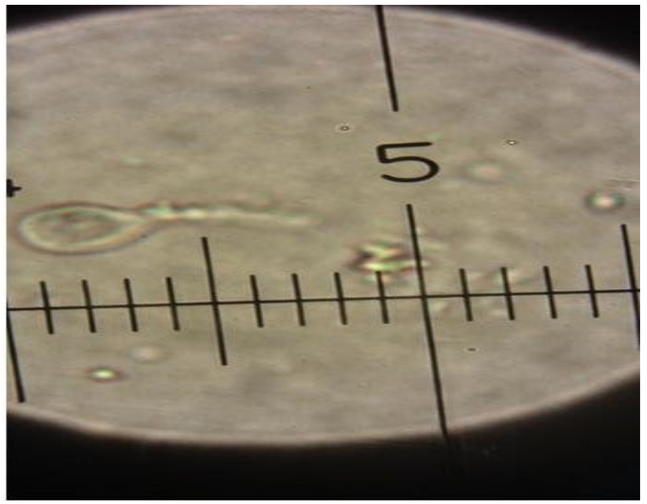
In Clinic Testing

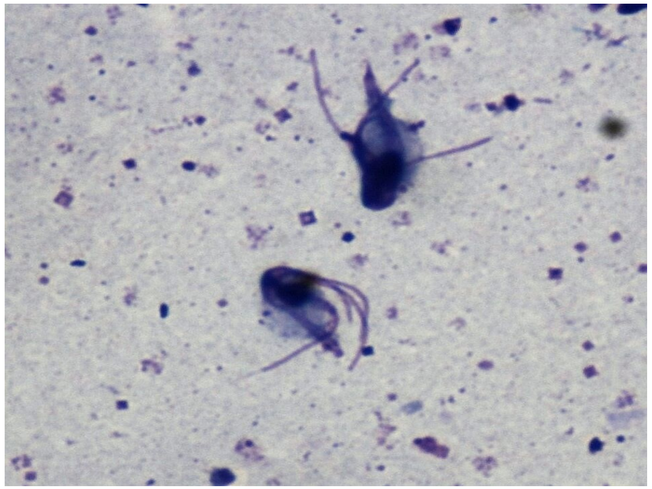
- Direct smear
- O&P-passive float
- O&P-centrifugation
- O&P-centrifugation + AI

In Clinic Testing

Direct smear

- **Least useful technique**. Should be used only on liquid feces to look for protozoal trophozoites.
- Small sample size, long time to examine properly, high propensity for false negatives.
- **High % of false negatives.**
- A positive is a positive, but a negative does not exclude infection.



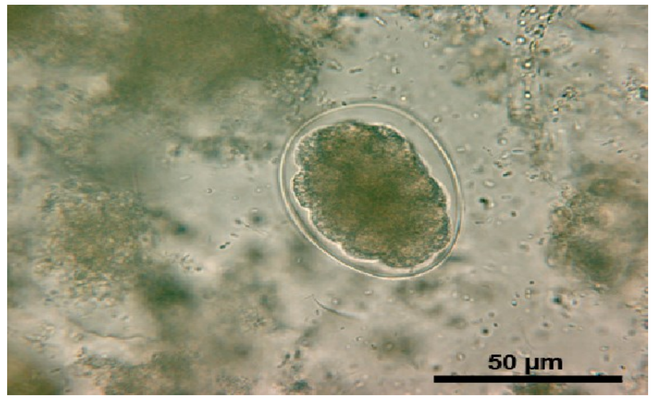


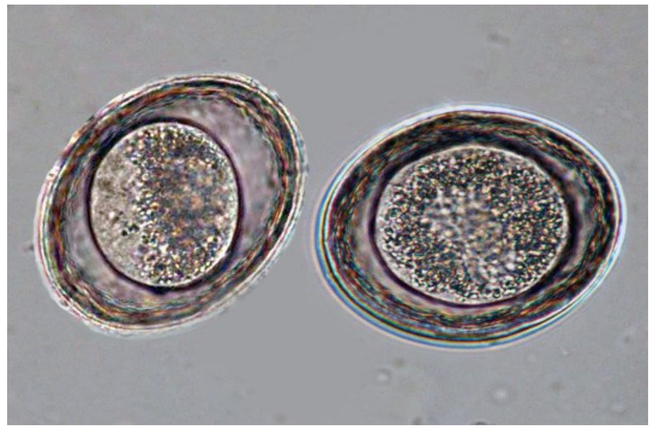
Top image source: www.researchgate.net/publication/280719898_Morphological_descriptive_study_of_giardia_lamblia_in_man_and_cow_at_basrah/citation/download; Bottom image: https://www.troccap.com/feline-guidelines/gastrointestinal-parasites/tritrichomonas-feline/

In Clinic Testing

O&P-passive float

- Saturated sucrose, saturated salt (sodium chloride or sodium nitrate), or zinc sulfate.
- Standard technique used in many veterinary clinics.
- Will miss most *Giardia* cases, many mild whipworm infections, and protozoal trophozoites.
- High degree of subjectivity.
- **Very high % of false negatives.**



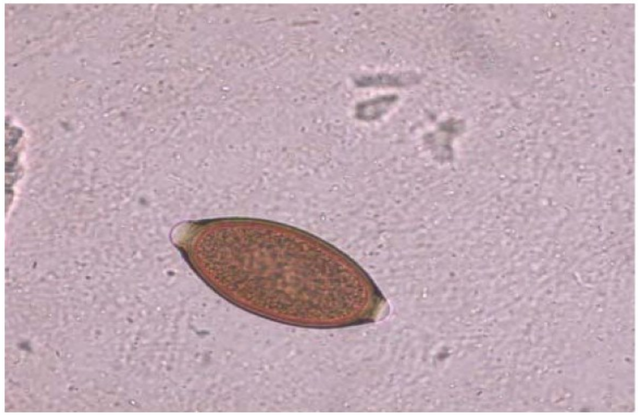


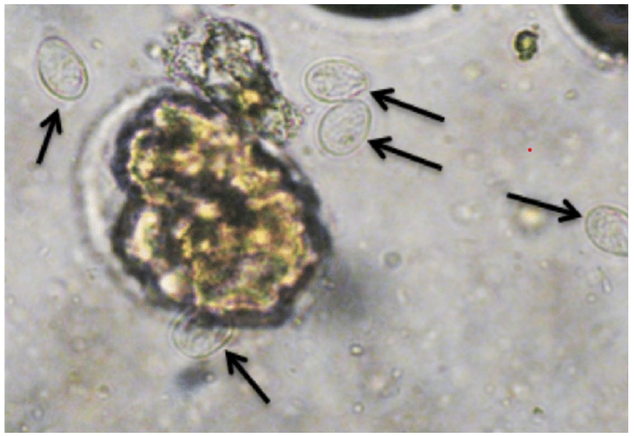
Top image source: www.researchgate.net/figure/Microscopic-image-of-a-hookworm-egg-under-500x-magnification-Scale-bar-50-mm_fig3_230804533; Bottom image: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/toxascaris

In Clinic Testing

O&P-centrifugation

- **Centrifuge for at least 10 minutes**. In clinic fecal testing with centrifugation takes ≈ 15 min.
- Small sample size, long time to examine properly.
- **Significantly improved egg recovery than passive O&P.**
- Parasites may be present but not shedding eggs.



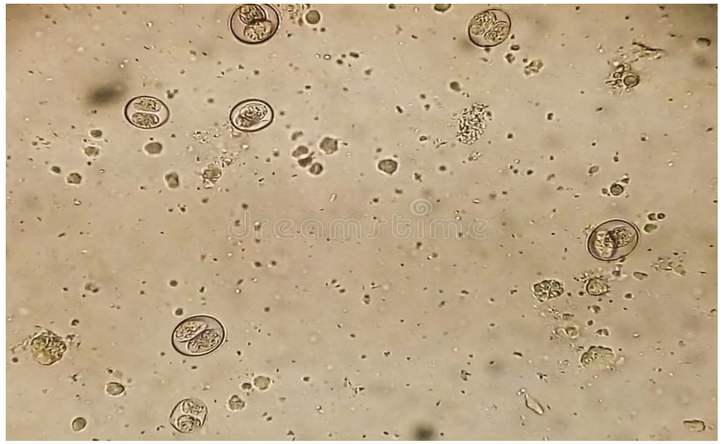


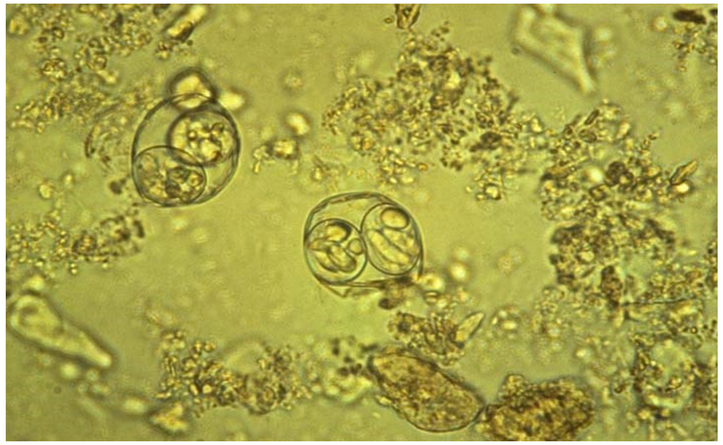
Top image source: www.researchgate.net/figure/Floatation-with-zinc-sulphate-Trichuris-vulpis-egg_fig2_50347597; Bottom image source: Giardia spp. cysts (arrow) as seen with microscopy (400× magnification) following the zinc sulfate flotation technique

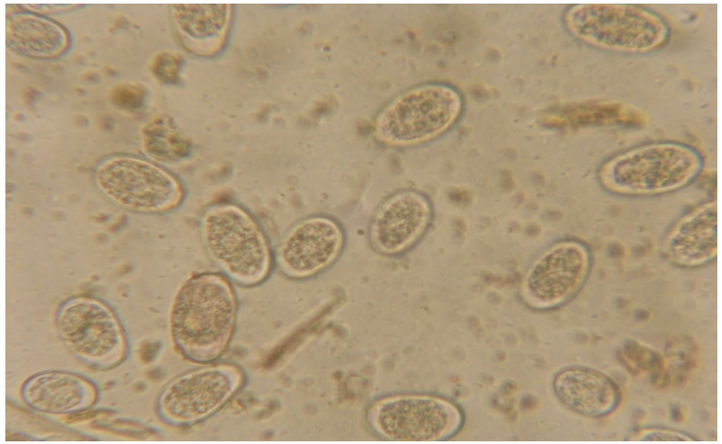
Can you tell the difference?

What we identify or misidentify matters. Why?

O&P-centrifugation







Left image sourcehttps://www.vetlexicon.com/treat/felis/bug/isospora-felis; /www.vetlexicon.com/treat/felis/bug/isospora-felis
Right image source: https://www.petvet1.com/category/eimeria/; https://www.troccap.com/canine-guidelines/gastrointestinal-parasites/canine-coccidia/)

In Clinic Testing

- **Uses AI to screen for GI parasite eggs.**
- Sample preparation is still necessary.
- Minimizes subjectivity.
- **May not improve efficiency.**
- Better documentation.
- Cost?

O&P-centrifugation + AI

Top image source: www.salon.com/2021/04/30/why-artificial-intelligence-research-might-be-going-down-a-dead-end/; Bottom image source: https://www.forbes.com/sites/bernardmarr/2020/05/08/5-reasons-why-artificial-intelligence-really-is-going-to-change-our-world/?sh=21126c4278b6

Reference Lab Testing

- Baermann
- O&P-centrifugation
- O&P-centrifugation + Antigen
- GI Parasite PCR

Reference Lab Testing

Baermann

- Used for recovering live nematode larvae for identification (most commonly lungworms).
- Not recommended as a primary diagnostic technique for evaluation of parasites in feces.
- **Takes a minimum of an hour to run and will recover only live nematode larvae.**
- Samples with only a few larvae in them may have to be run overnight.

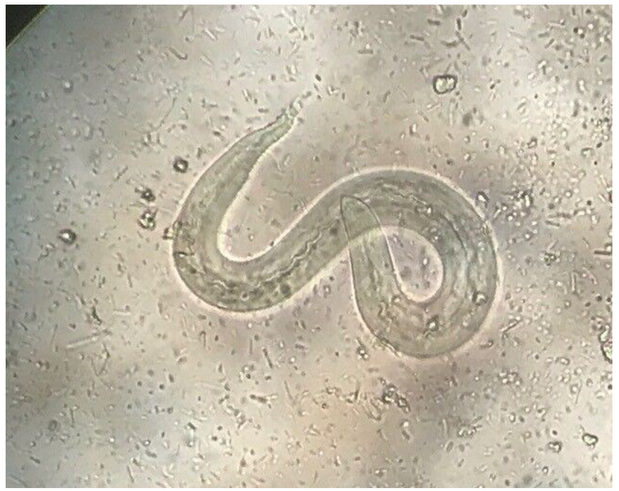
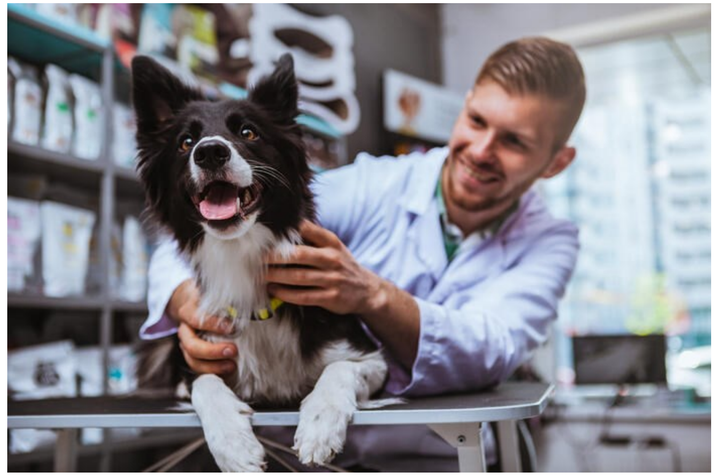


Image source: https://www.troccap.com/feline-guidelines/other-systems-feline/lungworms-feline/

Reference Lab Testing

O&P-centrifugation

- Fecals read by certified lab techs and pathologists.
- **Outsourcing O&P to the reference lab can improve efficiency and documentation.**
- Antech lab technicians participate in continuous proficiency training and are subjected to monthly reviews.
- Parasites may be present but not shedding eggs (prepatent infections; false negative).



Reference Lab Testing

- Antigen testing improves parasite detection by looking for specific proteins from the adult parasites.
- **More sensitive, more limited.**
- Outsourcing O&P to the reference lab can improve efficiency and documentation.
- Antigen testing should be done in combination with O&P testing.

