The Importance of Cryptosporidium Surveillance

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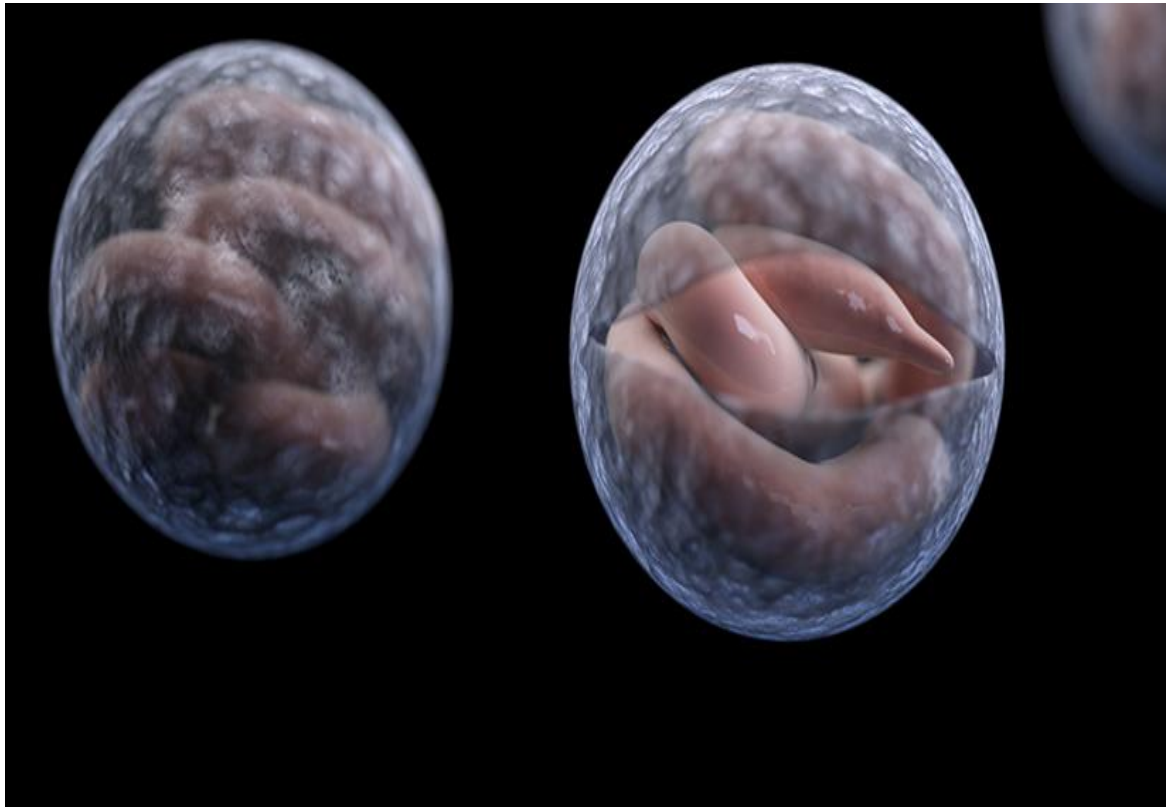
APHL-CDC Fellow

2022 WCLN Regional Meeting

Does your lab perform Cryptosporidium testing?

A. Yes
B. No



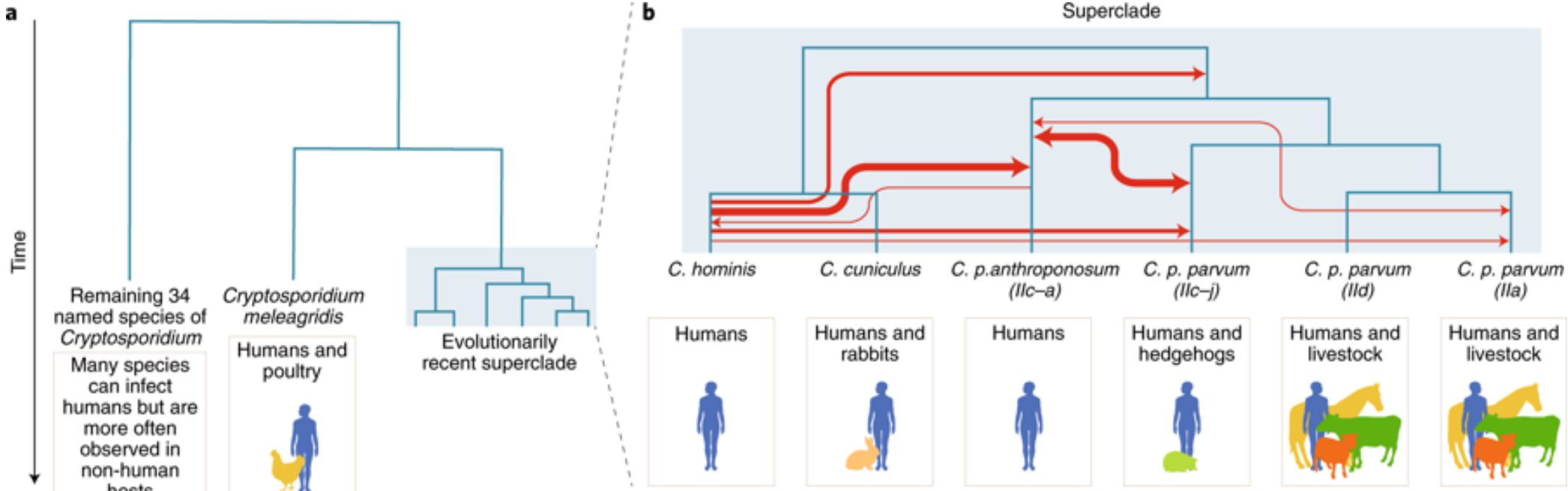
Cryptosporidium general information and diagnosis

All started at a summer retreat

- 5 people have became ill one week after attending a summer family gathering at a water park in Wisconsin.
- All of them experienced diarrhea for several days. Some of them also experienced abdominal cramps, headaches, and body aches.
- Biofire GI panel conducted in a local clinical lab detected presence of Cryptosporidium species in patient stool and negative for any other GI pathogenic infections.

