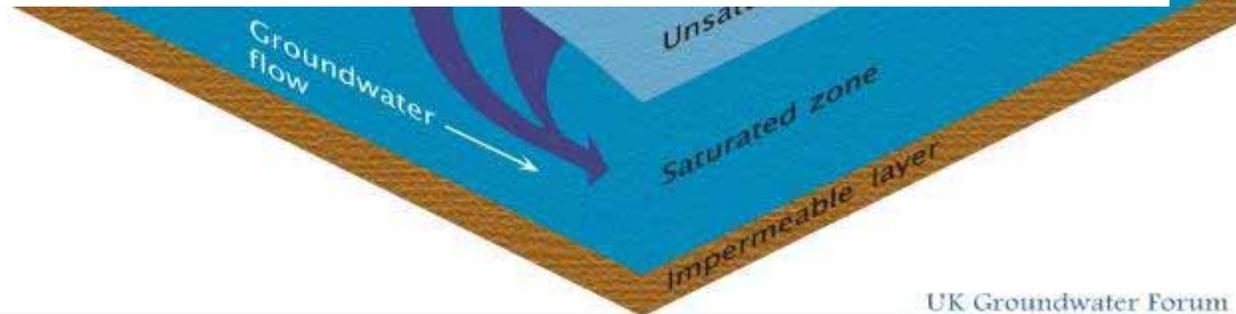


SOURCE TRACKING AND MICROBIAL WATER QUALITY IN THREE FLORIDA SPRINGS

Dale Griffin and Mike Gray
USGS, St. Petersburg, FL

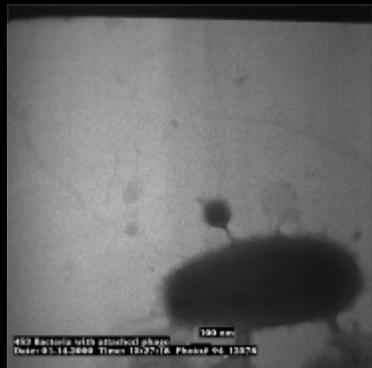
The Problem

- The susceptibility of Florida ground waters to recharge-borne microorganisms and pollutants (nutrients, herbicides, pesticides, hydrocarbons, etc.)
- Rising nitrate levels in Florida's springs
- The need to determine the source of pollutants
 - Agricultural versus residential – waste water, fertilizer, hydrocarbons, pesticides, etc.
 - Human versus animal (agricultural and indigenous)



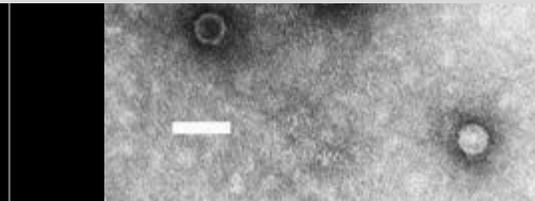
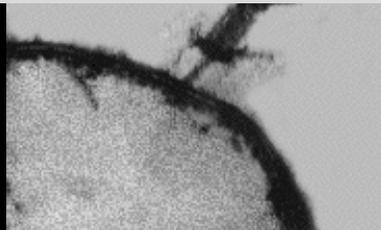
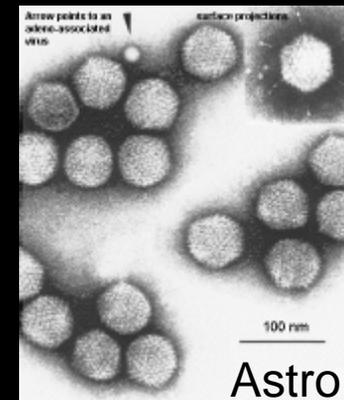
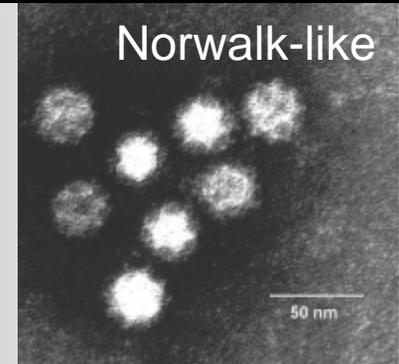
Enter the Virus

Bacteriophage



- Many viruses are host specific and within a host only infect specific types of tissue
- Host specific viruses can not replicate outside of their host – thus when they are found in the environment it means that its host is impacting that environment.
- Molecular techniques are available to detect low concentrations of viruses in water (Reverse transcriptase – Polymerase chain reaction, RT-PCR).