O&P-centrifugation + Antigen

[12–15]. Combining CAI for nematode antigens with CF in the present study resulted in detection of nearly 80% (78.4%) more nematode infections than CF alone, likely due to the CAI detecting non-patent infections [16, 17]. Detection of parasite ova by CF in instances when CAI was negative could be due to coprophagia or predation, resulting in a positive CF in the absence of infection. In

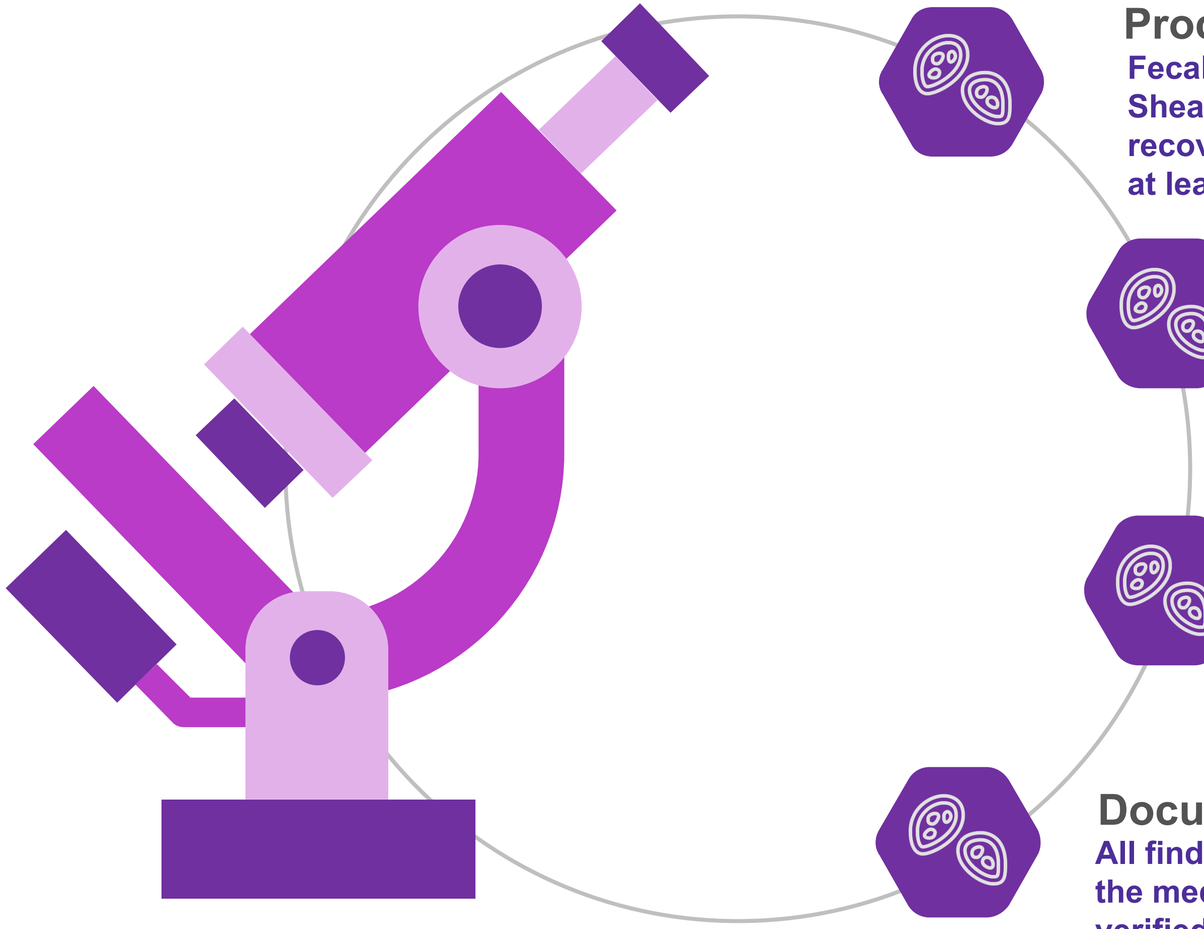
Reference Lab Testing

- Improved detection for a broader scope of gastrointestinal parasites.
- **Direct detection of parasitic DNA/RNA.**
- No subjective assessment of microscopy.
- Can detect genetic markers for anthelmintic resistance and zoonotic potential.
- Outsourcing O&P to the reference lab can improve efficiency and documentation.

GI Parasite PCR

Ova & Parasite Testing

O&P testing is the traditional screening method for detecting GI parasites. Centrifugation is required for improved egg/oocyst recovery.



Process

Fecal sample is mixed with zinc sulfate or Sheather's solution. For maximum egg/oocyst recovery the sample should then be centrifuged for at least 10 minutes.

Analyze

Accurate egg/oocyst detection requires time and experience. The entire slide should be methodically reviewed. Antech lab technicians participate in continuous proficiency training and are subjected to monthly reviews.

Identify

Parasite identification is challenging, especially for rare or complicated parasites. Proper identification and discerning between pathogenic and nonpathogenic variants requires significant proficiency.

Document

All findings must be well documented in the medical record. Results should be verified by a veterinarian.

Advancement in Molecular Diagnostics

Where is Molecular Diagnostics today?

Technical Advancement with Reagents, Equipment, and Protocols

- First step in workflow = extract DNA and RNA from sample
- **Fully enclosed** nucleic acid extraction robotic systems
    - Greatly reduces and prevents cross-contamination from sample to sample
- Optimized parasite protocols

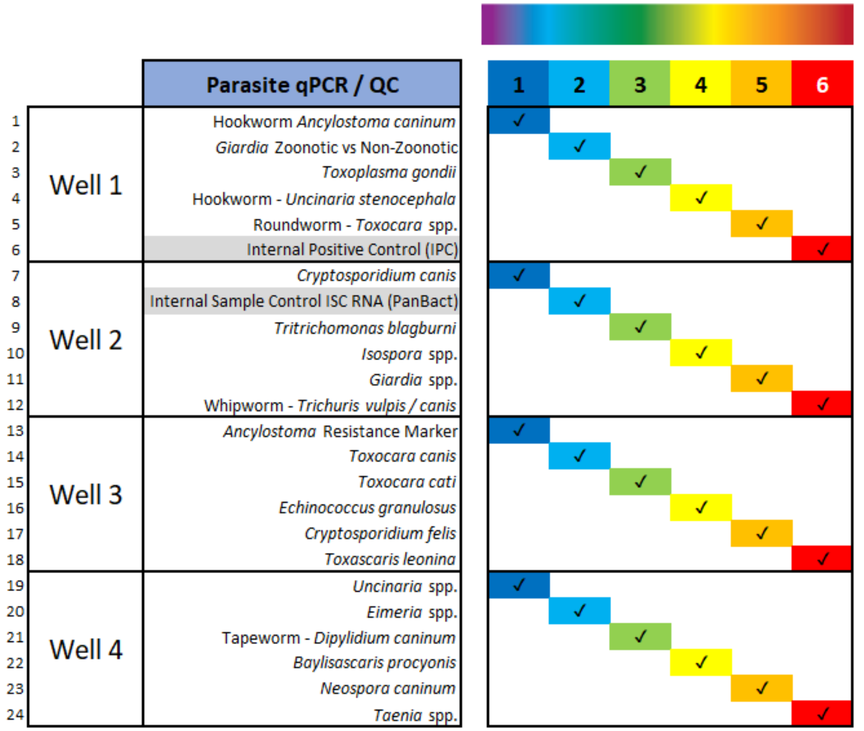
Image Credit: Pixabay/CC0 Public Domain

Advancement in Molecular Diagnostics

Where is Molecular Diagnostics today?

Technical Advancement with Reagents, Equipment, and Protocols

- Second step in workflow = PCR amplification step
- Real-time PCR thermocyclers with **6-plex capability**
    - **Cost control leading to affordable pricing**
- Reverse transcriptase step (previously manual step) added at the beginning
- 6 parasites detected in a single PCR reaction (vs. 1)
    - Reduced cost



		Parasite qPCR / QC	1	2	3	4	5	6
1	Well 1	Hookworm *Ancylostoma caninum*	✓					
2		*Giardia* Zoonotic vs Non-Zoonotic		✓				
3		*Toxoplasma gondii*			✓			
4		Hookworm - *Uncinaria stenocephala*				✓		
5		Roundworm - *Toxocara* spp.					✓	
6		Internal Positive Control (IPC)						✓
7	Well 2	*Cryptosporidium canis*	✓					
8		Internal Sample Control ISC RNA (PanBact)		✓				
9		*Tritrichomonas blagburni*			✓			
10		*Isospora* spp.				✓		
11		*Giardia* spp.					✓	
12		Whipworm - *Trichuris vulpis / canis*						✓
13	Well 3	*Ancylostoma* Resistance Marker	✓					
14		*Toxocara canis*		✓				
15		*Toxocara cati*			✓			
16		*Echinococcus granulosus*				✓		
17		*Cryptosporidium felis*					✓	
18		*Toxascaris leonina*						✓
19	Well 4	*Uncinaria* spp.	✓					
20		*Eimeria* spp.		✓				
21		Tapeworm - *Dipylidium caninum*			✓			
22		*Baylisascaris procyonis*				✓		
23		*Neospora caninum*					✓	
24		*Taenia* spp.						✓

Figure credit: Leutenegger 2022

Advancement in Molecular Diagnostics

Where is Molecular Diagnostics today?

Technical Advancement with Reagents, Equipment, and Protocols

- New generation **of DNA polymerases** (enzymes that copy the DNA
- Very stable
- More resistant against remaining PCR inhibition

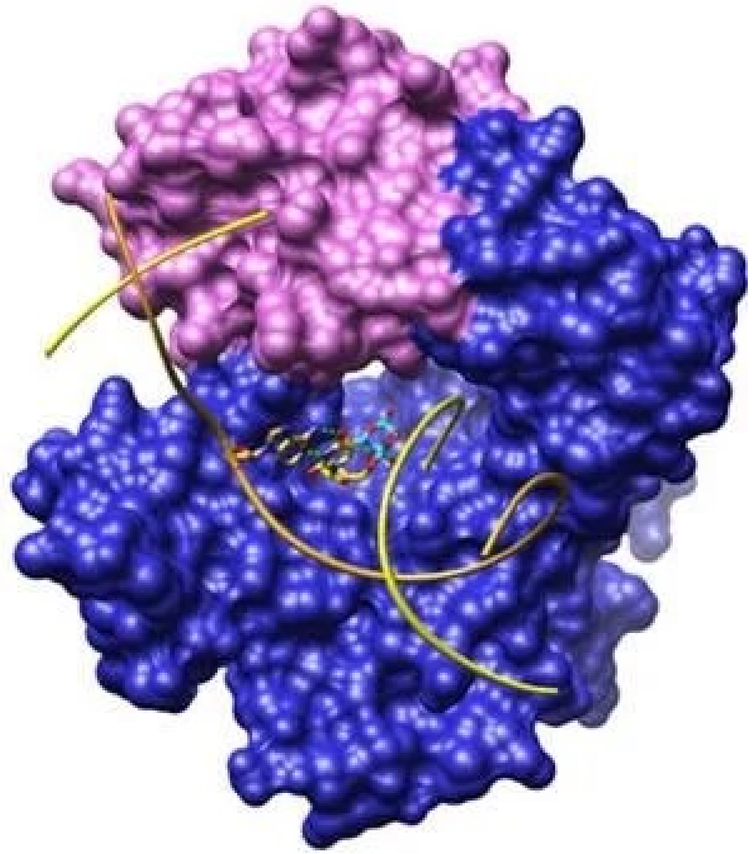


Image Credit: niehs.nih.gov

Advancement in Molecular Diagnostics

Where is Molecular Diagnostics today?

Technical Advancement with Reagents, Equipment, and Protocols

- **PCR product carry-over prevention:** UNG (Uracil N-glycosylase) available with different optimal temperatures
    - System destroys any PCR product contamination
- Newer UNG enzymes work for both direct DNA PCR and the added reverse transcriptase PCR

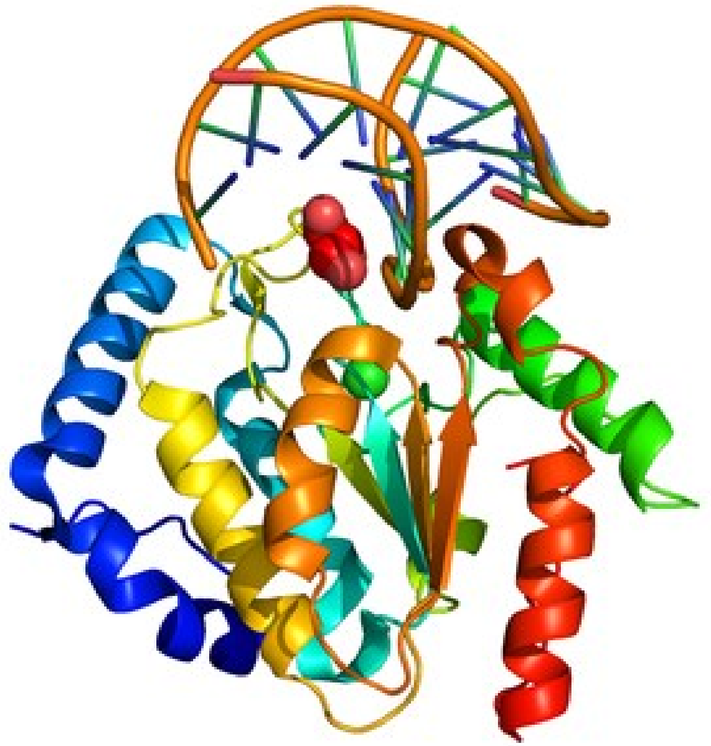


Image Credit: https://www.itqb.unl.pt/researchers/elinmoe/crystal-structures/uracil-dna-n-glycosylase

Advancement in Molecular Diagnostics

Where is Molecular Diagnostics today?

Technical Advancement with Reagents, Equipment, and Protocols

- **Hydrolysis probes** with significantly decreased background fluorescence
- Allow for highly complex multiplex qPCR reactions
- Essential to make the 6-plex work
    - Reduced cost

Image source: https://www.antechdiagnostics.com/keyscreen/

Advancement in Molecular Diagnostics

Where is Molecular Diagnostics today?

Technical Advancement with Reagents, Equipment, and Protocols

- **Post-genomic progress** in parasitology (post-human genome project in 2001)
    - Rapid genetic characterization of known and newly discovered parasites
    - More specific and sensitive real-time PCR
    - Characterization of anthelmintic drug resistance at genetic level

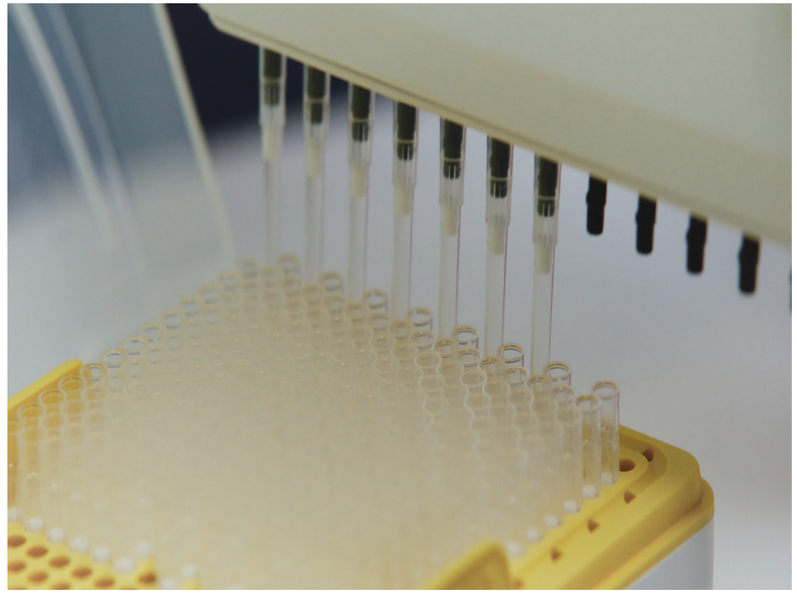


Image source: https://www.antechdiagnostics.com/keyscreen/

PCR Testing

The new Antech **KeyScreen™ GI Parasite PCR Panel** offers an advanced screening option to detect GI parasites in your patients.