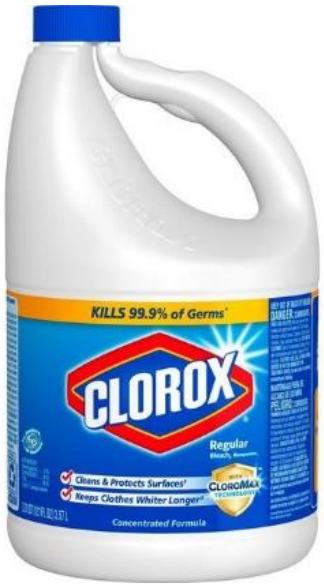
Cryptosporidium

- lives in the gut of infected humans or animals.
- ~40 species, but 2 in particular (*Cryptosporidium hominis* and *Cryptosporidium parvum*) account for most of infections.



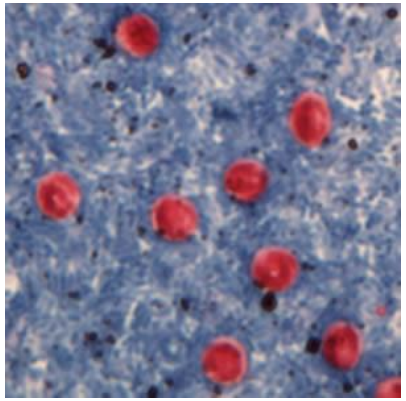
Jessica C. Kissinger. 2019. Nature Microbiology; 4: 730–731. https://www.nature.com/articles/s41564-019-0438-1

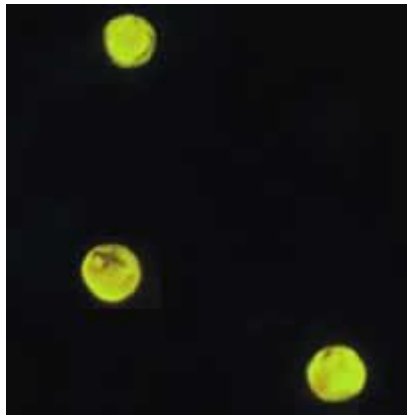
Cryptosporidium forms resistant oocysts



Cryptosporidium diagnosis methods

- Microscopy: Acid-fast stain, direct fluorescent antibody (DFA) assay.
- Rapid antigen cartridge test/microplate EIA tests
- PCR

