

Athlone, Ireland; October 28, 09

Evolving Epidemiology of *Cryptosporidium*

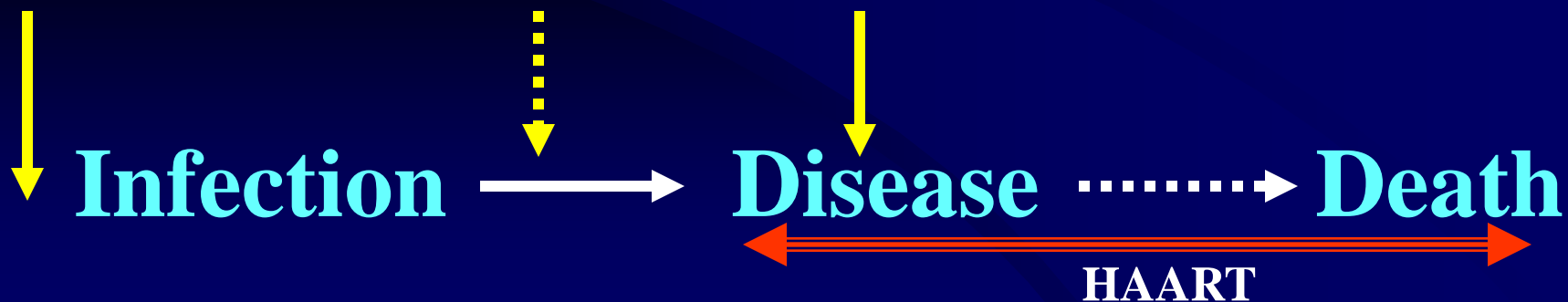
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Johns Hopkins Center for Water and Health

Department of Molecular Microbiology and Immunology

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA



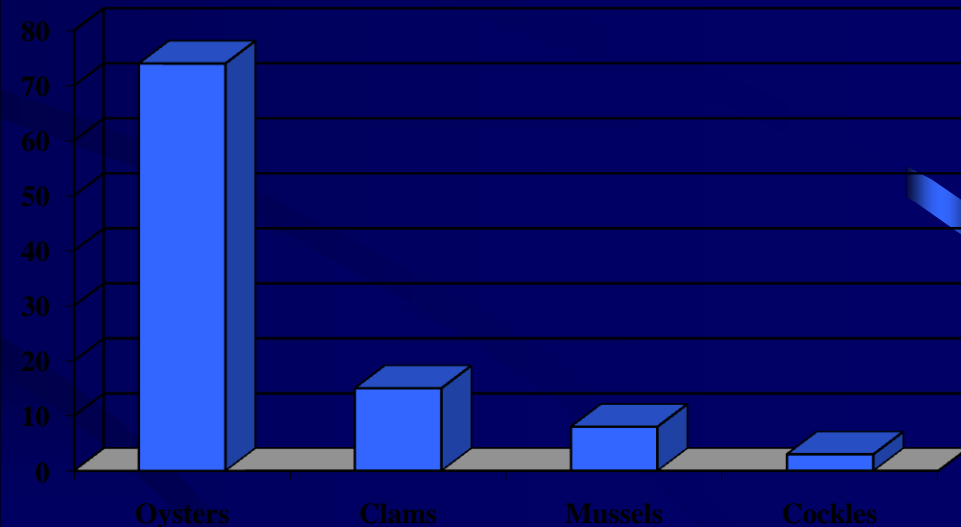
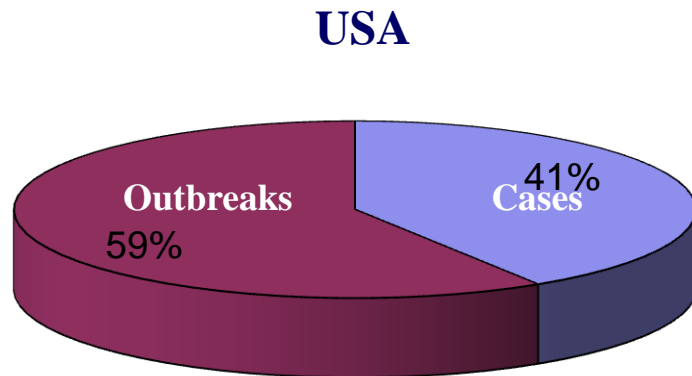
Contamination Sources for Illnesses Vectored by Molluscan Shellfish (USA)

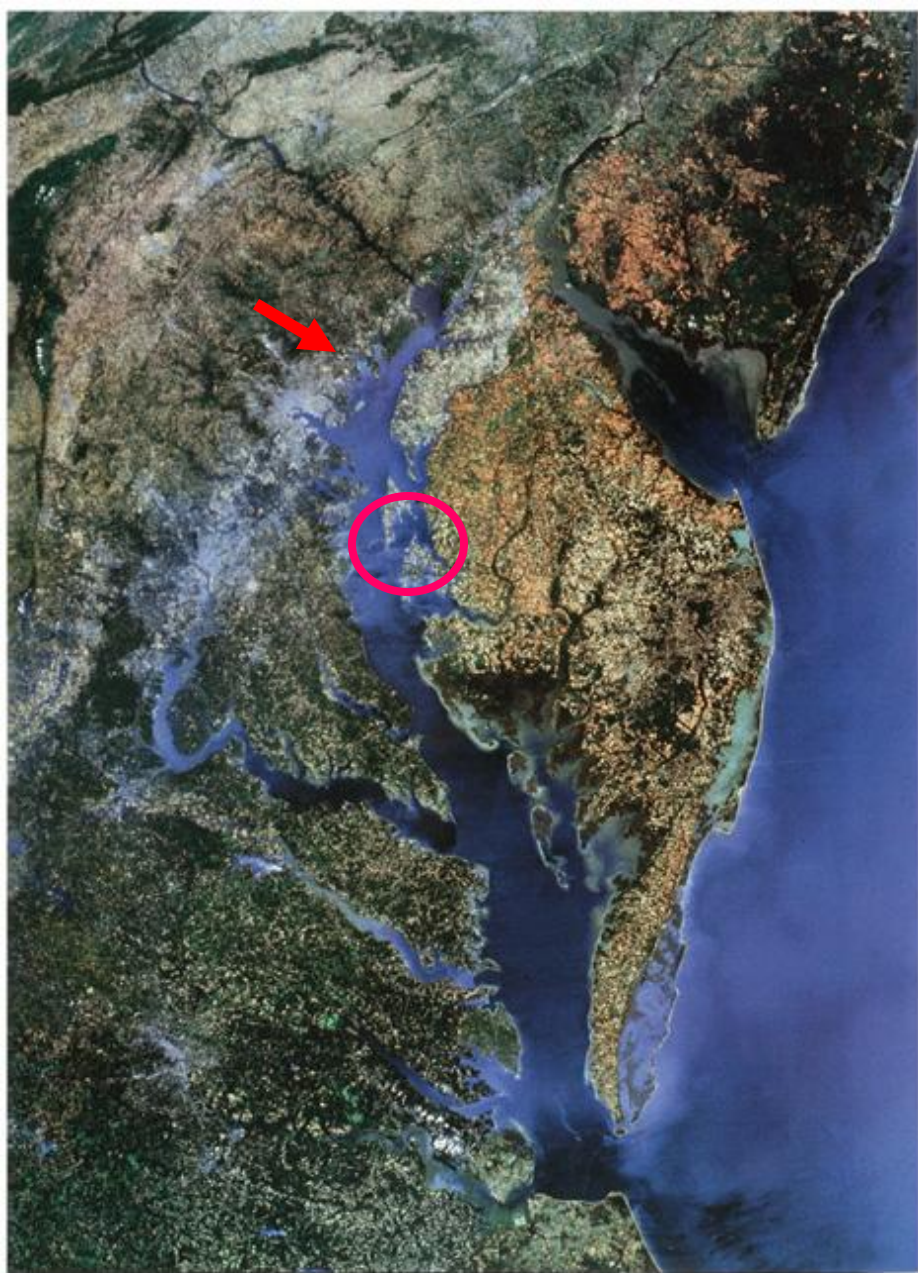
[Http://www.cdc.gov/epo/mmwr/preview/mmwrhtml](http://www.cdc.gov/epo/mmwr/preview/mmwrhtml)