Comprehensive Panel

Screening panel detects 20 different parasites from 6 parasite families.

Improved Parasite Detection

Direct detection of parasite DNA/RNA which is contained in *EVERY* cell of the parasite. PCR testing can detect parasites that O&P and ELISA can't.

Detection of Zoonotic Giardia

Detects A/B assemblages (potentially zoonotic) to better protect pet owners and guide pharmaceutical stewardship in asymptomatic patients. Supports One Health initiative.

A. caninum Benzimidazole Resistance

Automatically detects benzimidazole resistance for all *A. caninum* positive samples to provide effective treatment guidance.

Antech KeyScreen™ GI Parasite PCR Panel

Hookworms

- *Ancylostoma* spp.
- *Uncinaria stenocephala*
- ***A. caninum* Benzimidazole Resistance**

Roundworms

- *Toxocara canis*
- *Toxocara cati*
- *Toxascaris leonina*
- *Baylisascaris procyonis*

Whipworms

- *Trichuris vulpis*

Tapeworms

- *Dipylidium caninum*
- *Echinococcus granulosus*
- *Echinococcus multilocularis*
- *Taenia* spp.

Giardia

- *Giardia duodenalis*
- ***Giardia* Zoonotic Strains A & B**

Coccidia

- *Cystoisospora* spp.
- *Eimeria* spp.

Additional Protozoa

- *Cryptosporidium canis*
- *Cryptosporidium felis*
- *Toxoplasma gondii*
- *Neospora caninum*
- *Tritrichomonas blagburni*

ANTECH DIAGNOSTICS PRESENTS LARGEST MOLECULAR STUDY ON GI PARASITES IN PETS AT ACVIM, SHARING NEW INSIGHTS ON PREVALENCE IN NORTH AMERICA

NEWS PROVIDED BY
Antech Diagnostics →
16 Jun, 2023, 07:49 ET

*Results data drawn from a subset of samples tested in **KeyScreen® GI Parasite PCR's** first 10 months shows widespread problem with parasites in dogs and cats*

