

Molecular Testing for Enteric Parasites: Successes, Challenges, and Next Steps



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Toronto Invasive Bacterial Diseases Network (TIBDN) Education Day

Thursday, May 7, 2026, 9:50-10:20am

Conflict of interest

- No conflicts of interest to disclose.

Learning objectives

- By the end of this session, participants will be able to:
 - Understand the new PCR-enabled testing approach at PHO for enteric parasites.
 - Recognize the positive impacts and remaining gaps in testing for enteric parasites.
 - Look ahead at future innovations of enteric parasitology diagnostics.

Hypothetical scenario

- Three healthy household members develop watery diarrhea on the same day. After a week, the diarrhea is still significant. They have no travel or animal exposures. They are vegan and share the same food and water.

Question: Which of the following parasites would you test for, if any?

- A. Enteric protozoa
- B. Enteric helminths
- C. A and B
- D. None
- E. Not sure

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Enteric protozoan infections are not uncommon in Canada, yet their importance is often underrecognized

- Examples of recent public health outbreaks:

Cyclospora

Almost 700 sick in US and Canada in Fresh Express salad outbreak

Published June 29, 2020 • Updated July 28, 2020

By Jessi Devenyns
Contributing Reporter

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Entamoeba histolytica

29 cases of parasitic infection in Saskatoon Farm outbreak: AHS

By Michael Franklin

Published: August 05, 2025 at 12:55PM EDT



Alberta Health Services is continuing to investigate the source of a gastrointestinal outbreak at the Saskatoon Farm's restaurant in July. (File)

Cryptosporidium

Calgary petting farm hit with outbreak of 'diarrhea-causing parasite'

By Damien Wood

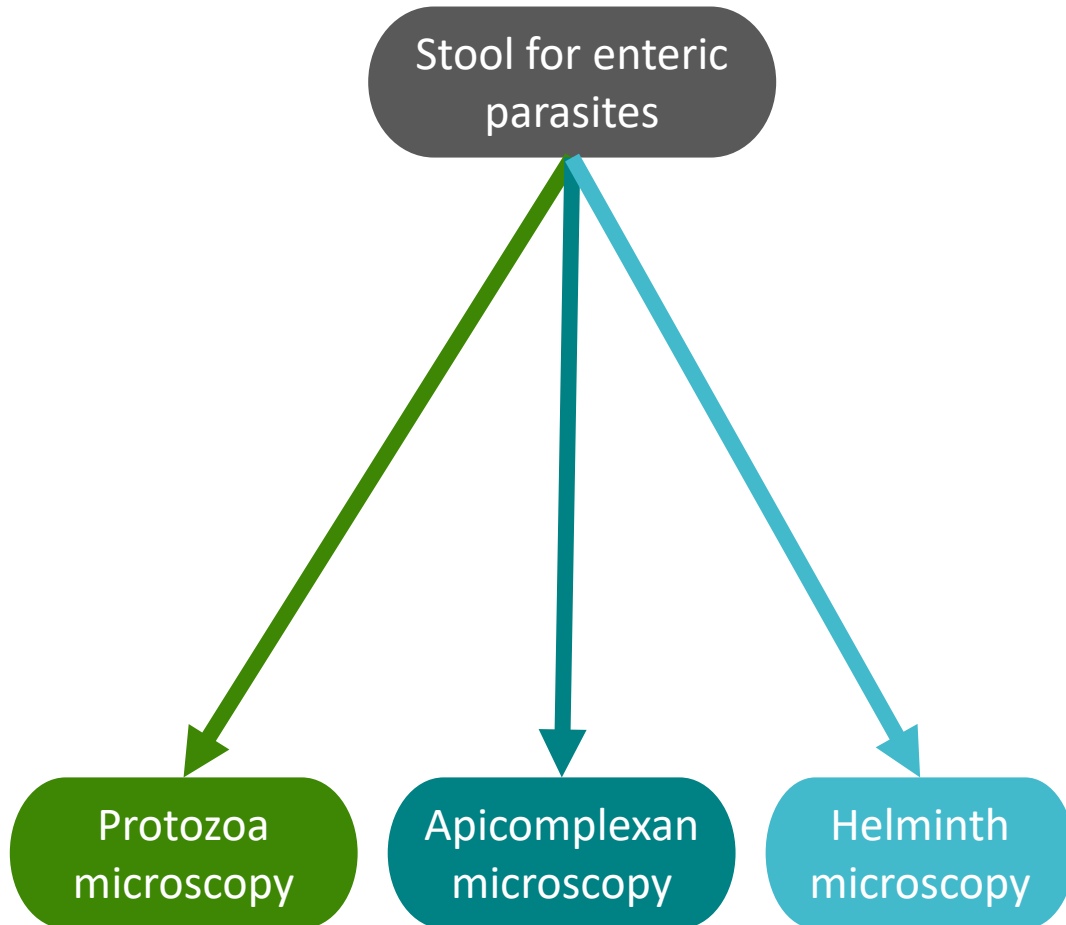
Published: April 28, 2026 at 5:37PM EDT



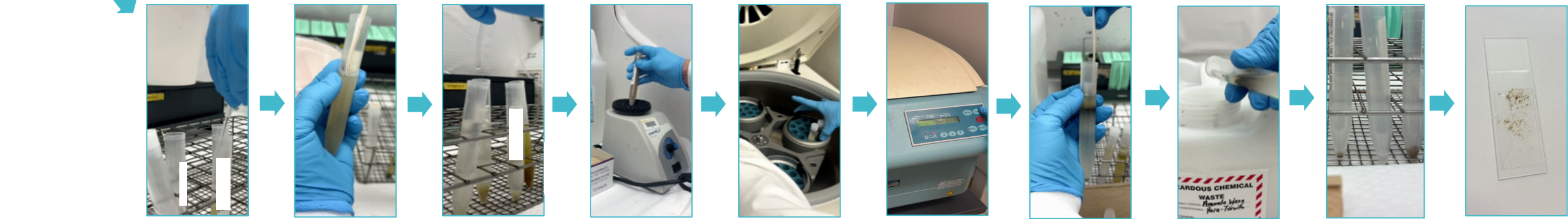
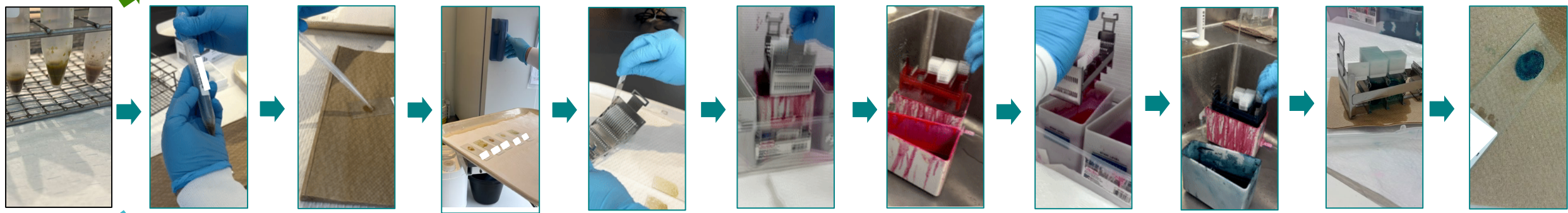
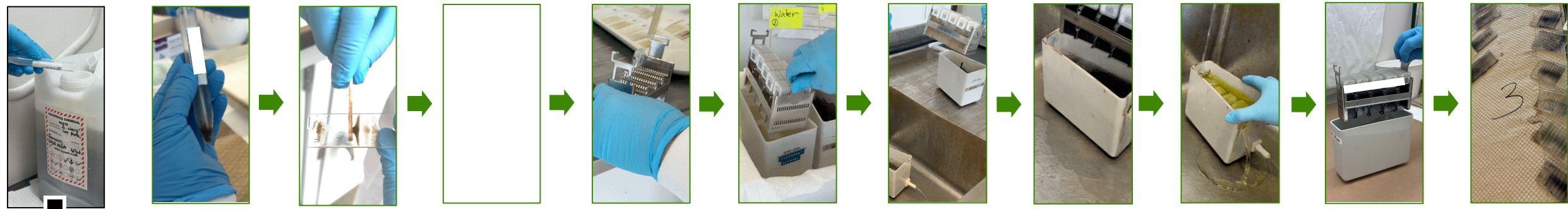
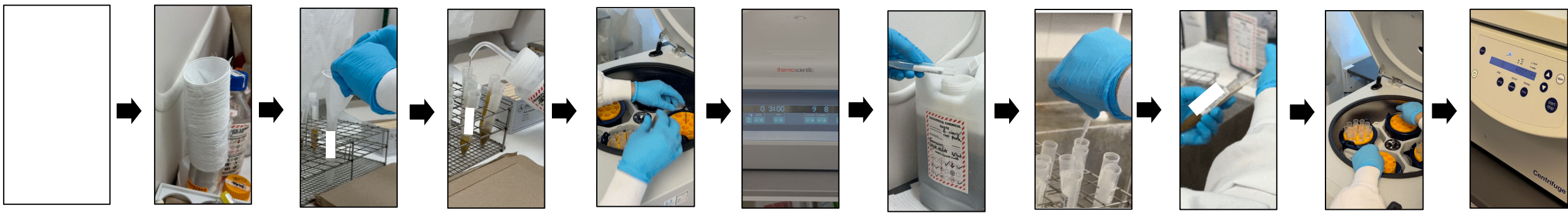
A cryptosporidium outbreak has been declared at the Butterfield Acres Petting Farm in Calgary.

