

CryptoNet: A DNA sequence and RFLP-based molecular surveillance network for cryptosporidiosis

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Microbiologist

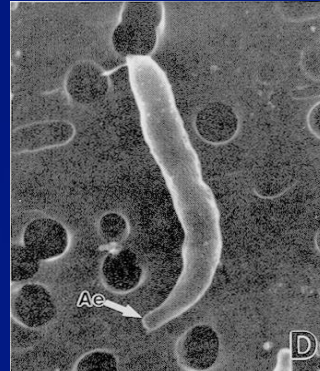
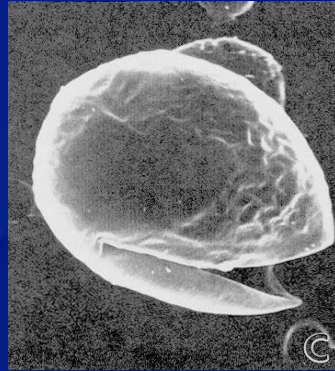
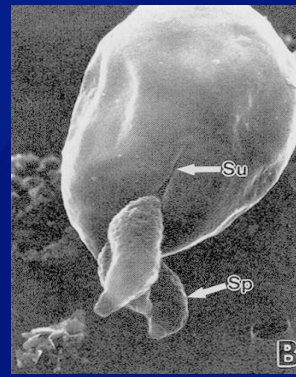
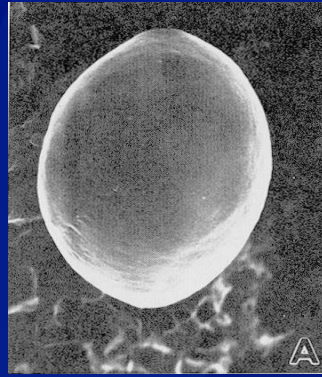
08/29/2012

Presentation Objectives

- ❑ **Introduce the life history *Cryptosporidium* spp.**
 - Infections in humans
 - Life cycle
- ❑ **Describe the epidemiology of cryptosporidiosis**
 - Incidence trends
 - Risk factors
 - Diagnostic methods
- ❑ **Provide a basic understanding of *Cryptosporidium* spp. genotyping methods**

Presentation Objectives

- ❑ **Present CryptoNet and the role it has in outbreak and case investigations**
- ❑ **Provide examples of genotyping revealing outbreak sources and risk factors undetermined by traditional epidemiologic methods**
 - North Carolina 2009
 - Italy 2011



LIFE HISTORY OF *CRYPTOSPORIDIUM* SPP.

From Reducker et al., J Protozool., 32, 708-711, 1985

Cryptosporidium spp.

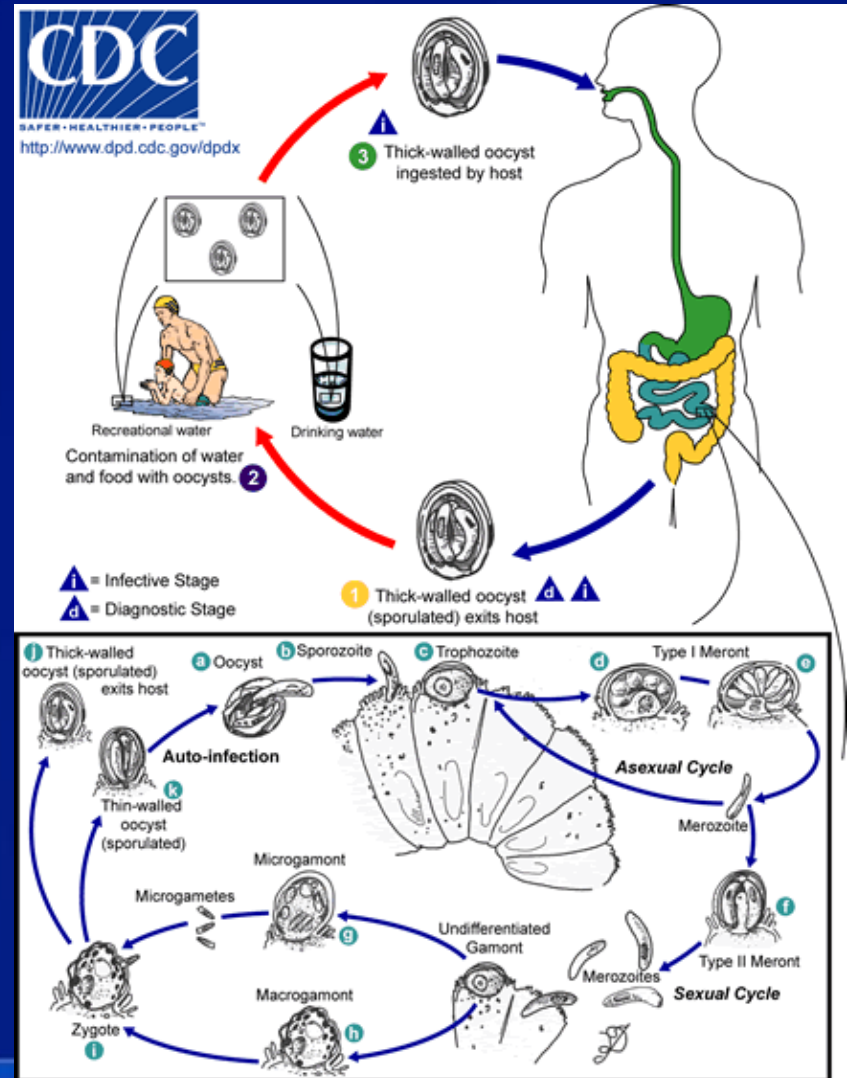
- ❑ **Environmentally-resistant, chlorine-tolerant protozoan parasite**
 - Can survive 2-6 months in a moist environment
- ❑ **Primary site of infection is within the distal region of the SI and proximal colon**
- ❑ **Transmission routes include:**
 - Contact with infected persons or animals
 - Contaminated water
 - Recreational or drinking
 - Contaminated food
- ❑ **Incubation period: 1-12 days, with 7 days being typical**

Cryptosporidium spp. life cycle

- Sporulated oocysts are shed in the feces of infected hosts



Approximately 30 different species, all indistinguishable from each other by microscopy



Cryptosporidiosis

❑ Clinical symptoms

- Profuse, watery diarrhea
- Cramping, abdominal pains, nausea
- Fever, malaise, weight loss

❑ Self-limiting in healthy individuals

❑ Immunosuppressed individuals may have chronic, debilitating and severe disease

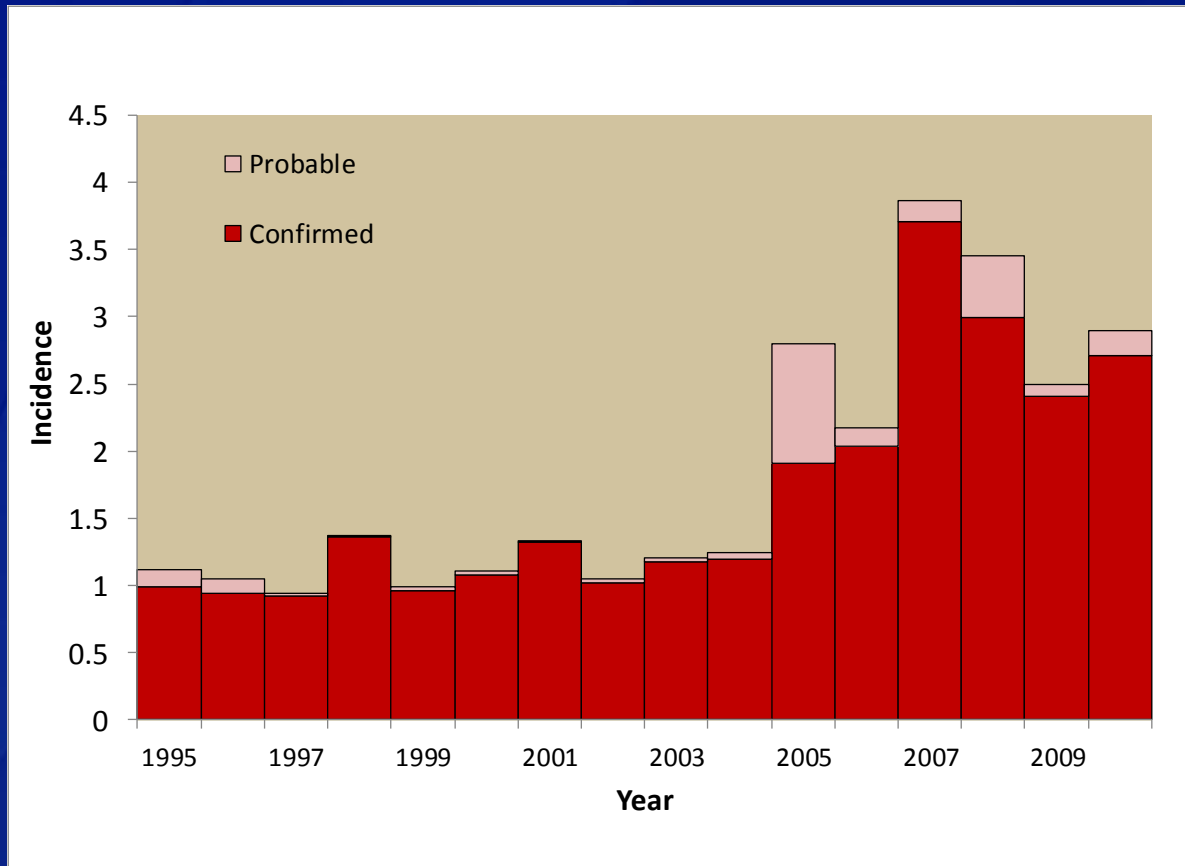
❑ Other high risk groups include children and pregnant women



Why is tracking *Cryptosporidium* spp. in the United States important?

EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS

Incidence* of human cryptosporidiosis by year- United States, 1995-2010

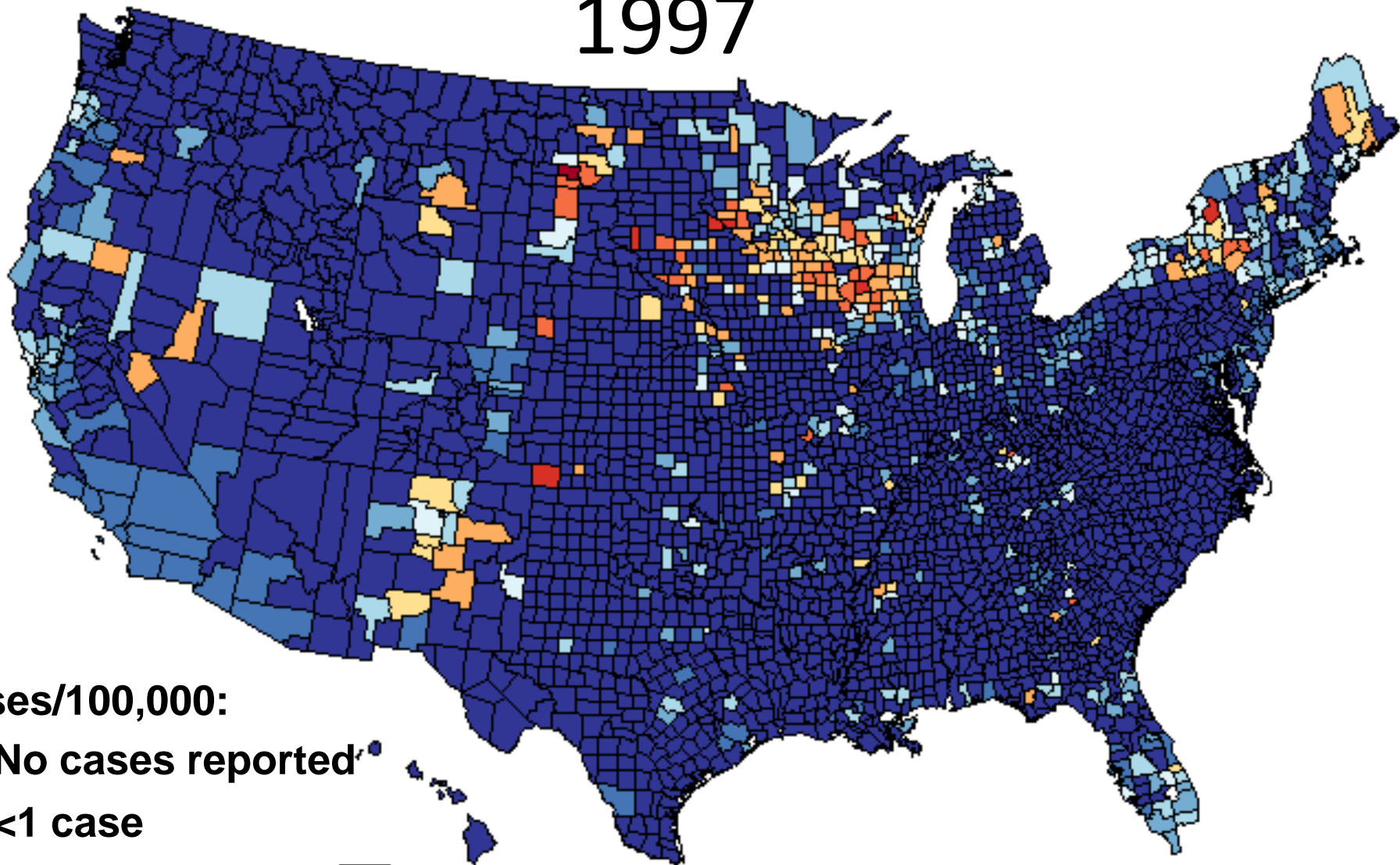


*Per 100,000 population.