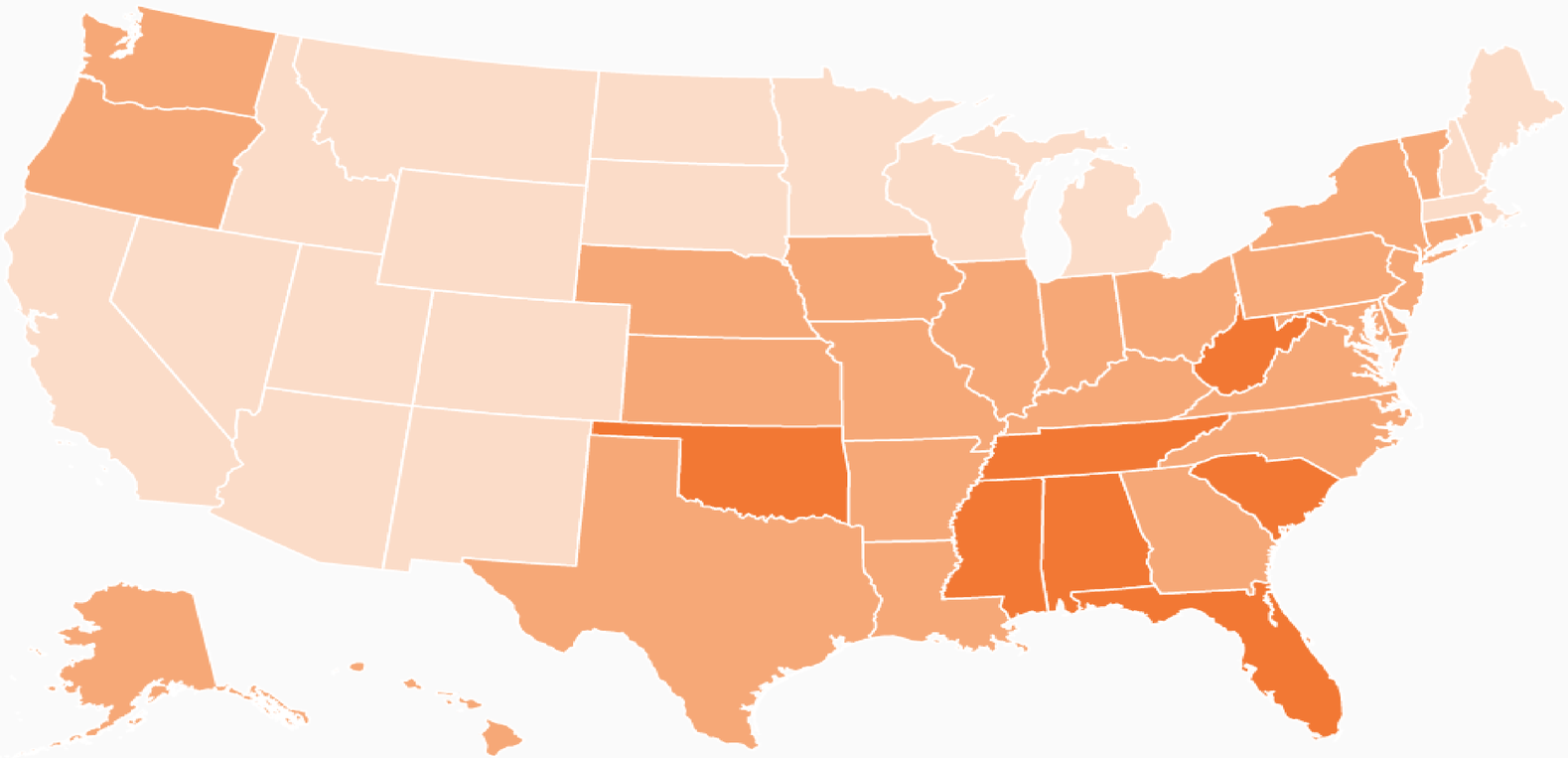
Hookworms

Image source: www.jcu.edu.au/news/releases/2016/october/worms-fight-the-wheeze

INTESTINAL PARASITES | HOOKWORM | DOG | 2012 | ALL YEAR

CLICK ON A REGION TO VIEW PREVALENCE DATA

SWITCH TO CANADA



HOOKWORM

UNITED STATES

TESTED POSITIVE

2.02%

1 IN 50

POSITIVE CASES

87,484

explain this data

TOTAL TESTED

4,327,474

explain this data

INFECTION RISK

LOW HIGH

learn about map data

CAPC Companion Animal Parasite Council

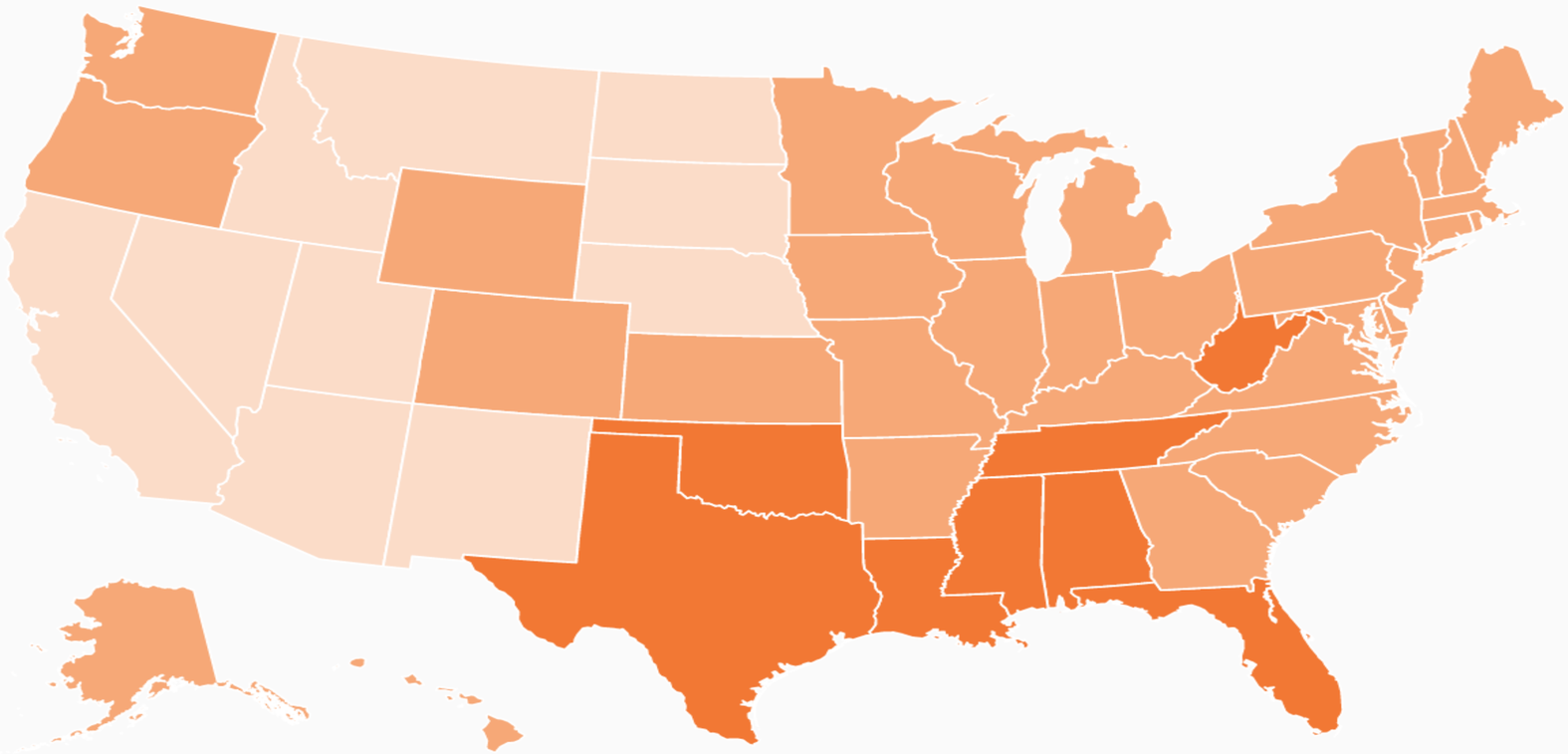
GET UPDATES

https://capcvet.org/maps/#/2021/all-year/hookworm/dog/united-states

INTESTINAL PARASITES | HOOKWORM | DOG | 2022 | ALL YEAR

CLICK ON A REGION TO VIEW PREVALENCE DATA

SWITCH TO CANADA



INFECTION RISK

LOW — HIGH

learn about map data

HOOKWORM

UNITED STATES

TESTED POSITIVE

2.73%

1 IN 30

POSITIVE CASES

274,831

explain this data

TOTAL TESTED

10,058,951

explain this data

CAPC Companion Animal Parasite Council

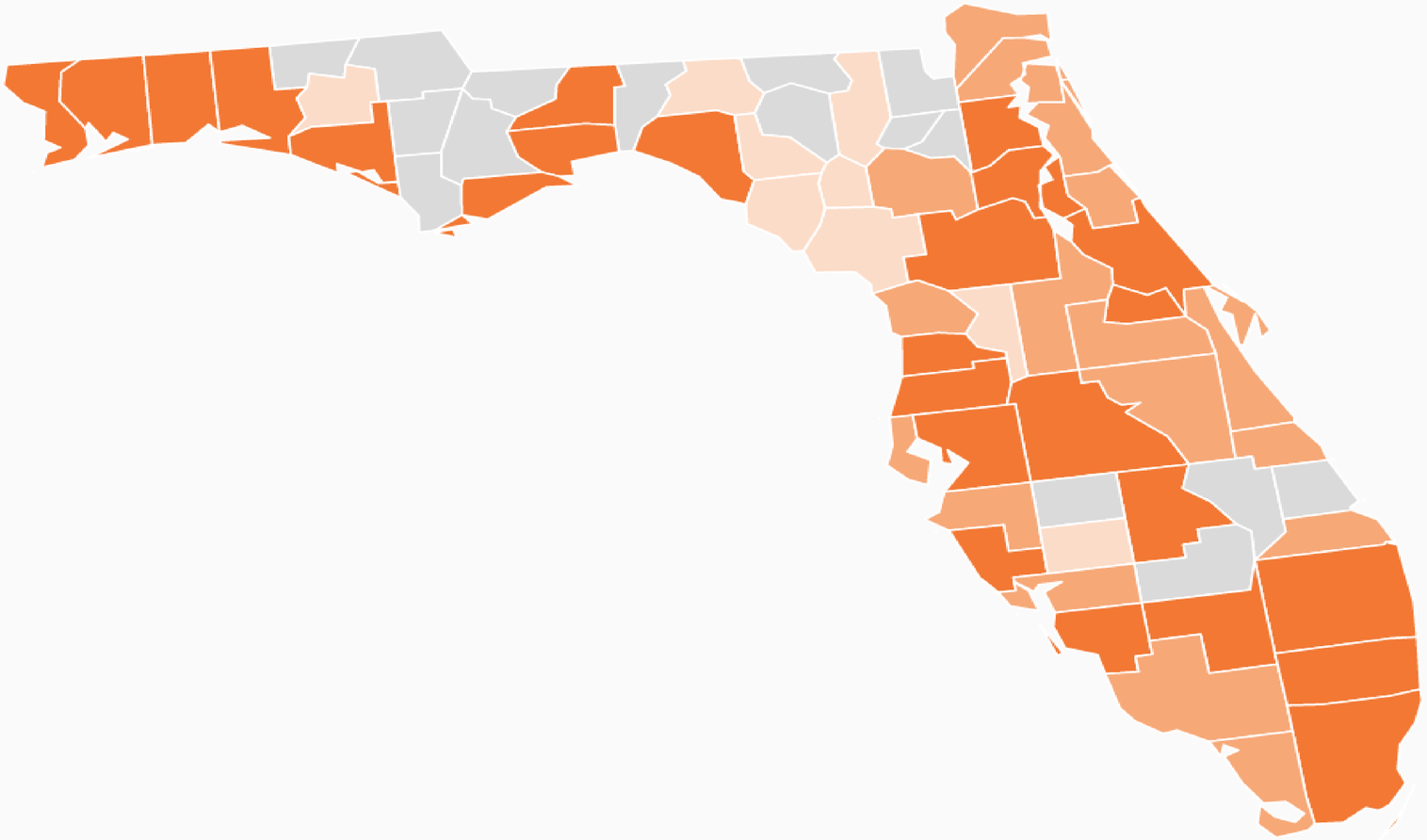
GET UPDATES

https://capcvet.org/maps/#/2022/all-year/hookworm/dog/united-states

INTESTINAL PARASITES | HOOKWORM | DOG | 2012 | ALL YEAR

UNITED STATES → FLORIDA

SWITCH TO CANADA



INFECTION RISK

LOW HIGH

learn about map data

HOOKWORM

UNITED STATES → FLORIDA

TESTED POSITIVE

4.13%

1 IN 30

POSITIVE CASES

10,155

explain this data

TOTAL TESTED

246,023

explain this data

CAPC Companion Animal Parasite Council

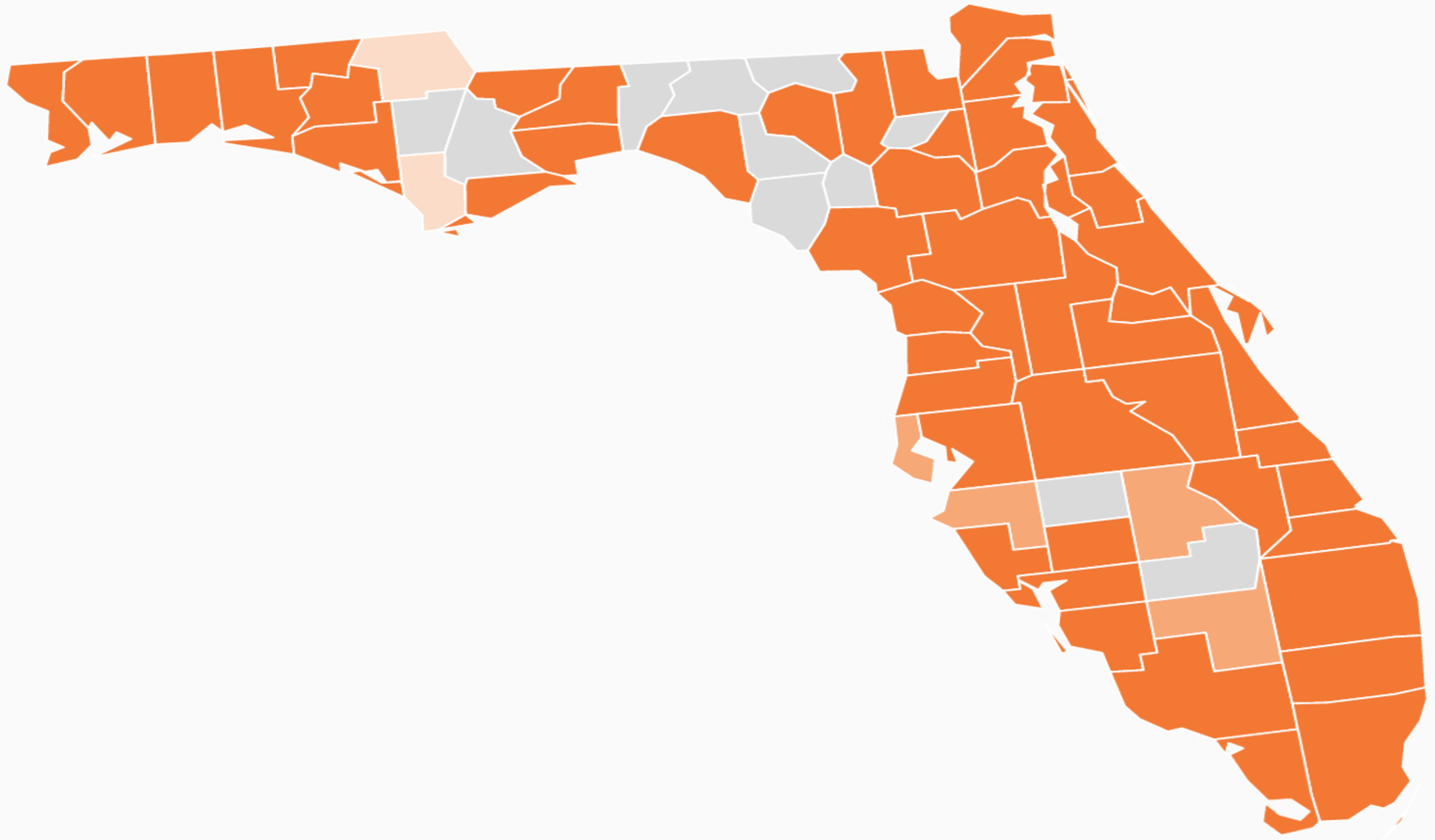
GET UPDATES

Source: https://capcvet.org/maps/#/2012/all-year/hookworm/dog/united-states/florida

INTESTINAL PARASITES | HOOKWORM | DOG | 2022 | ALL YEAR

UNITED STATES → FLORIDA

SWITCH TO CANADA



INFECTION RISK

LOW HIGH

learn about map data

HOOKWORM

UNITED STATES → FLORIDA

TESTED POSITIVE

5.85%

1 IN 15

POSITIVE CASES

45,824

explain this data

TOTAL TESTED

783,935

explain this data

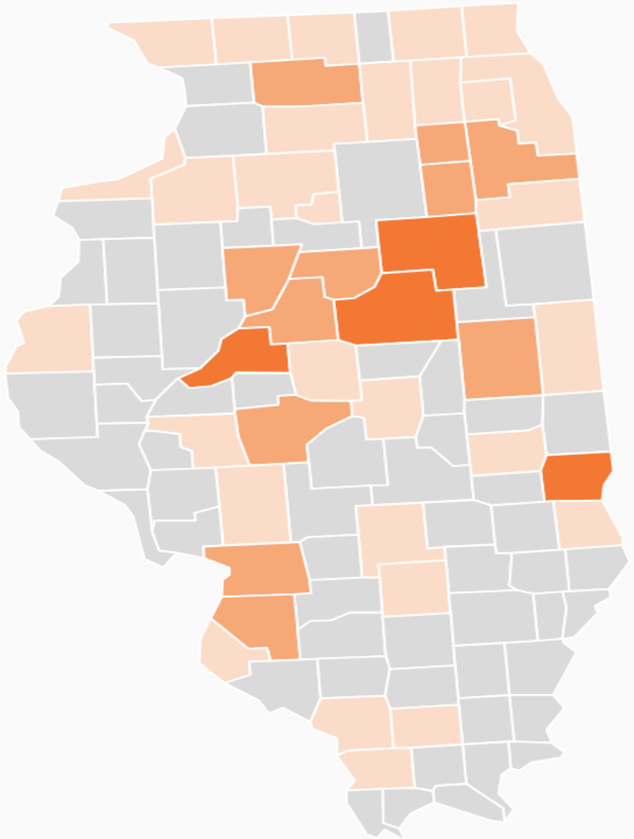
CAPC Companion Animal Parasite Council

GET UPDATES

INTESTINAL PARASITES | HOOKWORM | DOG | 2012 | ALL YEAR

UNITED STATES → ILLINOIS

SWITCH TO CANADA



INFECTION RISK

LOW HIGH

learn about map data

HOOKWORM

UNITED STATES → ILLINOIS

TESTED POSITIVE

1.56%

1 IN 50

POSITIVE CASES

3,274

explain this data

TOTAL TESTED

210,187

explain this data

CAPC Companion Animal Parasite Council

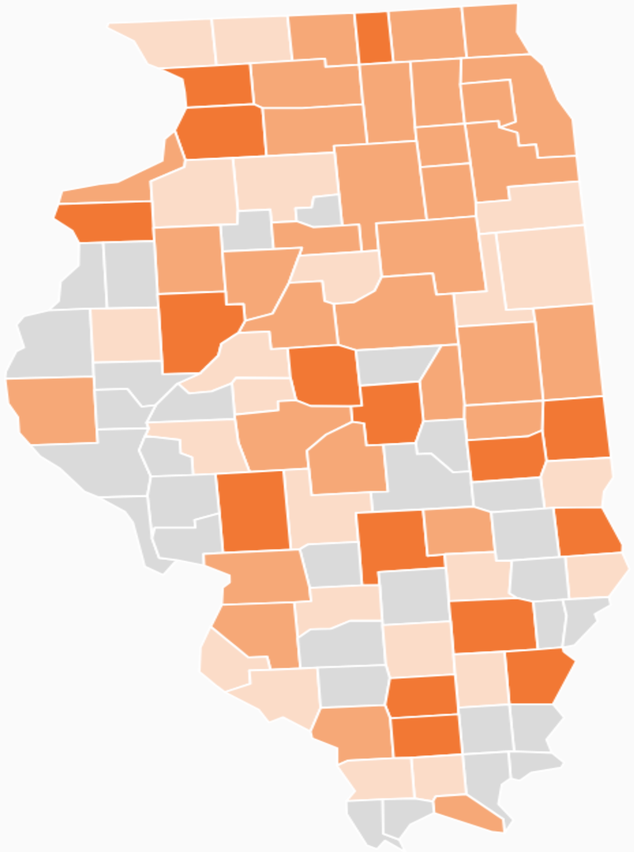
GET UPDATES

Source: https://capcvet.org/maps/#/2012/all-year/hookworm/dog/united-states/illinois

INTESTINAL PARASITES | HOOKWORM | DOG | 2022 | ALL YEAR

UNITED STATES → ILLINOIS

SWITCH TO CANADA



INFECTION RISK

LOW HIGH

learn about map data

HOOKWORM

UNITED STATES → ILLINOIS

TESTED POSITIVE

2.23%

1 IN 50

POSITIVE CASES

11,062

explain this data

TOTAL TESTED

495,593

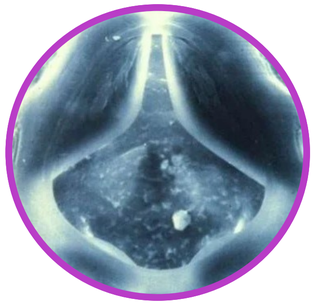
explain this data

CAPC Companion Animal Parasite Council

GET UPDATES

Source: https://capcvet.org/maps/#/2012/all-year/hookworm/dog/united-states/illinois

Uncinaria stenocephala



Clinical Relevance

- *Uncinaria* are a type of hookworm found in dogs and rarely in cats.
- Adult worms live in small intestine feeding on blood and nutrients.
- **Zoonotic potential**: cutaneous larval migrans is rare, but possible.

Infectivity

Transmission via the following mechanisms:

1. Ingestion of infective larvae from contaminated feces
2. Skin penetration of infected larvae
3. Ingestion of other animals that have infected larvae in their tissues

Treatment

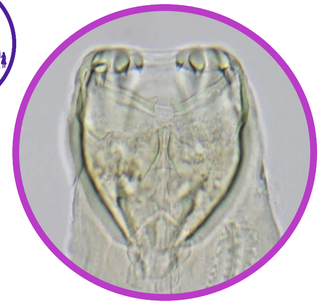
- Several medications: Fenbendazole, moxidectin (4^{th} stage larvae) & pyrantel pamoate.
- All medications for hookworms are adulticides and do not treat larval stages.
- Repeat treatment in 3-4 weeks.

Retesting

- **Retest 3 to 4 weeks post-treatment.**
- Dogs may retest positive due to reinfection from the environment.

Ancylostoma spp.

Ancylostoma caninum, A. tubaeforme, A. braziliense, A. ceylanicum, A. duodenale



Clinical Relevance

- Parasite lives in the intestines of dogs (rarely cats).
- Grasp intestinal lining to feed on blood and nutrients.
- Can cause **diarrhea, weight loss, failure to thrive and anemia.**
- May also cause respiratory disease/pneumonia.
- May cause dermatitis (feet) from skin penetration.
- **ZOONOTIC**

Infectivity

Transmission via the following mechanisms:

1. Ingestion of infective larvae from contaminated feces
2. Ingestion of other animals that have infected larvae in their tissues
3. Skin penetration of infected larvae from environment
4. Lactation - mothers can pass larvae to their offspring while nursing

Treatment

- Several medications: Fenbendazole, milbemycin oxime, moxidectin (4th stage larvae) & pyrantel pamoate.
- All medications for hookworms are adulticides and do not treat larval stages.
- **Repeat treatment in 3-4 weeks.**
- Puppies with severe clinical signs may require supportive care and possible hospitalization.
- Puppies should be dewormed at 2, 4, 6 and 8 weeks and then monthly until 6 months old.

Retesting

Retest 3 to 4 weeks post treatment.

Dog and cats may retest positive due to:

1. Reinfection from the environment
2. Reinfection from dormant larval stages
3. **Drug resistance**

An Emerging Threat

Jimenez Castro *et al. Parasites Vectors* (2019) 12:576
https://doi.org/10.1186/s13071-019-3828-6

Parasites & Vectors

RESEARCH Open Access

Multiple drug resistance in the canine hookworm *Ancylostoma caninum*: an emerging threat?

Pablo D. Jimenez Castro[1,2*], Sue B. Howell[1], John J. Schaefer[3], Russell W. Avramenko[4], John S. Gilleard[4] and Ray M. Kaplan[1]

Abstract

Background: The canine ho
tode parasite of dogs in the U
anthelmintics that are appro
canine hookworm infections
have evolved in this parasite.
also represented. The aim of
genetic and clinical testing t

Methods: Fecal samples co
a greyhound, one from a mir
were then used to establish
ment assays (LDA) for detect
and LDA were performed on
were performed to detect re
frequency of non-synonymo
isotype-1 β-tubulin gene.

Results: Resistance ratios fo
and LDA, respectively. Follow
amplicon sequencing of the
codon 167 in all three resista

Conclusions: These data co
of *A. caninum*, strongly sugge
these resistant hookworms c
to confirm this.

Keywords: *Ancylostoma car*

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† Department of Infectious Diseases, Coll
University of Georgia, Athens, GA 30602.
Full list of author information is available

CASE SERIES

Combination Anthelmintic Treatment for Persistent *Ancylostoma caninum* Ova Shedding in Greyhounds

Lindie B. Hess, BS, Laurie M. Millward, DVM, Adam Rudinsky, DVM, Emily Vincent, BS, Antoinette Marsh, PhD

ABSTRACT

Ancylostoma caninum **is a nematode of the canine gastrointestinal tract commonly referred to as hookworm. T study involved eight privately owned adult greyhounds presenting with persistent *A. caninum* ova shedding desp previous deworming treatments. The dogs received a combination treatment protocol comprising topical moxidec followed by pyrantel/febantel/praziquantel within 24 hr. At 7–10 days posttreatment, a fecal examination monitored parasite ova. Dogs remained on the monthly combination treatment protocol until they ceased shedding detecta ova. The dogs then received only the monthly topical moxidectin maintenance treatment. The dogs remained in study for 5–14 mo with periodical fecal examinations performed. During the study, three dogs reverted to posi fecal ova status, with two being associated with client noncompliance. Reinstitution of the combination treatm protocol resulted in no detectable ova. Use of monthly doses of combination pyrantel, febantel and moxide appears to be an effective treatment for nonresponsive or persistent *A. caninum* ova shedding. Follow-up fe examinations were important for verifying the presence or absence of ova shedding despite the use of anthelmi treatment. Limitations of the current study include small sample size, inclusion of only privately owned greyhoun and client compliance with fecal collection and animal care.** (*J Am Anim Hosp Assoc* 2019; 55:160–166. DOI 10.53 JAAHA-MS-6904)

Introduction

Ancylostoma caninum is a nematode of the canine gastrointestinal (GI) tract commonly referred to as hookworm. Transmission of infective larvae occurs primarily by transmammary or skin penetration routes. Infection of dogs with *A. caninum* can cause anemia and diarrhea and may be associated with cutaneous larva migrans, a zoonotic condition associated with third-stage larvae in the environment. Patent hookworm infection diagnosis is made by fecal examination and finding the typical parasite ova (63.92 ± 5.28 × 39.21 ± 1.52 µm, with an elliptical shape and smooth shell containing a cluster of cells referred to as a morula).[1] Several treatment options for treatment of dog gastrointestinal nematodes available include the following: moxidectin[a,b], milbemycin oxime[c], dazole[d], and/or pyrantel-containing products[e,f]. Some prod as pyrantel only treat the adult stages of the parasite and are adm istered orally.[2] A unique feature of *A. caninum* is the ability of immature larval stages to migrate to and encyst in somatic tissues, either remaining there or eventually finding their way to the GI tract. During pregnancy, encysted larvae in somatic tissues reactivate and migrate to the mammary gland.[3] These encysted or migrating larvae may also repopulate the GI tract.[4] Drugs that are poorly absorbed from the GI tract (e.g., pyrantel) only target the adult stages and will not affect the encysted or migrating somatic larvae, making treatment of *A. caninum* difficult because it is prone to reactivation.