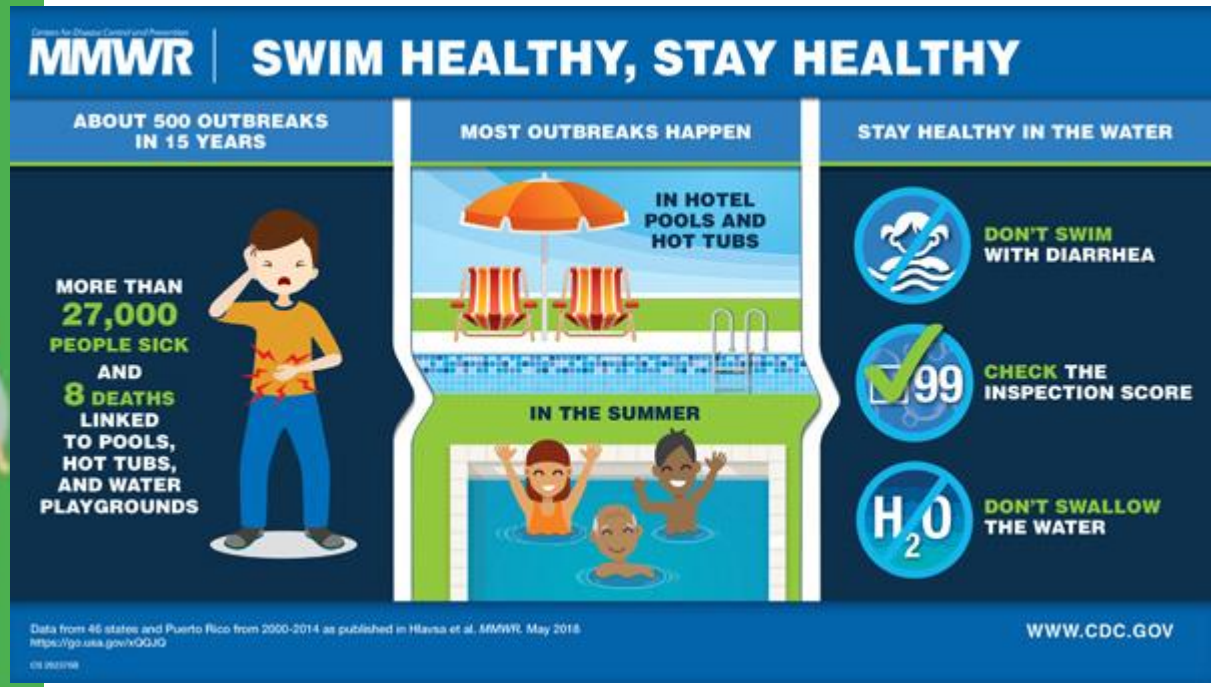




What kind of test does your lab do to detect *Cryptosporidium*

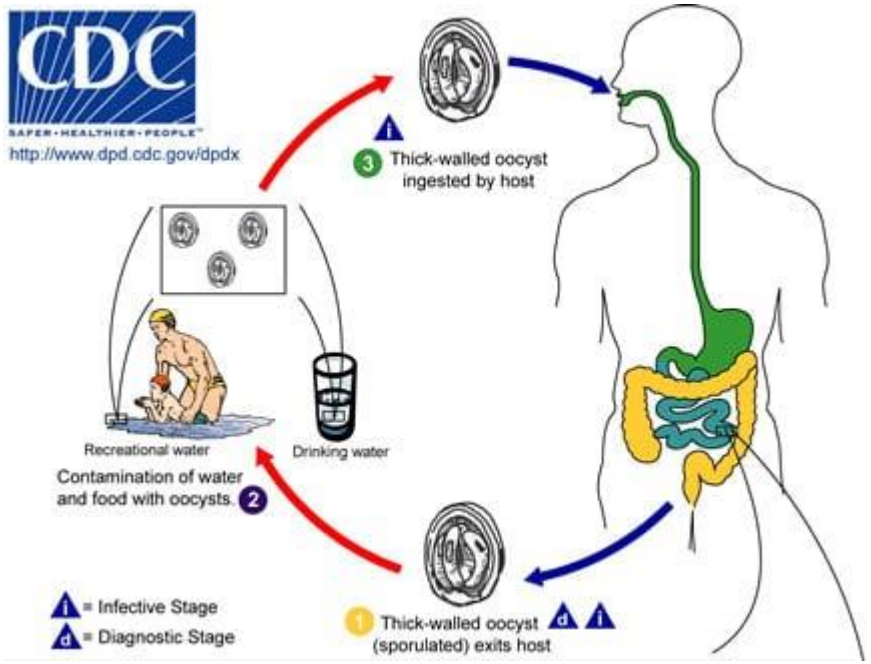
A. Microscopy
B. Antigen-based test
C. Molecular-based test

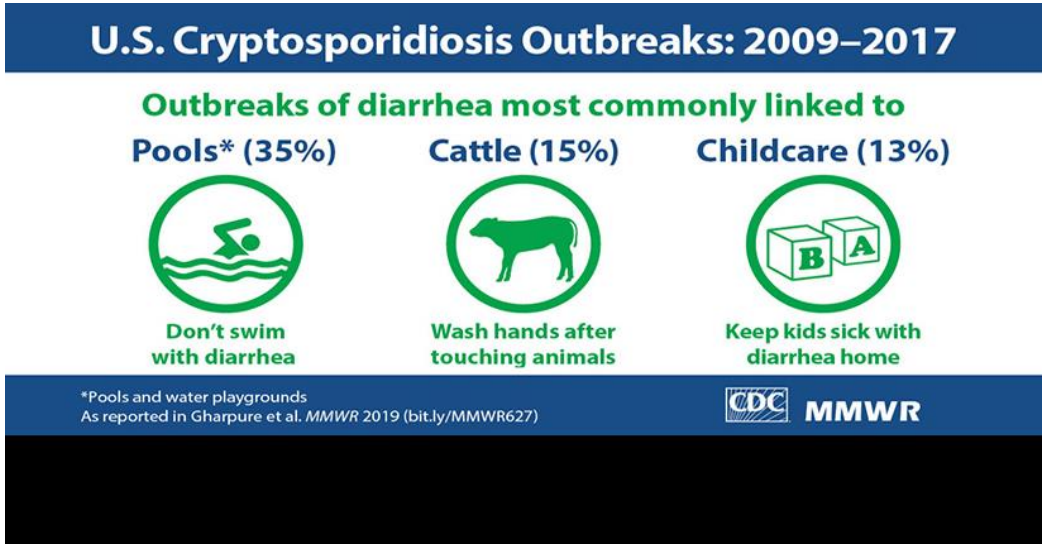
Risk factors



What risk factor causes the outbreak?