- 65%** • Pathogens associated with sewage disposal, wastewater effluents, overboard disposal, urban and agricultural runoff
- 28%** • Agents indigenous to coastal waters
- 7%** • Post-harvest contamination

% outbreaks; worldwide; 1996 - 2008

Unknown Etiology

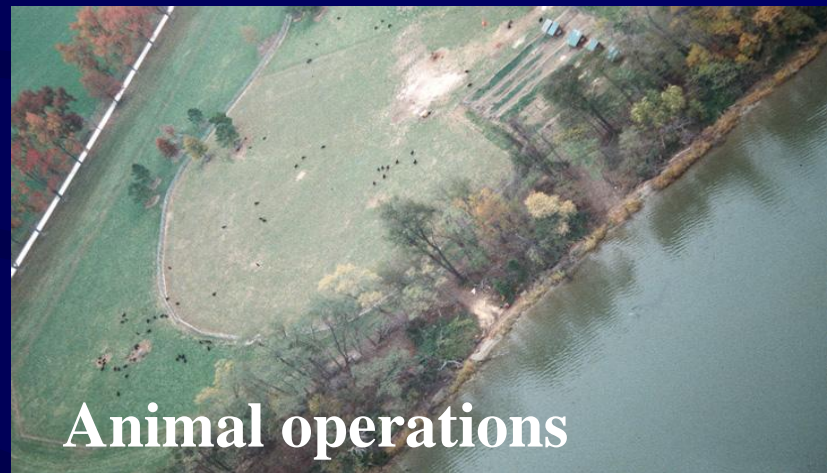




CHESAPEAKE BAY
A Satellite View



Septic tanks

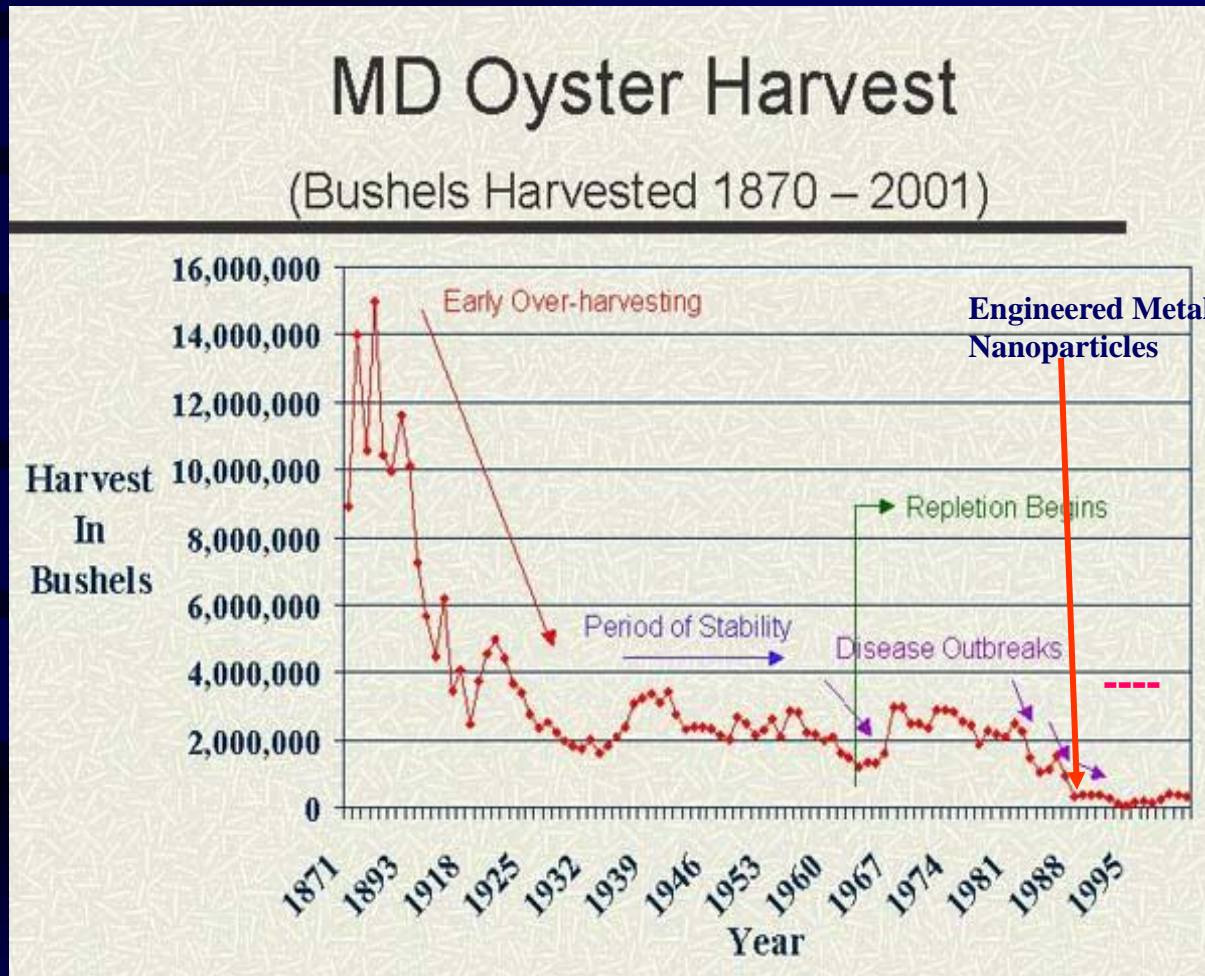


Animal operations



Wastewater discharges

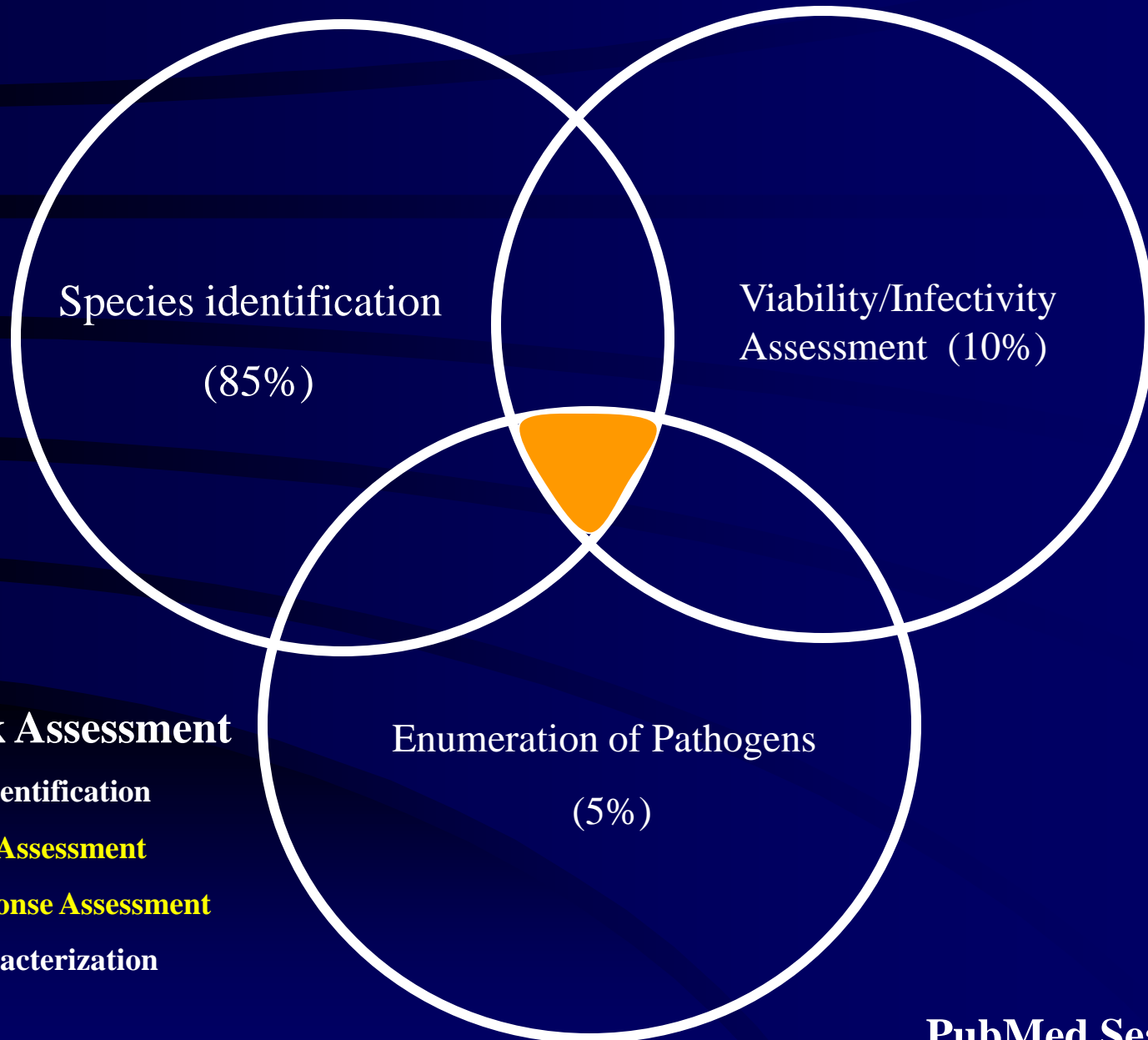
Cheesepiooc = “Great Shellfish Bay”



(MSX and Dermo)

Presently, the Chesapeake Bay supports only 1% of its historic native oyster populations!