†N=85,514.

§First full year of national reporting.

1997



Cases/100,000:

No cases reported

<1 case

1-<2 cases

2-<4 cases

4-<7 cases

7-<10 cases

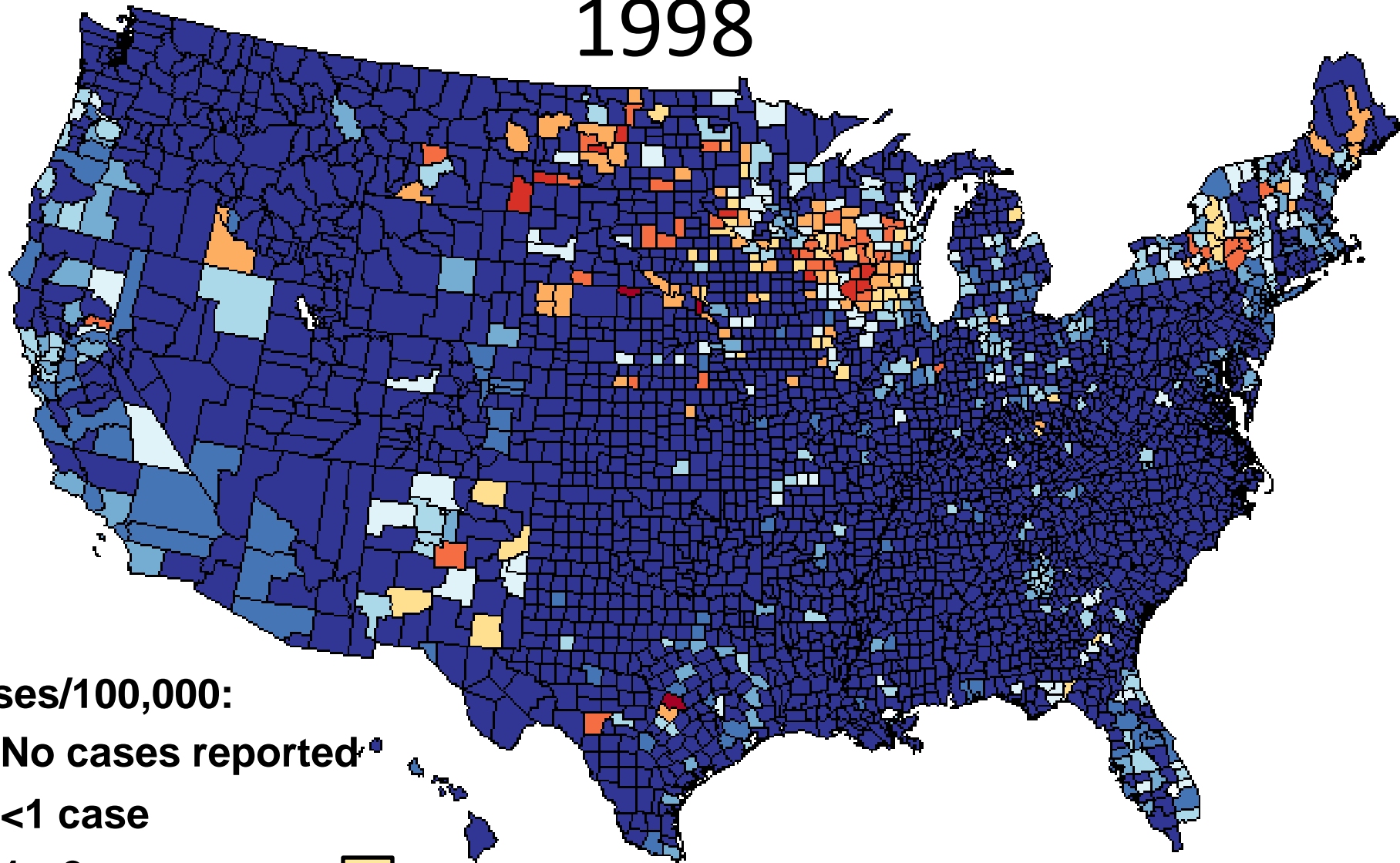
10-<20 cases

20-<40 cases

40-<100 cases

100+ cases

1998



Cases/100,000:

No cases reported

<1 case

1-<2 cases

2-<4 cases

4-<7 cases

7-<10 cases

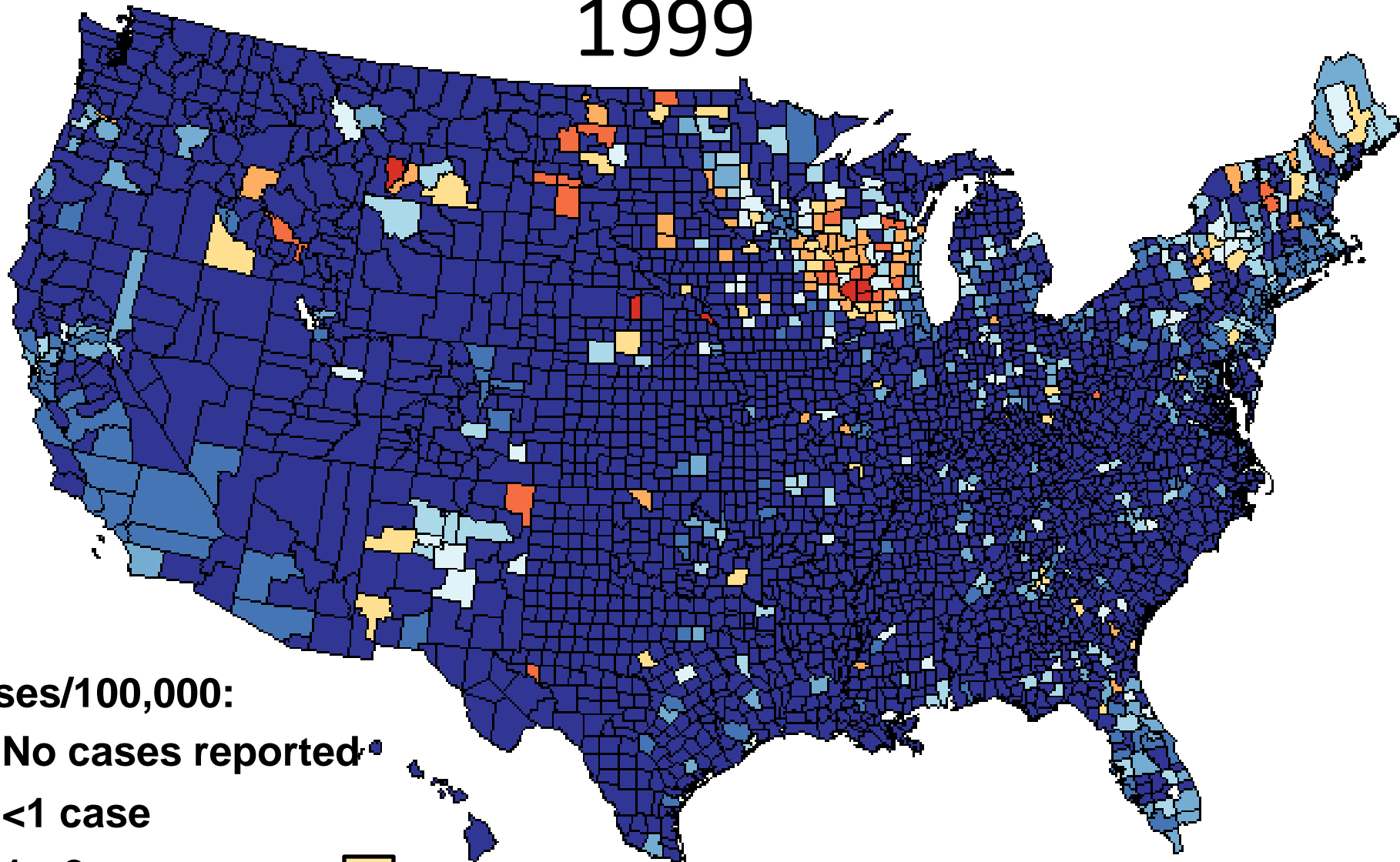
10-<20 cases

20-<40 cases

40-<100 cases


100+ cases

1999



Cases/100,000:

 No cases reported


 <1 case

 1-<2 cases

 2-<4 cases

 4-<7 cases

 7-<10 cases

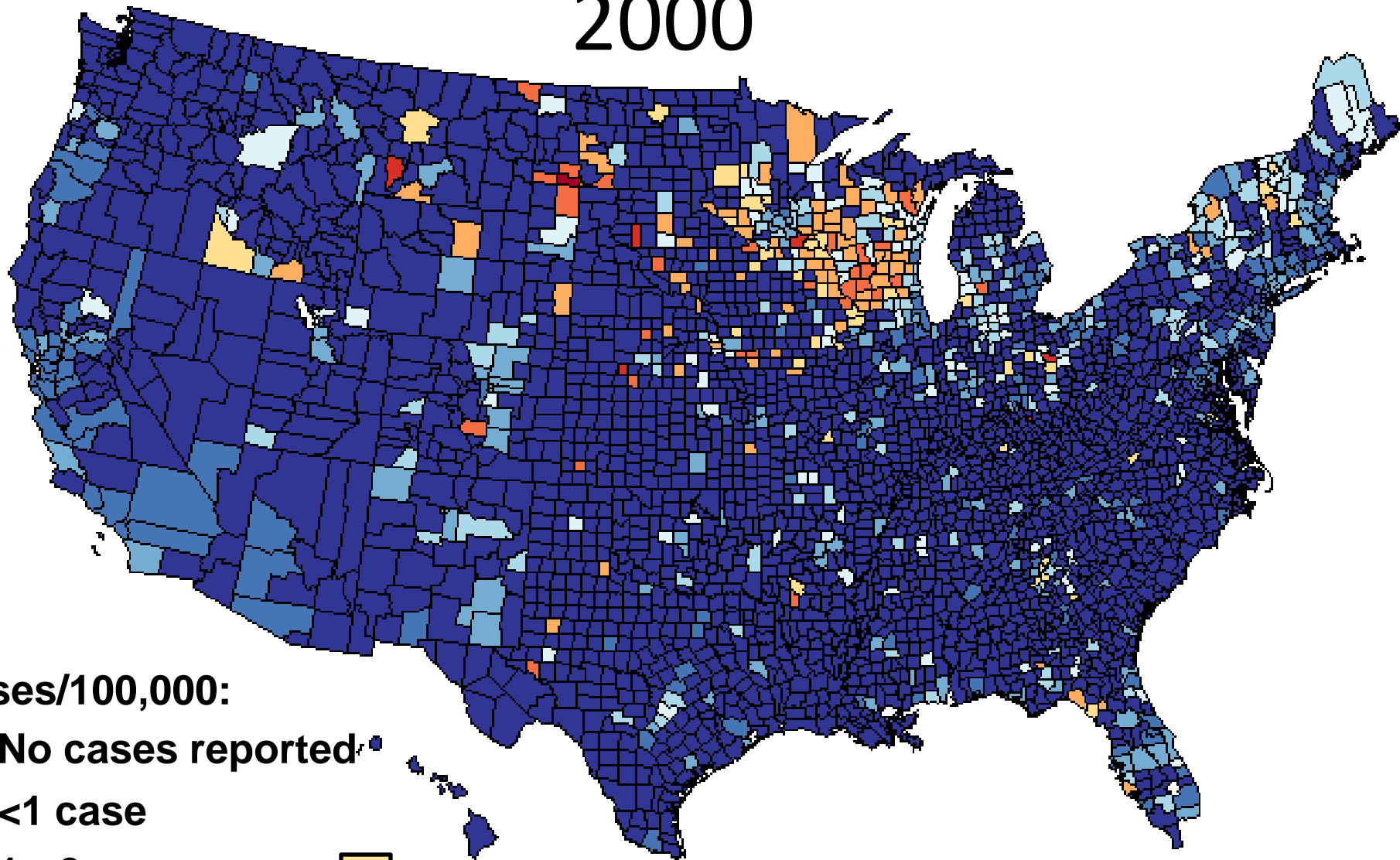
 10-<20 cases

 20-<40 cases

 40-<100 cases

 100+ cases

2000



Cases/100,000:

No cases reported

<1 case

1-<2 cases

2-<4 cases

4-<7 cases

7-<10 cases

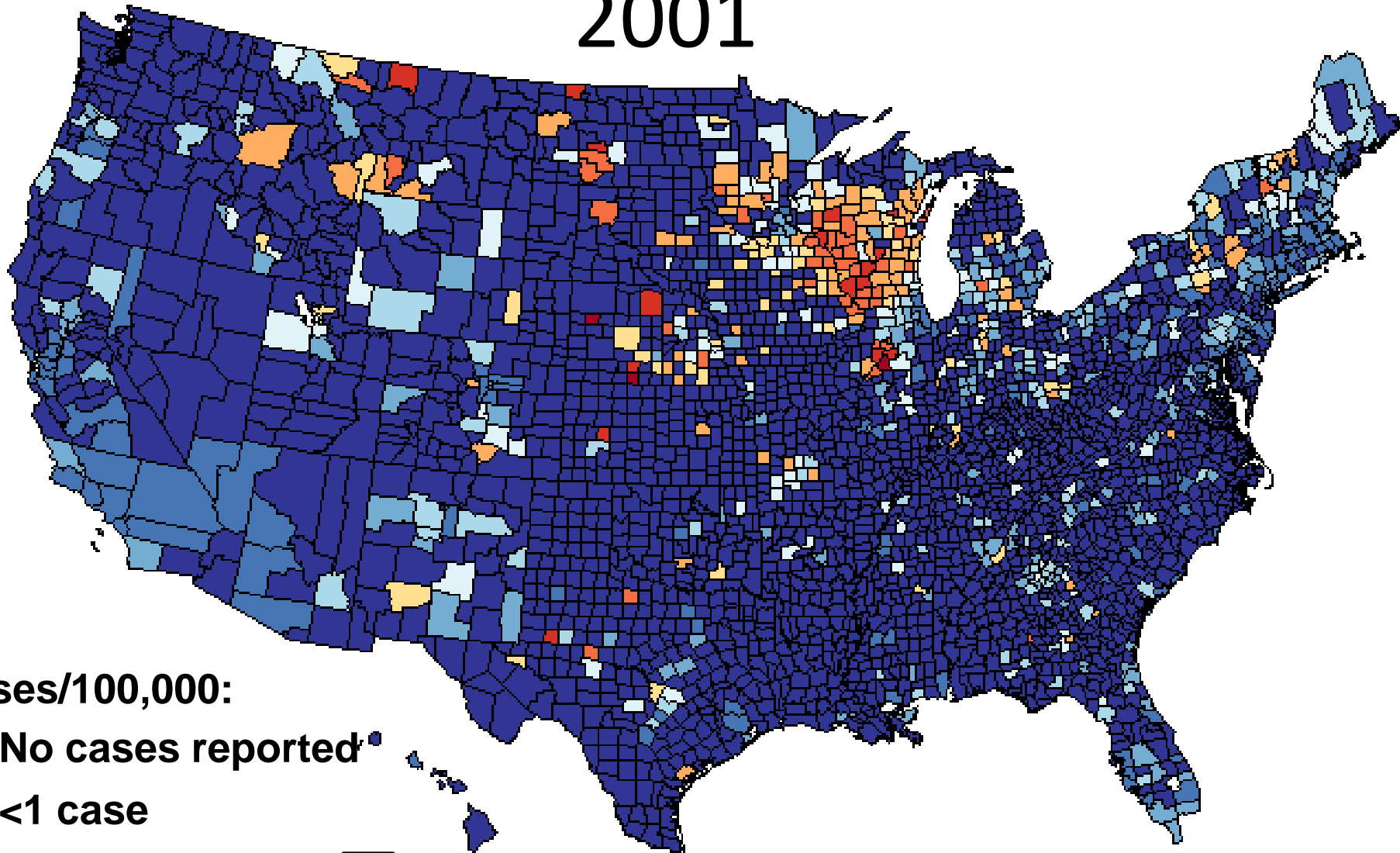
10-<20 cases

20-<40 cases

40-<100 cases

100+ cases

2001



Cases/100,000:

No cases reported

<1 case

1-<2 cases

2-<4 cases

4-<7 cases

7-<10 cases

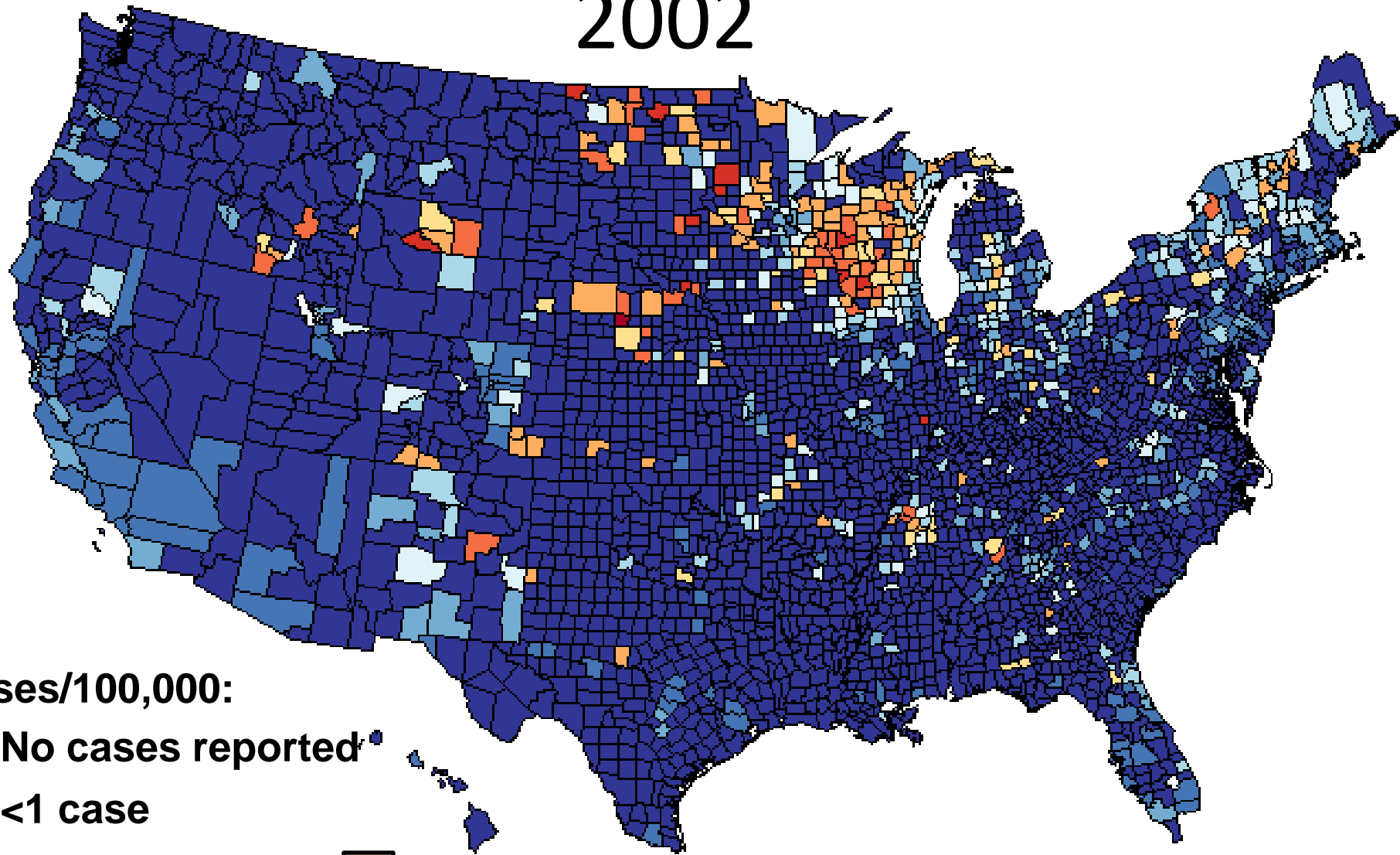
10-<20 cases

20-<40 cases

40-<100 cases

100+ cases

2002



Cases/100,000:

 No cases reported


 <1 case

 1-<2 cases

 2-<4 cases

 4-<7 cases

 7-<10 cases

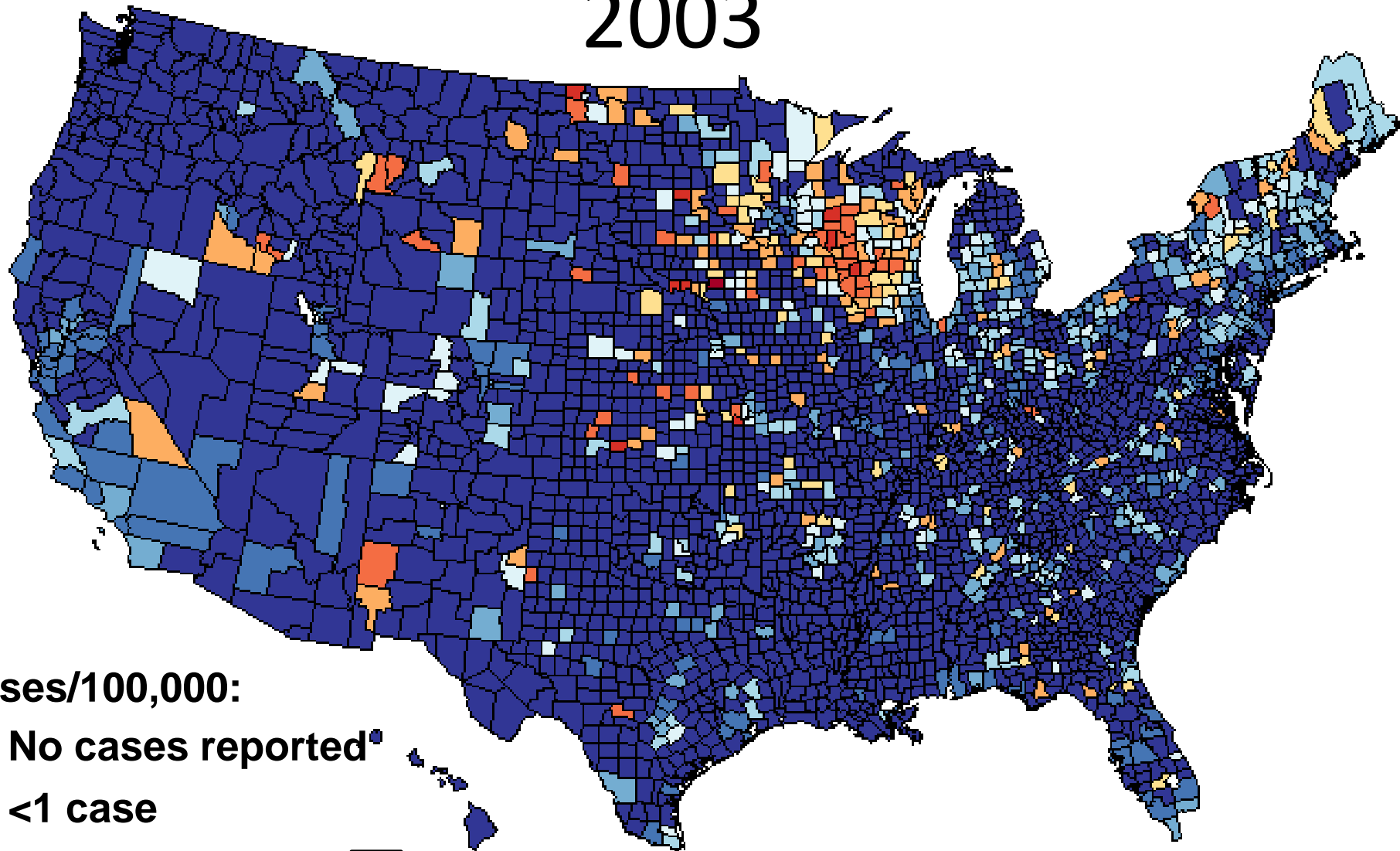
 10-<20 cases

 20-<40 cases

 40-<100 cases

 100+ cases

2003



Cases/100,000:

No cases reported

<1 case

1-<2 cases

2-<4 cases

4-<7 cases

7-<10 cases

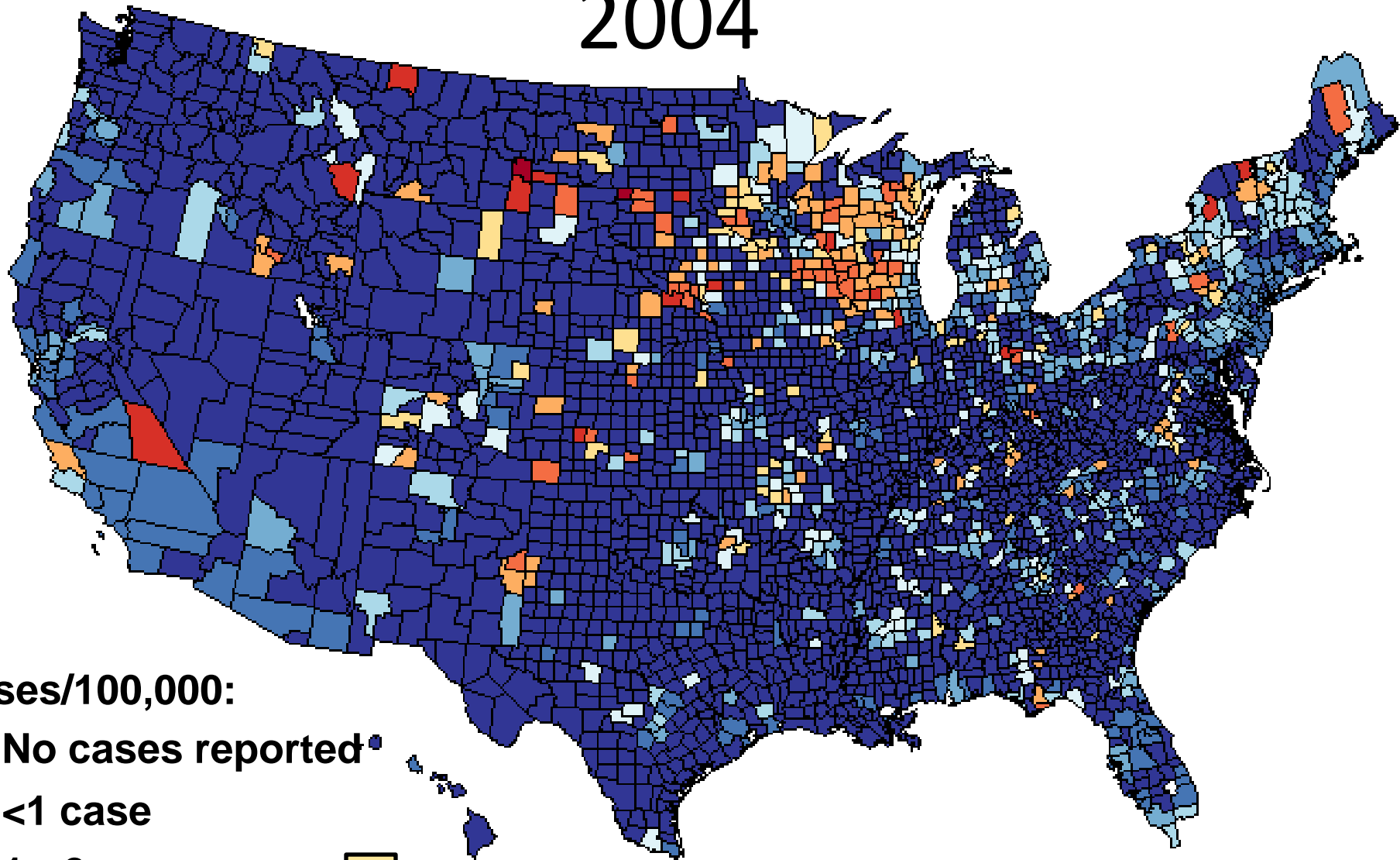
10-<20 cases

20-<40 cases

40-<100 cases


100+ cases

2004



Cases/100,000:

 No cases reported


 <1 case

 1-<2 cases

 2-<4 cases

 4-<7 cases

 7-<10 cases

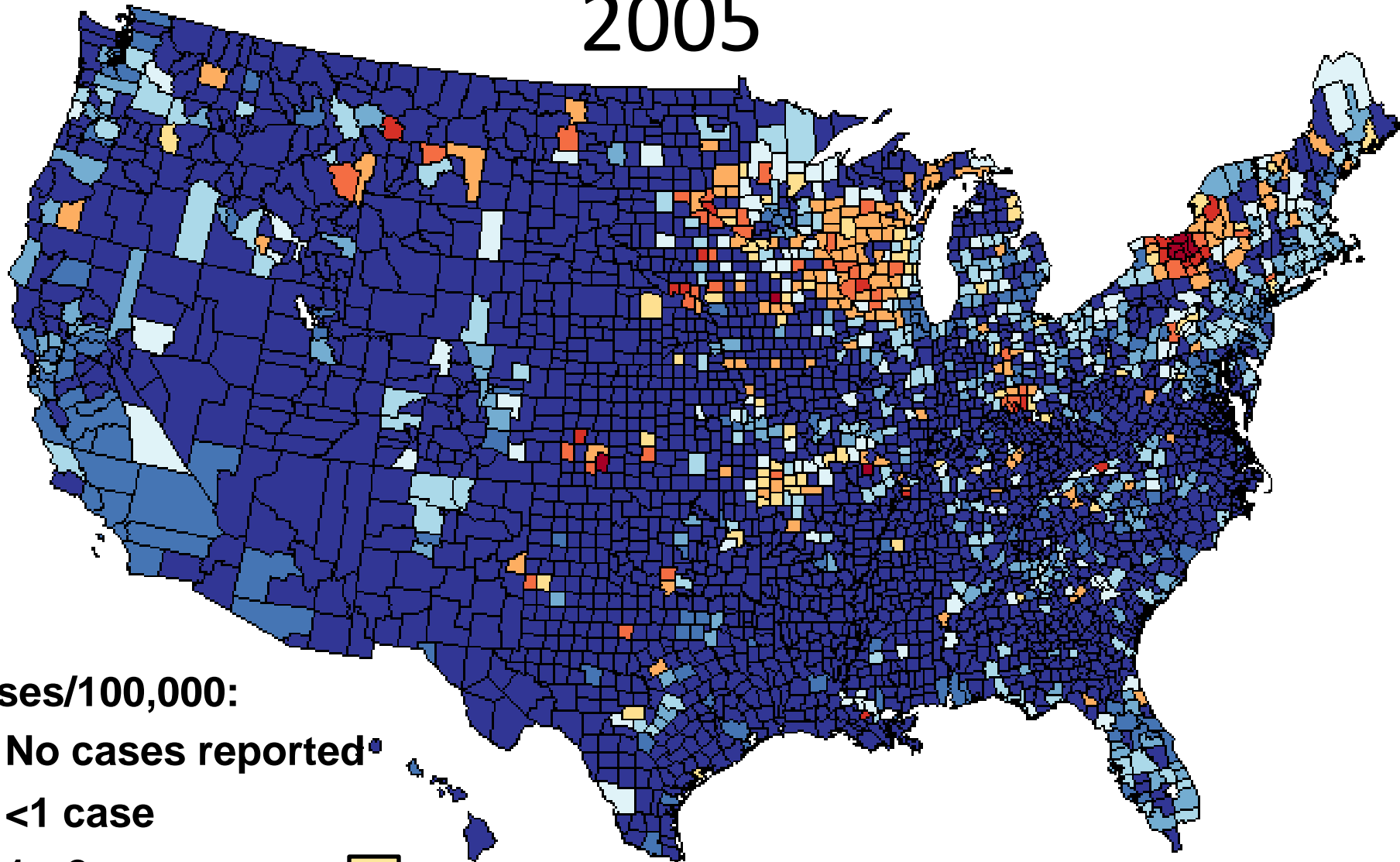
 10-<20 cases

 20-<40 cases

 40-<100 cases

 100+ cases

2005



Cases/100,000:

No cases reported

<1 case

1-<2 cases

2-<4 cases

4-<7 cases

7-<10 cases

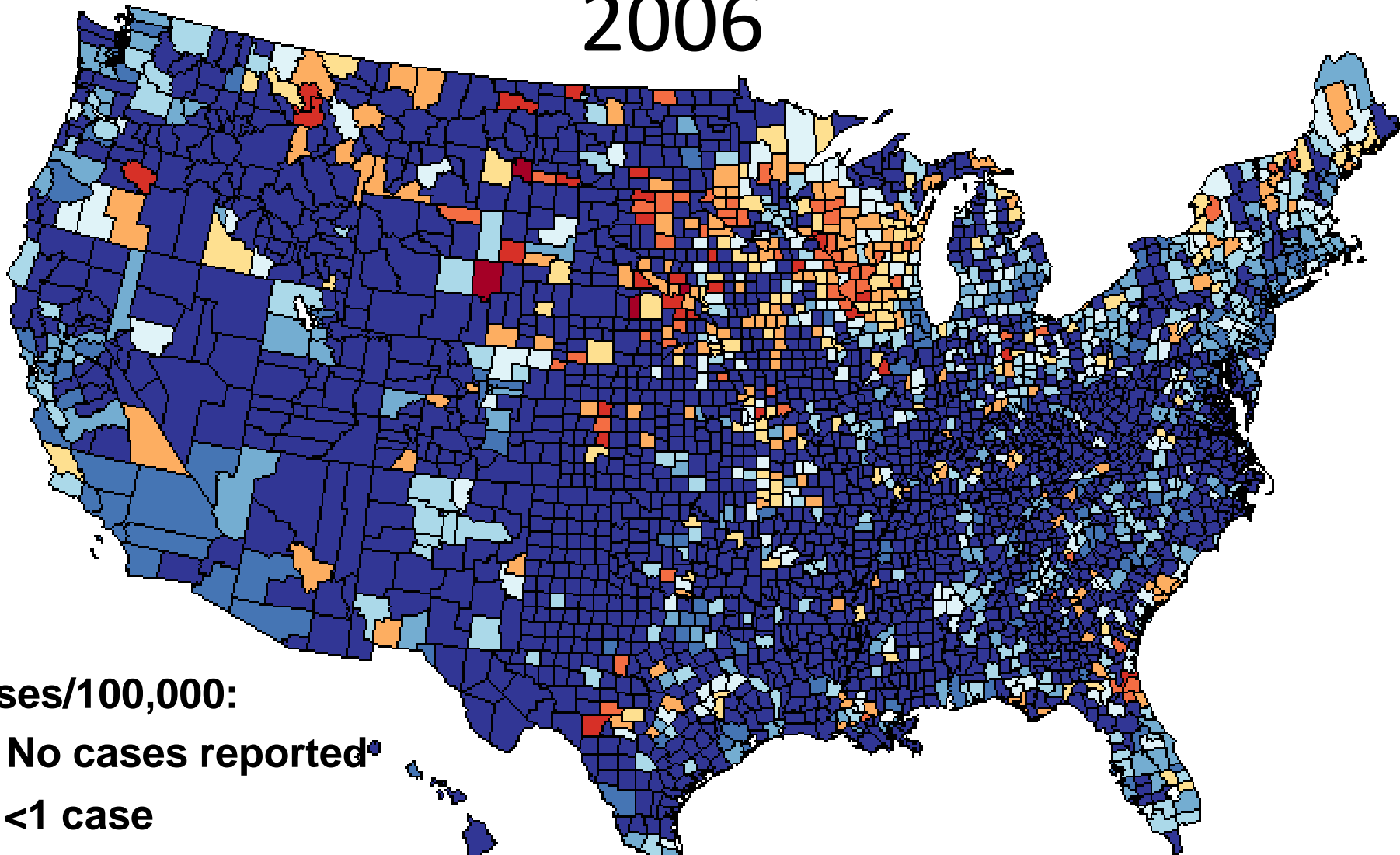
10-<20 cases

20-<40 cases

40-<100 cases


100+ cases

2006



Cases/100,000:

 No cases reported

 <1 case

 1-<2 cases

 2-<4 cases

 4-<7 cases

 7-<10 cases

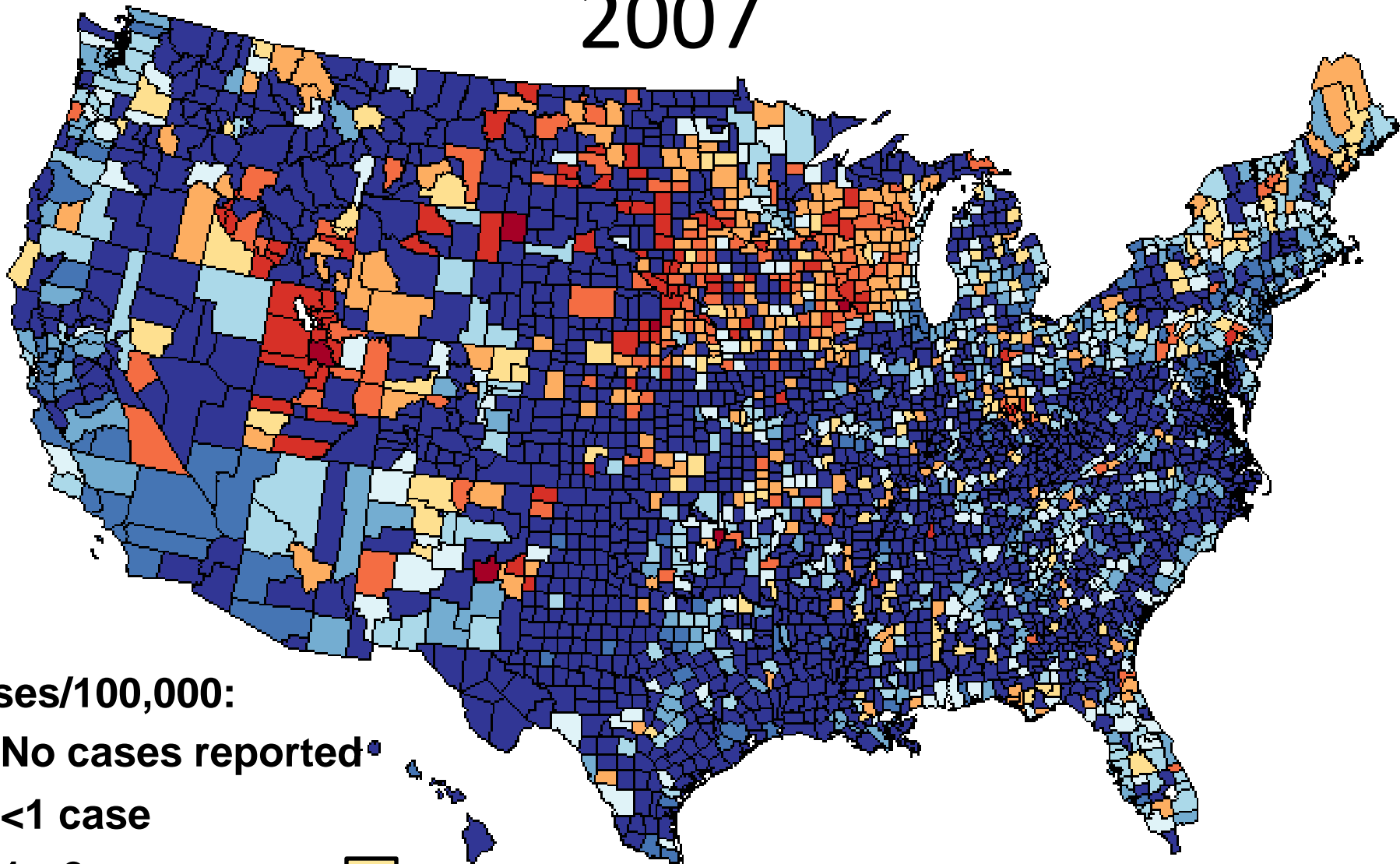
 10-<20 cases

 20-<40 cases

 40-<100 cases

 100+ cases

2007



Cases/100,000:

No cases reported

<1 case

1-<2 cases

2-<4 cases

4-<7 cases

7-<10 cases

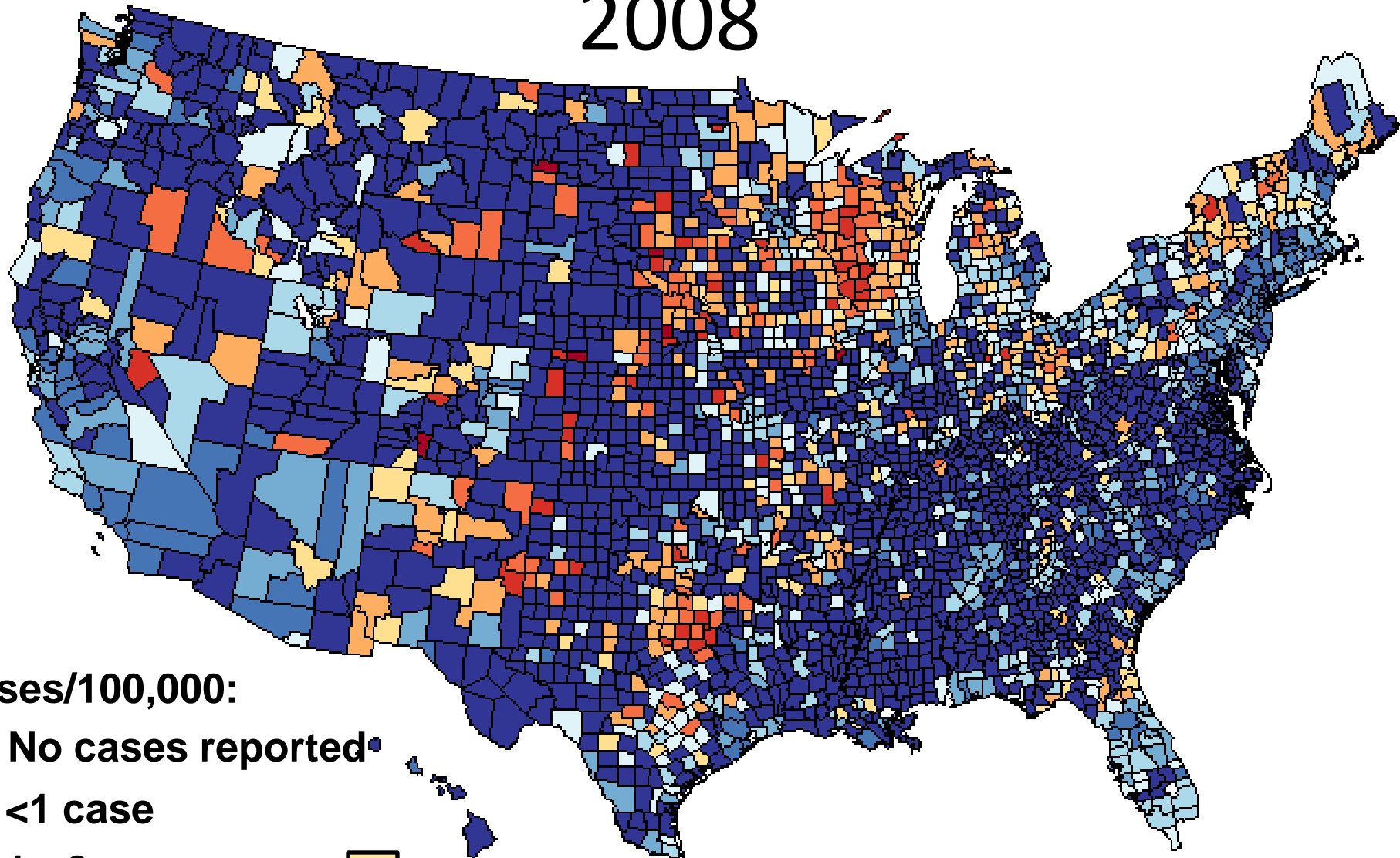
10-<20 cases

20-<40 cases

40-<100 cases


100+ cases

2008



Cases/100,000:

 No cases reported

 <1 case

 1-<2 cases

 2-<4 cases

 4-<7 cases

 7-<10 cases

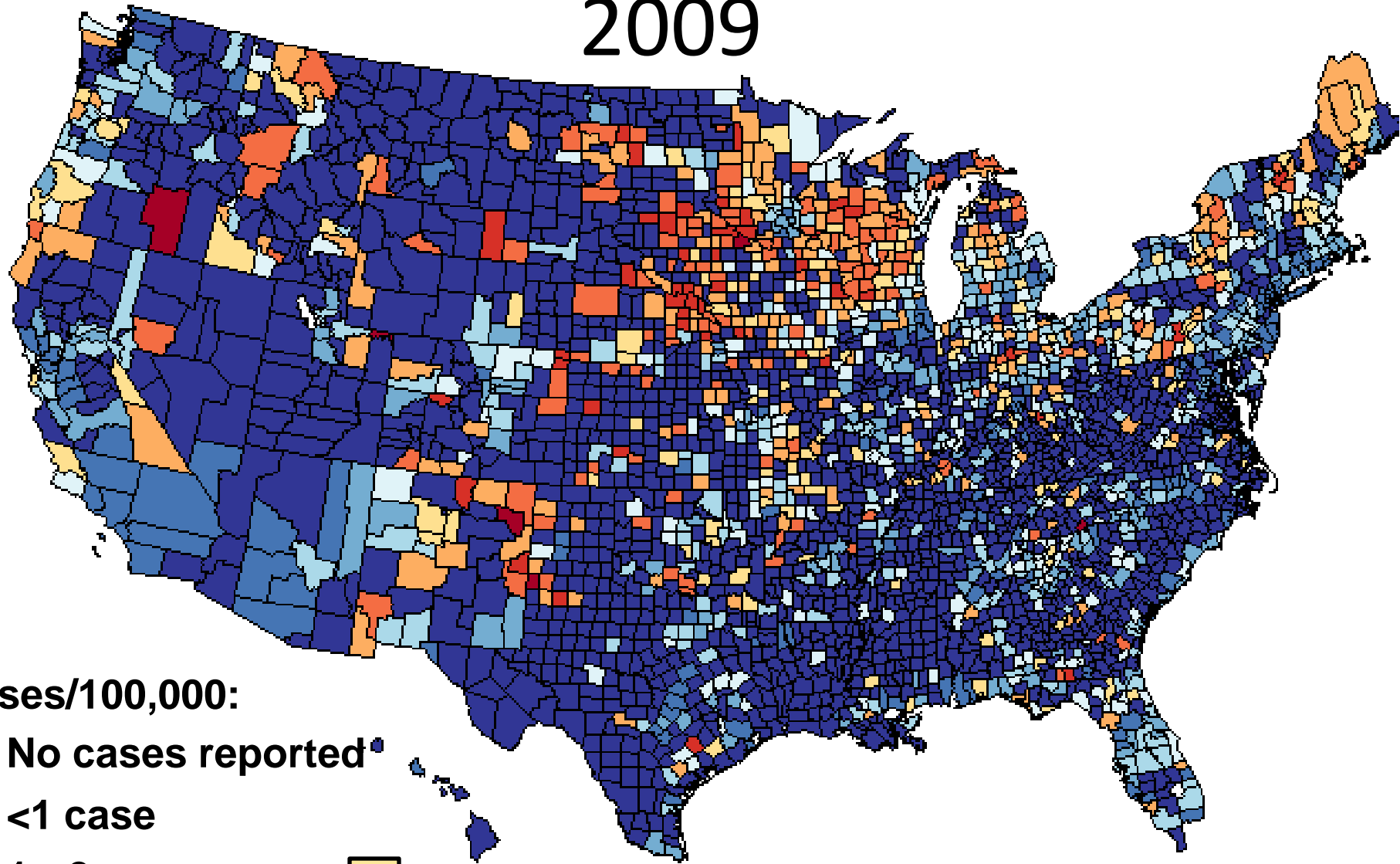
 10-<20 cases

 20-<40 cases

 40-<100 cases


 100+ cases

2009



Cases/100,000:

 No cases reported

 <1 case

 1-<2 cases

 2-<4 cases

 4-<7 cases

 7-<10 cases

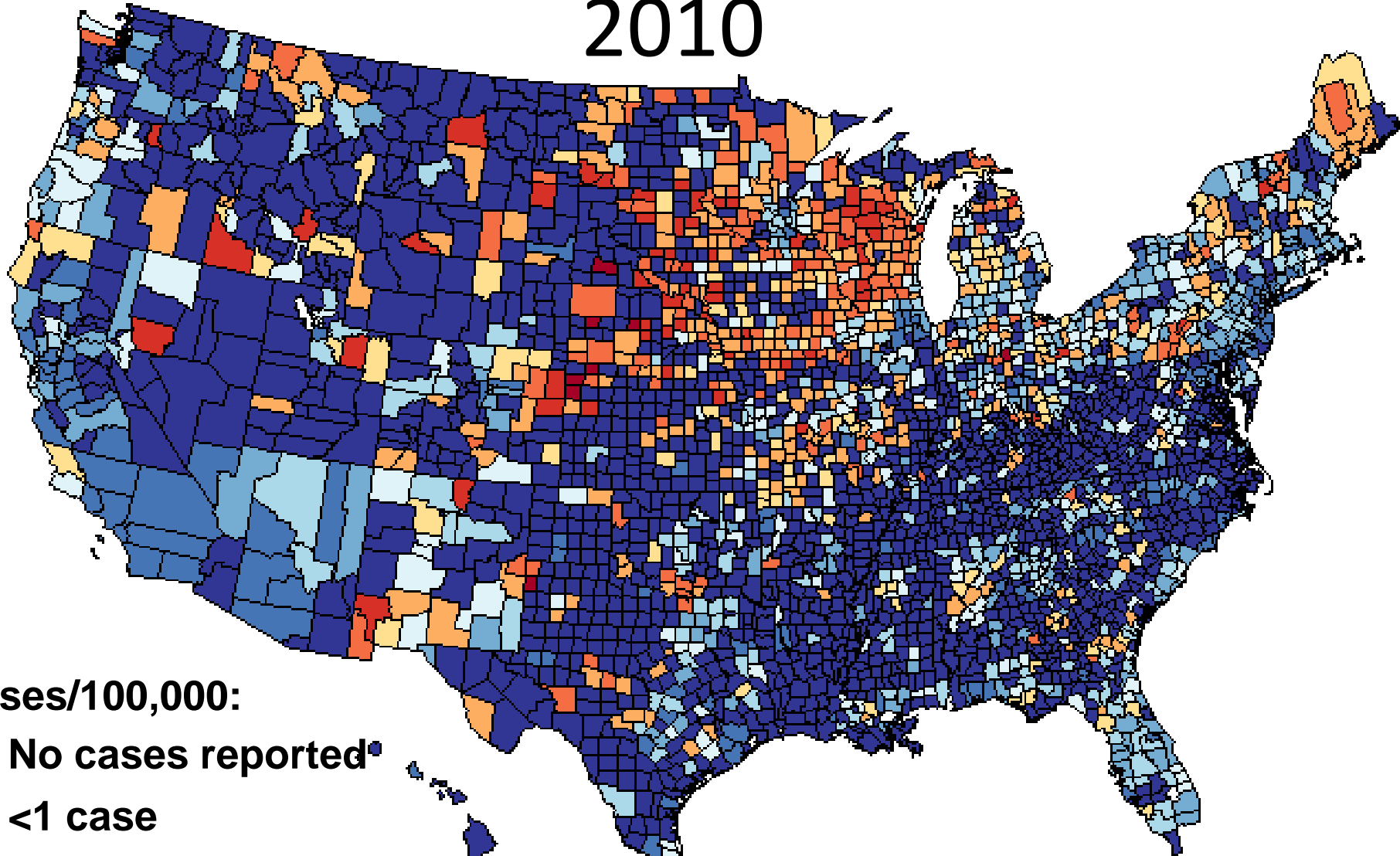
 10-<20 cases

 20-<40 cases

 40-<100 cases


 100+ cases

2010



Cases/100,000:

 No cases reported


 <1 case

 1-<2 cases

 2-<4 cases

 4-<7 cases

 7-<10 cases

 10-<20 cases

 20-<40 cases

 40-<100 cases

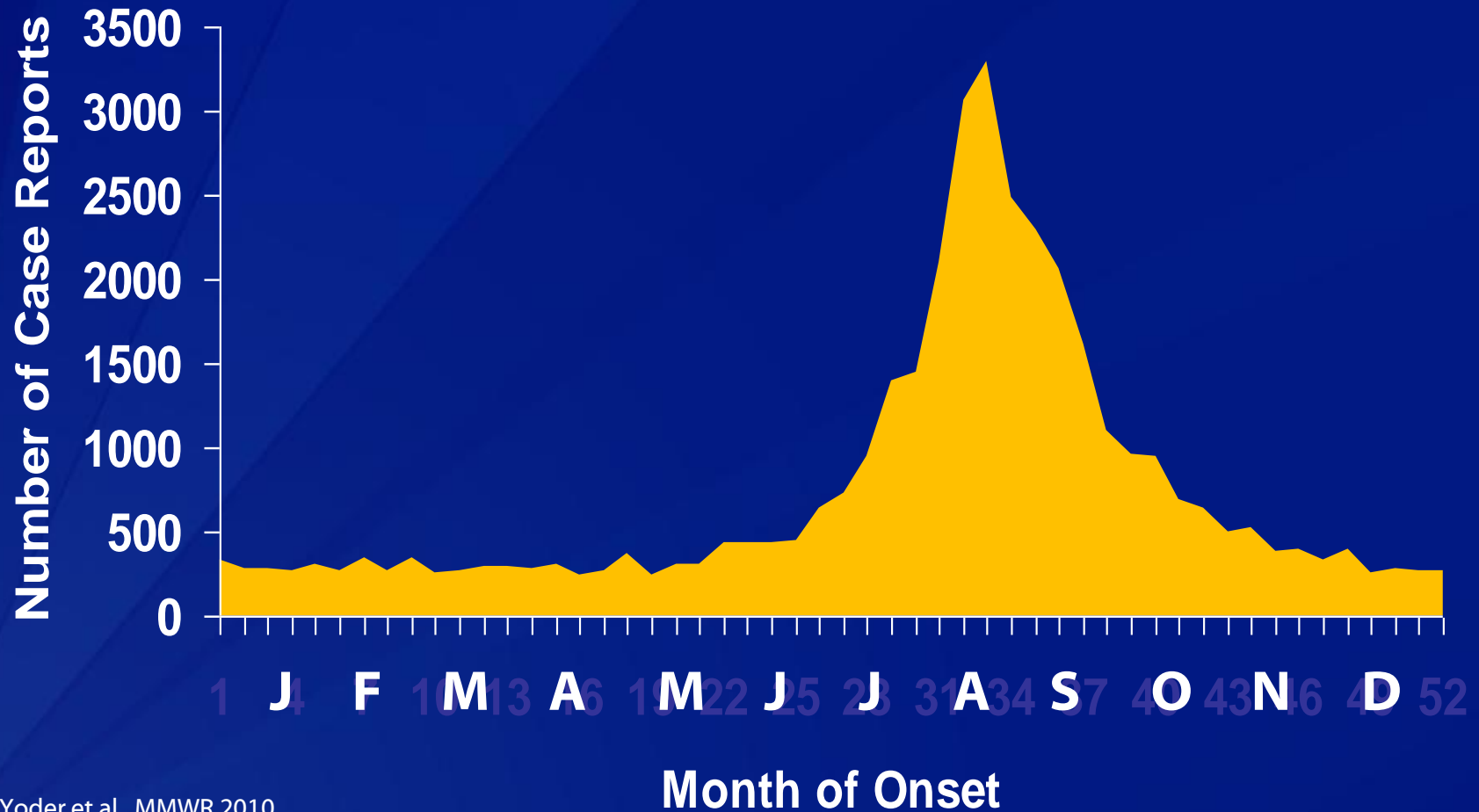
 100+ cases

Risk Factors

- ❑ Exposure to recreational water venues
- ❑ Child-care center attendance, contact with attendees
- ❑ Consumption of contaminated produce from farm/farm stand*
- ❑ Drinking untreated water from lakes, rivers, or streams
- ❑ Contact with cows, sheep, goats
- ❑ International travel



Cryptosporidiosis seasonality: United States 1995-2008



Yoder et al., MMWR 2010

Clinical diagnostic methods

- ❑ Detection of *Cryptosporidium* organisms in stool samples
- ❑ Detection of *Cryptosporidium* antigen by immunodiagnosics (EIA, DFA)
- ❑ Detection by rapid cartridge assay kits
- ❑ **Detection of *Cryptosporidium* DNA in stool samples**

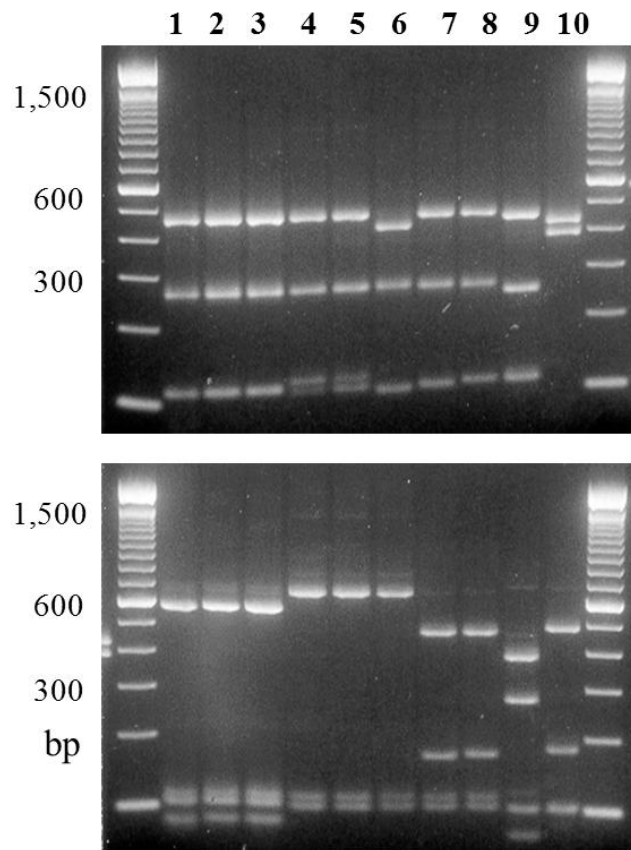


Two methods: PCR-RFLP and DNA sequencing

□ PCR-RFLP of 18S rRNA gene

- Used for speciation of human, animal, and environmental samples
- 18S rRNA gene
 - High copy number gene target
 - Semi-conserved and hyper-variable regions
- Amplification of ~830 bp fragment used in restriction enzyme reactions

18S PCR-RFLP



1-3: *C. hominis*

4&5: *C. parvum*

6: *C. canis*

7&8: *C. meleagridis*

9: *C. meleagridis* (heterogeneous)

10: *C. felis*

Upper panel: SspI digestion

Lower panel: VspI digestion

From Xiao et al., 2001

Two methods: PCR-RFLP and DNA sequencing

□ DNA sequencing of GP60

- Used for subtyping of *C. hominis*, *C. parvum*, and *C. meleagridis*
- GP60 (aka GP40/15)
 - Contains tandem repeats at the 5' end
 - Variations also present in non-repeat regions group *C. hominis* and *C. parvum* into subtype families

Species	Subtype family	
<i>C. hominis</i>	Ia	
	Ib	
	Id	
	Ie	
	If	
	Ig	
	<i>C. parvum</i>	IIa
IIb		
IIc		
		II d
		IIe
		II f
		II g
		III h
		III i
		III k
		III l

Resources and costs

❑ **Equipment**

- Thermal cycler
- Water bath
- Electrophoresis system
- Microcentrifuge
- ABI 3130xl Genetic Analyzer

❑ **Estimated cost: ~\$9.50/sample**

Advantages of using molecular genotyping

❑ Identify outbreaks

- Differentiates clusters from sporadic cases

❑ Track sources of infection

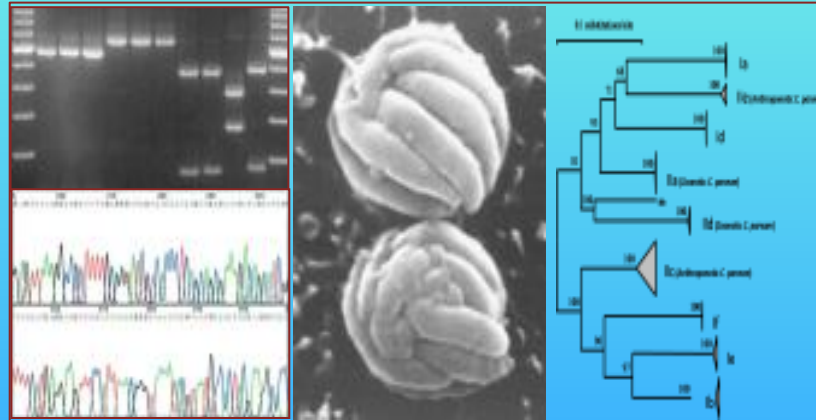
- Person-to-person vs animal-to-person
- Association of environmental, food, water samples to cases

❑ Differentiation between outbreaks

- Concurrent outbreaks in one geographical area
- Secondary spread of strains

❑ Identification of geographical and temporal trends in outbreaks and cases

Crypto-Net USA



National Molecular Surveillance for *Cryptosporidium*

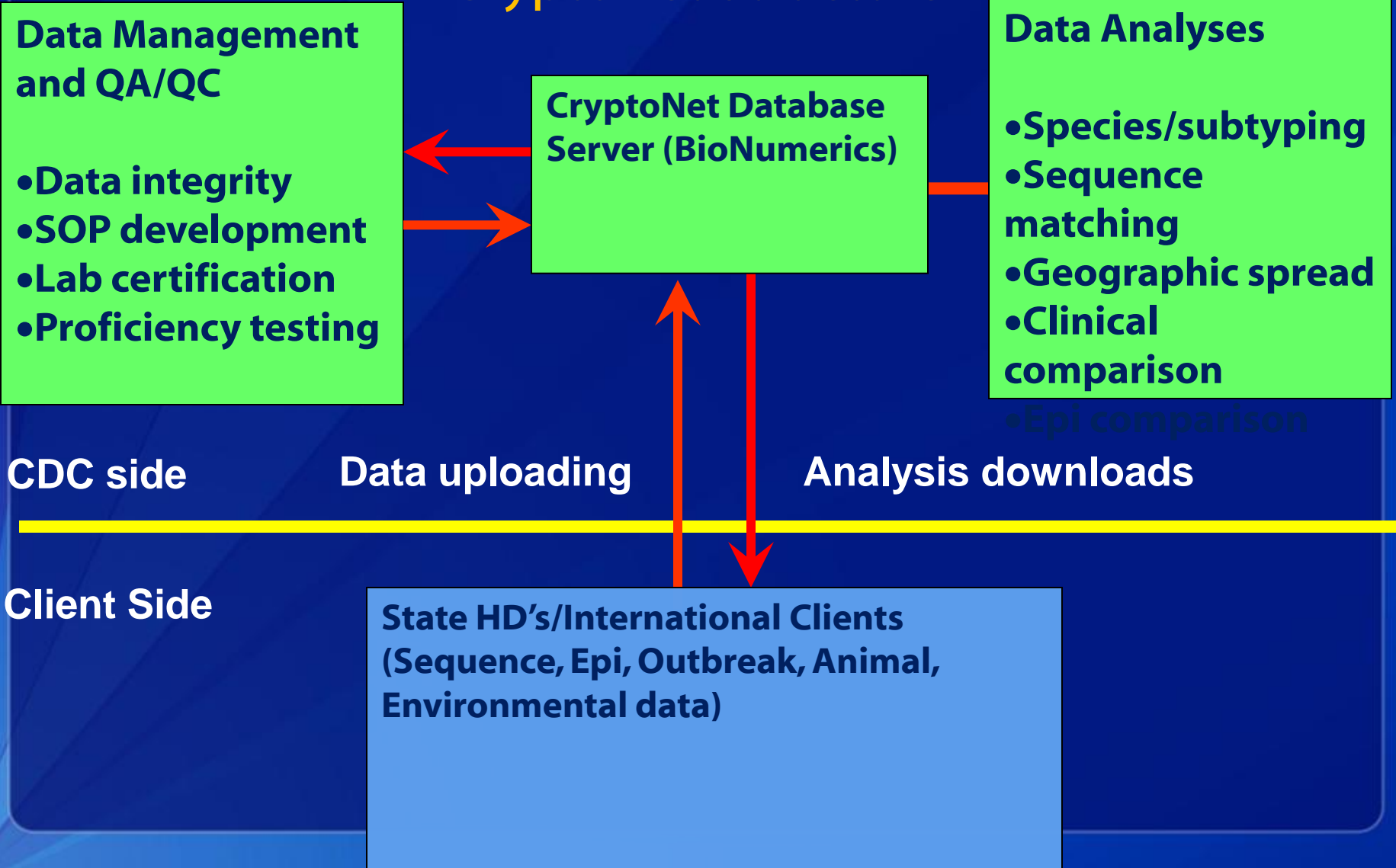
CRYPTONET



Objectives of CryptoNet

- ❑ **Build our understanding *Cryptosporidium* transmission and the epidemiology of cryptosporidiosis**
- ❑ **Collaborate to develop, test, and implement standardized lab methods for Crypto investigations**
- ❑ **Build a network for tracking *Cryptosporidium* strains in humans, animals, and environmental samples**
- ❑ **Share this info in real time with partners to identify outbreaks and source infections**