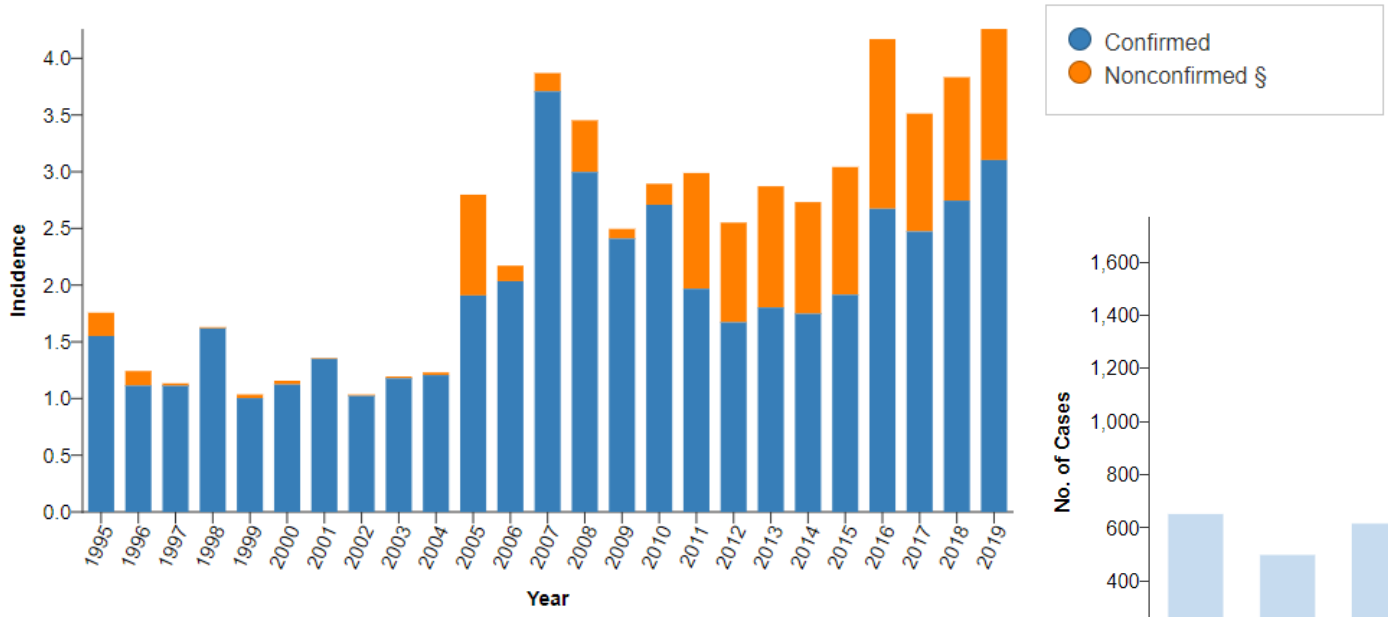
- There was an fecal incident reported in the kiddie pool by parents of one of the ill children.
- All of the ill children attended primary school before the family gathering.
- One of the children also attended a swimming lesson at a local YMCA.


CDC
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx
3 Thick-walled oocyst ingested by host
Recreational water
Drinking water
Contamination of water and food with oocysts 2
i = Infective Stage
d = Diagnostic Stage
1 Thick-walled oocyst (sporulated) exits host

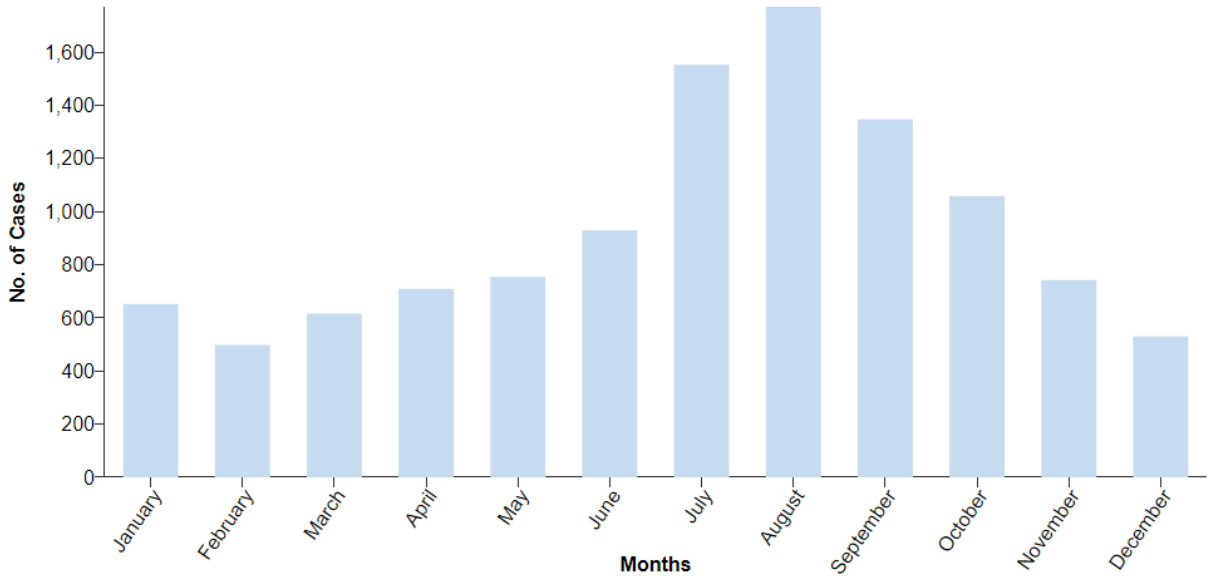

U.S. Cryptosporidiosis Outbreaks: 2009–2017
Outbreaks of diarrhea most commonly linked to
Pools* (35%)
Cattle (15%)
Childcare (13%)
Don't swim with diarrhea
Wash hands after touching animals
Keep kids sick with diarrhea home
*Pools and water playgrounds
As reported in Gharpure et al. MMWR 2019 (bit.ly/MMWR627)
CDC MMWR