“Either Fish or Aquarium” concept

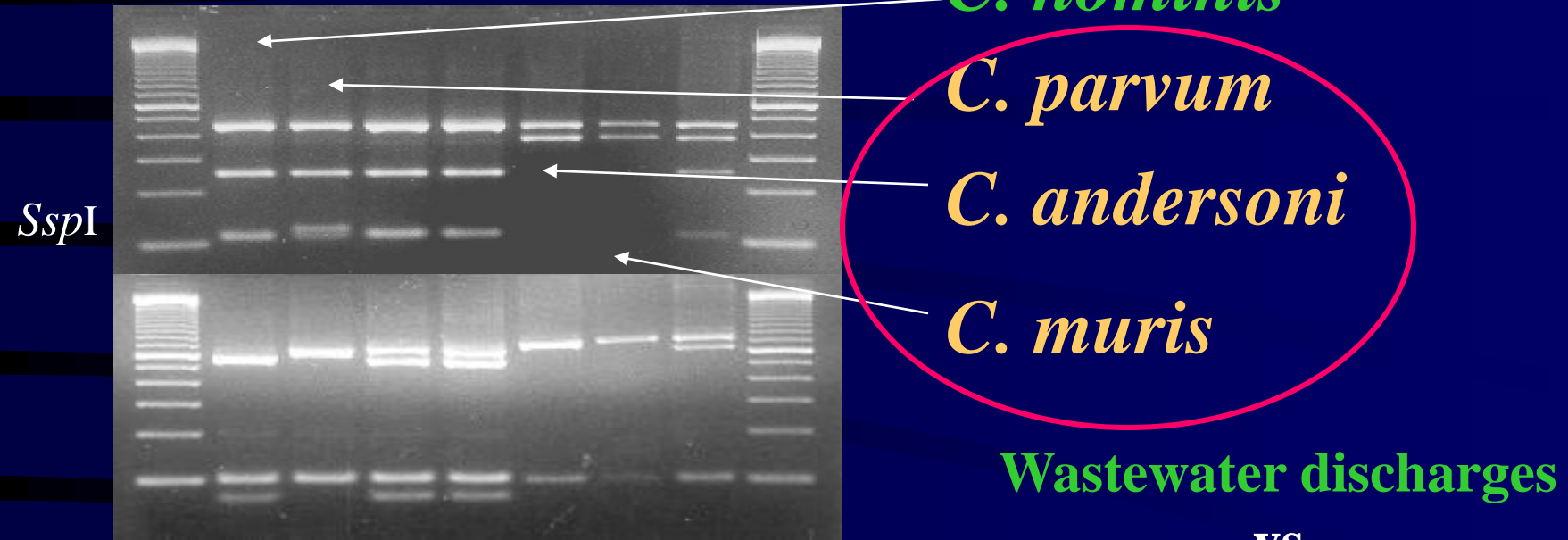


Risk Assessment

1. Hazard Identification
2. Exposure Assessment
3. Dose-response Assessment
4. Risk Characterization

Identification of *Cryptosporidium*; PCR, RFLP

A



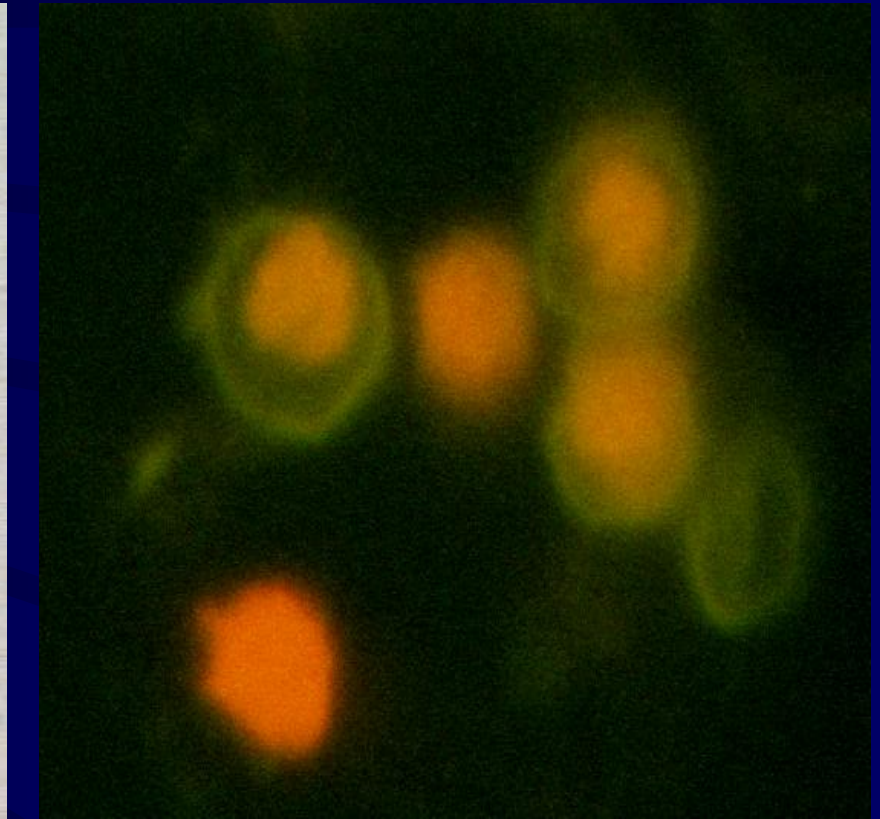
VS

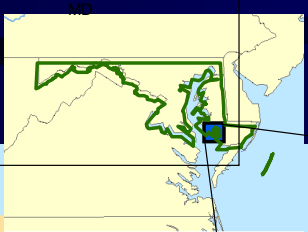
Agricultural runoff

B

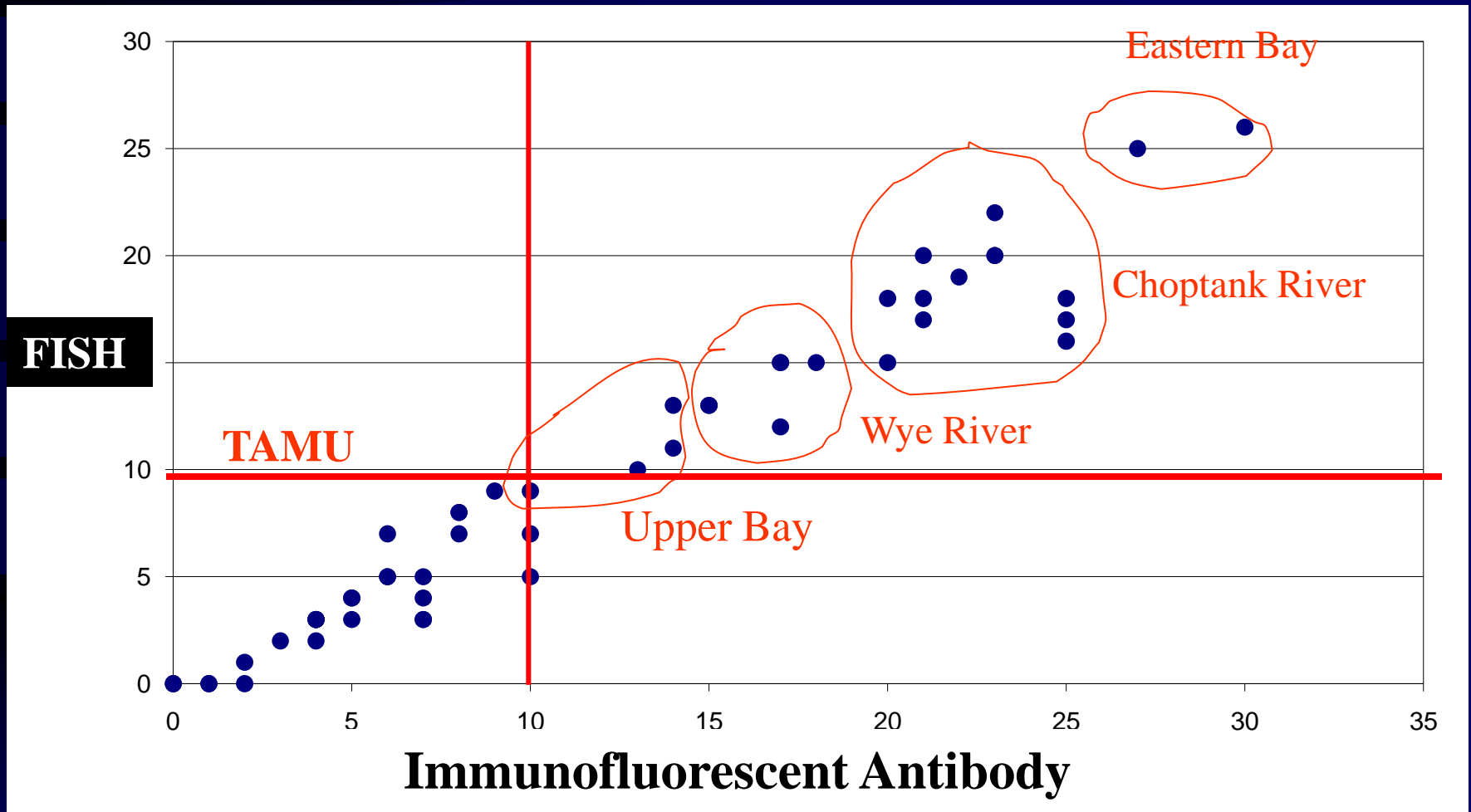


The Graczyk's Lab: Fluorescence *In Situ* Hybridization (FISH)