From the Department of Veterinary Preventive Medicine (L.B.H., A.M.), and Department of Veterinary Clinical Sciences (L.M.M., A.R., E.V.), College of Veterinary Medicine, The Ohio State University, Columbus, Ohio.

Correspondence: marsh.2061@osu.edu (A.M.)

EPG (eggs per gram); FEC (fecal egg count); GI (gastrointestinal)

The online version of this article (available at jaaha.org) contains supplementary data in the form of one table.

Accepted for publication: November 30, 2018.

International Journal for Parasitology: Drugs and Drug Resistance 17 (2021) 107–117

Contents lists available at ScienceDirect

International Journal for Parasitology: Drugs and Drug Resistance

journal homepage: www.elsevier.com/locate/ijpddr

Multiple drug resistance in hookworms infecting greyhound dogs in the USA

Pablo D. Jimenez Castro [a, b, *], Abhinaya Venkatesan [c], Elizabeth Redman [c], Rebecca Chen [c], Abigail Malatesta [a], Hannah Huff [a], Daniel A. Zuluaga Salazar [d], Russell Avramenko [c], John S. Gilleard [c], Ray M. Kaplan [a]

[a] Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA, 30602, USA
[b] Grupo de Parasitología Veterinaria, Universidad Nacional de Colombia, Colombia
[c] Department of Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine, Host-Parasite Interacti Canada
[d] Laboratorio de Parasitología Veterinaria, Corporación Universitaria Santa Rosa de Cabal-UNISARC, Santa Rosa de Cal

ARTICLE INFO

Keywords:
Ancylostoma caninum, hookworms
Multiple-drug resistance (MDR)
Deep-amplicon
Greyhounds

ABSTRACT

Ancylostoma caninum is the most prevalent nemat
(MDR) in several *A. caninum* isolates to all anthelmi
dogs in the USA. Cases of MDR hookworms appear
study were to evaluate the drug-resistant phenoty
Fecal samples from greyhounds of the USA were
greyhound racing kennel, and three veterinary p
samples from 219 greyhounds, and despite treatme
(EPG). Resistance to benzimidazoles and macrocyc
and the larval development assay (LDA), respective
pooled feces, representing 54 animals. Mean and m
and 24.5 µM, 23.4 µM, respectively. For the LDA,
62–81 times higher than our susceptible laborato
samples collected <10 days post-treatment with all
moxidectin, the mean FEC were 349, 333, and 83
isolated from 70 fecal samples, comprised of 60 ins
β-tubulin gene only revealed the presence of the F
samples. These clinical, *in vitro*, and genetic data p
infected with MDR *A. caninum* at very high levels

1. Introduction

The canine hookworm, *Ancylostoma caninum*, is the most prevalent and important intestinal nematode parasite of dogs in the USA, with the prevalence depending on age, level of care and geographic location of the dog (Little et al., 2009). A recent study evaluating over 39 million fecal samples from 2012 to 2018, found that the prevalence of hookworms remained very stable from 2012 to 2014 at around 2%, but then from 2015 onwards, there was a steady yearly increase, with an overall increase of 47% by 2018 (Drake and Carey, 2019). Moreover, in a study assessing intesti 288 off-leash d *A. caninum* was lence is more tha Carey, 2019), an 2017–2019 repo suggest that hoo that visit dog pa Anthelmintic *A. caninum* in t

* Corresponding author. Wildlife Health Building, College of Veterinary Medicine, University of Geo
E-mail address: pdj38559@uga.edu (P.D. Jimenez Castro).

https://doi.org/10.1016/j.ijpddr.2021.08.005
Received 24 April 2021; Received in revised form 15 August 2021; Accepted 19 August 2021
Available online 2 September 2021
2211-3207/

IJP: Drugs and Drug Resistance 13 (2020) 22–27

Contents lists available at ScienceDirect

IJP: Drugs and Drug Resistance

journal homepage: www.elsevier.com/locate/ijpddr

Efficacy evaluation of anthelmintic products against an infection with the canine hookworm (*Ancylostoma caninum*) isolate Worthy 4.1F3P in dogs

Pablo D. Jimenez Castro[a,b,*], Abdelmoneim Mansour[c], Samuel Charles[d], Joe Hostetler[d], Terry Settje[d], Daniel Kulke[e], Ray M. Kaplan[a]

[a] Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA, 30602, USA
[b] Grupo de Parasitología Veterinaria, Universidad Nacional de Colombia, Colombia
[c] TRS Labs, Inc, Athens, GA, USA
[d] Bayer US LLC, Animal Health, Research and Development, Shawnee, KS, USA
[e] Bayer Animal Health GmbH, Drug Discovery & External Innovation, Leverkusen, Germany

ARTICLE INFO

Keywords:
Ancylostoma caninum
Hookworms
Lack of efficacy
Multiple-drug resistance (MDR)
Treatment

ABSTRACT

Ancylostoma caninum is the most prevalent intestinal nematode of dogs, and has a zoonotic potential. Multiple-drug resistance (MDR) has been confirmed in a number of *A. caninum* isolates, including isolate Worthy 4.1F3P, against all anthelmintic drug classes approved for hookworm treatment in dogs in the United States (US). The cyclooctadepsipeptide emodepside is not registered to use in dogs in the US, but in a number of other countries/regions. The objective of this study was to evaluate the efficacy of emodepside + praziquantel, as well as three commercial products that are commonly used in the US for treatment of hookworms, against a suspected R *A. caninum* isolate Worthy 4.1F3P. 40 dogs infected on study day (SD) 0 with 300 lomly allocated to one of five treatment groups with eight dogs each: pyrantel lazole (Panacur® C), milbemycin oxime (Interceptor®), emodepside + praziquantel ol. Fecal egg counts (FEC) were performed on SDs 19, 20, 22, 27, 31 and 34. All as per label requirements on SD 24 to dogs in Groups 1 through 4. Two additional d on SDs 25 and 26 to dogs in Group 2 as per label requirements. Dogs were digestive tract was removed/processed for worm recovery and enumeration. The counts for the control group was 97.4, and for the pyrantel pamoate, fenbendazole, lepside + praziquantel groups were 74.8, 72.0, 88.9, and 0.4, respectively. These 6.1%, and 8.8%, and 99.6%, respectively. These data support previous findings of 1F3P as treatments with pyrantel pamoate, fenbendazole and milbemycin oxime ntrast, Worthy 4.1F3P was highly susceptible to treatment with emodep-

moxidectin and milbemycin oxime of the macrocyclic lactone class (sub-class milbemycins), and pyrantel of the tetrahydropyrimidine class. In registration studies, febantel, moxidectin and milbemycin oxime all demonstrated efficacies > 99% (F.D.A, 1994, 1998, 2006), fenbendazole demonstrated an efficacy > 98% (F.D.A, 1983) and pyrantel demonstrated a somewhat variable efficacy, with a mean across studies of approximately 94% with more than half of those studies with efficacies greater than 99% (F.D.A, 1993).

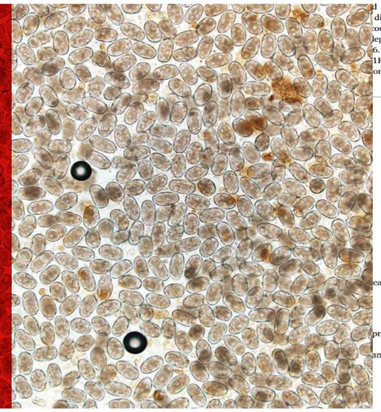
Hookworms are blood-feeding nematodes that use a cutting apparatus to attach to the intestinal mucosa and submucosa, and contract their muscular esophagus to create negative pressure, which sucks a

eases, College of Veterinary Medicine, University of Georgia, Athens, GA, 30602,

pril 2020

Persistent or Suspected-Resistant Hookworm Infections

Pablo David Jimenez Castro, DVM
Ray M. Kaplan, DVM, PhD, DEVPC, DACVM (Parasitology)
University of Georgia



Numerous cases of canine hookworm (ie, *Ancylostoma caninum*) with multidrug resistance to all 3 major anthelmintic classes have been identified.[1]

Background & Pathophysiology

Diagnostic surveillance performed at the authors' laboratory over the past few years suggests the presence of multidrug-resistant (MDR) hookworms (ie, *A caninum*) likely evolved on greyhound breeding farms and in racing kennels. Most, if not all, actively racing and/or recently adopted greyhounds appear to be infected with MDR hookworms; however, many cases of MDR hookworms have been diagnosed in non-greyhound breeds, suggesting MDR hookworms are spreading to the general canine population.

Hookworms (***Figure 1***, next page) have a direct life cycle, with adult females releasing a large number of eggs (up to 10,000/day). Once passed in the feces, development of eggs to third-stage infective larvae (L3) typically takes ≈5 days, although this will vary depending on temperature. Dogs may be infected via both the oral and percutaneous routes. L3 larvae are ingested either directly or by ingestion of paratenic hosts carrying L3 tissue larvae. After penetrating the skin, L3 larvae migrate via the bloodstream to the lungs, penetrate the alveoli, migrate up the bronchial tree to the trachea, are expectorated via coughing, are swallowed, and enter the small intestine, where they complete development into the adult stage. The prepatent period

L3 = third-stage infective larvae
MDR = multidrug-resistant

An Emerging Threat

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American Veterinary Medical Association

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HOME > JAVMA NEWS > SEPTEMBER 15, 2021 > DRUG-RESISTANT HOOKWORMS SPREADING IN DOGS, PARASITOLOGISTS WARN

JAVMAnews

September 15, 2021

Drug-resistant hookworms spreading in dogs, parasitologists warn

Some isolates seem resistant to all anthelmintic drug classes approved to treat hookworm infections

By Greg Cima
Published on August 25, 2021

info@aavp.org

AAVP

AAVP Forms Hookworm Task Force

Schaumburg, Illinois, September 15, 2021 - The American Association of Veterinary Parasitologists (AAVP) recently formed a national task force to address an emerging issue in canine and human health: multi-anthelmintic drug resistant (MADR) *Ancylostoma caninum*, which are canine hookworms resistant to two or more drug classes of FDA-approved dewormers (benzimidazoles, macrocyclic lactones, tetrahydropyrimidines (e.g. pyrantel)). This AAVP Hookworm Task Force, which is composed of 25 members, including veterinarians and parasitologists from academia, industry and government, is developing guidance for veterinary practitioners related to the standard of care for the diagnosis and treatment of MADR *A. caninum*.

"Multiple anthelmintic resistance in *Ancylostoma caninum* is much worse than we feared," said Dr. Ray Kaplan, Professor of Parasitology and Associate Dean for Graduate Studies at the School of Veterinary Medicine, St. George's University. "It has been found in almost every Greyhound fecal sample we recently evaluated from breeding farms and racing tracks, and clinical reports and preliminary molecular data suggest it has entered the pet dog population where we are now finding suspected cases in many non-Greyhound dogs as well."

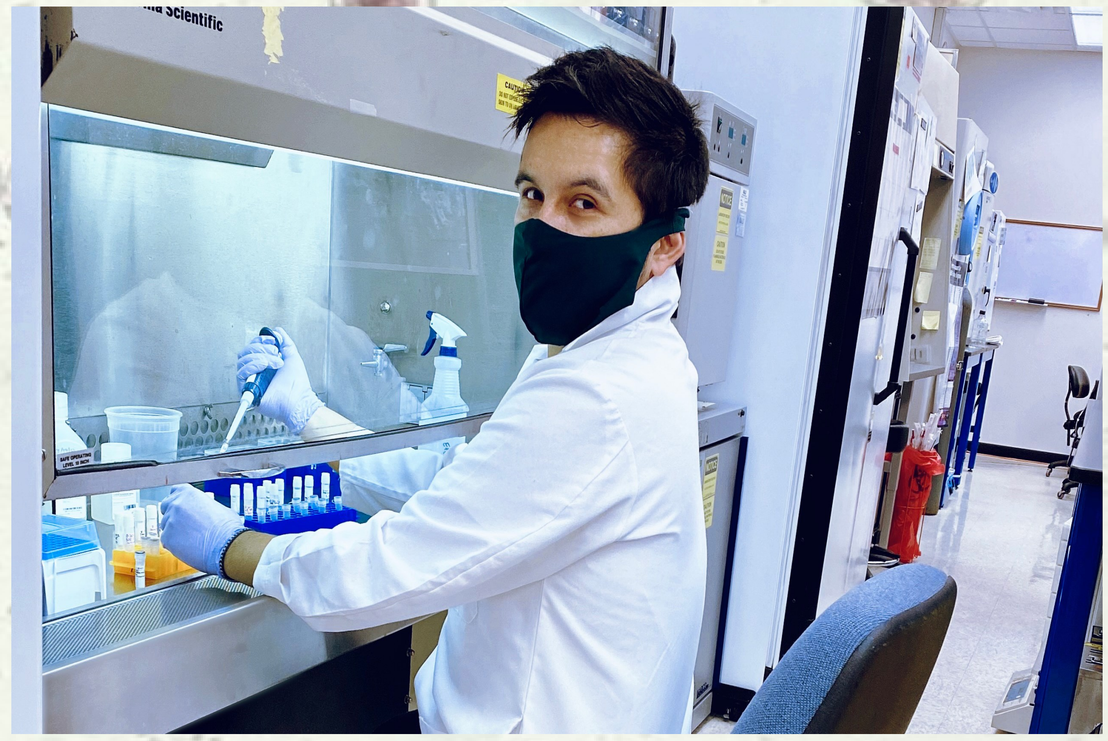
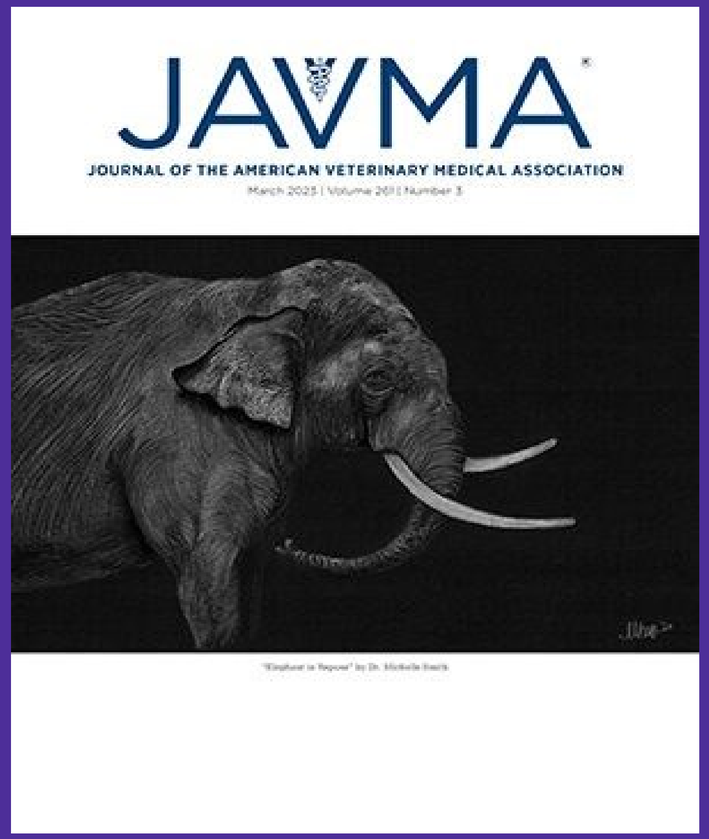


Image source: https://vet.uga.edu/handling-hookworms/

Anthelmintic resistance occurs “when a greater frequency of individuals in a parasite population, usually affected by a dose or concentration of compound, are no longer affected, or a greater concentration of drug is required to reach a certain level of efficacy.”