Disease Trend

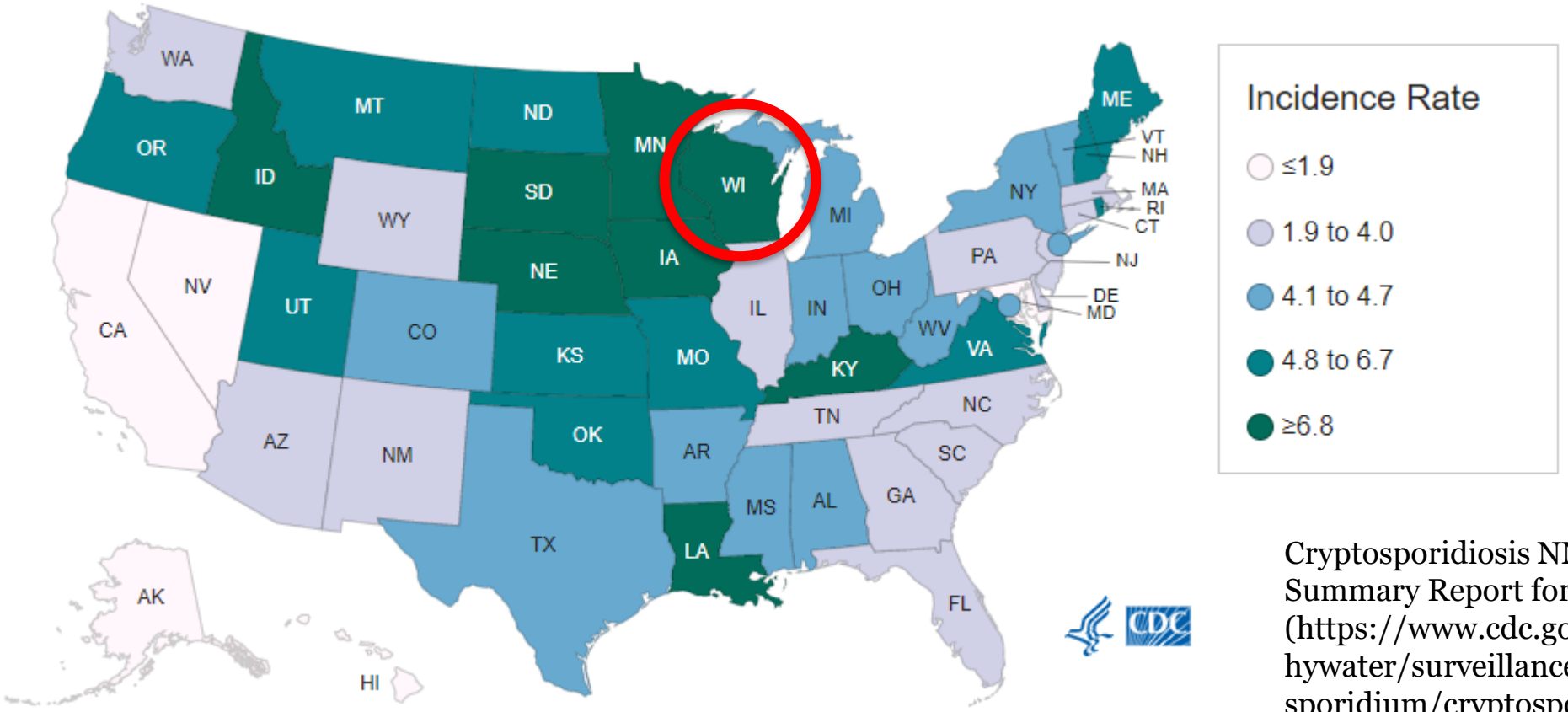
Supplemental Figure 1. Incidence* of reported cryptosporidiosis cases, by year and case classification — National Notifiable Diseases Surveillance System, United States, 1995¶–2019 (N=181,621)



Cryptosporidiosis NNDSS Summary Report for 2019 (https://www.cdc.gov/healthywater/surveillance/cryptosporidium/cryptosporidium-2019.html)

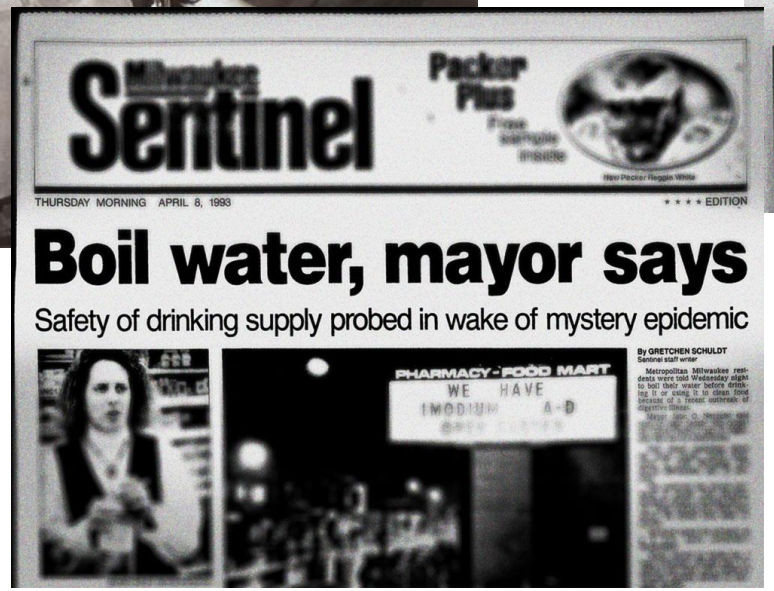


Geographic Distribution (per 100000)



Cryptosporidiosis NNDSS Summary Report for 2019 (https://www.cdc.gov/healthywater/surveillance/cryptosporidium/cryptosporidium-2019.html)

1993 Waterborne Cryptosporidium Outbreak in Milwaukee



Milwaukee Sentinel

Packer Plus

THURSDAY MORNING APRIL 8, 1993

★★★★ EDITION

Boil water, mayor says

Safety of drinking supply probed in wake of mystery epidemic

By GRETCHEN SCHULDT
Sentinel staff writer

PHARMACY · FOOD MART

WE HAVE IMODIUM A-D

SATURDAY MORNING April 10, 1993

WATER CRISIS: DAY 3

Chemist, city fight over early signs of impurities

'Boy, is that water raunchy'