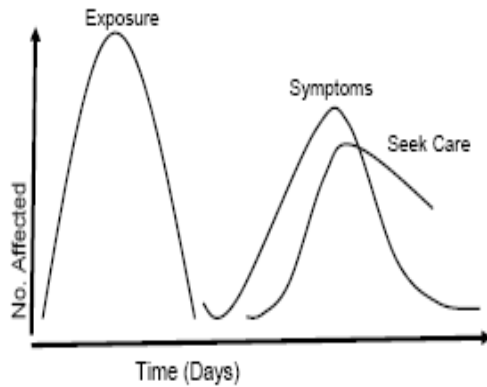
Cumulative number of viable *C. parvum* oocysts found in six oysters



Bars, 20 of 54 (37%)

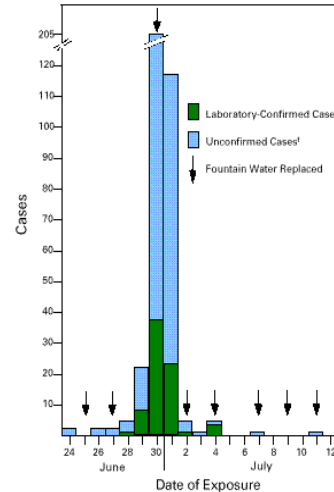
Meals 130 of 324 (40%)

Infectious Disease Outbreak



Center for Food Security and Public Health
Iowa State University - ISU

FIGURE 1. Reported cases of cryptosporidiosis associated with a water sprinkler fountain, by date of exposure — Minnesota, 1997*