Photo credit: Nicki Kohl (Telegraph Herald via AP, File)



Multiple anthelmintic drug resistance in hookworms (*Ancylostoma caninum*) in a Labrador breeding and training kennel in Georgia, USA

Pablo D. Jimenez Castro DVM, PhD, Kendra Durrence BS, Stephen Durrence BA, Leonor Sicalo Gianechini DVM, James Collins PhD, Kayla Dunn BS, and Ray M. Kaplan DVM, PhD

View Less —

DOI: https://doi.org/10.2460/javma.22.08.0377

Volume/Issue: Volume 261: Issue 3 Online Publication Date: 15 Dec 2022

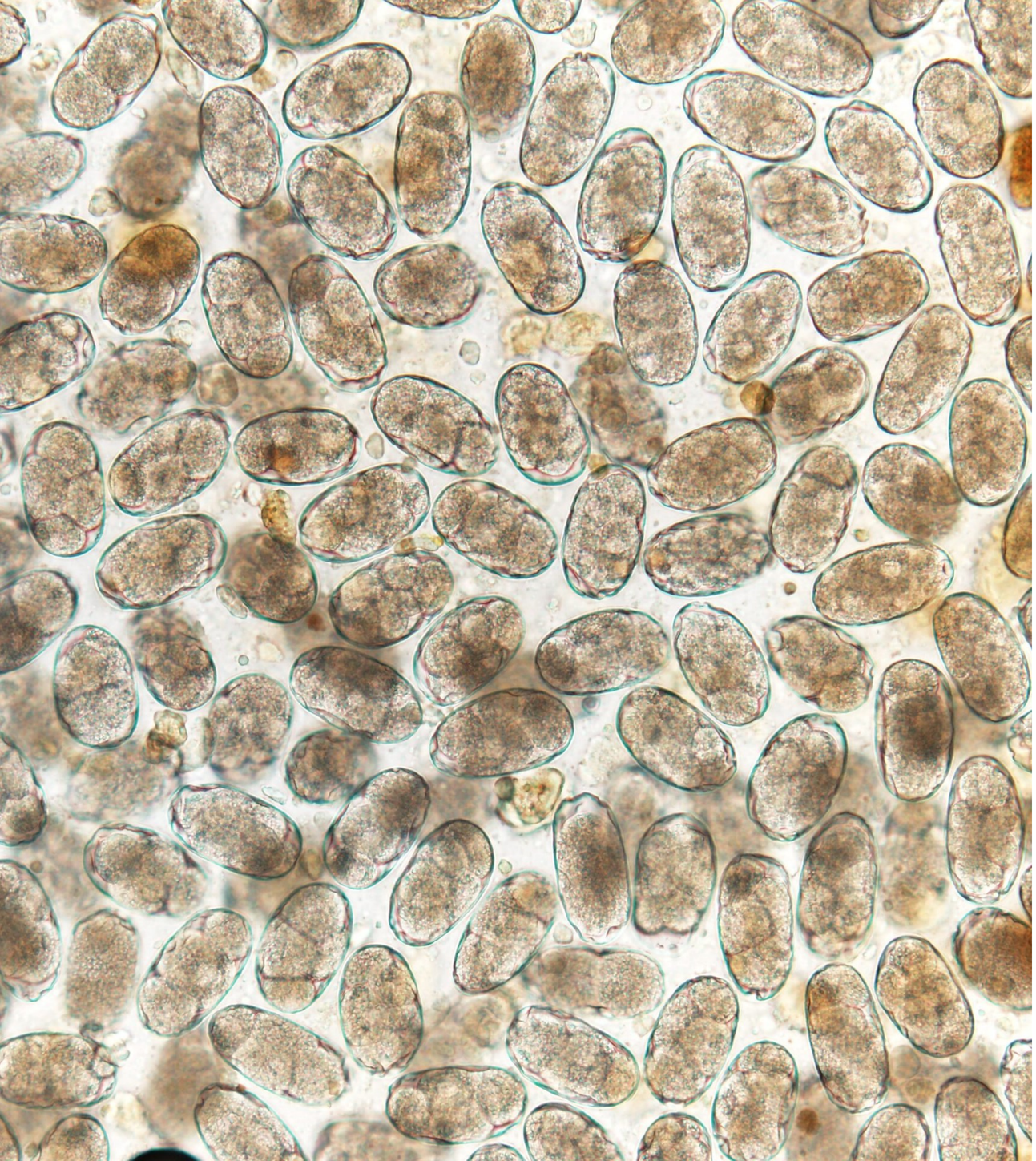
Peer reviewed: no longer "just a Greyhound" concern

- **Study population**: 22 dogs housed in a single kennel; no Greyhounds
- Objective: evaluate efficacy of:
  - Three classes FDA approved anthelmintics
  - Extra-label treatment
- Results:
  - ***A. caninum* highly resistant to all major anthelmintic classes**
  - **Susceptible to emodepside**

- "First report of multiple anthelmintic drug-resistant *A. caninum* in a dog kennel that does not involve Greyhounds."

Source: March 2023 JAVMA study (Castro et al. 2023)

Source: https://doi.org/10.2460/javma.22.08.0377



Multi-drug resistance

Three drug classes are currently FDA approved for treatment of hookworms in the United States.

These include:

- **Benzimidazoles** (febantel and fenbendazole)
- **Tetrahydropyrimidines** (pyrantel)
- **Macrocyclic lactones** (ivermectin, moxidectin and milbemycin oxime)

When initially registered with the FDA, these drugs demonstrated exceptional efficacy.

Hookworms resistant to ≥1 FDA approved anthelmintic drug classes are well documented and are becoming an **emerging threat** in veterinary medicine.

These are referred to as **multi-drug resistant (MDR) hookworms.**

A. caninum Benzimidazole Resistance

Allele-specific qPCR

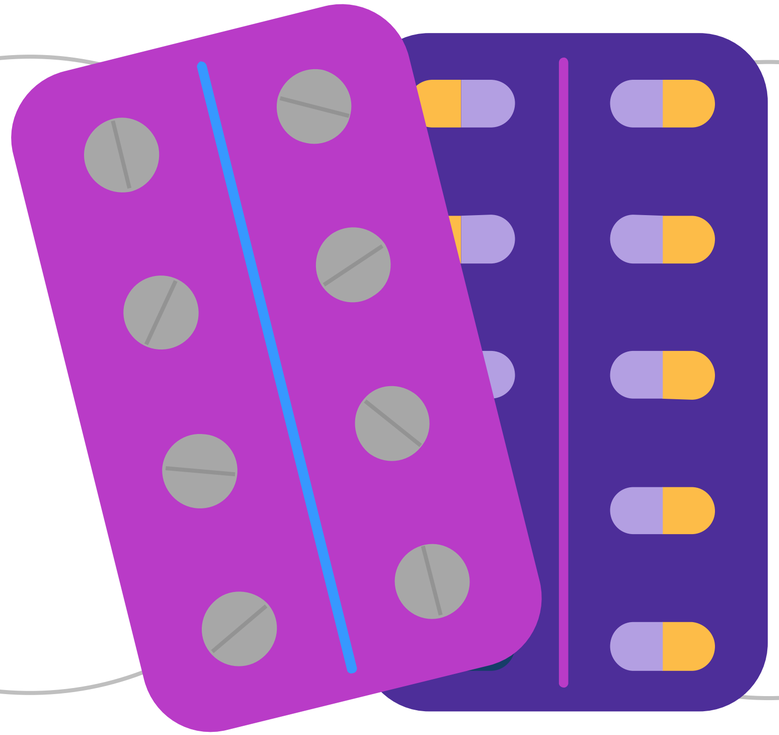
F167Y single nucleotide polymorphism.

Emerging Threat

MDR Hookworms are a major concern in small animal medicine.

Prevalence

Antech will be sharing results data with CAPC for mapping.



Identify

Results automatically provided with positive *Ancylostoma* results.

Not just Greyhounds

Despite first cases in a particular breed, now seen in general canine population.

Informed Treatment Decisions

Identification of resistance at time of diagnosis helps aid appropriate treatment.

Antech Diagnostics to Introduce New Parasite Detection Capabilities to KeyScreen™ GI Parasite PCR Test

Screening for a second mutation increases detection of treatment-resistant hookworm by 25.8 percent

By mid-April, KeyScreen will detect new mutation for treatment-resistant hookworm in dogs

Treatment-resistance in canine hookworm is a growing problem across North America

February 20, 2023 10:00 AM Eastern Standard Time

FOUNTAIN VALLEY, Calif.--(BUSINESS WIRE)--Antech Diagnostics, North America's largest network of veterinary diagnostic reference laboratories, today announced that KeyScreen™ GI Parasite PCR will detect a new mutation for treatment-resistant canine hookworm by mid-April. KeyScreen is a molecular diagnostic test that detects 20 intestinal parasites in cats and dogs, including *Giardia* with zoonotic potential and treatment-resistant canine hookworm.

"Getting around to endoparasites: panel perspectives on GI parasite preventative care"

Tweet this

Following researchers' discovery of a new mutation for treatment-resistant hookworm, Antech determined that screening for both mutations increased KeyScreen's detection rate for this emerging threat by more than 25 percent. As the only parasite test that can quickly deliver information about treatment-resistant hookworm, KeyScreen's enhanced detection capabilities give veterinary teams the most complete diagnostics information available, allowing for more precise and effective treatment. Antech will discuss this at the WVC Annual Conference in Las Vegas at a panel presentation titled, "*Getting around to endoparasites: panel perspectives on GI parasite preventative care*," on Monday, Feb. 20 at 12:45 pm PST at Oceanside B, Level 2.

A Legit Reason to be “Beach Bummed”

HEALTH

This Teen Got Hookworms After His Friends Buried Him In The Sand At The Beach

A church trip turned into a nightmare for this Memphis family.

Lauren Strapagiel
BuzzFeed News Reporter

Posted on July 27, 2018, 5:42 pm

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What started as a fun stop at a beach during a church trip has turned into a nightmare of a hookworm infection for this Tennessee teen.

Kelli Mulhollen Dumas

Michael Dumas, 17, visited Florida in June while on a mission trip with his church. During the trip, they stopped by Pompano Beach where Michael was buried in the sand, as one does at the beach.

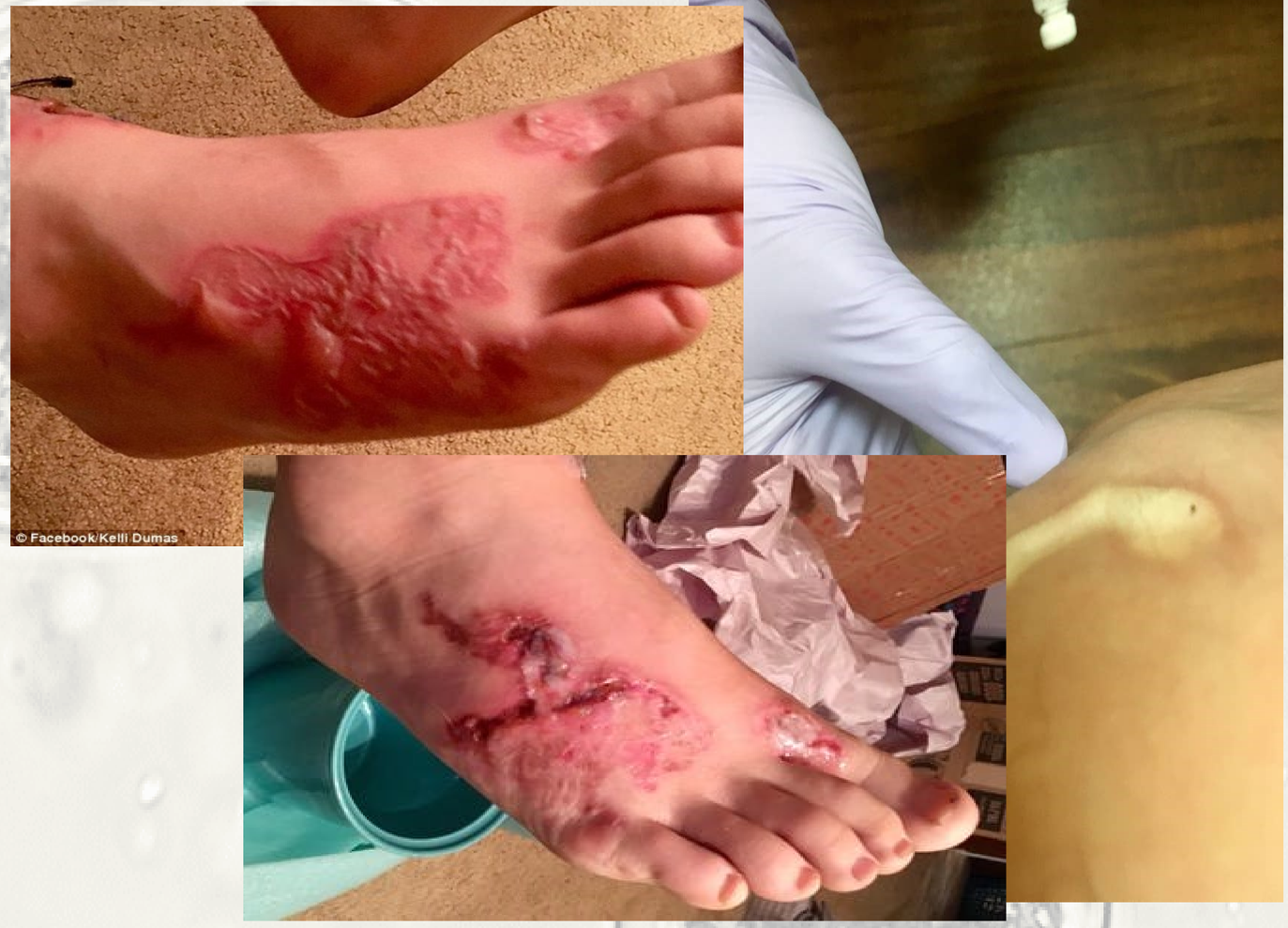


Photo credit: Annie Spratt

Source: https://www.buzzfeednews.com/article/laurenstrapagiel/hookworm-infection-florida-beach-teen

Roundworms

Image source: https://todaysveterinarypractice.com/parasitology/roundworms-in-dogs/

Toxocara and *Toxascaris*

Toxocara canis, Toxocara cati*, Toxascaris leonina*

Clinical Relevance

- Common GI parasites that live in the small intestines of dogs and cats.
- **Puppies and kittens are most severely affected.**
- Can cause **distended, pot-belly appearance, diarrhea, poor growth, dull haircoat** and potential respiratory signs and seizures.
- ALL kittens and puppies should be assumed positive for roundworms.
- ZOONOTIC

Infectivity

Cats and dogs can become infected by many mechanisms:

1. Ingestion of infective larvae from contaminated feces or soil
2. Ingestion of other animals that have infected larvae in their tissues
3. Transplacental – larvae can be passed from mother to offspring across the placenta
4. Lactation – mothers can pass larvae to their offspring while nursing

Treatment

- Several medications: fenbendazole, milbemycin oxime, moxidectin & pyrantel pamoate.
- Repeat treatment in 3-4 weeks.
- Pregnant animals should be treated to prevent transplacental and trans-mammary transmission.
- Puppies and kittens should be dewormed at 2, 4, 6 and 8 weeks and then monthly until 6 months old.