The traditional “ova & parasites” test looks for all stool parasites

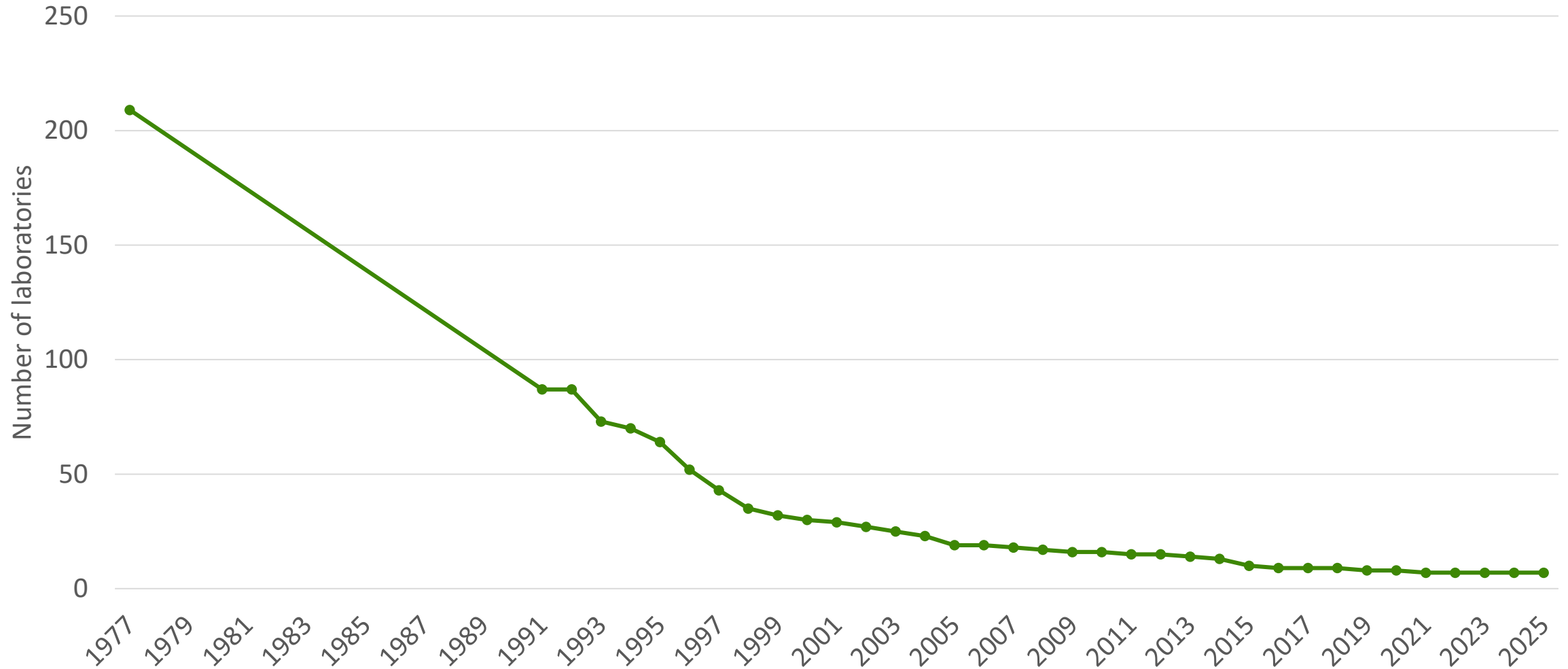
- Traditional “O&P” approach:



- Yet, in Canada...
 - Enteric protozoa (including apicomplexans) are common and usually cause acute to subacute diarrheal illnesses
 - Enteric helminths are rare without risk factors and usually cause a wide diversity of chronic illnesses



The number of laboratories in Ontario providing enteric parasitology services has decreased significantly over the past 50 years

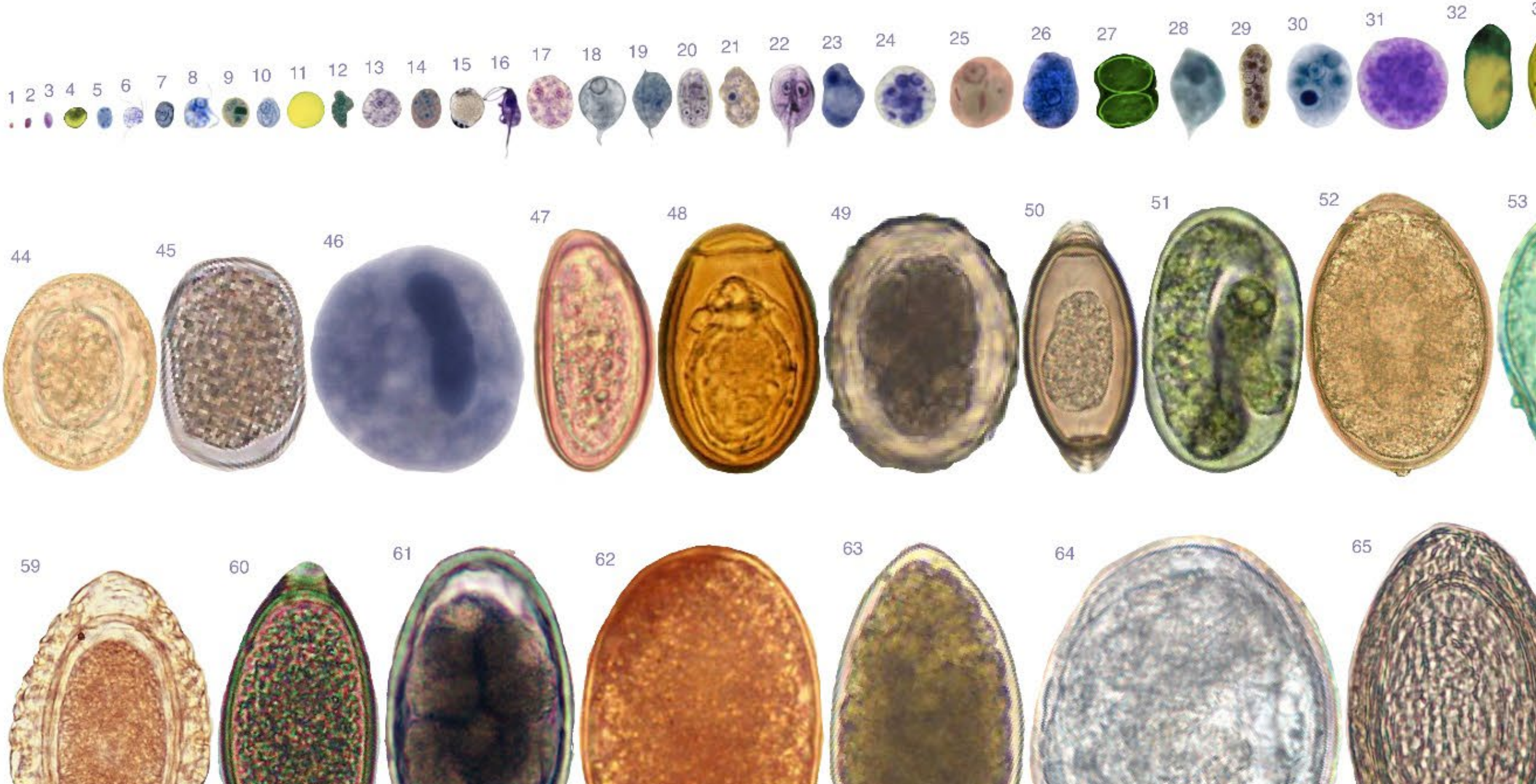


Source: Richardson H et al. Can J Infect Dis 1996; Fleming CA. Accred Qual Assur 2012; IQMH Data.

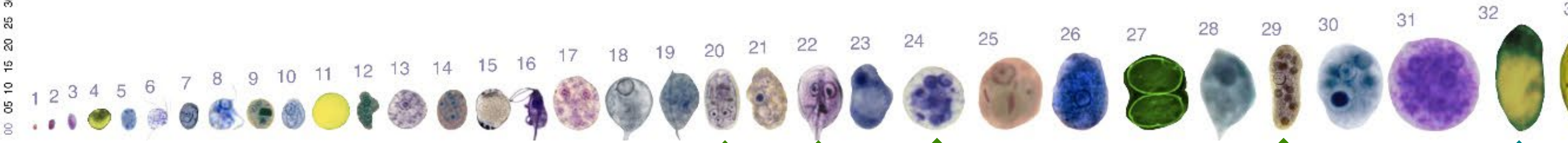
Parasites are hard to identify



Protozoa are harder to identify



Pathogenic protozoa are hardest to identify



Cryptosporidium

Cyclospora

Dientamoeba

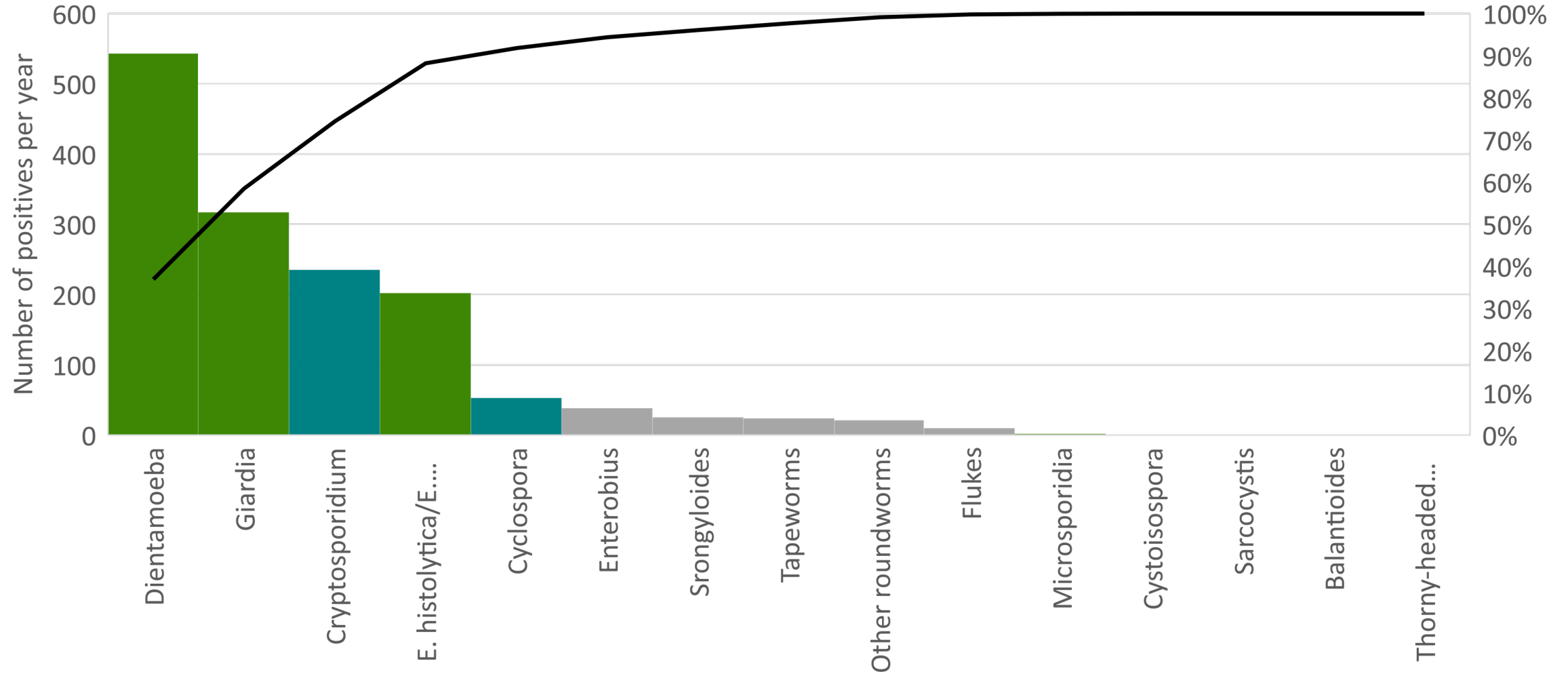
Giardia

Entamoeba histolytica

Sarcocystis

Cystoisospora

Pathogenic protozoa have represented over 90% of positive enteric microscopy results at PHO from 2009-2025



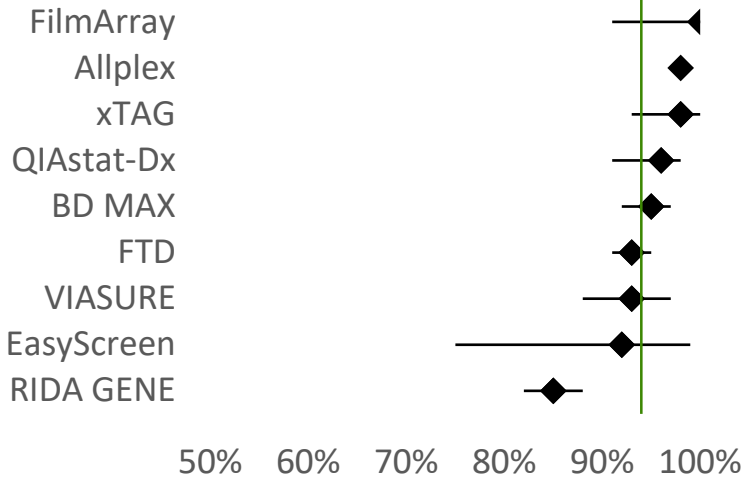
*Cannot distinguish species by microscopy

Source: PHO Laboratory Data.