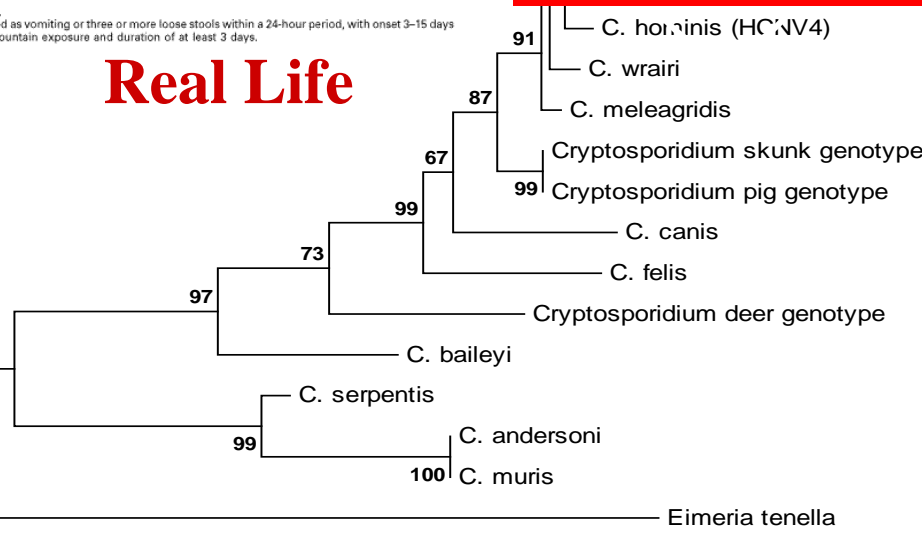
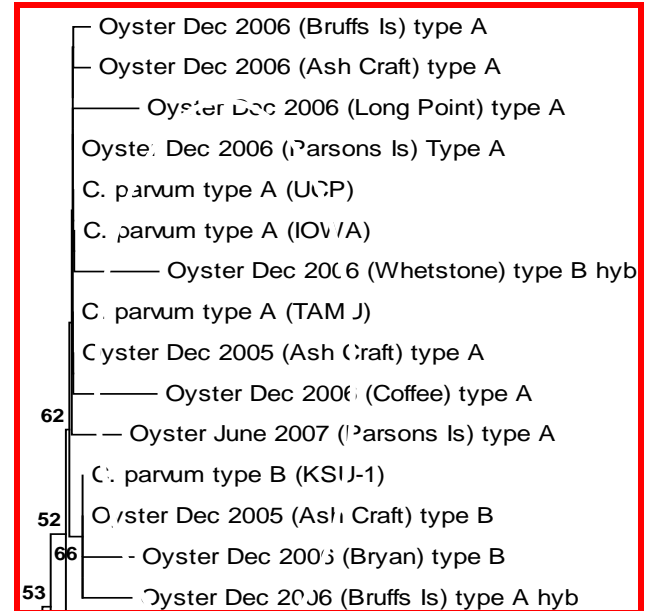
*n=369.

†Defined as vomiting or three or more loose stools within a 24-hour period, with onset 3–15 days after fountain exposure and duration of at least 3 days.

Common Sense

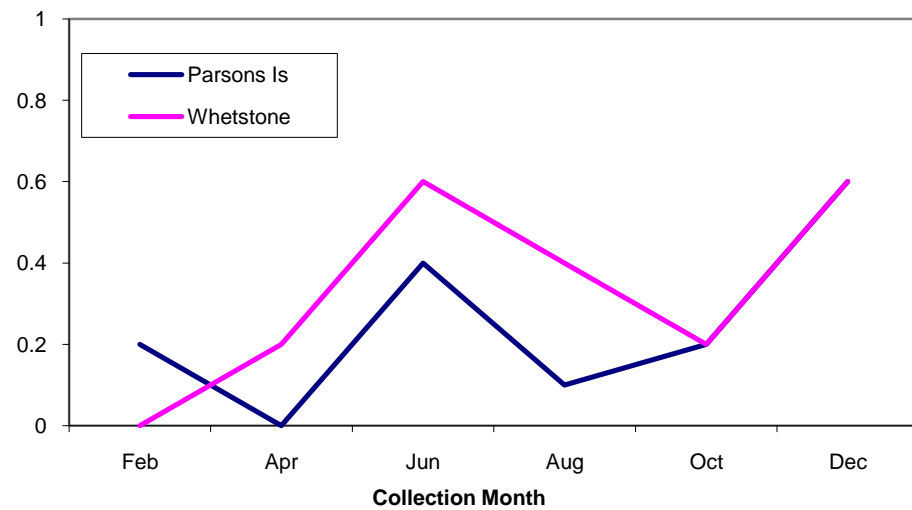
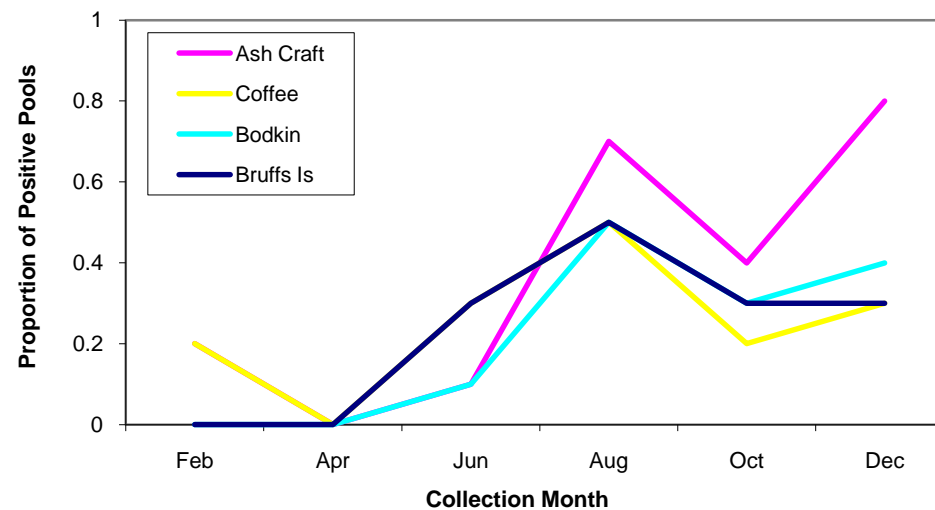
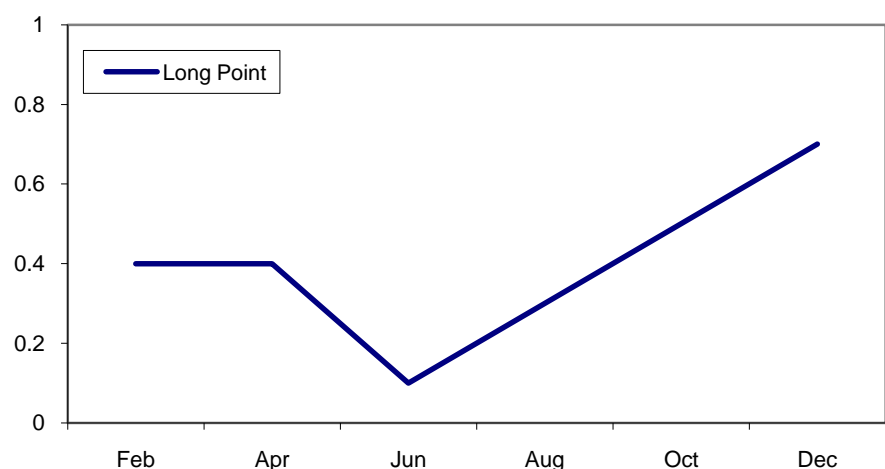
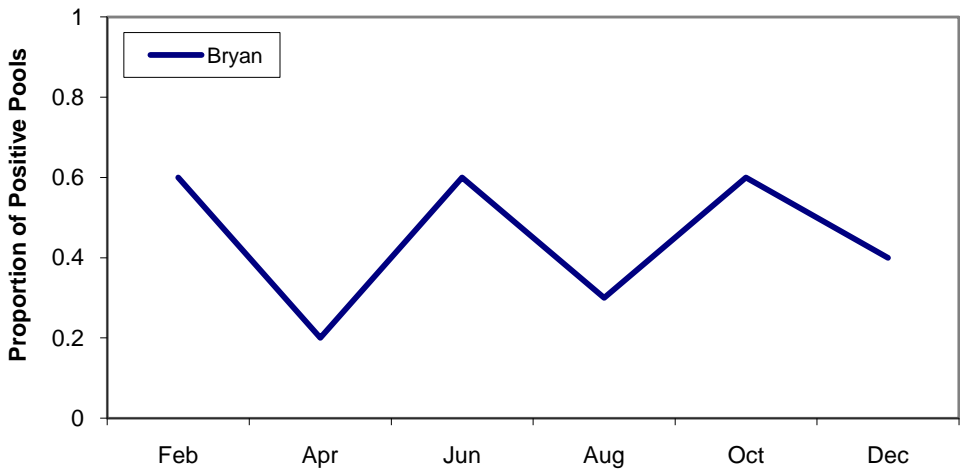
Real Life