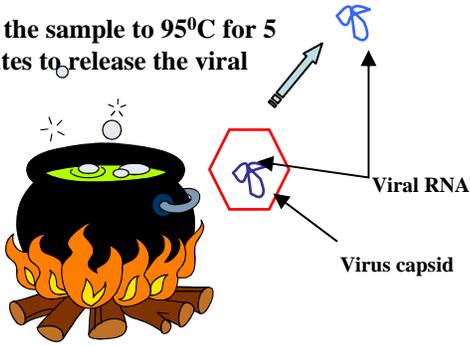
Animal viruses



Reverse Transcriptase - Polymerase Chain Reaction

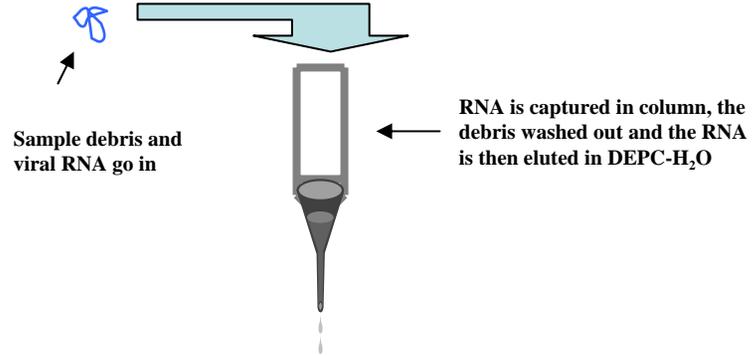
I

Heat the sample to 95°C for 5 minutes to release the viral RNA



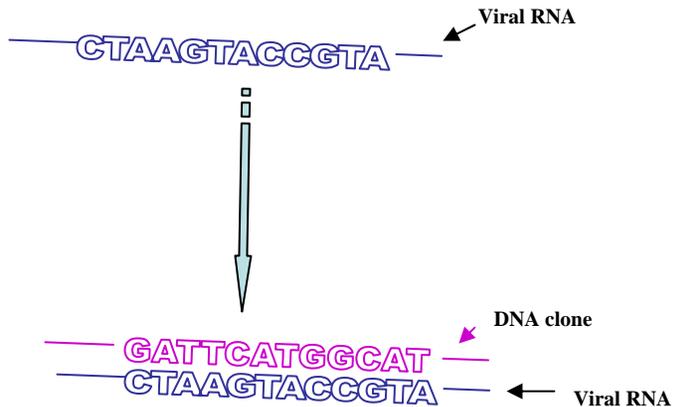
II

Use Qiagen Rneasy Kit to isolate the released viral RNA (column capture via centrifugation)