Water was cloudy in March

Discolored water with illness-causing microbes likely seeped through the Howard Avenue water purification plant in late

6A EPA action

Plants to be told to reanalyze records

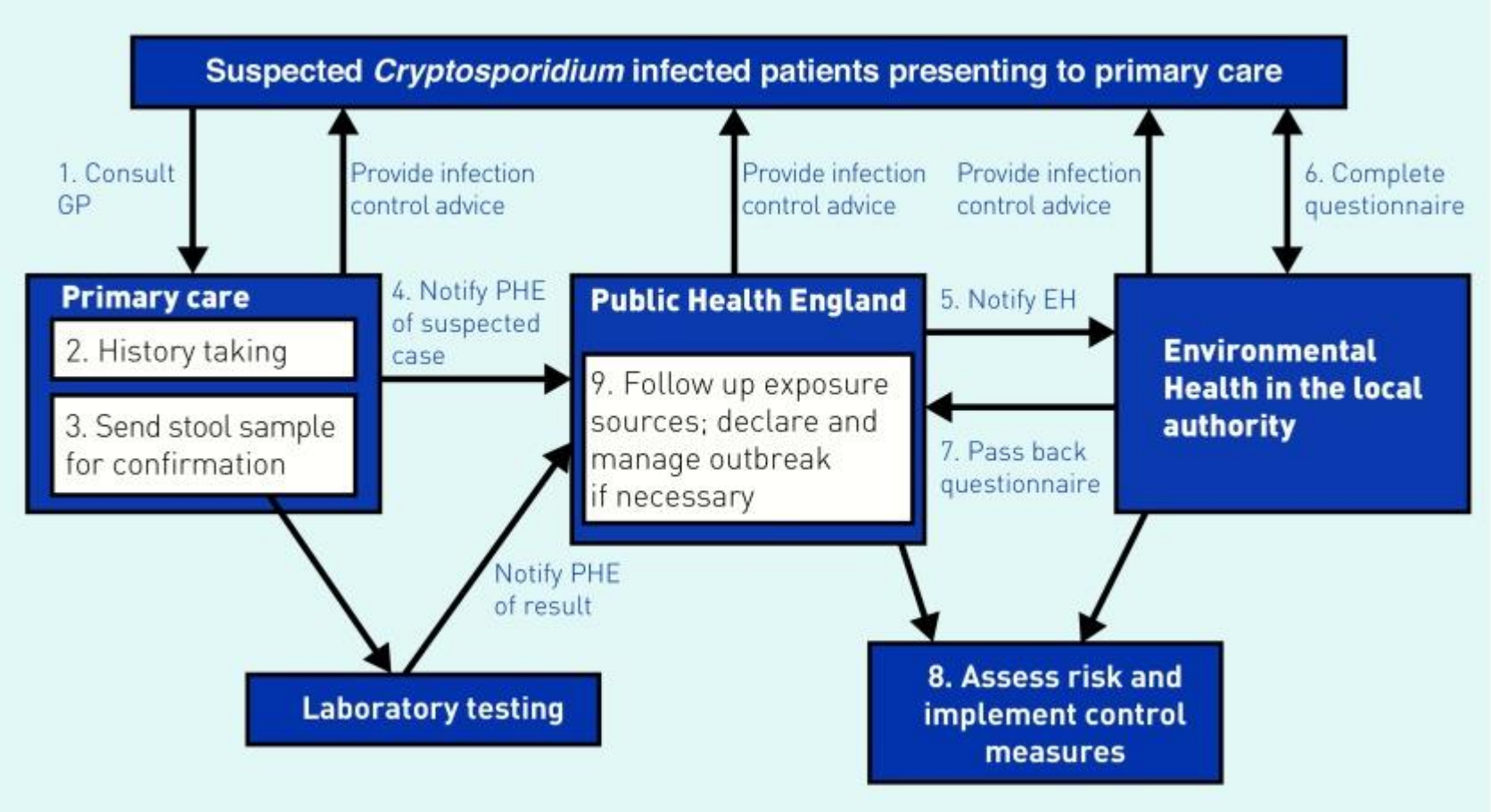
Marks said, "The warning signs were not recognized or responded to."

The plant could have been shut down when the turbidity level was higher than normal

Largest Cryptosporidiosis outbreak in the world.

Identify the source of outbreaks

How do sources of outbreaks are identified



Horne S, Sibal B, Sibal N, Green HK. Cryptosporidium outbreaks: identification, diagnosis, and management. Br J Gen Pract. 2017 Sep;67(662):425-426. doi: 10.3399/bjgp17X692501. PMID: 28860302; PMCID: PMC5569737.