Genotype



0.005

Neighbor-joining phylogenetic tree based on evolutionary distances between sequences calculated using the Tamura-Nei method



SEASONAL TRENDS OF OYSTER CONTAMINATION PREVALENCE OVER A TWO-YEAR COLLECTION PERIOD

Incidence of Cryptosporidiosis

reported by the

Foodborne Diseases Active Surveillance Network (FoodNet)

Dietz et al., 2000; Wallace et al., 2000

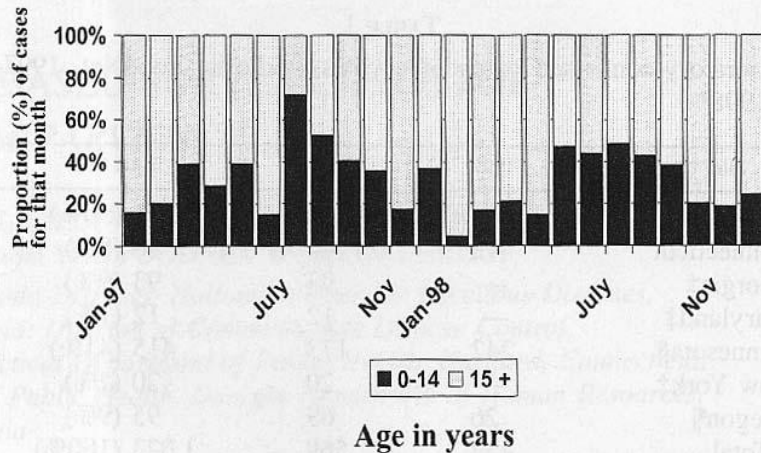


FIGURE 4. Age distribution for each month of surveillance for cases of cryptosporidiosis by month of collection. Collected by FoodNet, 1997–1998.

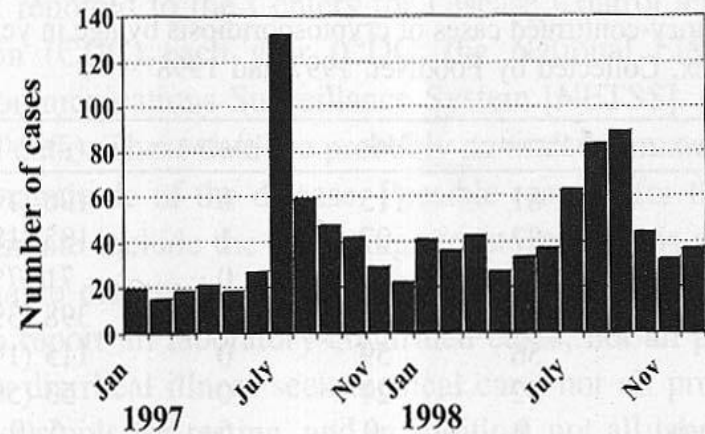
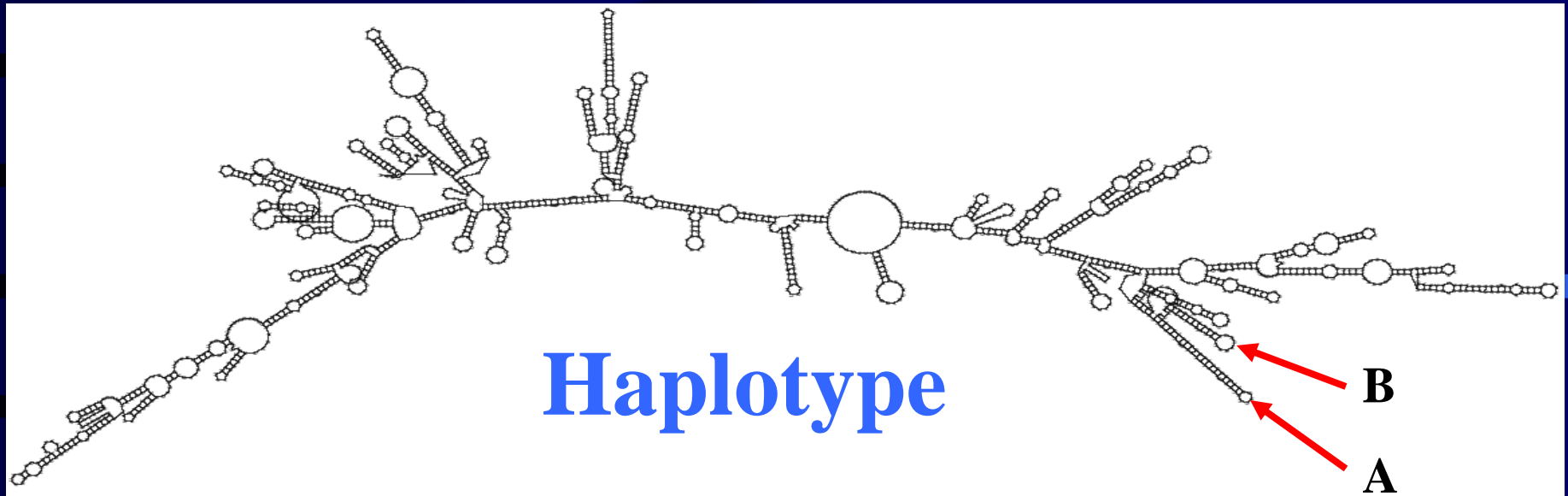
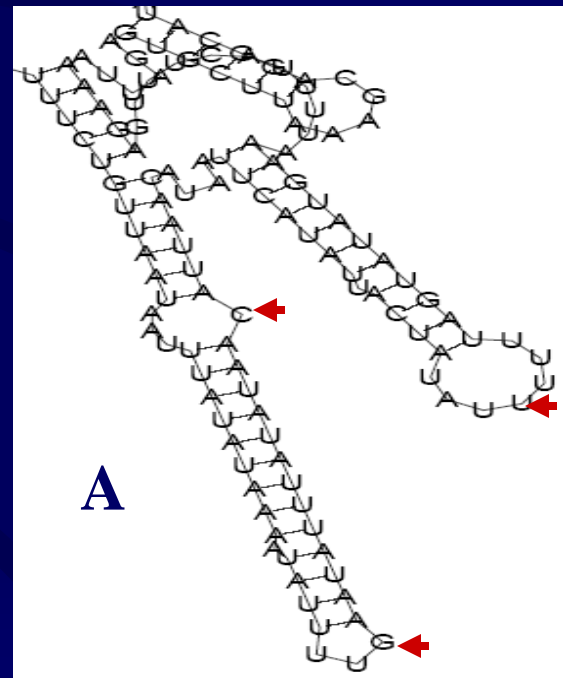
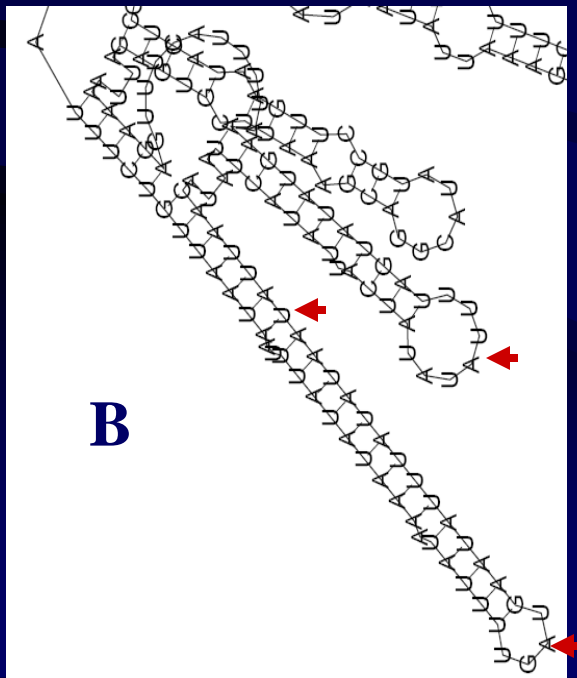