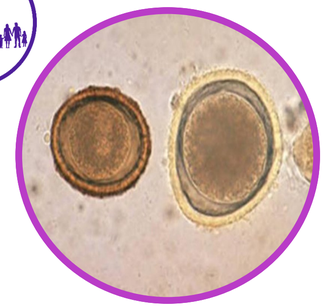
Retesting

- **Retest 3 to 4 weeks post treatment.**
- Pets may retest positive due to:

1. Ineffective treatment
2. Reinfection from the environment
3. Reinfection from dormant larval stages

Baylisascaris procyonis

The Raccoon Roundworm



Clinical Relevance

- Found in the small intestine of raccoons.
- While **usually subclinical**, dogs harboring adult worms in their small intestine can exhibit **mild diarrhea**.
- While rare in dogs, significant disease can occur when ingested larva migrate through tissue or the CNS.
- Zoonotic (visceral and ocular larval migrans).

Infectivity

- Dogs can become infected with roundworms by the following mechanisms:

1. Ingestion of infective larvated eggs from contaminated environment
2. Ingestion of tissue from other animals containing immature stages of the parasite (various mammals and birds)

- Eggs passed from racoon feces take 2-4 weeks to become infective

Treatment

- Most medications effective against *Toxocara* spp. are effective against *B. procyonis*, but **no medications are specially approved for use for this parasite.**
- Fenbendazole, milbemycin oxime, moxidectin & pyrantel pamoate.
- Retreatment in 3-4 weeks may be needed to eliminate infections.

Retesting

- **Retest 3 to 4 weeks post treatment.**
- Dogs and children should be prevented from having access to raccoons and/or raccoon feces.
- Raccoon feces in the environment should be promptly removed (wear gloves and use caution).

https://www.cdc.gov/parasites/baylisasccaris/resources/raccoonlatrines.pdf

Whipworms

Image source: Thierry Berrod, Mona Lisa Production

Trichuris vulpis

Clinical Relevance

- Whipworms live in the large intestines.
- Can cause watery, bloody diarrhea, dehydration and weight loss.
- Many cases are subclinical.
- Extreme cases can lead to anemia and death.
- **Pre-patent period up to 3 months.**
- Not zoonotic.

Infectivity

- Dogs can only become infected with whipworms by ingesting infective larvae.
- Whipworm eggs are extremely tough and can survive in the environment for years.

Treatment

- Several medications: febantel, fenbendazole, milbemycin oxime & moxidectin are approved for use.
- Repeat treatment in 3-4 weeks.
- Persistent reinfection from environmental contamination can become an issue.

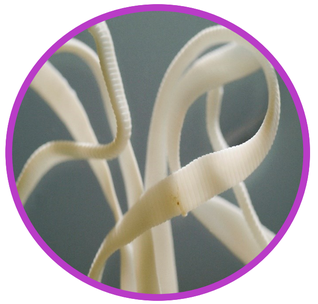
Retesting

- Retest 3 to 4 weeks post treatment.
- Reinfection from a heavily contaminated environment may contribute to treatment failure.
- Some preventative medications (e.g., Simparica Trio) are not labeled for the treatment of whipworms.

Tapeworms

Image source" https://www.microscopyu.com/gallery-images/tapeworm-echinococcus-granulosus-hydatid-cyst

Dipylidium caninum



Clinical Relevance

- Common tapeworms that live in the small intestines of cats and dogs.
- Each proglottid contains up to 30 tapeworm eggs.
- The passing of proglottids can cause irritation and scooting.
- True prevalence is unknown since ***Dipylidium*** **eggs are rarely detected via O&P.**

Infectivity

- Dogs and cats become infected with tapeworms by ingesting a flea or louse that contains the infective stage.
- The flea problem may not be apparent to the pet owner.

Treatment

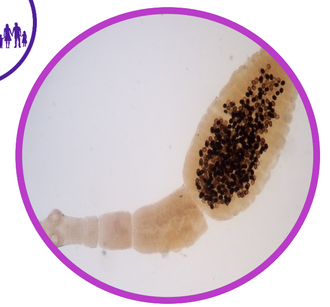
- Praziquantel (5 mg/kg PO or SQ) and epsiprantel (5.5 mg/kg PO in dogs, 2.75 mg/kg PO in cats) are approved for use and are highly effective against *Dipylidium caninum.*
- Repeat treatment in 3-4 weeks may be needed.
- The following medication is not approved for use:
- **Nitazoxanide** (**off-label**: 100 mg/kg) may be helpful in the rare refractory case – see CAPC for more details

Retesting

- **Retest 3 to 4 weeks post treatment using KeyScreen GI Parasite PCR.**
- Treat the fleas.

Echinococcus spp.

Echinococcus granulosus, Echinococcus multilocularis**



Clinical Relevance

Small tapeworms that live in the intestines of wild canids (*E. granulosus*) or rodents (*E. multilocularis*)

1. Wild and domestic canids and rodents are definitive hosts for adult *Echinococcus* tapeworms.
2. Definitive host shed oocysts which are ingested by intermediate hosts.
3. Definitive host have no evidence of clinical disease.
4. Humans/dogs can be accidental intermediate hosts with severe sequelae depending on infecting organism (alveolar and cystic echinococcosis).

Infectivity

Echinococcus life cycles are complex and involve various definitive and intermediate hosts.

Treatment

- Praziquantel is approved at 5 mg/kg PO or SQ for the treatment of intestinal stages of *Echinococcus* spp. in dogs.
- Canine alveolar echinococcosis caries a guarded prognosis.

Retesting

- Retest 3 to 4 weeks post treatment using KeyScreen PCR GI Parasite PCR.
- To limit opportunities for reinfection, treatment must be combined with management changes to prevent ingestion of prey species.
- Without behavior modification, reinfection is likely to occur.

Taenia spp.

T. crassiceps, T. hydatigena, T. multiceps, T. pisiformis, T. serialis, T. taeniaeformis

Clinical Relevance

- There are several species of *Taenia* tapeworms that commonly infect cats and dogs.
- *Taenia* spp. have indirect life cycles requiring specific intermediate hosts.
- Disease in cats and dogs due to infection with adult *Taenia* species is rare.

Infectivity

- Cats and dogs become infected when they eat (hunting or scavenging) an infected prey species.
- Pets cannot become infected by consuming eggs or tapeworm segments.

Treatment

- Praziquantel and epsiprantel are approved for the treatment of *Taenia* spp. infections in dogs and cats.
- Praziquantel is formulated with many heartworm preventatives to provide broad-spectrum internal parasite control in dogs.
- Praziquantel is formulated with emodepside to provide broad-spectrum internal parasite control in cats (topical).

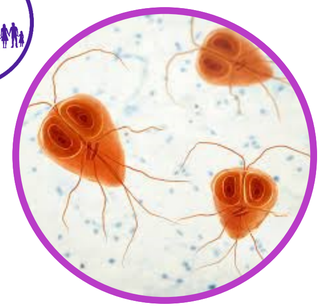
Retesting

- Retest 3 to 4 weeks post treatment using KeyScreen PCR.
- Treatment in dogs and cats must be combined with behavior modification to prevent ingestion of vertebrae prey species.
- Without behavior modification, reinfection is likely to occur.

Giardia

Image source: ://thenativeantigencompany.com/products/giardia-lamblia-cysts/

Giardia duodenalis



Clinical Relevance

- *Giardia* is a common single-celled parasite found in the small intestine of many animals – including dogs, cats and humans.
- **Most infected animals do not show any clinical signs.**
- Can cause diarrhea, dehydration and weight loss.
- **Only *Giardia* A/B assemblages are reported to be potentially zoonotic** to humans.

Infectivity

- **Pets become infected by ingesting infective cysts which are intermittently passed** in the feces of an infected animal.
- Cysts can be ingested from contaminated food or water.
- Cats and dogs do not appear to be able to infect each other although reinfection can occur from self-grooming.

Treatment

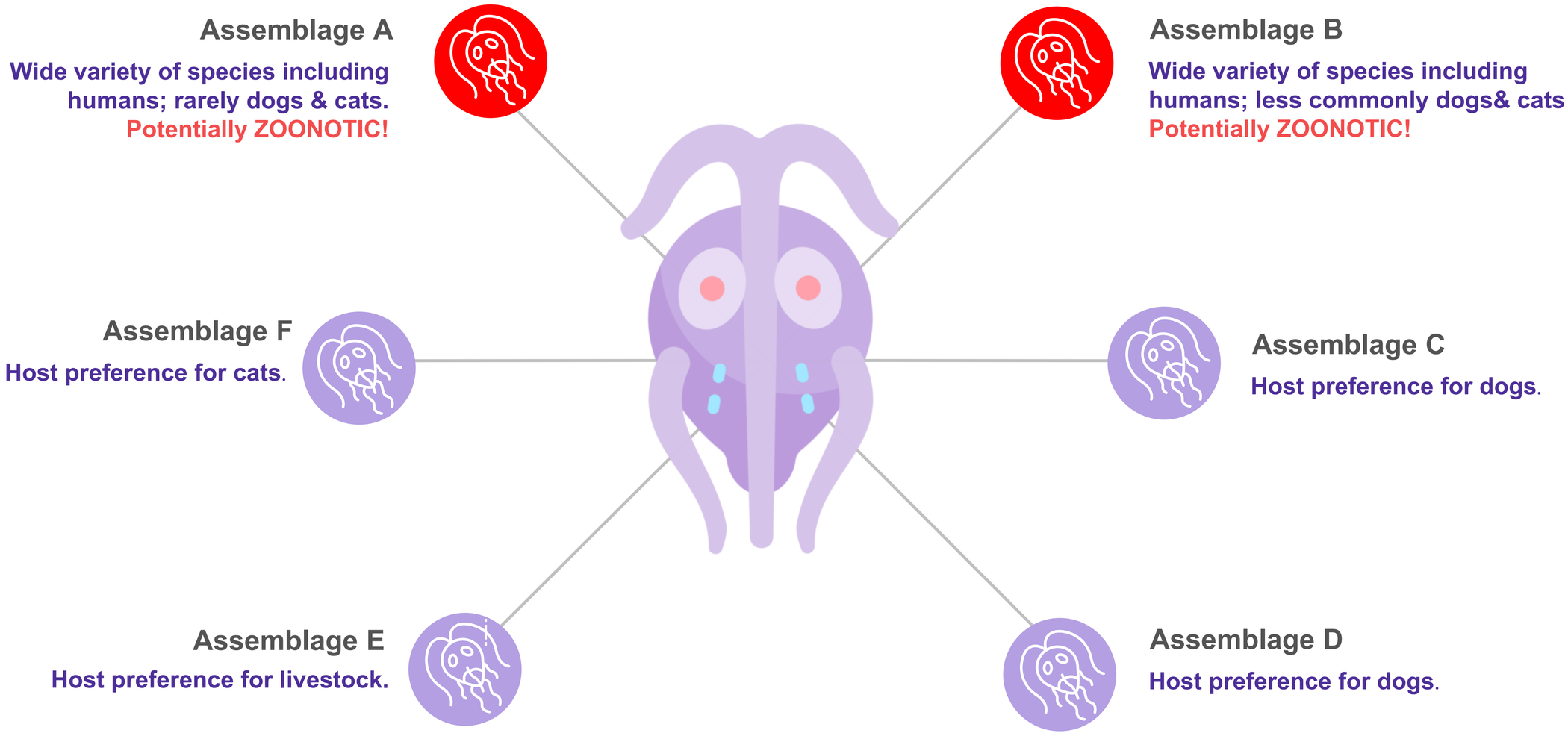
- **Treatment is recommended** for pets with **clinical signs consistent with giardiasis.**
- Treatment **may not be indicated** in dogs and cats without clinical signs of giardiasis.
- If the potentially zoonotic A/B assemblage is detected in a subclinical pet, zoonotic risk should be assessed, and treatment may be warranted to protect the pet owners.

Retesting

- **With Keyscreen, retest 3 to 4 weeks post treatment.**
- For O&P, CAPC recommends retesting in 24-48 hours post treatment.
- Environmental contamination should be addressed – especially at dog parks and other communal pet areas.

Not All *Giardia* are the Same

Dogs are primarily infected with C & D assemblages whereas cats tend to get the F assemblage.
The C, D & F *Giardia* assemblages have not been reported to infect people!



Assemblage A

Wide variety of species including humans; rarely dogs & cats.
Potentially ZOONOTIC!

Assemblage B

Wide variety of species including humans; less commonly dogs& cats
Potentially ZOONOTIC!

Assemblage F

Host preference for cats.

Assemblage C

Host preference for dogs.

Assemblage E

Host preference for livestock.

Assemblage D

Host preference for dogs.

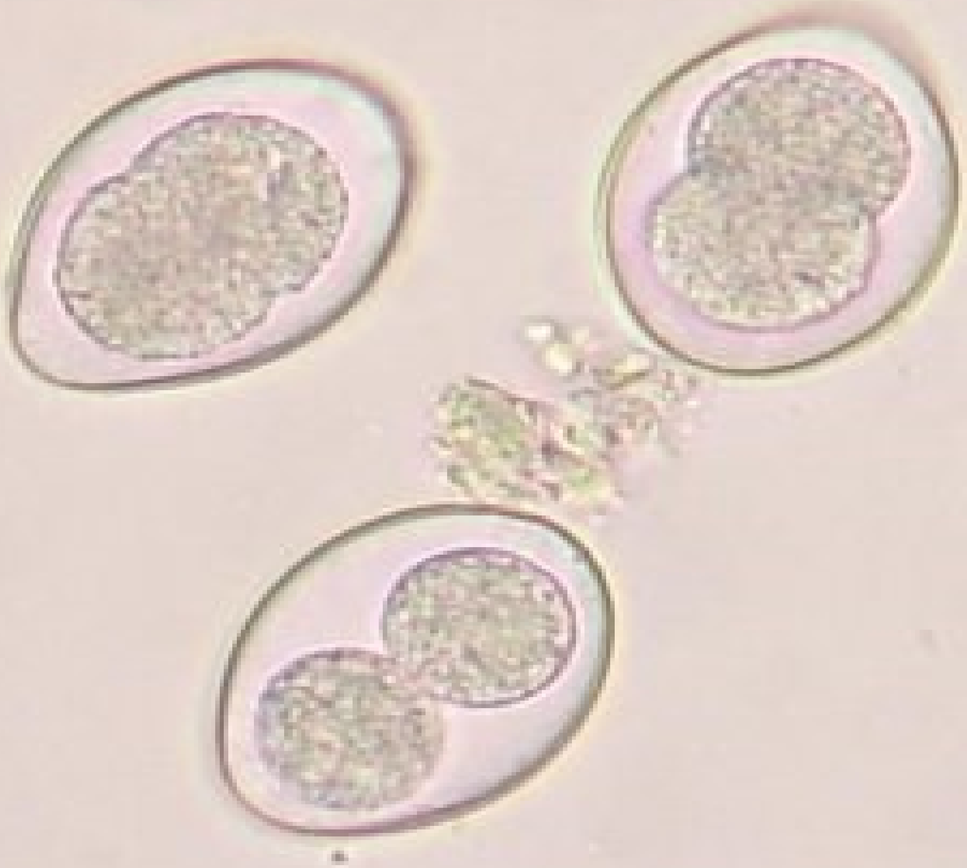
To complicate things more - there are *Giardia* sub-assemblages.