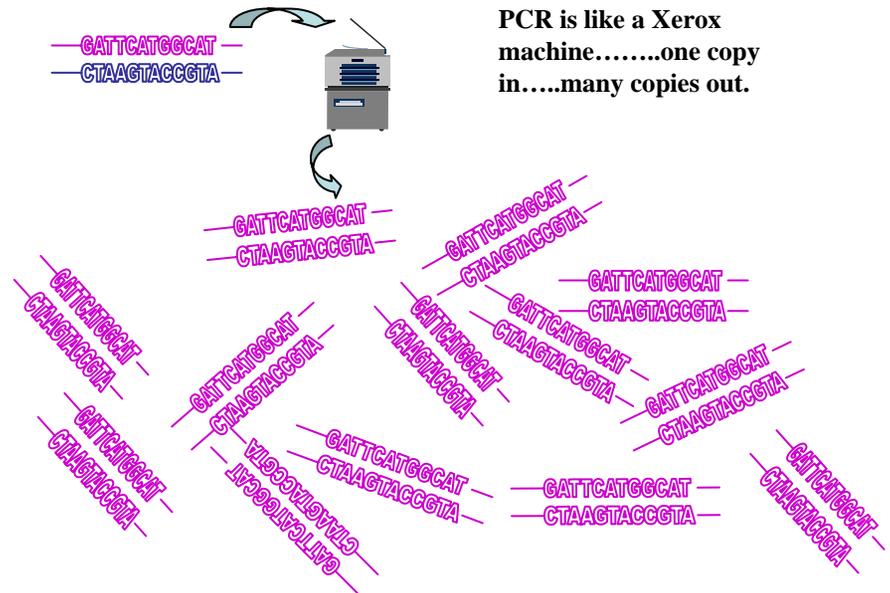
III

Use reverse transcriptase to make a DNA copy of the viral RNA genome