There are now many commercial options available for these protozoa

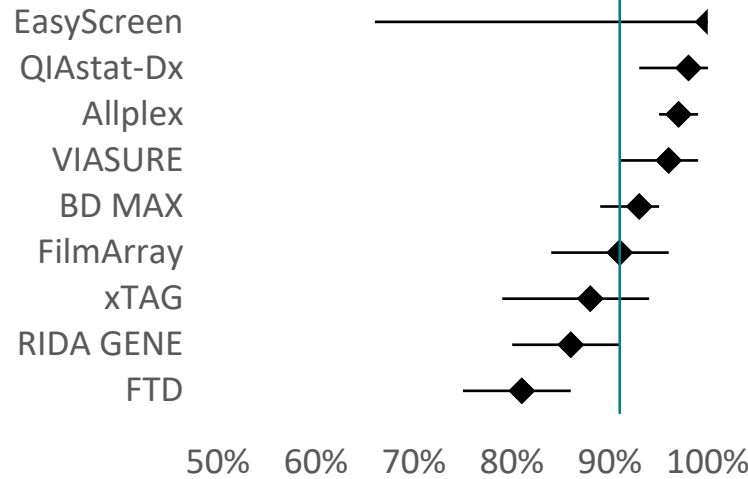
Giardia

94%



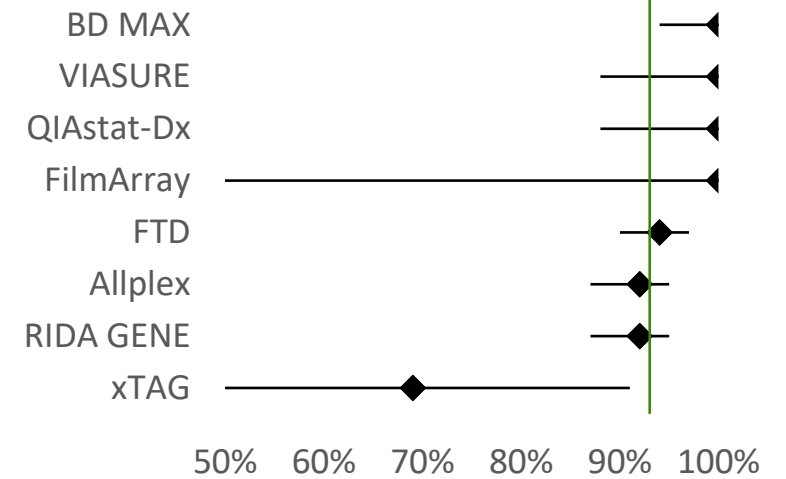
Cryptosporidium

91%



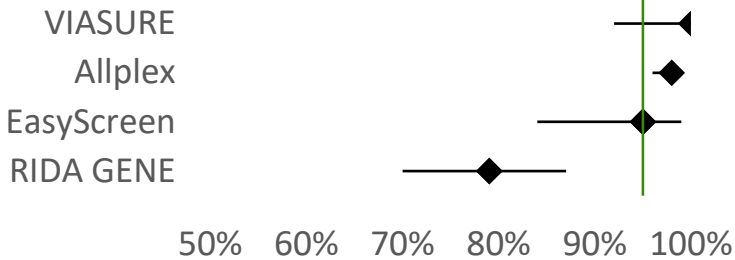
E. histolytica

93%



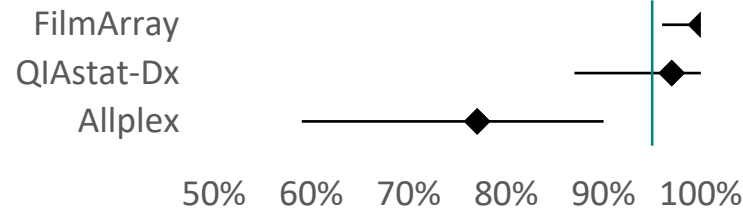
Dientamoeba

95%



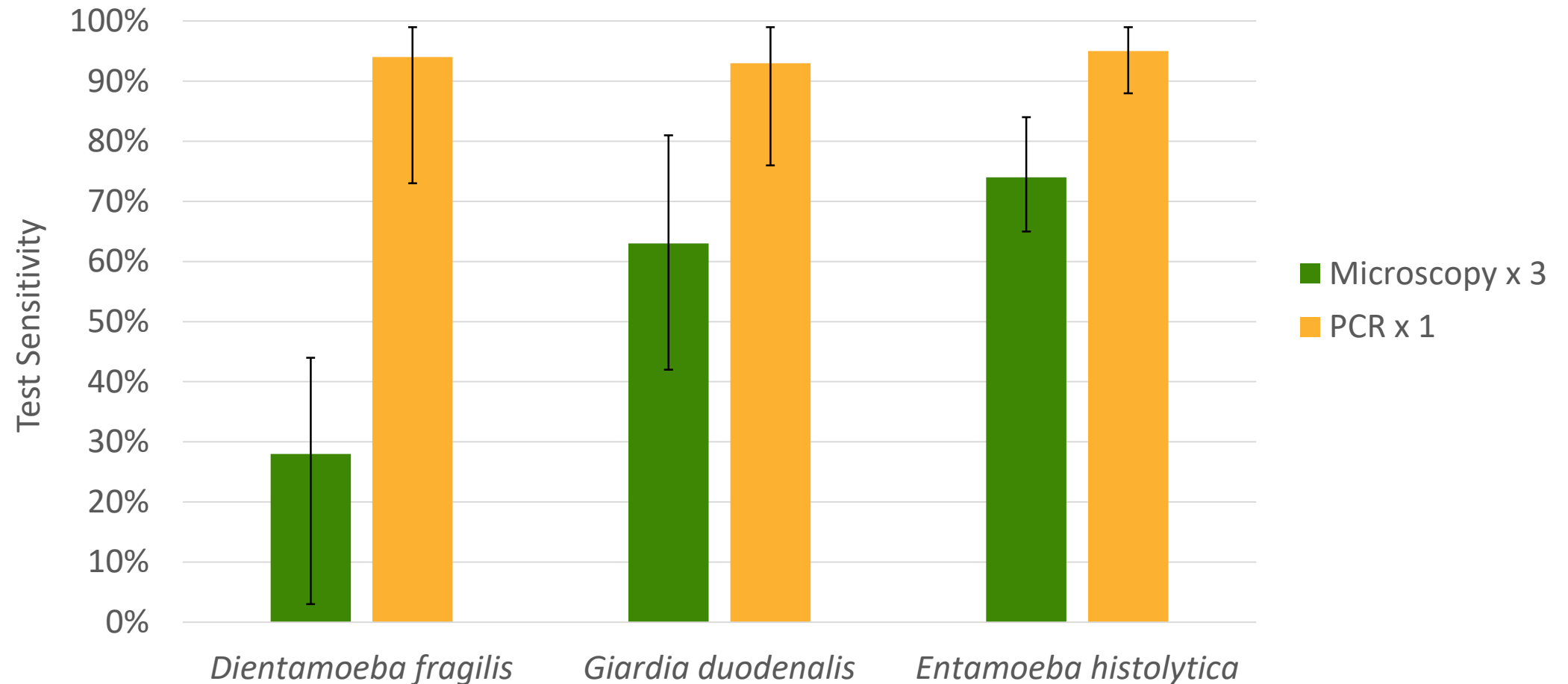
Cyclospora

95%



Source: Akgun 2020, Alejo-Cancho 2017, Argy 2022, Autier 2018, Autier 2020, Basmaciyan 2021, Bateman 2020, Batra 2016, Beckmann 2014, Boggild 2025, Buss 2015, Calderaro 2018, Caza 2025, Claas 2013, Coppens 2024, Formenti 2018, Fricmann 2021, Giffen 2023, Halligan 2013, Hannet 2019, Hartmeyer 2017, Khare 2014, Koller 2020, Madison 2016, McAuliffe 203, Mergen 2020, Molling 2016, Navidad 2013, Oliva 2025, Parcina 2017, Park 2025, Patel 2014, Paulos 2019, Perry 2014, Perry 2017, Robert-Gangneux 2025, Sobczyk 2020, Spina 2015, Stark 2014, Szymczak 2025, Wessels 2013, Yoo 2019, Zboromyrska 2014, Zhan 2020