Not all *Giardia* A/B is zoonotic, these are just the assemblages that are *potentially* zoonotic. Only certain sub-assemblages, in these groups, are actually zoonotic!

Step 1: Determine if the pet has *Giardia*.

Step 2: For every sample where *Giardia* was detected, determine if that *Giardia* is A/B assemblage to assess the zoonotic risk.

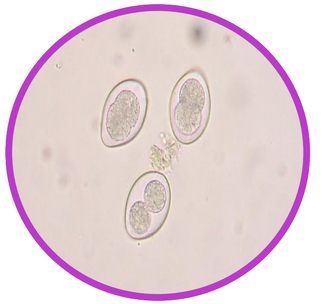
Step 3: More commonly, the Giardia A/B assemblage will **NOT** be detected. In the face of minimal zoonotic risk, a discussion about not treating subclinical patients will be easier.



Coccidia

Cystoisospera spp.

C. canis, C. ohioensis, C. neorivolta, C. burrows; Formerly *Isospora* spp.



Clinical Relevance

- Common single-celled parasites that infect the small intestine and can cause diarrhea.
- Young puppies and kittens are most affected.
- Clinical signs include **diarrhea (possibly bloody), vomiting, weight loss, and dehydration.**

Infectivity

Dogs and cats become infected from ingesting an infective oocyst

- From environment contaminated with feces.
- Eating a prey animal that has a stage of the parasite in its tissues.

Treatment

The following medication is approved for use:

Sulfadimethoxine (50-60 mg/kg PO daily for 5-20 days) is the only drug that is label approved for treatment of enteritis associated with coccidiosis.

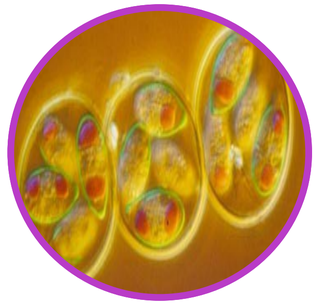
The following medication has not been approved:

Ponazuril (20 mg/kg PO daily for 1-3 days) appears to be effective, especially in refractory cases as per CAPC.

Retesting

- The decision when or if to retest for this parasite involves a lot of factors.
- In addition to treatment, **appropriate sanitation is helpful in preventing spread of coccidiosis.**
- **Infective oocysts are resistant to most commonly used disinfectants and can survive for many months in the environment.**

Eimeria spp.



Clinical Relevance

- Single-celled parasites (a type of coccidia) that is sometimes detected in dogs.
- It **does not** cause clinical disease but does indicate coprophagic behavior.
- *Eimeria* is a GI parasite of a variety of species including cattle, goats, sheep and birds.

Infectivity

- Indicates coprophagic behavior.
- This parasite will pass through the dog and will not cause infection.

Treatment

- No treatment is indicated.
- Coprophagia may require behavior modification.

Retesting

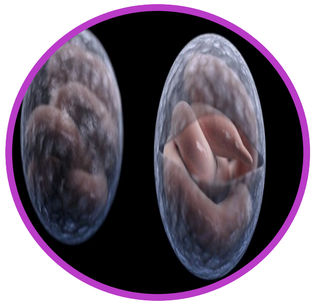
Unnecessary

Other Protozoa

Image source: https://thenativeantigencompany.com/products/cryptosporidium-parvum-oocysts/

Cryptosporidium

Cryptosporidium canis, Cryptosporidium felis



Clinical Relevance

- Single-celled parasites that infect the small intestine
- **Very common for dogs and cats to shed this organism in the absence of ANY clinical signs.**
- ***C. canis*** can cause watery diarrhea and dehydration although **often there are no clinical signs.**
- ***C. felis*** can cause watery, foul-smelling diarrhea, dehydration, vomiting and fever in cats although **often there are no clinical signs.**

Infectivity

- Fecal-oral infection: pets become infected with *Cryptosporidium* spp. from ingesting an infective oocyst
- This parasite is small and does not settle out in water. Water contaminated with fecal material is often a source of infection.

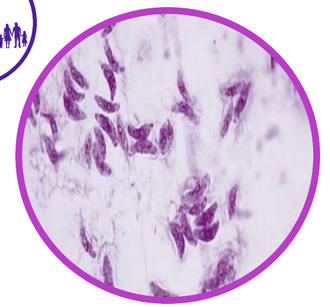
Treatment

- **There are no medications specially approved for treating *Cryptosporidium* in dogs or cats.**
- **Most infections will resolve without specific treatment!**
- While no medication is consistently effective against Cryptosporidium, the following has been used with varying degrees of success as per CAPC:
- Paromonycin: 150 mg/kg SID for 5 days (dogs & cats)
- Tylosin: 10-15 mg/kg TID for 14-21 days (cats)
- Azithromycin: 5-10 mg/kg BID for 5-7d (dogs); 7-15 mg/kg for 5-7d (cats)

Retesting

- The decision when or if to retest for this parasite involves many factors.
- ***Cryptosporidium* oocysts are very resistant in the environment.**
- *Cryptosporidium* oocysts are immediately infective if ingested.

Toxoplasma gondii



Clinical Relevance

- Single-celled parasite that can affect almost all warm-blooded animals and people.
- Cats are an essential part of the parasite's life cycle but rarely show clinical signs of illness.
- Kittens or immunosuppressed cats may have lethargy, decreased appetite and fever.
- Wide variety of clinical signs associated with extraintestinal phase of life cycle.

Infectivity

Cats become infected with this parasite from:

1. Ingesting an animal (birds, rodents, etc.) with bradyzoites (infective cysts) in its tissues
2. Environmental contamination - ingesting an infective oocyst from fecal contaminated soil or water

Treatment

There is no approved treatment for toxoplasmosis in cats or dogs.

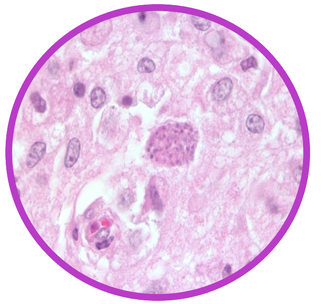
The following medications have been used successfully as per CAPC:

1. Clindamycin (10-12 mg/kg PO BID q 2-4w)
2. Pyrimethamine (0.25-0.5 mg/kg) plus a sulfonamide (30 mg/kg BID q 2-4 w) – for disseminated toxoplasmosis/reduce oocyst shedding
3. TMS 15 mg/kg PO BID q 4 weeks

Retesting

- Fecal shedding is usually self limiting, 1-2 days to a maximum of 20 days. Shed oocysts are not immediately infective.
- **Positive pets should be retested to ensure shedding is complete because of zoonotic risk.**
- Cats should not be fed raw meat.
- Zoonotic. Pregnant or immunosuppressed individuals should avoid contact with fecal matter or potentially contaminated environments.

Neospora caninum



Clinical Relevance

- Single-celled parasite of dogs.
- Infected dogs often show no clinical signs.
- Clinical signs are more common in puppies and affect the neuromuscular system.
- Disease is most severe in congenitally infected puppies (beginning 3-9 weeks after birth): rear limb paralysis (hyperextension), muscle atrophy, cervical weakness, dysphagia.

Infectivity

- Dogs can become infected after ingesting *Neospora* cysts from the tissue of an infected host species (especially the placenta or fetal tissue of infected cattle).
- Infections can be passed from mother to puppies in utero.

Treatment

- There is no approved treatment for canine neosporosis.
- Treatment is most successful when initiated *before* the occurrence of contracture or paralysis.

1. Clindamycin (12-25 mg/kg PO or IM BID q 4w)
2. TMS 15-20 mg/kg PO BID q 4 weeks with pyrimethamine (1mg/kg PO once daily q 4 weeks)

- If clinical improvement is slow, treatment should be extended beyond 4 weeks

Retesting

- The decision when or if to retest for this parasite involves many factors.
- Raw meat – especially from cattle or deer – should be avoided or fully cooked.
- The period of shedding can be months.

Tritrichomonas blagburni

Formerly *T. foetus*

Clinical Relevance

Single-celled parasite that infects the large intestine of cats (especially kittens).

It can cause chronic diarrhea (sometimes with blood and/or mucus) that may improve only to recur again.

Tritrichomonas blagburni is rarely found in dogs.

Infectivity

- Cats likely become infected by the fecal-oral route (ingestion of trophozoites from a contaminated litterbox or environment).
- Infection is most seen in breeding colonies and shelters.

Treatment

- **There is no approved treatment for *Tritrichomonas blagburni*.**
- Treatment has been successfully reported from CAPC with:

1. **Ronidazole (30 mg/kg once daily q 14 d)**

- Resistance to ronidazole has been reported.
- Consider treating all cats in a household as some cats can be asymptomatic shedders that reinfect the infected cat again after treatment.

Retesting

- The decision when or if to retest for this parasite involves many factors.
- This parasite is commonly detected in cats with co-infections.

Why the Antech KeyScreen GI Parasite PCR is the best intestinal parasite screening test

Superior sensitivity

- Improved detection for the most common and important gastrointestinal parasites
- Direct detection of parasitic DNA/RNA
- Multiplex platform enables more sensitive detection of intestinal parasites with improved TAT at a lower cost to clinic
- No subjective assessment of microscopy

Comprehensive panel

- **Tests for 20 parasites:**
- Hookworms
- Roundworms
- Whipworms
- Tapeworms
- *Giardia*
- Coccidia
- *Cryptosporidium*
- *Toxoplasma*
- *Neospora*
- *Tritrichomonas*

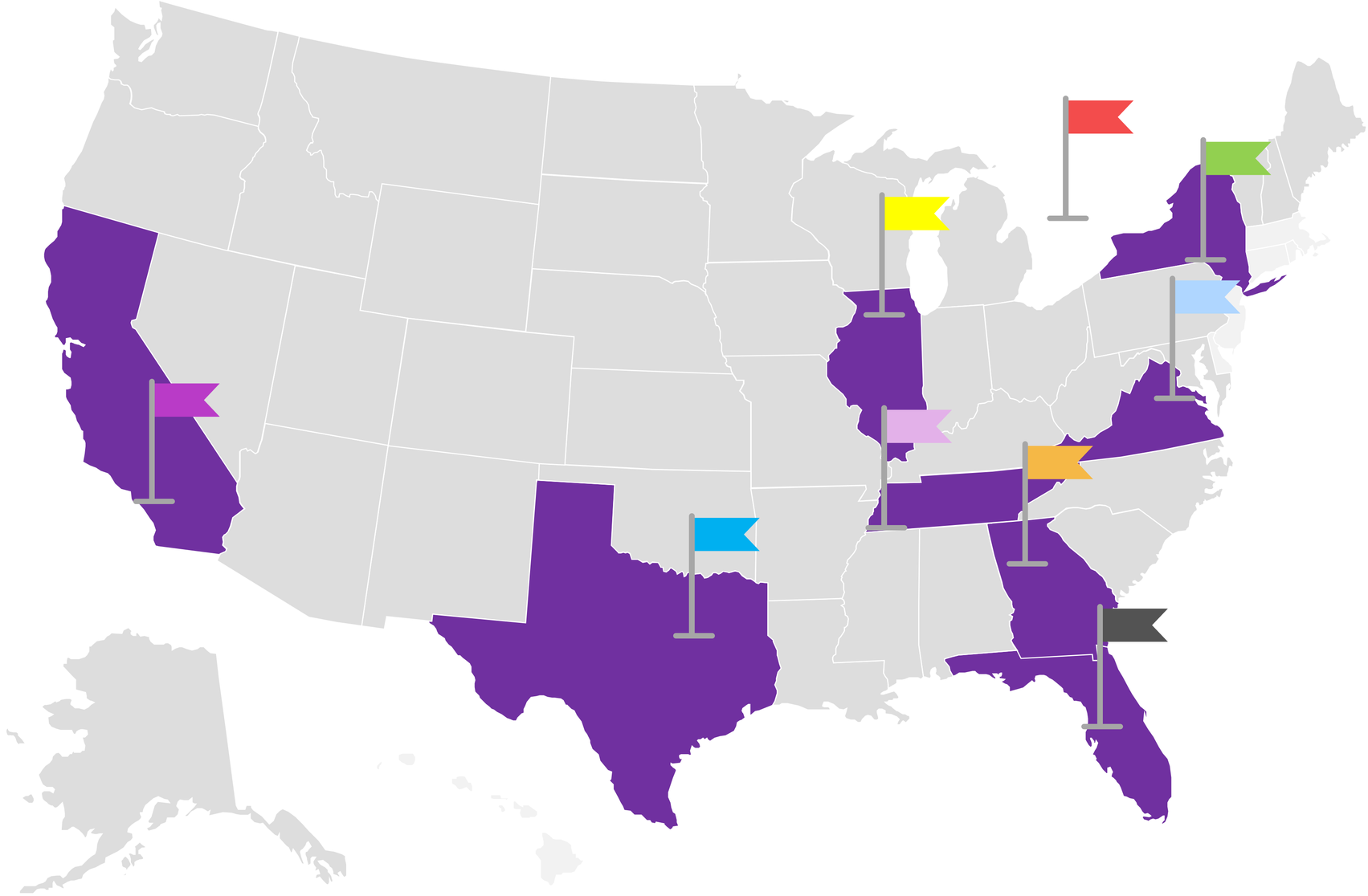
Anthelmintic hookworm resistance

- Anthelmintic resistance in hookworms is an emerging threat in veterinary medicine
- The panel automatically identifies the presence of resistance genes for all hookworm positive samples
- Helps guide appropriate and effective treatment at time of diagnosis

Giardia zoonotic potential

- Zoonotic assessment of Giardia determines how infective a particular strain of Giardia is to humans
- All *Giardia* positive samples will be automatically tested for presence of potentially zoonotic A/B assemblages
- Allows veterinarians to educate and protect pet owners

Antech PCR Labs



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> 85% of customers will get next day TAT!

Antech KeyScreen™ GI Parasite PCR Panel

Test Code T991/CT991

Hookworms

- *Ancylostoma* spp.
- *Uncinaria stenocephala*
- ***A. caninum* Benzimidazole Resistance**

Roundworms

- *Toxocara canis*
- *Toxocara cati*
- *Toxascaris leonina*
- *Baylisascaris procyonis*

Whipworms

- *Trichuris vulpis*

Tapeworms

- *Dipylidium caninum*
- *Echinococcus granulosus*
- *Echinococcus multilocularis*
- *Taenia* spp.

Giardia

- *Giardia duodenalis*
- ***Giardia* Zoonotic Strains A & B**

Coccidia

- *Cystoisospora* spp.
- *Eimeria* spp.

Additional Protozoa

- *Cryptosporidium canis*
- *Cryptosporidium felis*
- *Toxoplasma gondii*
- *Neospora caninum*
- *Tritrichomonas blagburni*

Questions?

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