Genotyping

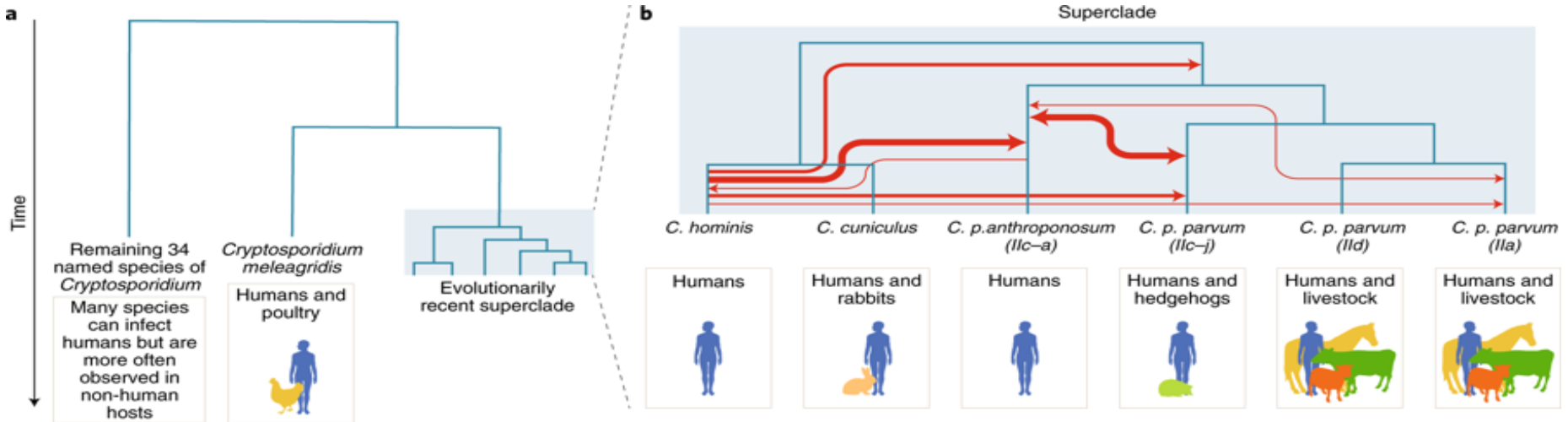
- Genotyping of Cryptosporidium can
  - Identify the Cryptosporidium species and subtypes that most commonly cause human cryptosporidiosis.
  - Identify the demographic and temporal-spatial distribution of common Cryptosporidium species and subtypes.
  - Identify the source of outbreaks and contamination.

CDC
CENTERS FOR DISEASE™
CONTROL AND PREVENTION

CryptoNet

Identify the correct Crypto species

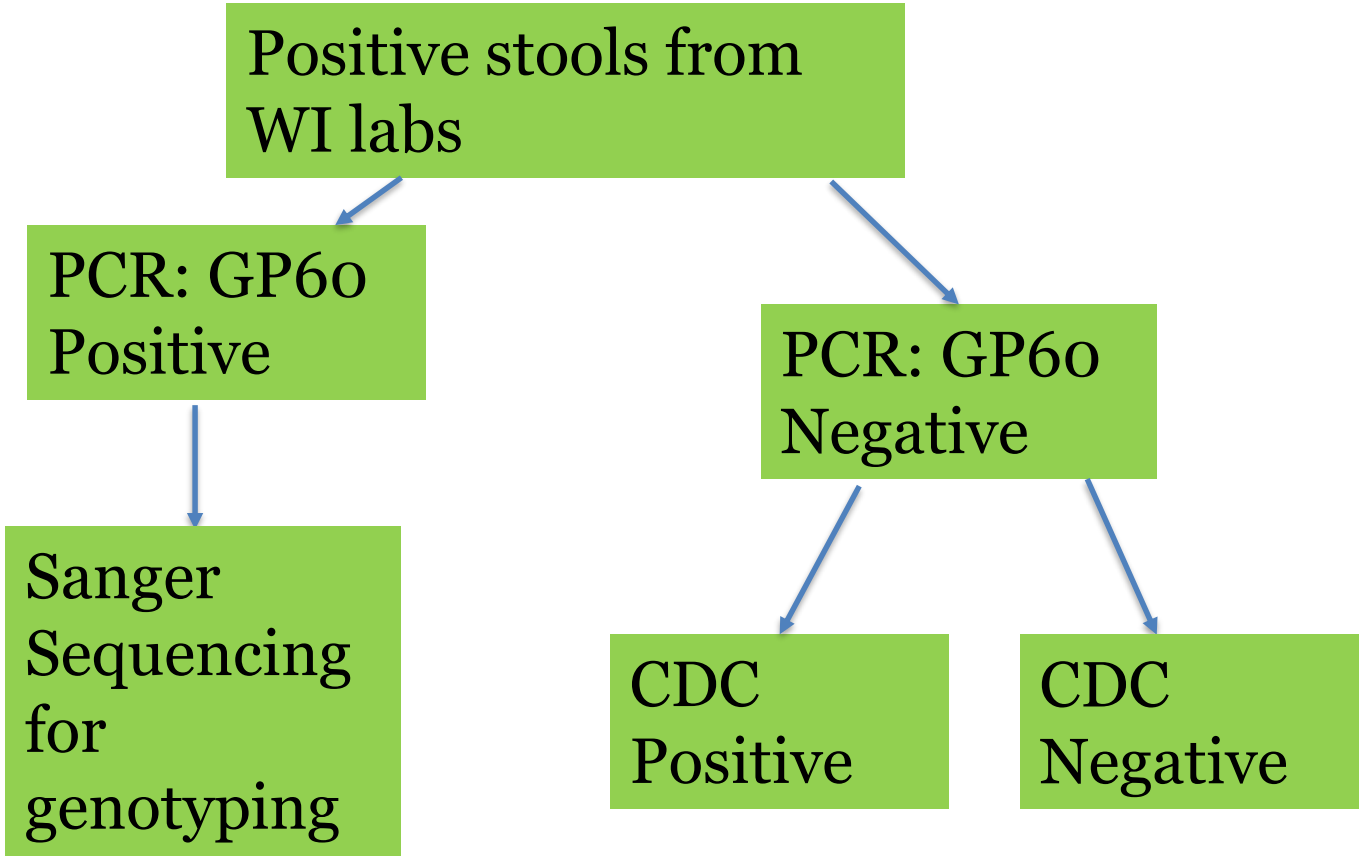
- CDC subtyping revealed that the infected individuals were infected with *Cryptosporidium hominis* subtype IfA12G1
- This strain was also isolated from patients during a recreational water associated Cryptosporidium outbreak in the North Shore Milwaukee area in the same year.