FIGURE 3. Number of laboratory-confirmed cases of cryptosporidiosis by month of collection. Collected by FoodNet, 1997–1998.

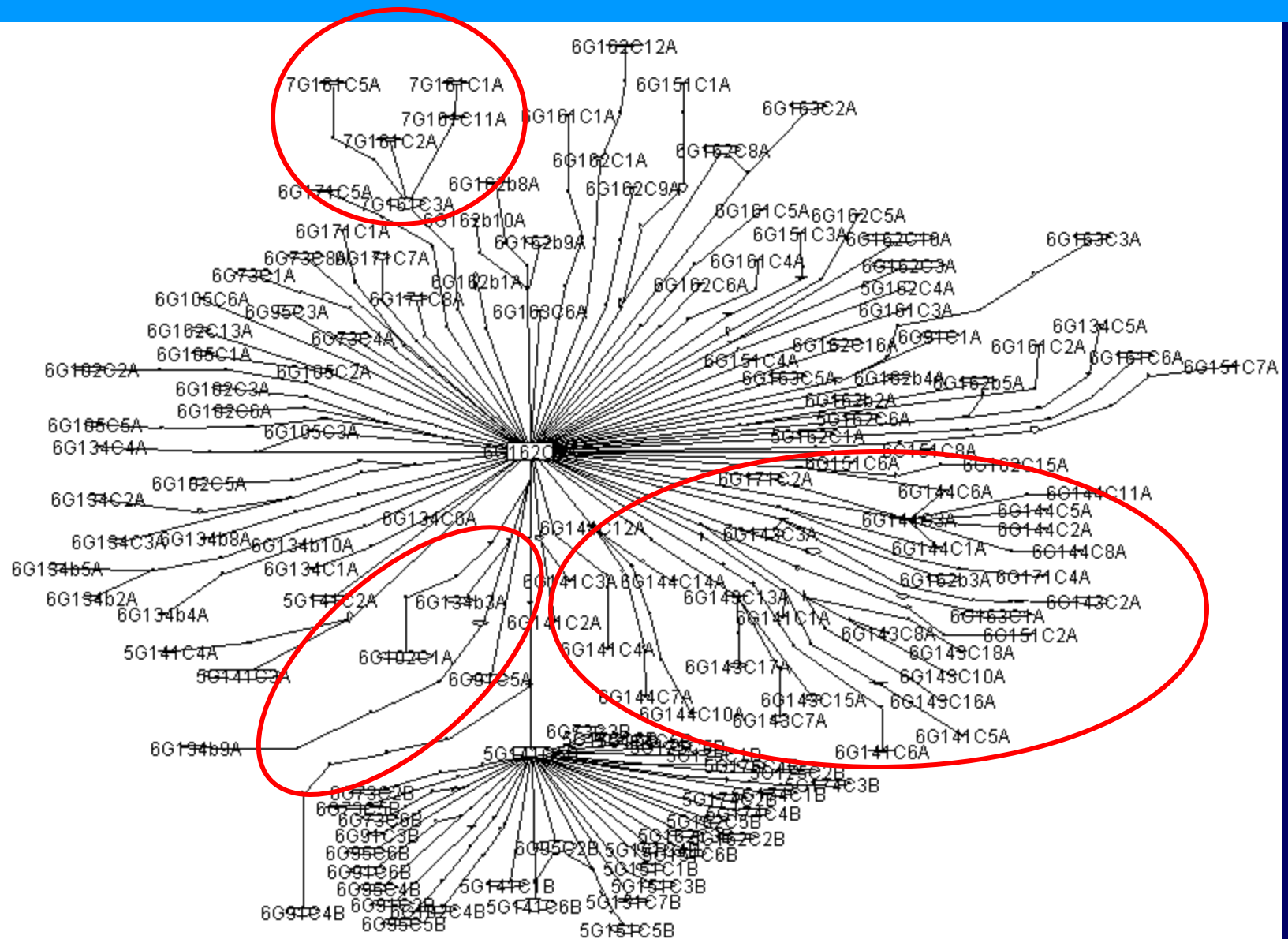
...“Data reported here suggest a seasonal trend *C. parvum* infections. This has been observed at the national level as well (CDC, unpublished data)... Dietz et al., 2000 *Am J Trop Med Hyg* 62(3), 368



Predicted secondary structure of the complete SSUrRNA molecule from *C. parvum*. The loops containing the A-versus B-type defining residue indicated by arrows.