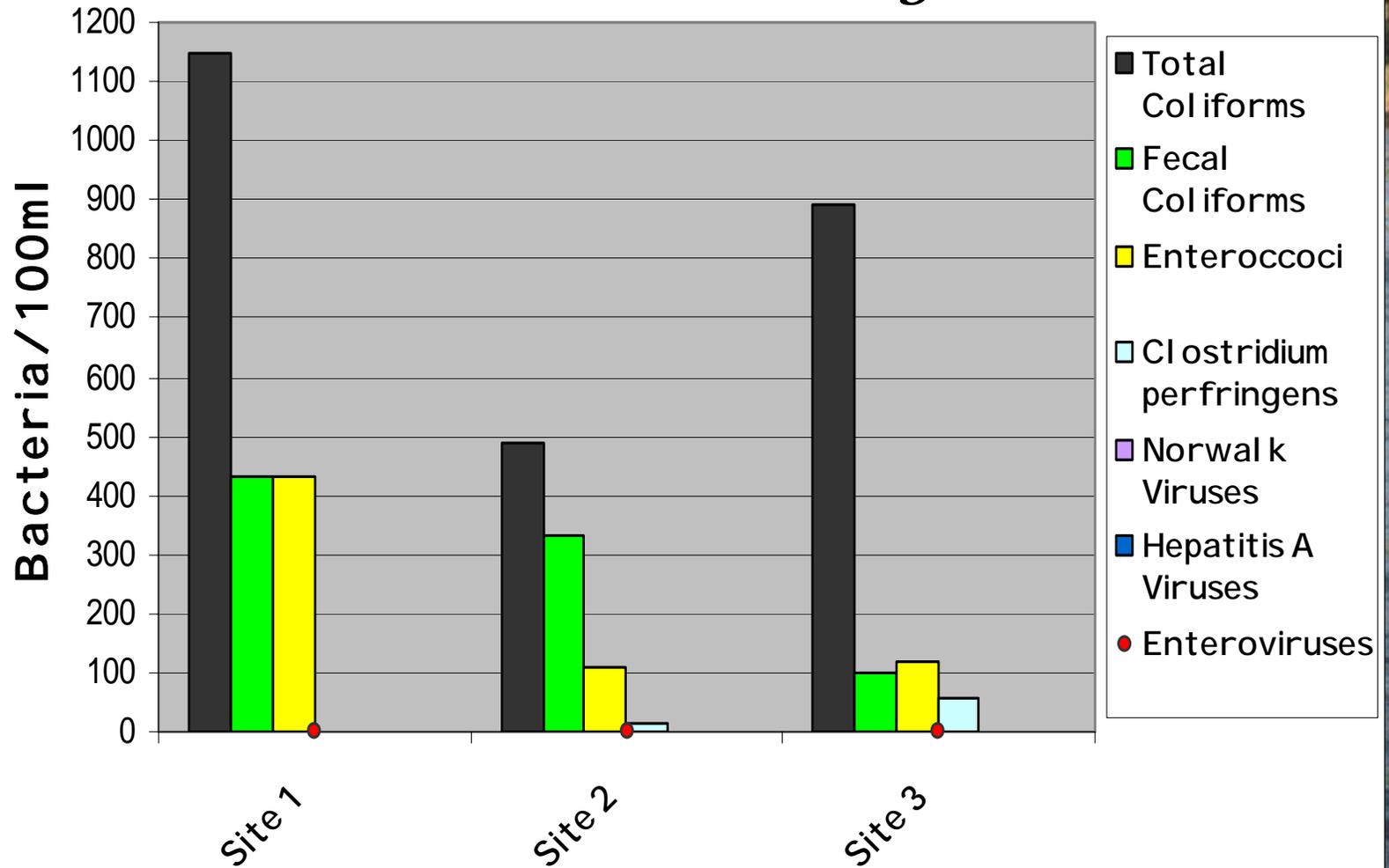


IV

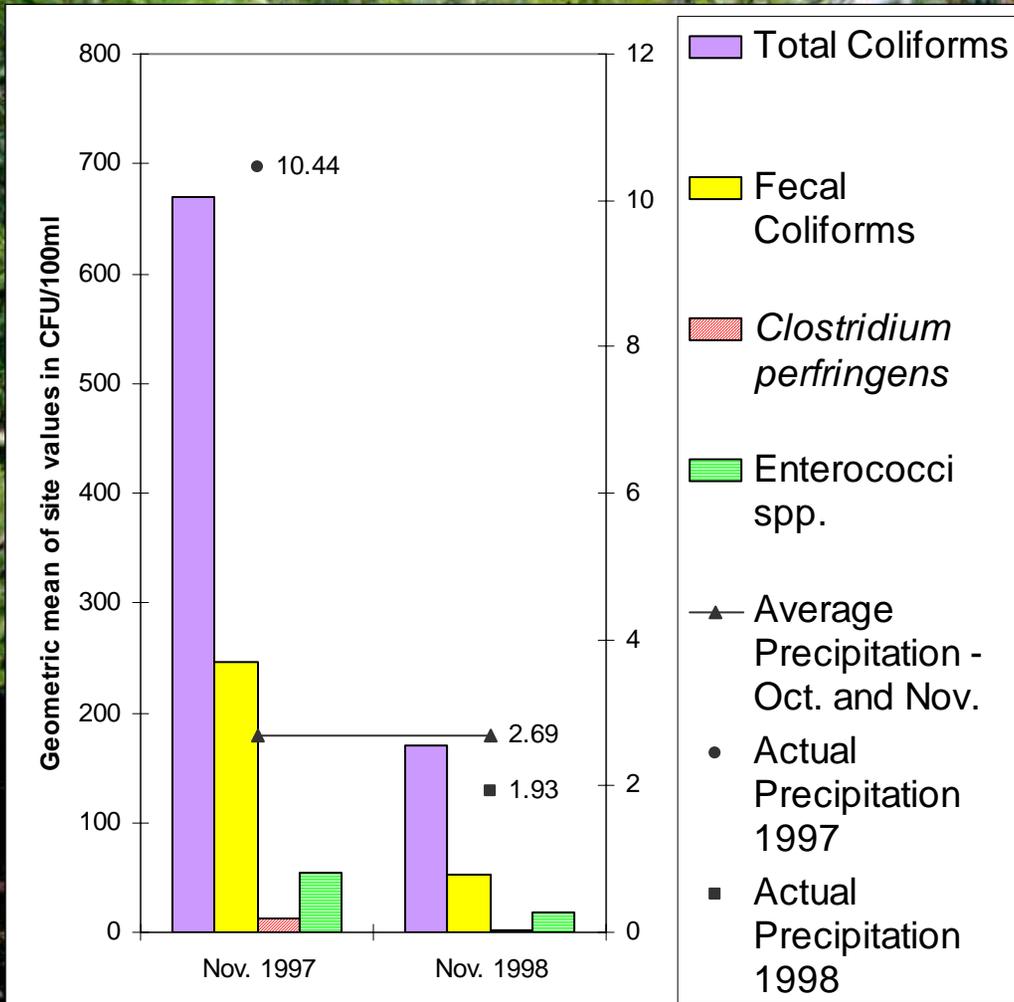
PCR is like a Xerox machine.....one copy in.....many copies out.