CryptoNet Structure



Data Management

❑ Epidemiologic data

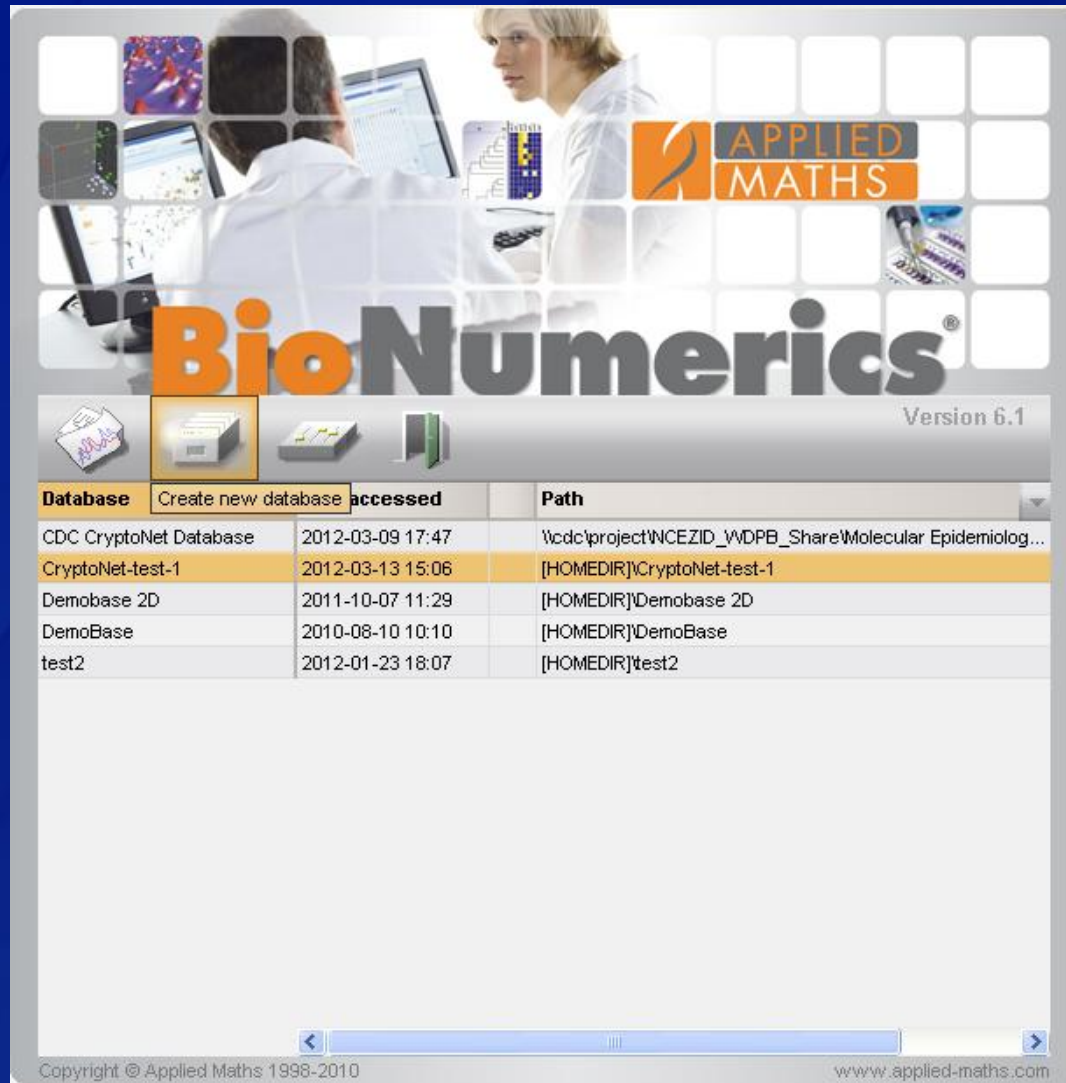
- Host, geographic origin, clinical manifestations, potential exposures
- No Personal Identifiable Information (PII)

❑ Experiments

- PCR-RFLP image of 18S rRNA gene
- DNA sequence of GP60 gene target (fasta)

❑ **Hope to link to existing data systems, such as NDDS, to reduce redundant data entry**

BioNumerics Platform



The screenshot displays the BioNumerics software interface. At the top, there is a header banner with the text "APPLIED MATHS" and "BioNumerics®". Below the banner, the version "Version 6.1" is indicated. The main area features a table with columns for "Database", "accessed", and "Path". The table lists several databases, with "CryptoNet-test-1" highlighted in yellow. At the bottom of the interface, there is a copyright notice "Copyright © Applied Maths 1998-2010" and the website "www.applied-maths.com".

Database	accessed	Path
CDC CryptoNet Database	2012-03-09 17:47	Vcdc\project\NCEZID_VWDPB_Share\Molecular Epidemiolog...
CryptoNet-test-1	2012-03-13 15:06	[HOMEDIR]\CryptoNet-test-1
Demobase 2D	2011-10-07 11:29	[HOMEDIR]\Demobase 2D
DemoBase	2010-08-10 10:10	[HOMEDIR]\DemoBase
test2	2012-01-23 18:07	[HOMEDIR]\test2

Copyright © Applied Maths 1998-2010 www.applied-maths.com

Reference strains

BioNumerics

File Edit Database Subsets Experiments Comparison Identification CryptoNet Scripts Help Window

Complete view

Database entries

Key	Category	LocalID	CryptoNetOBID	1	2	3	4	5	6	7
Angelfish genotype F...	Reference									
Avian genotype I DG6...	Reference									
Avian genotype IV D...	Reference									
Bear genotype	Reference									
Bear genotype AF24...	Reference									
Beaver genotype	Reference									
Beaver genotype 121...	Reference									
C. andersoni	Reference									
C. andersoni AF093496	Reference									
C. baileyi AF093495	Reference									
C. baileyi AF262324	Reference									
C. bovis 2622	Reference									
C. bovis AY120911	Reference									
C. bovis-like EU825742	Reference									
C. canis 9566	Reference									
C. canis AF112576	Reference									
C. canis coyote genot...	Reference									
C. canis fox genotyp...	Reference									
C. cuniculus 2246	Reference									
C. cuniculus AY120901	Reference									
C. fayeri	Reference									
C. fayeri AF112570	Reference									
C. fayeri-IVaA10G3T...	Reference									
C. fayeri-IVaA9G4T1...	Reference									
C. fayeri-IVbA9G1T1...	Reference									
C. fayeri-IVcA8G1T1...	Reference									
C. fayeri-IVdA7G1T1...	Reference									
C. fayeri-IVeA7G1T1...	Reference									
C. fayeri-IVfA11G1T1...	Reference									
C. fayeri-IVgA12G1T1...	Reference									

Experiments

Name	Type
1 RFLP_Ddel	Fingerprint types
2 RFLP_MbolI	Fingerprint types
3 RFLP_SspI	Fingerprint types

Files

Name	Created	Modified
Ddel_andersoni_muri...	2012-03-13 15h27m1...	2012-03-13
New Standard Marke...	2012-03-13 15h27m1...	2012-03-13
Standard Ddel	2012-03-13 15h27m1...	2012-03-13

Comparisons

Name	Created	Modified
------	---------	----------

Alignments

Name	Created	Modified
------	---------	----------

All levels

Database: CryptoNet-test-2 (connected, _DefaultUser_) 356 entries 7 experiments \\\cdc.gov\private\W121\GYO7\BioNumerics Data\data\CryptoNet-test-2

Inputting Data

- Differentiate outbreak clusters from sporadic cases

Add new entries [X]

Number of entries to add: 1

Sample Collection Year: 2010

Sample Category: Outbreak

Related to a new outbreak

Existing outbreak

State/LabID: GA

Country (3 letters): USA-UNITED STATES

OK Cancel

Inputting Epidemiologic Data

The screenshot displays the BioNumerics software interface. The main window shows a 'Database entries' table with columns for Key, Category, LocalID, and CryptoNetOBID. An 'Entry edit' dialog box is open, showing the details for a specific entry. The dialog box has tabs for 'Database fields', 'Experiments', and 'Attachments'. The 'Database fields' tab is active, showing a list of fields and their values. The 'Experiments' tab shows a list of experiments associated with the entry.

Key	Category	LocalID	CryptoNetOBID
2010-0001-HS-GA-U...	Outbreak		2010-001-OB-USA
Angelfish genotype F...	Reference		
Avian genotype I DC...			
Avian genotype IV D...			
Bear genotype			
Bear genotype AF2...			
Beaver genotype			
Beaver genotype 12...			
C. andersoni			
C. andersoni AF093...			
C. baileyi AF093495			
C. baileyi AF262324			
C. bovis 2622			
C. bovis AY120911			
C. bovis-like EU825...			
C. canis 9566			
C. canis AF112576			
C. canis coyote gen...			
C. canis fox genoty...			
C. cuniculus 2246			
C. cuniculus AY120...			
C. fayeri			
C. fayeri AF112570			
C. fayeri-IVaA1003...			

Entry edit

File Edit Attachments CryptoNet Window

Database fields

Field	Value
Key	2010-0001-HS-GA-USA
Category	Outbreak
LocalID	
CryptoNetOBID	2010-001-OB-USA
CDCLabID	
OtherReportID	
City	
State	GA-Georgia
Country	USA-UNITED STATES
ZIP	

Experiments

- RFLP_Ddel
- RFLP_Mboll
- RFLP_Sspl
- RFLP_Vspl
- SEQ_18s
- SEQ_gp60
- SEQ_MultiLocus

Database entries

Name	Type
1 RFLP_Ddel	Fingerprint types
2 RFLP_Mboll	Fingerprint types
3 RFLP_Sspl	Fingerprint types

Experiments

Name	Created	Modified
Ddel_andersoni_muri...	2012-03-13 15h27m1...	2012-03-13
New Standard Marke...	2012-03-13 15h27m1...	2012-03-13
Standard Ddel	2012-03-13 15h27m1...	2012-03-13

Inputting RFLP Data

Database entries

Key	Category	LocalID	CryptoNetOBID	1	2	3	4	5	6	7
2010-0001-HS-GA-U...	Outbreak		2010-001-OB-USA							

Import fingerprint file

Look in: Test

- RFLP-07282011-1.tif
- RFLP-10212010-1.tif

File name: RFLP-07282011-1.tif

Files of type: Bitmap files

Open Cancel

Experiments

Name	Type
1 RFLP_Ddel	Fingerprint types
2 RFLP_Mboll	Fingerprint types
3 RFLP_Sspl	Fingerprint types

Experiments Entry relations

Files

Name	Created	Modified
Ddel_andersoni_muri...	2012-03-13 15h27m1...	2012-03-13
New Standard Marke...	2012-03-13 15h27m1...	2012-03-13
Standard Ddel	2012-03-13 15h27m1...	2012-03-13

Comparisons

Name	Created	Modified
------	---------	----------

Comparisons Libraries Decision Networks

Alignments

Name	Created	Modified
------	---------	----------

Alignments Chromosome comparisons

Database: CryptoNet-test-2 (connected, _DefaultUser_) 357 entries 7 experiments %cd\gov\private\W121\GYO7\BioNumerics Data\data\CryptoNet-test-2

Ability to Edit and Normalize



Completed Experiment Input

BioNumerics

File Edit Database Subsets Experiments Comparison Identification CryptoNet Scripts Help Window

Sample

Database entries

Key	Category	LocalID	CryptoNetOBID	1	2	3	4	5	6	7
2010-0001-HS-GA-U...	Outbreak		2010-001-OB-USA							