Haplotype Networks – Exploratory analysis

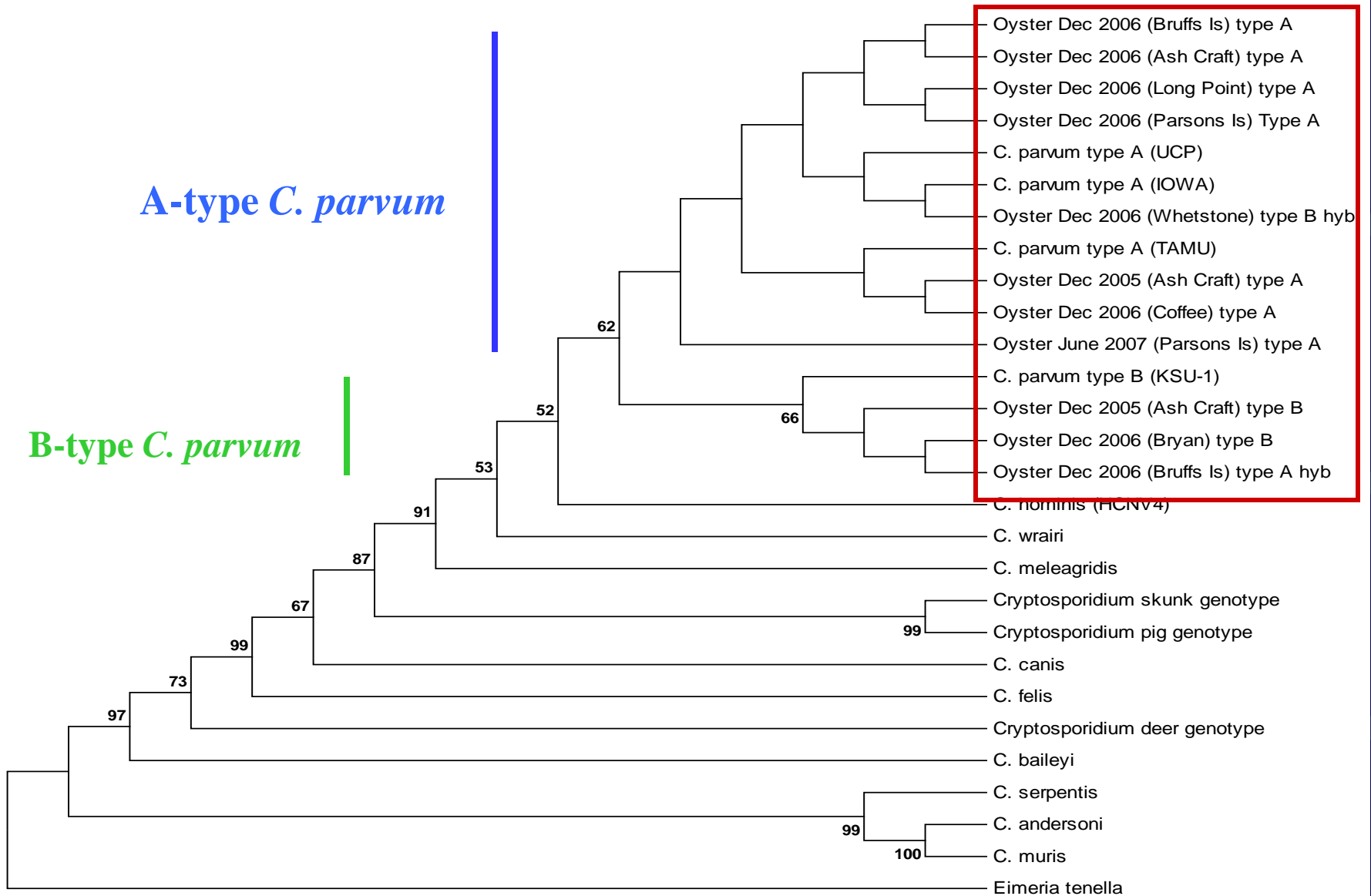


A

B

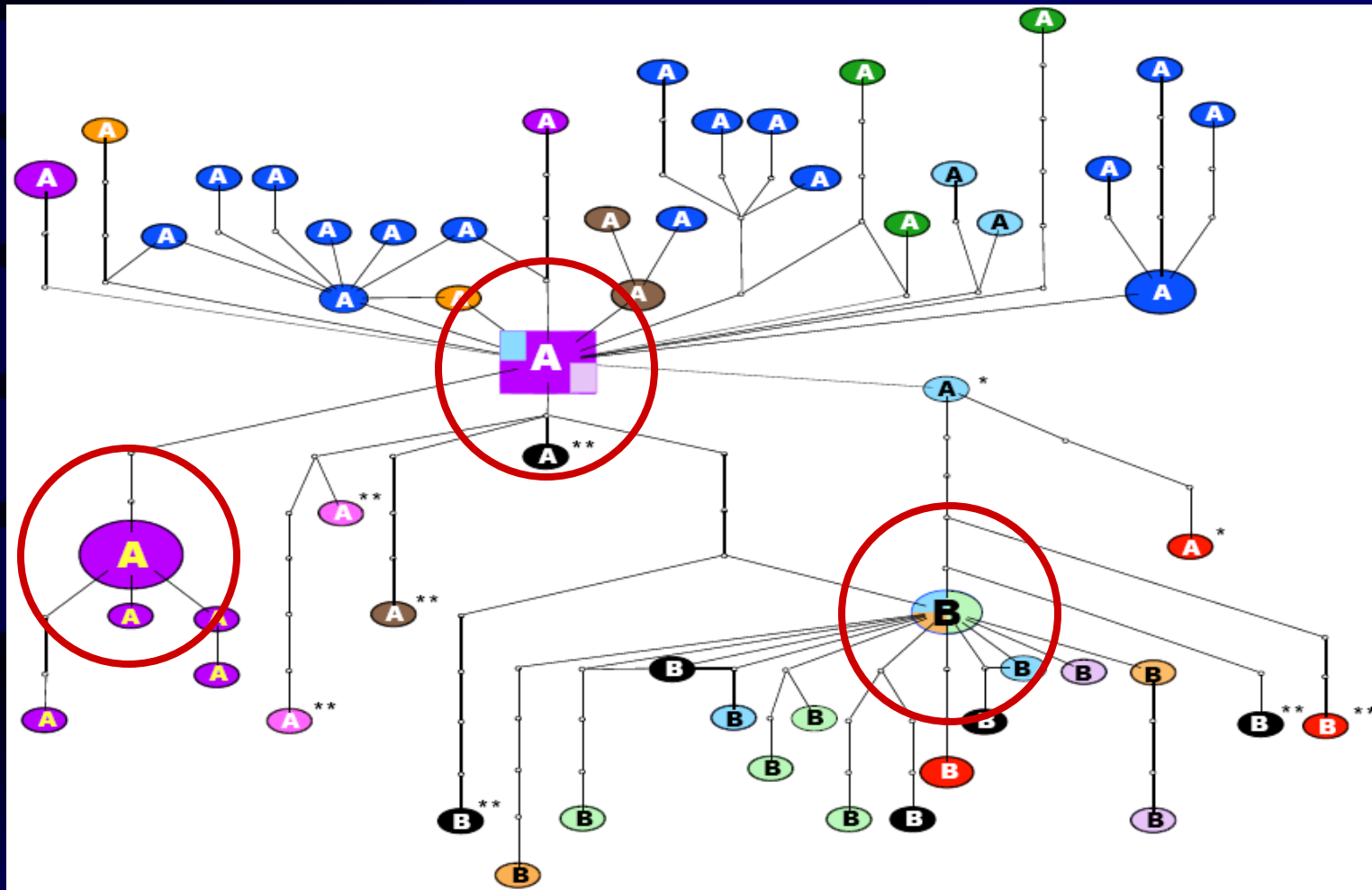
A-type *C. parvum*

B-type *C. parvum*



Neighbor-joining phylogenetic tree without genetic distance to show tree topology

AMOVA shows sequence differences by oyster collection date contribute to total variation



Statistical parsimony haplotype network with 95% connection limits constructed from a representative subset of *Cryptosporidium* SSU rRNA sequences from 8 oyster bars and three collection times

Precipitation and specific conductance are significantly associated with *Cryptosporidium* contamination

Precipitation:

- Known to affect *Cryptosporidium* levels in water and oysters due to surface runoff
- Lags are important
- Time for transport of oocysts to oyster beds
 - Low sedimentation rates

Specific conductivity:

- Strongest association with contamination
- Measure of ionic content
- Indicator of freshwater input
- Sources of freshwater
 - Precipitation runoff
 - Irrigation water runoff
 - Groundwater intrusion
 - Wastewater discharges
 - Freshwater Influx from rivers

Conclusions from environmental surveillance study

- ~35% of oyster pools contaminated with *Cryptosporidium* and estimated prevalence ~7%. Only *Cryptosporidium parvum* identified.
- Seasonal but no spatial variation in contamination prevalence, with highest risks in December and August. December is in the middle of oyster harvesting season.
- Precipitation and other freshwater inputs are risk factors for *Cryptosporidium* contamination of oysters
- Proximity to known potential sources like cattle farms and sewage outfalls are significant risk factors and indicate need for targeted prevention efforts
- Monitoring of water for fecal coliforms is not sufficient to indicate the presence of human waterborne pathogens
- Oysters can cause cryptosporidiosis if consumed raw

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**WHO 2008; 2.3 million children under 5 yr of age die
from diarrhea-related causes**