Chassahowitzka Monitoring Data

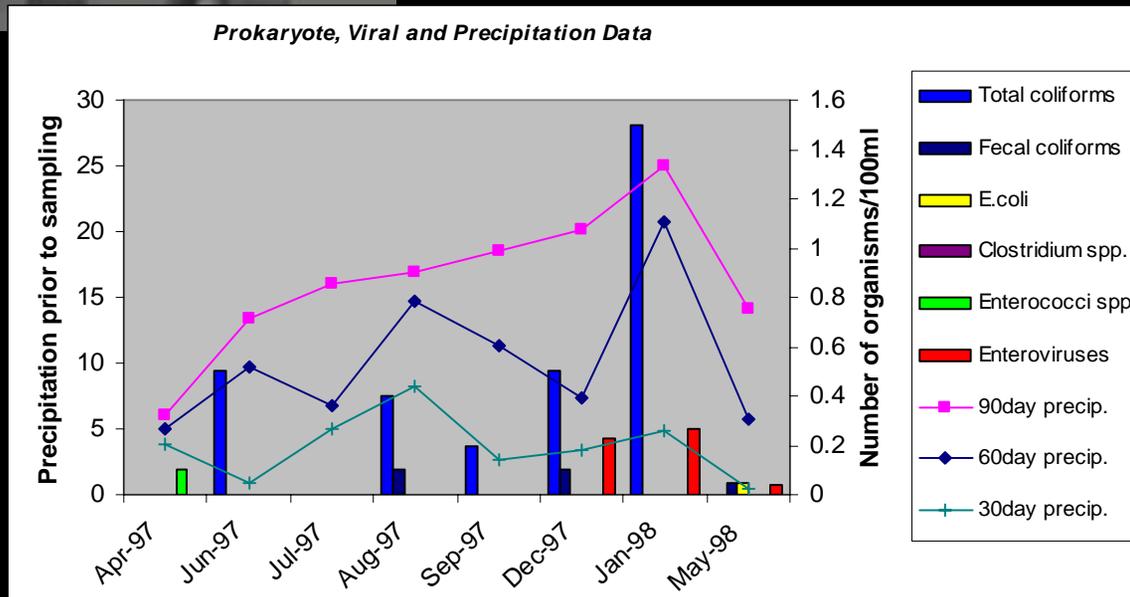


Homosassa Springs





Mystery Spring



Average spring vent bacteria count/ml = 5,870
 Average spring vent virus count/ml = 29,900

Table 1. Microbiology data (spring sample data for 9-25-03)

Spring	pH/Temperature-°C/EC-µS/TDS-ppm	Indicator Data – Colony Forming Units/100ml (duplicate average)				Virus Polymerase Chain Reaction data (Equivalent volume assayed)			
		Enterococci	<i>Clostridium perfringens</i>	Fecal coliforms	<i>E.coli</i>	Enteroviruses	Hepatitis A viruses	Adenoviruses	Cattle enteroviruses
Hornsby	6.9/22.8//298/150	0	0	0.2	0.1	- (0.04L)	-	-	-
Poe	7.4/-/287/143	0.1	0	0	0	- (0.5L)	-	-	-
Manatee	7.3/-/329/164	0.2	0	0.2	0.1	- (0.1L)	-	-	-

Table 2. Microbiology data (spring sample data for 12-17-03)

Spring	pH/Temperature-°C/EC-µS/TDS-ppm	Indicator Data – Colony Forming Units/100ml (duplicate average)				Virus Polymerase Chain Reaction data			
		Enterococci	<i>Clostridium perfringens</i>	Fecal coliforms	<i>E.coli</i>	Enteroviruses	Hepatitis A viruses	Adenoviruses	Cattle enteroviruses
Hornsby	7.1/22.0/271/131	0.2	0	0	0	- (0.3L)	-	-	-
Poe	7.1/22.1/266/133	0	0	0	0	- (0.3L)	-	-	-
Manatee	7.0/22.0/311/155	2.0	0	4.7	4.7	- (0.3L)	-	-	-

Table 3. Microbiology data (spring sample data for 4-20-04)

Spring	pH/Temperature-°C/EC-µS/TDS-ppm	Indicator Data – Colony Forming Units/100ml (duplicate average)				Virus Polymerase Chain Reaction data			
		Enterococci	<i>Clostridium perfringens</i>	Fecal coliforms	<i>E.coli</i>	Enteroviruses	Hepatitis A viruses	Adenoviruses	Cattle enteroviruses
Hornsby	7.2/22.5/276/139	1.2	0	3.0	3.0	+ (0.5L)	-	-	-
Poe	7.2/22.5/273/137	0.1	0	4.5	4.5	++ (0.5L)	-	-	-
Manatee	7.1/22.5/320/158	0.4	0	14.7	14.7	+++ (0.5L)	-	-	-

SRWMD District rain data – Sept. 03, actual 3.17 (avg. 5.46) – Manatee 2.36
 Dec. 03, actual 1.43 (avg. 3.17) – Manatee 1.23
 May 04, actual 1.69 (avg. 3.39) – Manatee 3.53

+ = light signal via genetic probe/dot blot
 ++ = medium signal via genetic probe/dot blot
 +++ = heavy signal via genetic probe/dot blot

Conclusions

- Surface associated waste water and pollutants can impact Florida's ground water
- Human viruses have been detected in Florida ground water and emanating from spring vents
- Host specific virus assays are a useful tool in determining sources of pollutants
 - Agricultural versus residential
 - Human versus animal