Experiments

Name	Type
1 RFLP_Ddel	Fingerprint types
2 RFLP_Mboll	Fingerprint types
3 RFLP_Sspl	Fingerprint types

Experiments Entry relations

Files

Name	Created	Modified
Ddel_andersoni_muri...	2012-03-13 15h27m1...	2012-03-13
New Standard Marke...	2012-03-13 15h27m1...	2012-03-13
RFLP-07282011-Sspl	2012-03-13 16h39m0...	2012-03-13

Comparisons

Name	Created	Modified
------	---------	----------

Comparisons Libraries Decision Networks

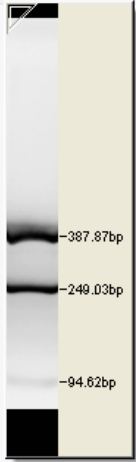
Alignments

Name	Created	Modified
------	---------	----------

Alignments Chromosome comparisons

All levels

Database: CryptoNet-test-2 (connected, _DefaultUser_) 357 entries 7 experiments Wdc.gov\private\W121\GYO7\BioNumerics Data\data\CryptoNet-test-2



Inputting Sequencing (fasta) Data

The screenshot displays the BioNumerics software interface. A window titled "2010-0001-HS-GA-USA (Sequence Viewer)" is open, showing a sequence editor and a sequence viewer. The sequence editor contains the following text:

```
attttgggtg actcataata actttacgga tcacactatg tgacatatca ttcaggttcc 60
tgacctatca gcttttagacg gtagggattt ggccctaccgt ggctatgacg ggtaacgggg 120
aattaggggt cgatttcgga gagggagcct gagaaacgcg taccacatct aaggaaggca 180
gcaggcgcgc aaattaccca atcctaatac agggaggtag tgacaagaaa taacaatata 240
gagccttacg gttttgtaat tggaatgagt taagtataaa ccccttaaca agtatcaatt 300
gaaaaaaa tctaatacca acacccccaa taattccaac tccaatatac tatattaaaa 360
```

The sequence viewer below shows a scale from 0 to 600. The annotation section is currently empty. The status bar at the bottom indicates: "Sequence: 2010-0001-HS-GA-USA | Experiment: SEQ_18s | 1 | 781 bp".

The background interface shows a "Database entries" table with the following data:

Key	Category	LocalID	CryptoNetOBID	1	2	3	4	5	6	7
2010-0001-HS-GA-U...	Outbreak		2010-001-OB-USA							

The "Experiments" section shows a table with columns "Name" and "Type":

Name	Type
1	Fingerprint types
2	Fingerprint types
3	Fingerprint types

The status bar at the bottom of the main window shows: "Database: CryptoNet-test-2 (connected, _DefaultUser_) | 357 entries | 7 experiments | vcdd.gov/private\M121\GYO7\BioNumerics Data\data\CryptoNet-test-2".

Query

CryptoNet Data Query Form



Subset Information

Subset

Case Information

Onset from: Tuesday , March 13, 2012

Onset to: Tuesday , March 13, 2012

Host

Age between To

Sex

Race

Transmission

Outbreak Information

OB from: Tuesday , March 13, 2012

OB to: Tuesday , March 13, 2012

Setting

Transmission

Location Information

City

State

Country

ZIP

GPS range: From To

Latitude

Longitude

Sample and Lab results

Sample type

Category

Species (RFLP)

Species (Melting Curve)

SubTypeFamily (gp60)

Subtype (gp60)

Add to list

Replace list

Search in list

Negative Search

NOTE: Wildcard (*) cannot be used

Data Analysis by Comparison

The screenshot displays the 'Comparison' software interface. The main window is titled 'Comparison' and features a menu bar with options: File, Edit, Layout, Groups, Clustering, Statistics, Fingerprints, Characters, Sequence, TrendData, Composite, and Window. Below the menu bar is a toolbar with various icons for file operations and analysis. The interface is divided into several panes:

- Experiments:** A list of experiments including RFLP_Ddel (selected), RFLP_Mboll, RFLP_Sspl, RFLP_Vspl, SEQ_18s, SEQ_gp60, and SEQ_MultiLocus.
- Analyses:** A section with a 'Name' dropdown and a list of analysis results.
- Groups:** A table with columns 'Size' and 'Name', showing a list of groups with a size of 0.
- Dendrogra...:** A pane for dendrogram visualization.
- Experiment data:** A pane for viewing experimental data.
- Information fields:** A pane showing a list of information fields under a 'Key' dropdown. The list includes:
 - 2010-0001-HS-GA-USA
 - 2010-0002-HS-GA-USA
 - 2010-0003-HS-GA-USA
 - 2011-0001-HS-GA-USA
 - Angelfish genotype FJ769050
 - Avian genotype I DQ650339
 - Avian genotype IV DQ650344
 - Bear genotype
 - Bear genotype AF247535
 - Beaver genotype
 - Beaver genotype 12161
 - C. andersoni
 - C. andersoni AF093496
 - C. baileyi AF093495
- Similarities:** A pane for viewing similarity results.

The status bar at the bottom left indicates '14 entries'.

RFLP-Comparisons (Comparison)

File Edit Layout Groups Clustering Statistics Fingerprints Characters Sequence TrendData Composite Window



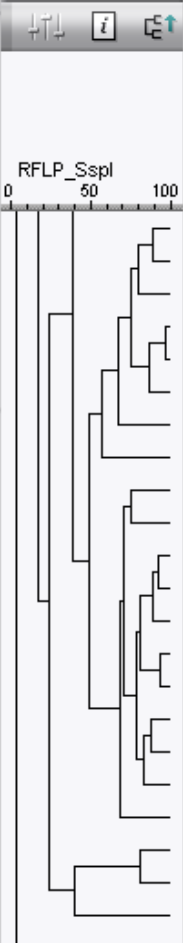
- Experiments**
- RFLP_Ddel
 - RFLP_MbolI
 - RFLP_Sspl**
 - RFLP_Vspl
 - SEQ_18s
 - SEQ_gp60
 - SEQ_MultiLocus

- Analyses**
- Name
- RFLP_Sspl

Groups

Size	Name
0	
0	
0	
0	
0	
0	
0	
0	

Dendrogra...



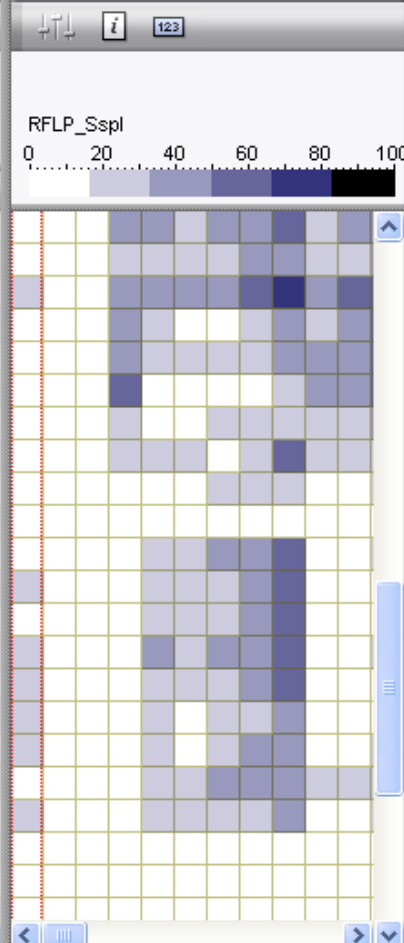
Experiment data



Information fields

- Key
- C. hominis AF159110
 - C. meleagridis B AF112574
 - C. meleagridis A 3758
 - C. cuniculus 2246
 - Horse genotype 6481
 - Beaver genotype
 - Mink genotype
 - Duck genotype 6876
 - 2010-0002-HS-GA-USA
 - Pig genotype II
 - C. parvum 9037
 - C. ubiquitum
 - C. bovis 2622
 - Rat genotype I AY737578
 - Rat genotype III AY737582
 - C. canis 9566
 - C. ryanae 771
 - C. varani
 - Rat genotype IV 8420
 - 2010-0003-HS-GA-USA
 - Bear genotype
 - C. muris

Similarities



62 entries

How useful is it?

OUTBREAK INVESTIGATIONS USING GENOTYPING AND CRYPTONET

Potential Public Health Impacts of a *Cryptosporidium* Genotype Tracking System

- ❑ Identify unknown epidemiologic links**
- ❑ Identify unknown species that may cause human cryptosporidiosis**
- ❑ Increase laboratory data sharing among national, state, and local public health laboratories**
- ❑ Promote improved laboratory-based tracking of cryptosporidiosis in the United States**

- ❑ **Improved efficiency in detection and investigation of foodborne, waterborne, person-to-person, and zoonotic outbreaks of cryptosporidiosis**
- ❑ **Improve understanding of *Cryptosporidium* transmission in the United States**

North Carolina Outbreak-2009

- ❑ **Youth Summer camp**
 - livestock, recreational water venues, well-supplied drinking water, meals from a central kitchen and garden that provided >50% of produce for meals
- ❑ **46 lab-confirmed and probable cases of cryptosporidiosis**
- ❑ **Analysis of data from retrospective cohort study was used to ID risk factors and implement control measures**
 - Ham from a sandwich bar significantly associated with illness

NC outbreak 2009-Sampling

❑ Stool samples

- Animals: calves, goats, kid, and piglet
- Human cases: 12 confirmed by PCR and genotyping

❑ Environmental samples

- Water sources
 - All negative
- Composite soil samples from the garden
 - *Cryptosporidium* spp. detected but typing could not be performed



Attention: Please pet only one calf and spray your hands before and after. If there are bottles out, please don't feed the calves unless a staff member has asked you to do so.
😊 Thanks,
The Farm Staff 😊

NC outbreak 2009-Molecular Epidemiology

	<i>Cryptosporidium parvum</i>	
	#	Subtype
Human	8	IlaA17G2R1 (8)
Calf	3	IlaA17G2R1(2) IlaA15G2R1
Goat kid	1	IlaA17G2R1 (1)
Piglet	1	IlaA17G2R1 (1)

Utility of genotyping in NC outbreak

- ❑ **Traditional epidemiologic methods identified eating ham from a sandwich bar as the main risk factor**
- ❑ **Genotyping revealed identical genotypes between human cases and livestock on the property**
- ❑ **Source of infection was likely the livestock and contamination of lettuce picked with dirty hands was linked to disease**
- ❑ **Enabled accurate directives to prevent new infections**
 - Previously a diluted chlorine spray was used for “washing” after handling animals
 - New prevention efforts included running water , soap, and appropriate hand-washing techniques

Zoonotic case in Italy-2011

- ❑ **October outbreak of cryptosporidiosis identified in lambs of mixed livestock/sheep farm in Italy**
 - 50% of lambs affected, mortality rate of 80%
- ❑ **November, 18 mo old child of farm owner presented acute enteritis**
 - Free of common bacterial species
 - *Cryptosporidium* sp. identified by microscopy



Findings-Italy 2011

- ❑ **Child attended day care**
- ❑ **No direct contact with animals on farm**
- ❑ **Molecular Epidemiology**
 - DNA sequencing from lab samples and human sample revealed presence of *C. parvum*
 - GP60 sequencing
 - Genotype IIaA20G2R1 present in sheep and human sample
- ❑ **Presence of rare genotype in both sheep and human case samples suggest exposure on the farm**
 - Indirect contact with oocysts on father's clothing

CryptoNet Proof of Utility

- ❑ **Differentiation and/or connection of outbreaks**
 - Two distinct outbreaks of cryptosporidiosis in neighboring counties of OK in the same month (2007)
- ❑ **Source tracking**
 - Linkage between cases and suspected swimming pool in SC outbreak
 - Identification of a common zoonotic exposure between TN cases and a rare *C. parvum* subtype (IIaA15G2R1)
- ❑ **Improved epidemiologic understanding**
 - Finding a predominant *C. hominis* subtype IaA28R4
 - Common occurrence of zoonotic species in sporadic cases in ME, TN, VT, and Wi

CryptoNet timeline

□ Currently completed goals

- User scripts
- Over 350 reference sequences included in the database
- Over 1000 sporadic and outbreak cases in the database
- QA and QC of lab methodologies

□ 2012

- Revise user scripts
- Complete admin and server scripts

❑ **2013 launch**

- Training and certification of 3 state partners

❑ **2014 roll-out**

- Train and certify 5 additional partners
- Revise any scripts based on trials with 2013 partners

❑ **2015 final roll-out**

- Train and certify all interested partners

Acknowledgements

- ❑ **Molecular epi laboratory
CDC/DFWED/PDB**
- ❑ **Water epi team**
 - Michele Hlavsa
 - Jonathan Yoder
 - Michael Beach
 - EISO
- ❑ **CryptoNet, DFWED/PDB**
 - Michael Beach
 - Lihua Xiao
 - Vince Hill
- ❑ **CaliciNet**
 - Duping Zheng
 - Jan Vinjie
- ❑ **PulseNet**
 - Brenda Brown
- ❑ **State and local health
departments**

We need partners!!

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