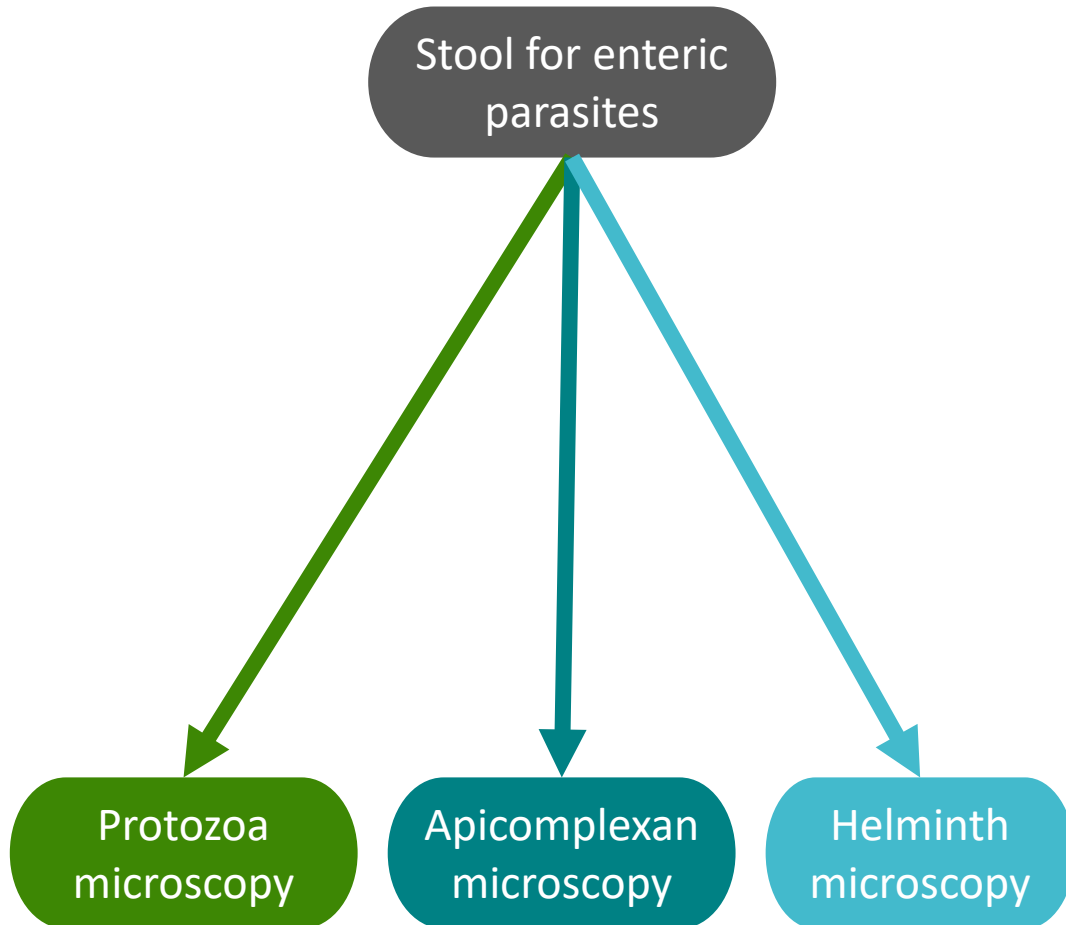
A single PCR is often more sensitive than 3 x serial stool microscopy, and can distinguish *Entamoeba histolytica* vs. non-pathogenic species



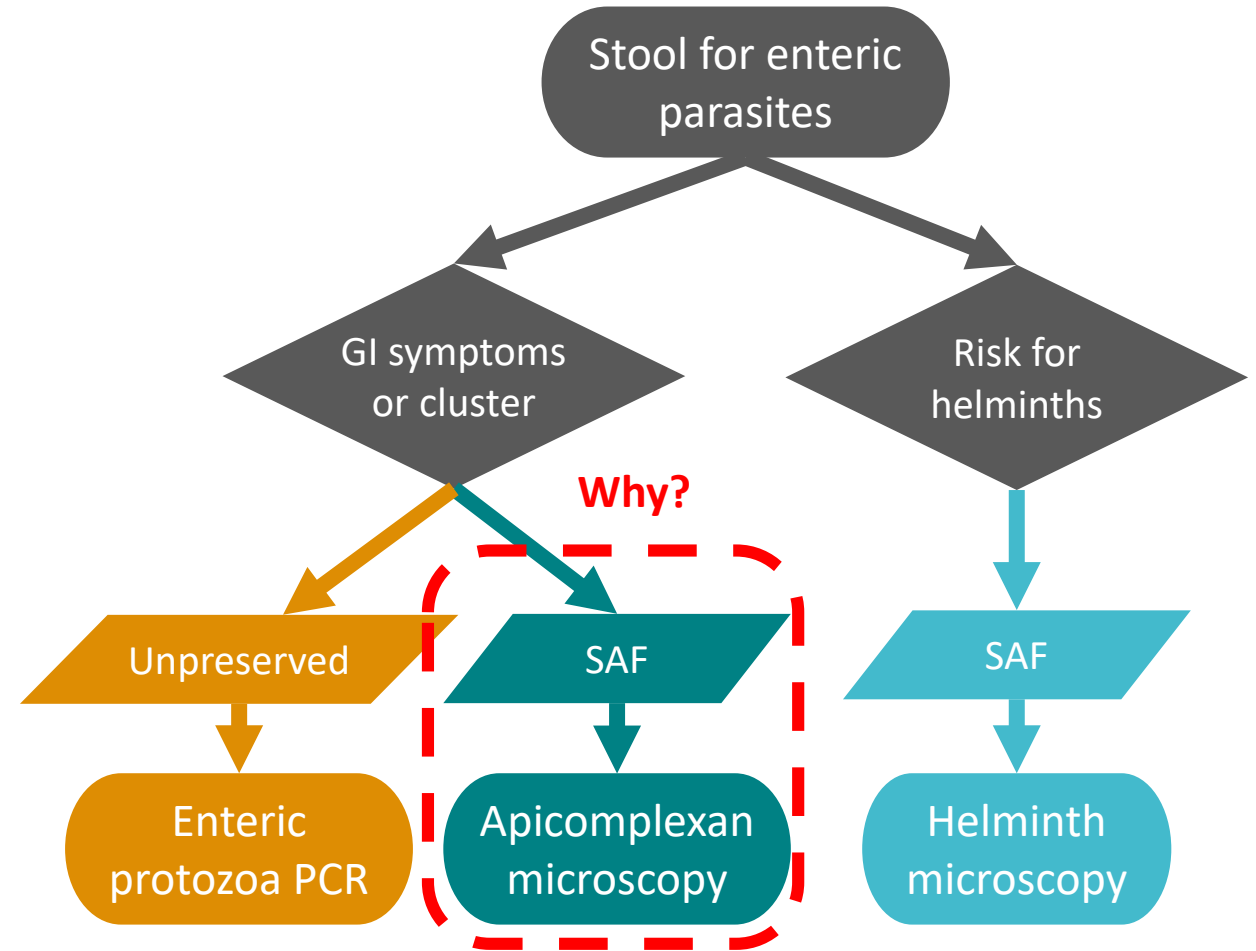
Source: Formenti F et al. Front Microbiol 2017; Meurs L et al. PLoS Negl Trop Dis 2017; Autier B et al. Microorganisms 2020.

Since January 2026, PHO has implemented a new testing approach using PCR and clinical criteria for enteric parasites

- Traditional “O&P” approach:



- New approach:



Two apicomplexans don't readily have PCR yet



↑
Cryptosporidium

↑
Cyclospora

↑
Dientamoeba

↑
Giardia

↑
Entamoeba histolytica

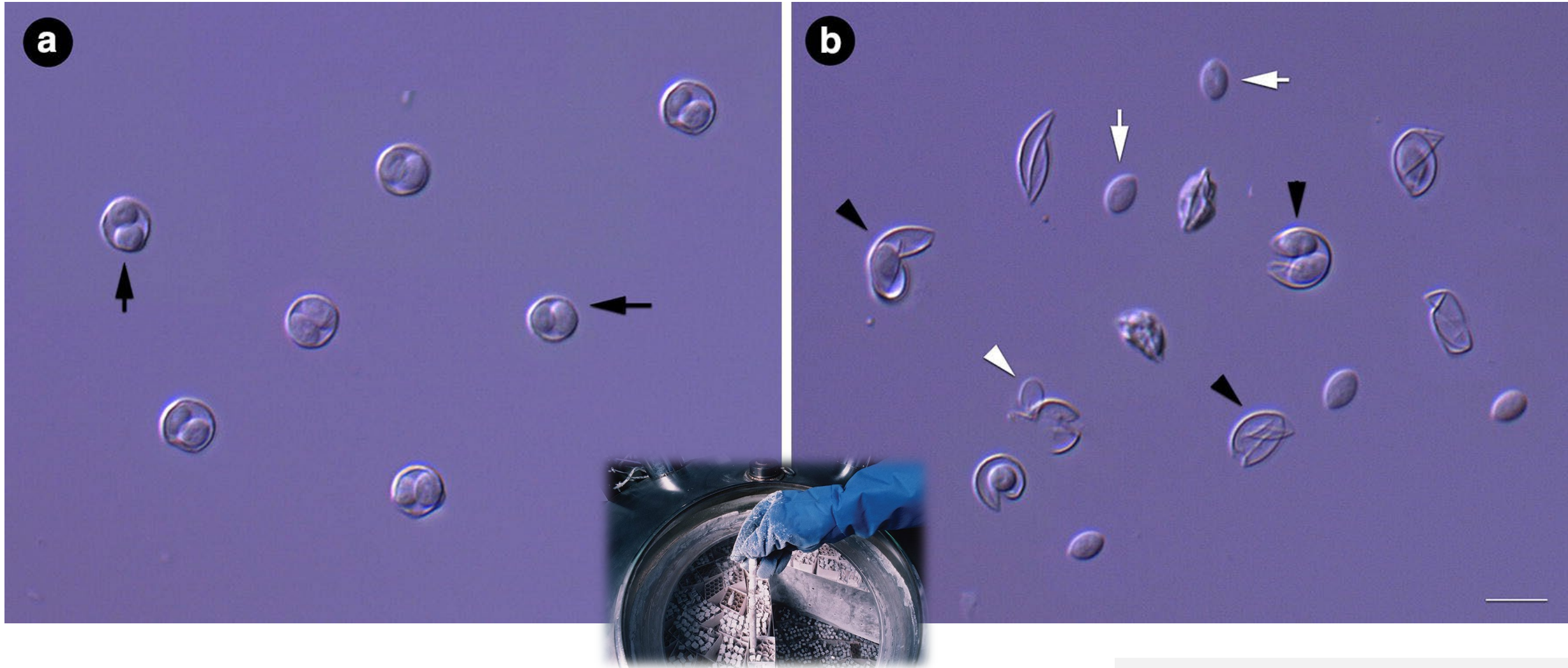
↑
Sarcocystis
No PCR yet

↑
Cystoisospora
No PCR yet

**Apicomplexans have
hardy oocyst walls**

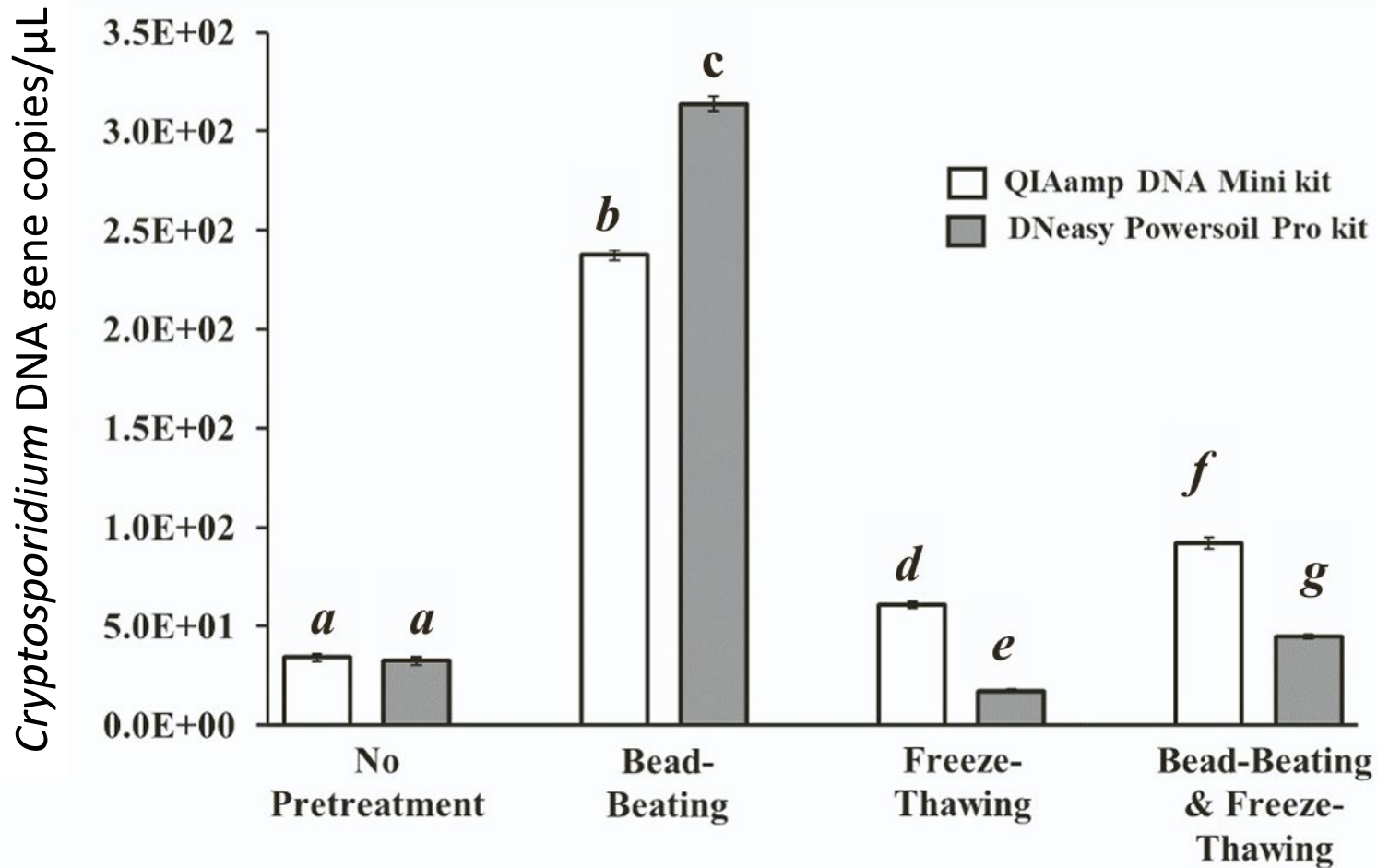


Additional pre-processing is often recommended for apicomplexans



Source: Qvarnstrom Y et al. Gut Pathog 2018.

There is a lot variability in pre-processing approaches and outcomes



2 min Bead-beating

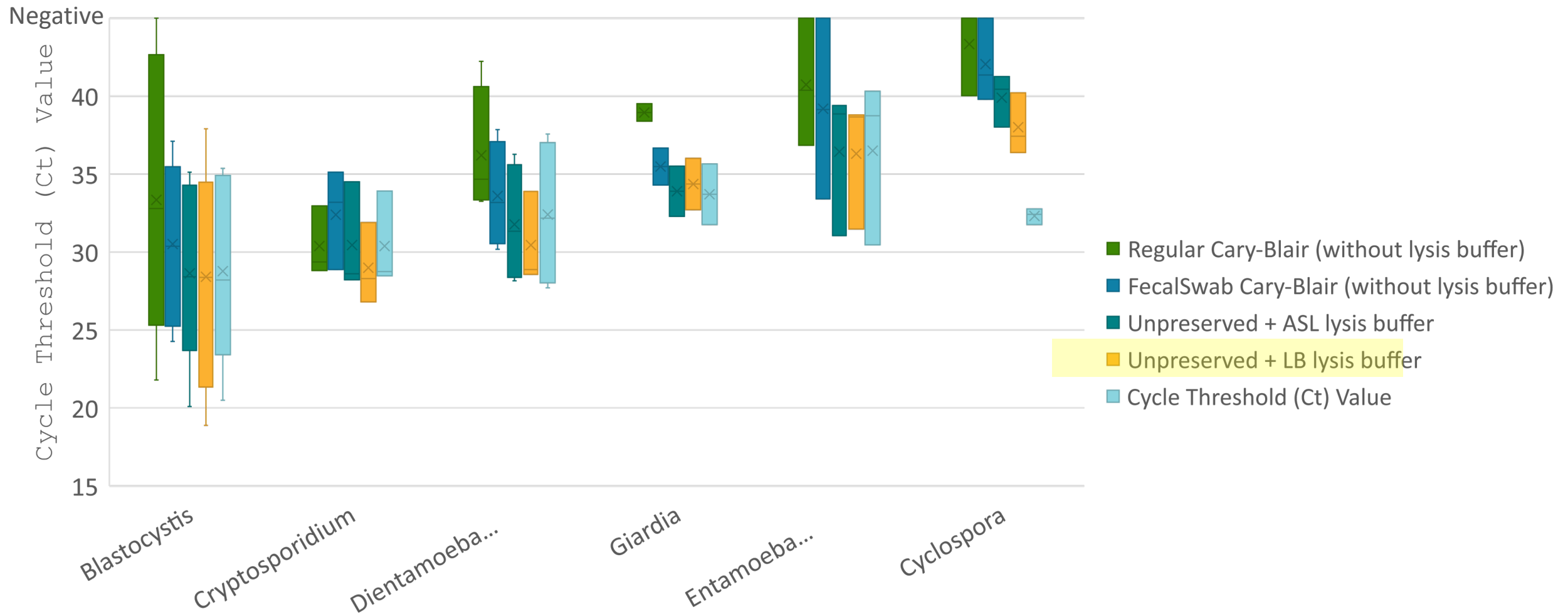
versus



5 cycles of freeze-thaw

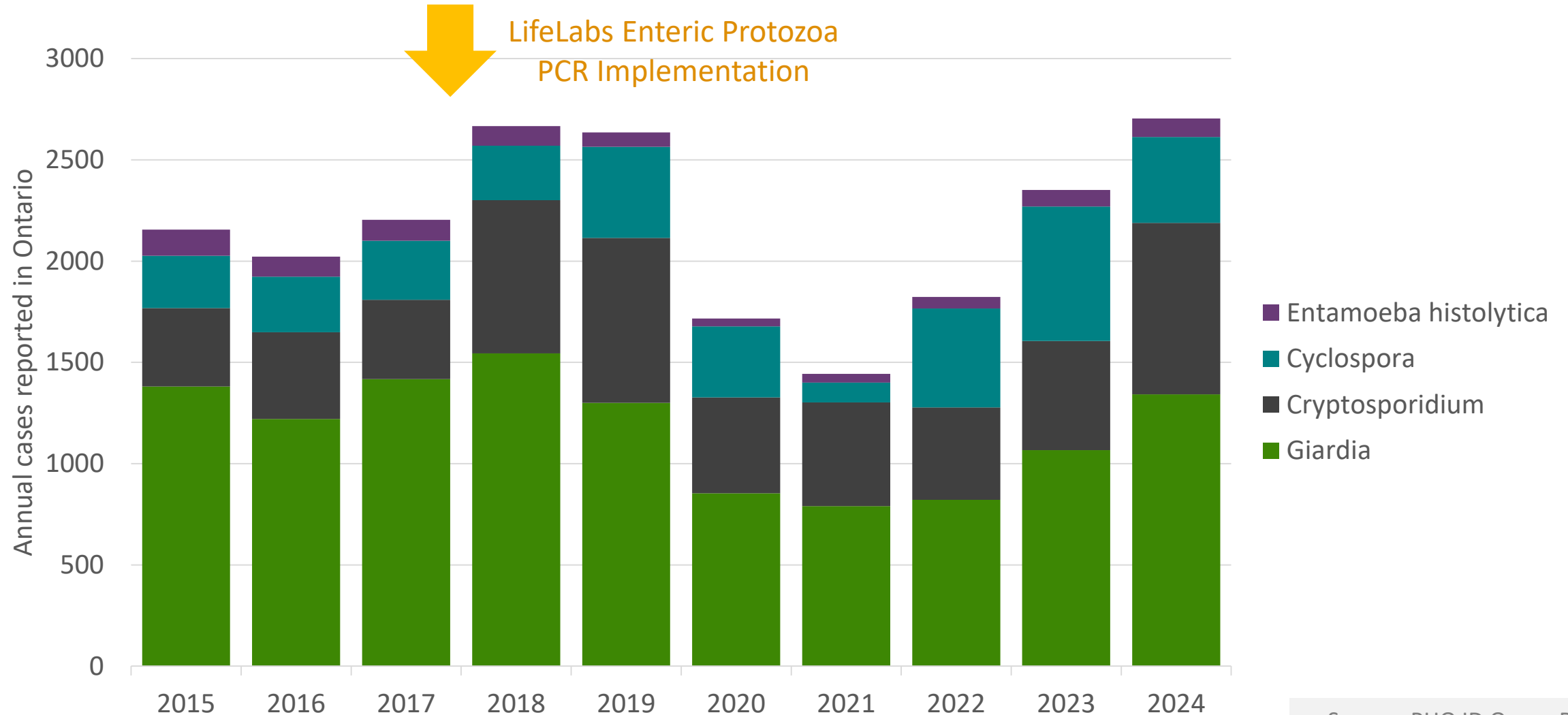
Source: Hachimi O et al. Sci Total Environ 2024.

At PHO, lysis buffers have shown to improve analytical sensitivity for Cary-Blair and unpreserved samples



Source: PHO Laboratory Data.

We should expect an increase in detections in Ontario following PCR implementation



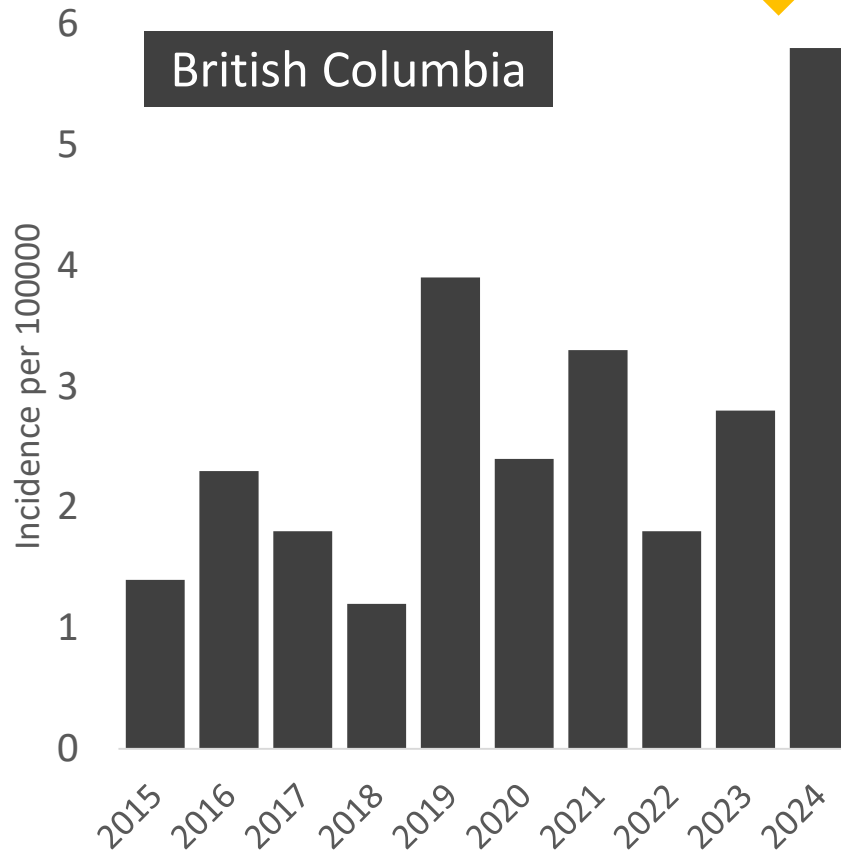
Source: PHO ID Query Data.