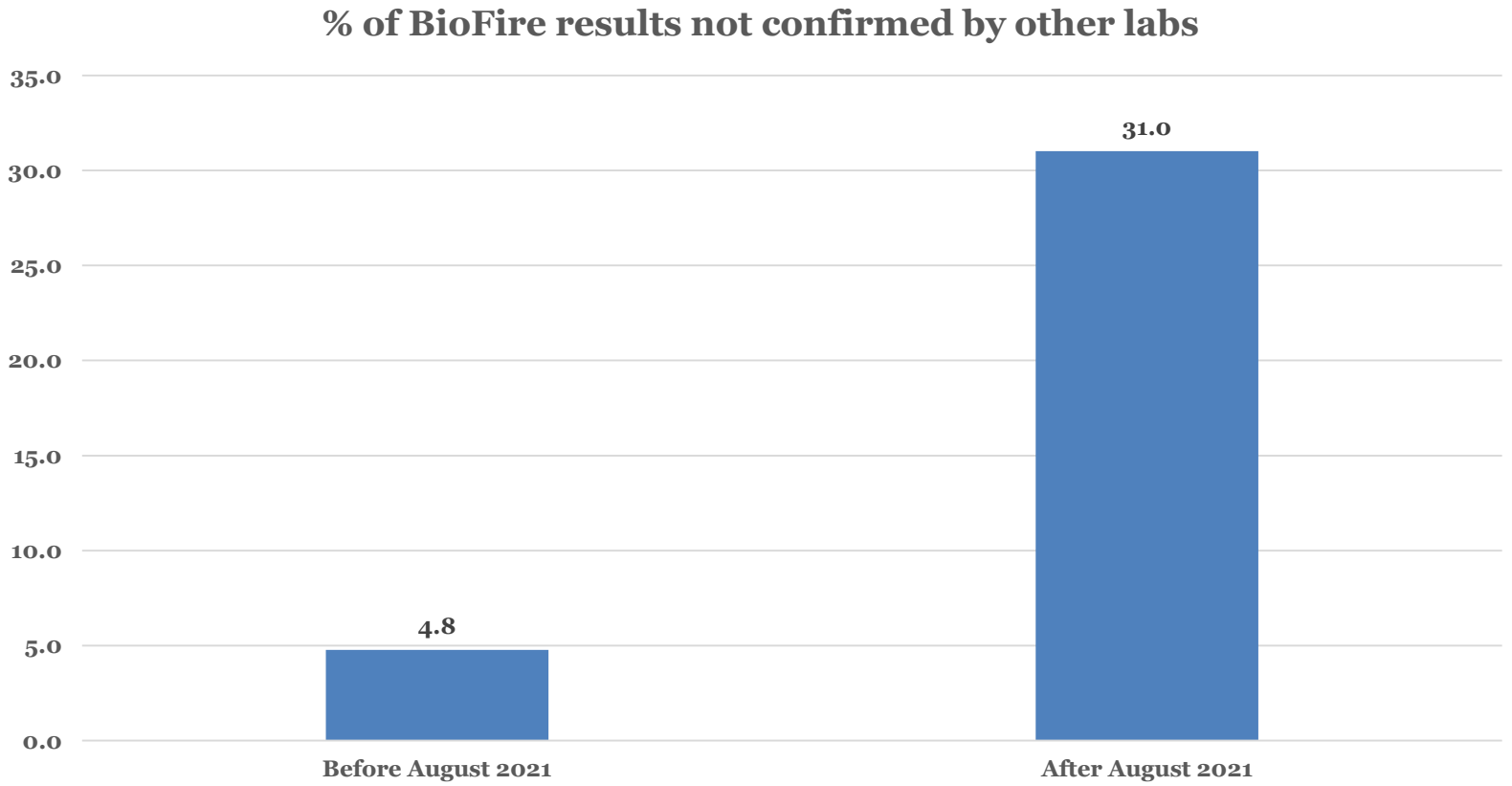
Sanger Sequencing

- Sanger sequencing is most ideal for short sequences and provides high accuracy.
- Sanger sequencing part of the gp60 gene is most commonly used for further discriminating *C. parvum* and *C. hominis* at WSLH.

WSLH genotyping pipeline



Importance of Cryptosporidium surveillance to identify issues.



- We are working closely with CDC, CryptoNet labs, and the manufacturer.
- The Biofire false positive discovery would not be possible without the networking of state public health labs and clinical labs.
- We would like the clinical labs to engage in conversations with the manufacturer (Cary-Blair lot#/Biofire GI kit lot#/raw Biofire run files).
- We can take Biofire positive samples from labs for fee-exempt confirmatory DFA testing.

NGS Sequencing

- Next generation sequencing (NGS) is a high-through put process that can rapidly sequence whole genomes of a species and allows us to study multiple regions of genome.

- NGS can be used to identity additional Cryptosporidium species and outbreak relatedness that could not be identified from Sanger sequencing.

- WSLH was chosen to CDC to validate Cryptosporidium NGS protocols.

Conclusion

- It is crucial for WI clinical labs to maintain good Cryptosporidium surveillance.
- The partnership of WSLH and WI clinical labs, and the voluntary submission of Cryptosporidium positive specimens for genotyping at WSLH, are a valuable asset to Cryptosporidium case and outbreak surveillance in Wisconsin.

Please send us more formalin-free residual stools for sequencing!

Questions?