A similar increase was seen in other jurisdictions

Enteric Protozoa PCR Implementation



British Columbia

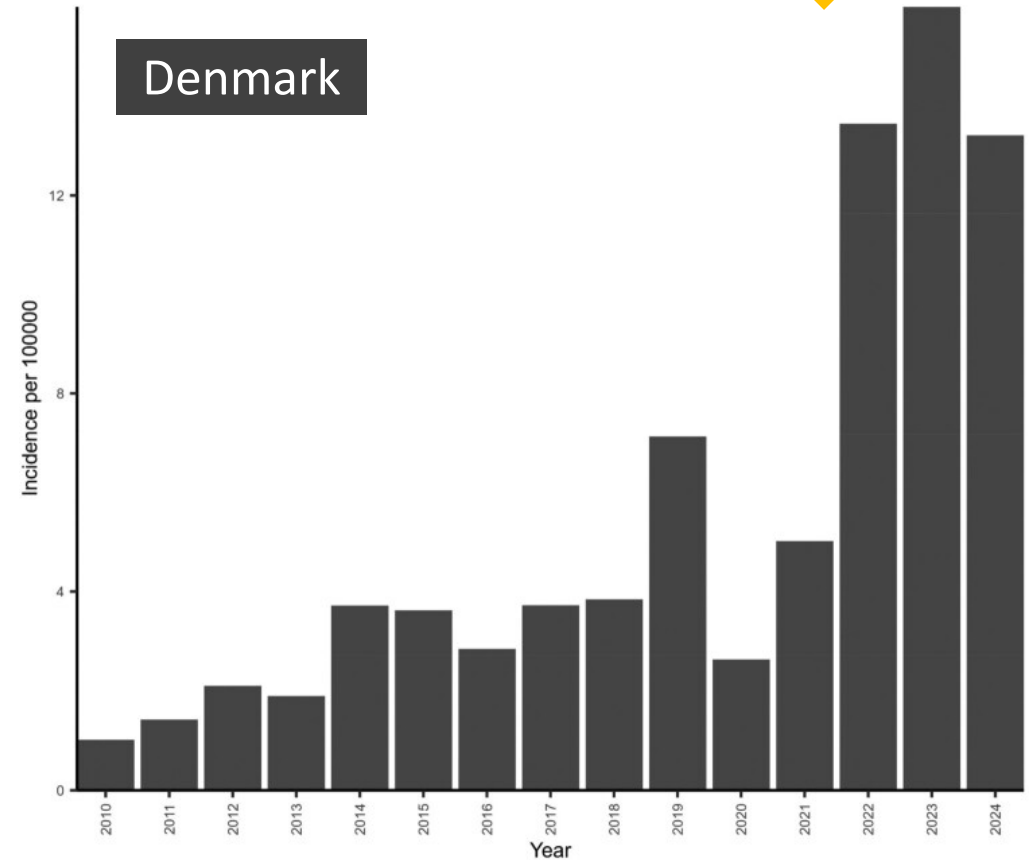


■ Cryptosporidium

Enteric Protozoa PCR Implementation

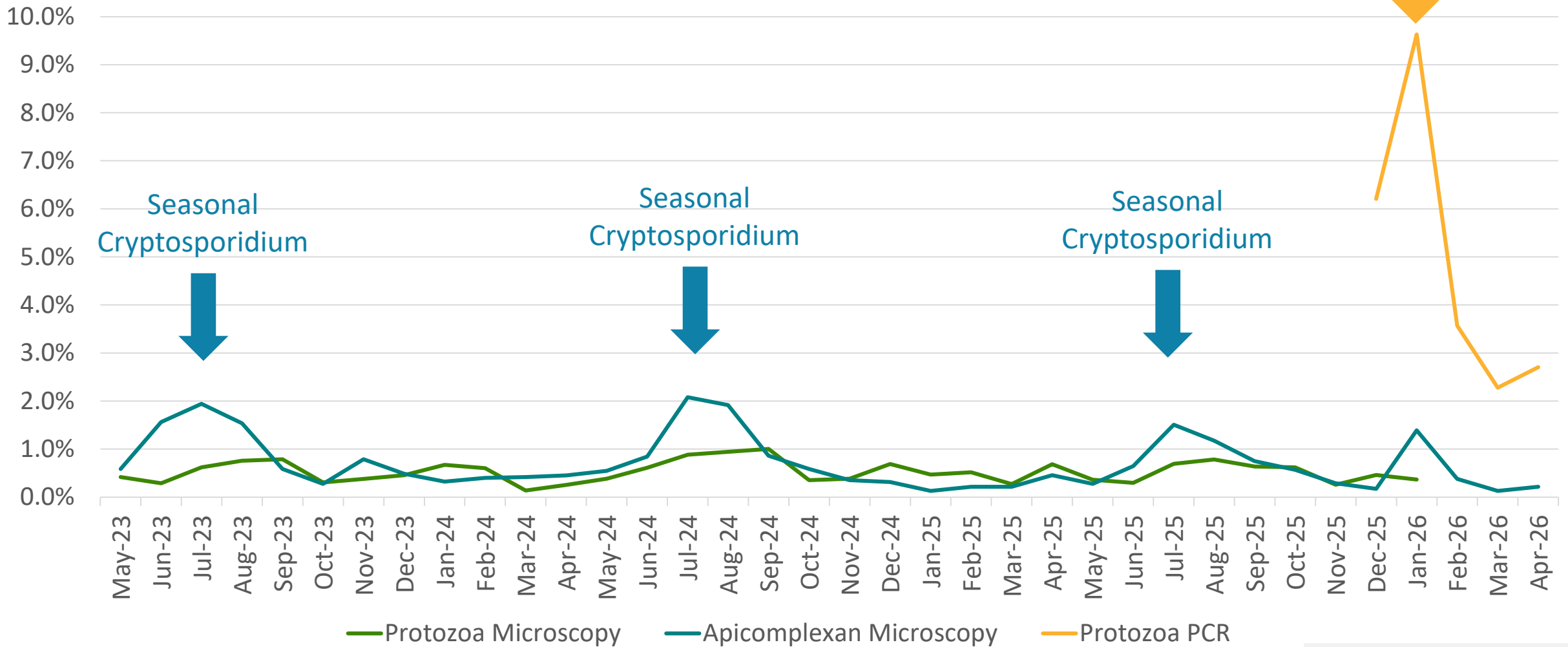


Denmark



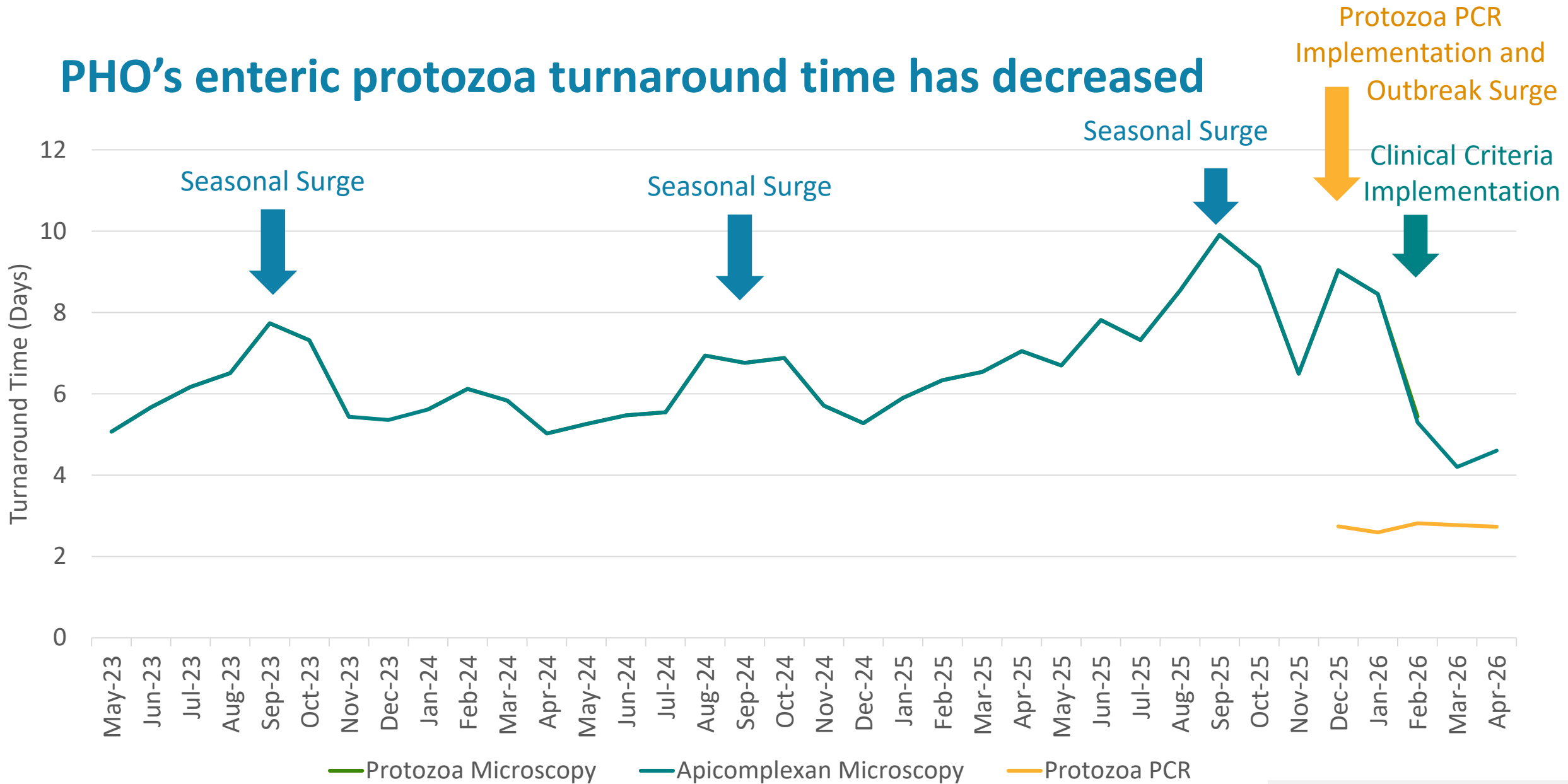
Source: BCCDC Communicable Disease Dashboard Data. Larsen TG et al. Emerg Microbes Infect 2025.

PHO's enteric protozoa positivity rate has increased



Source: PHO Laboratory Data.

PHO's enteric protozoa turnaround time has decreased



Source: PHO Laboratory Data.

Routine use of unpreserved/Cary-Blair samples will enable the use of other molecular tests

- For example, *Cyclospora* genotyping may help identify the source of outbreaks:

Almost 700 sick in US and Canada in Fresh Express salad outbreak

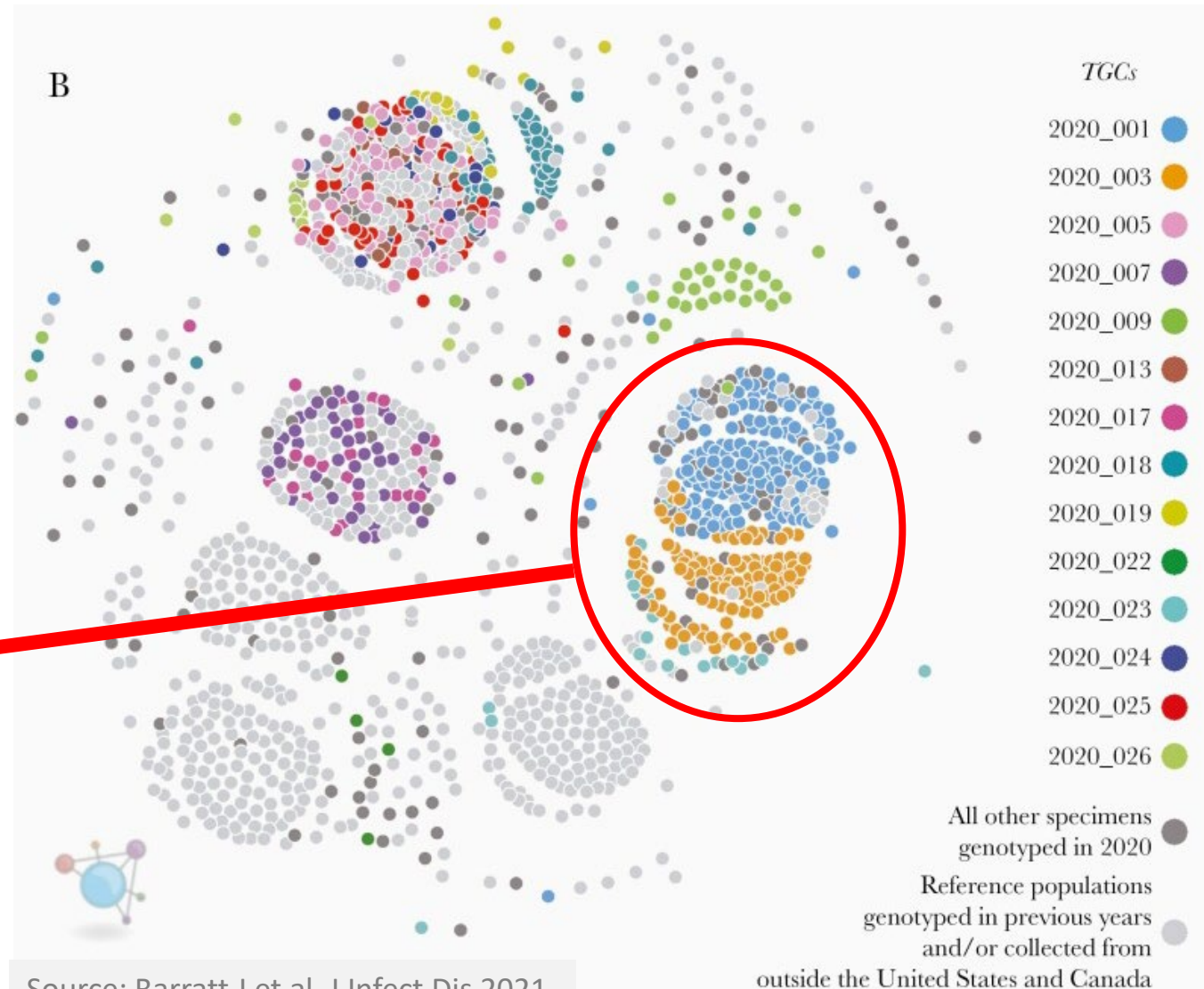
Published June 29, 2020 • Updated July 28, 2020

By Jessi Devenyns
Contributing Reporter

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< 30% success rate with SAF samples

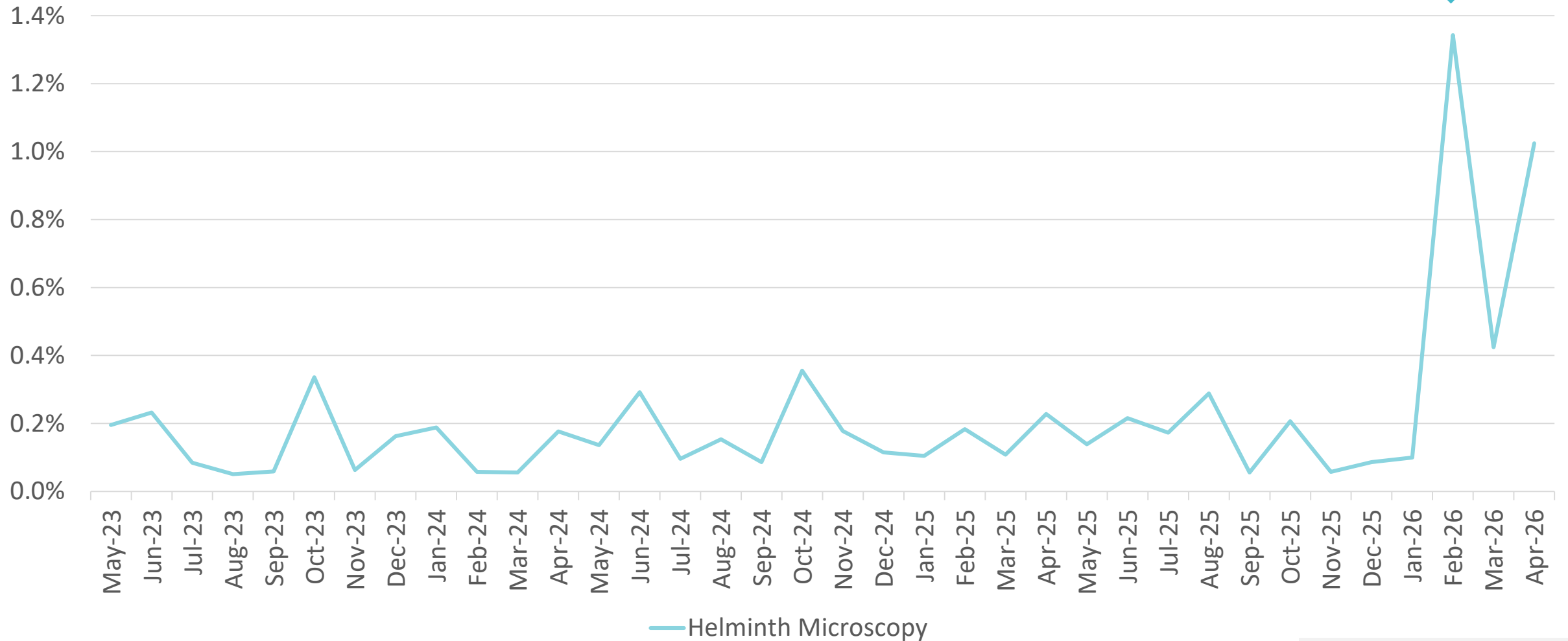


Early benefits of the new testing approach on protozoa diagnoses

- Improved sensitivity and high-yield testing from **1%** → **3%**
- Reduced test turnaround times from **7 days** → **3 days**
- Reduced hands-on work per sample from **35-40 min.** → **1-5 min.**
- Improved daily surge capacity from **270 samples** → **360 samples**
- Reduced training requirements from **12 weeks** → **2 weeks**
- Other benefits:
 - **Earlier outbreak detection and management**
 - **More accurate identification of *Entamoeba histolytica***
 - **Access to additional molecular tests (e.g., species identification, genotyping, AMR)**
 - **Lower barrier of entry for other labs wishing to do enteric protozoa testing**

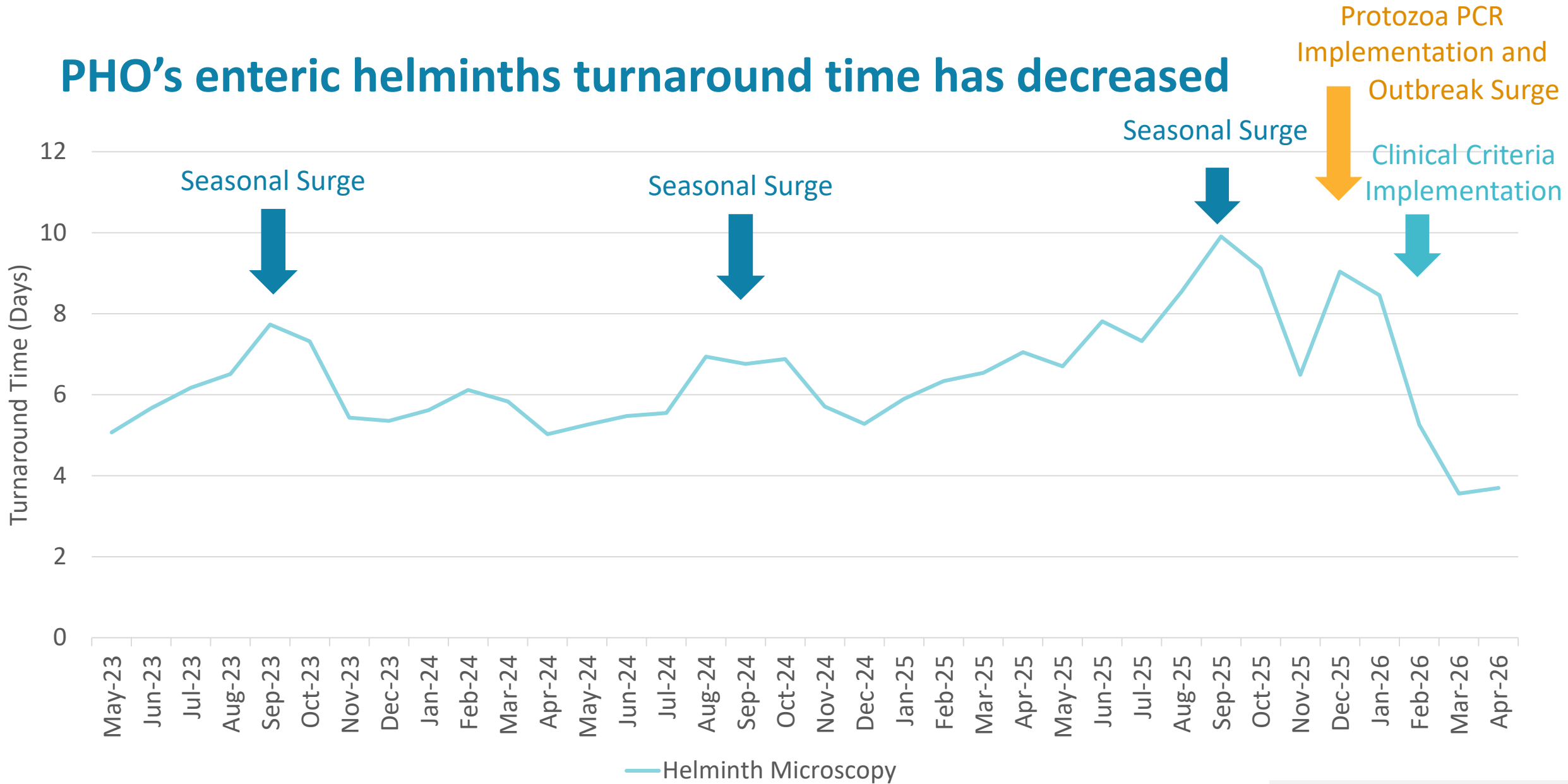
PHO's enteric helminths positivity rate has also increased

Clinical Criteria Implementation



Source: PHO Laboratory Data.

PHO's enteric helminths turnaround time has decreased



Source: PHO Laboratory Data.

Early benefits of the new testing approach on helminth diagnoses

- Improved high-yield testing (positivity rate) from **0.2%** → **1%**
- Reduced test turnaround times from **7 days** → **4 days**
- Reduced hands-on work per sample from **35-40 min.** → **10-15 min.**
- Other benefits:
 - **Improved microscopy expertise specifically for helminths**
 - **Potential to explore innovative solutions specifically for helminths**

There are > 50 enteric helminths identifiable by microscopy

- Broad multiplexing needed
- PCR accuracy is variable and heavily reliant on pre-processing methods:



RESEARCH ARTICLE

Scrambled eggs: A highly sensitive molecular diagnostic workflow for *Fasciola* species specific detection from faecal samples

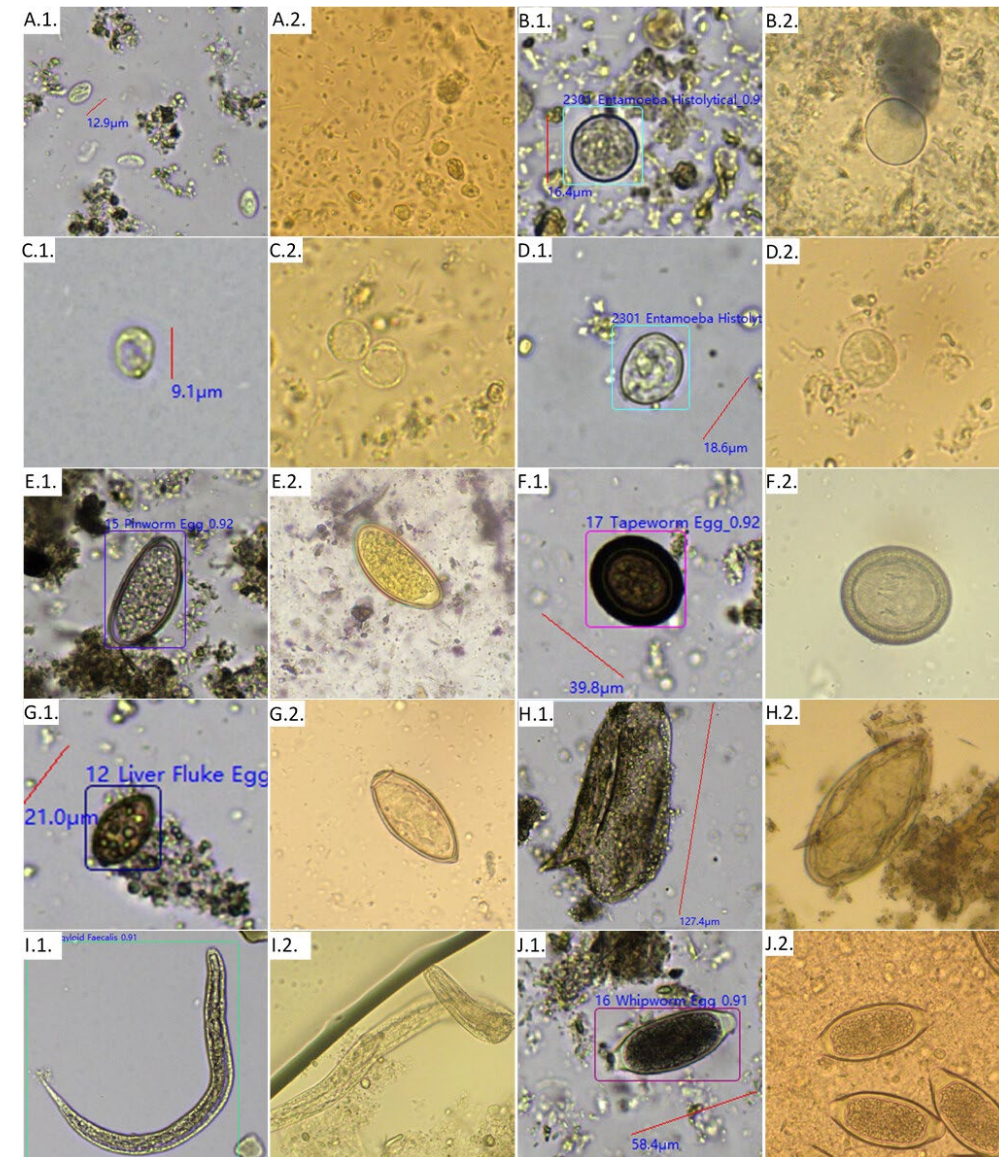
Nichola Eliza Davies Calvani^{1,2}, Peter Andrew Windsor², Russell David Bush², Jan Šlapeta^{1*}

¹ Laboratory of Veterinary Parasitology, Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, Camperdown, New South Wales, Australia, ² Mekong Livestock Research Group, Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, Camperdown, New South Wales, Australia

* jan.slapeta@sydney.edu.au

Multiple AI-assisted enteric microscopy options are now available

- Usually more **accurate** with helminths compared to protozoa
- Examples:
 - Techcyte Fusion Parasitology
 - KEYU KU-F40
 - AVE Diagnostics AVE-562
 - Orienter FA280
 - Menarini SediMAX 2
 - Antek HESKA Element AIM
 - Techion FECPAKG2

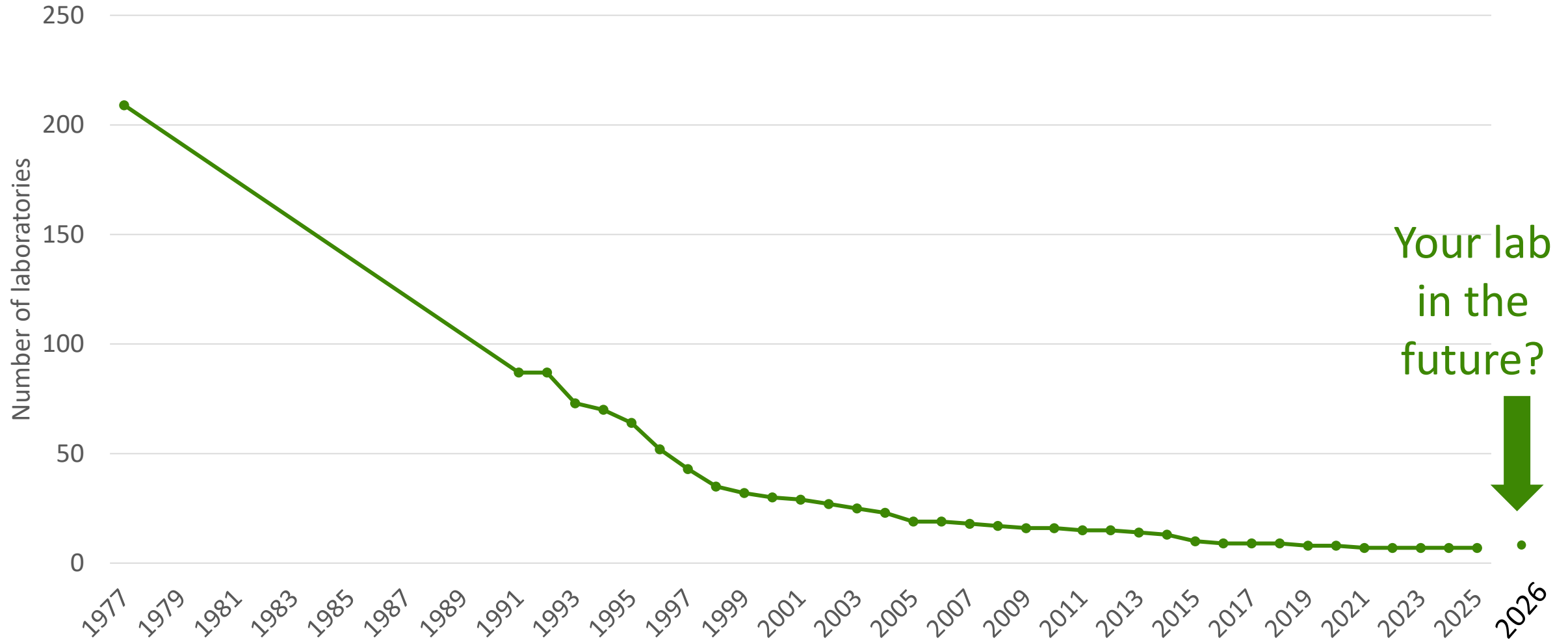


Source: Aupaix A et al. J Clin Microbiol 2026.

Summary and next steps

- So far, the new PCR-enabled enteric testing approach has provided **improvements** for lab utilization, service delivery, and clinical care
- Monitoring of **positivity rates** will remain important in the months ahead
- We will review PCR **adoption** rates and appropriate use of clinical **criteria**
- We will explore additional workflow optimizations
 - Simpler, better pre-processing methods to enhance *Cyclospora* detection
 - Additional molecular assays for species identification, genotyping, and resistance
 - Stability of unpreserved/Cary-Blair stool for apicomplexan and helminth microscopy
 - AI-assisted microscopy screening?
 - Eventual discontinuation of SAF vial requirements for enteric parasitology?

Take-home message: testing for enteric parasites (especially protozoa) is now more accessible than ever



Source: Richardson H et al. Can J Infect Dis 1996; Fleming CA. Accred Qual Assur 2012; IQMH Data.

Acknowledgments

- Dr. Andrea Boggild and Rachel Lau for their pioneering work
- Sarah Teatero for her hard work and excellent ideas
- PHO's enteric protozoa PCR implementation planning and support team
- Our dedicated parasitology staff across PHO
- The audience for your participation!



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