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

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08/29/2012

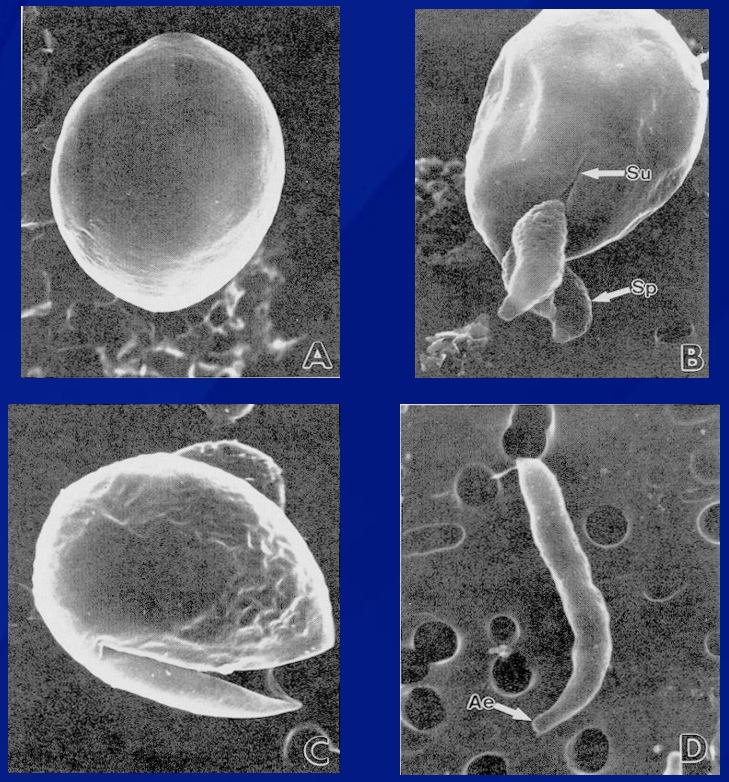
National Center for Emerging and Zoonotic Infectious Diseases
Waterborne Disease Prevention Branch

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.

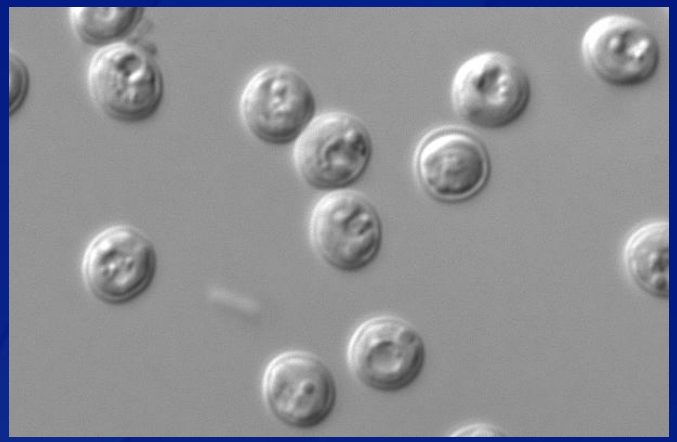
From Reducker et al., J Protozool., 32, 708-711, 1985

Cryptosporidium spp.

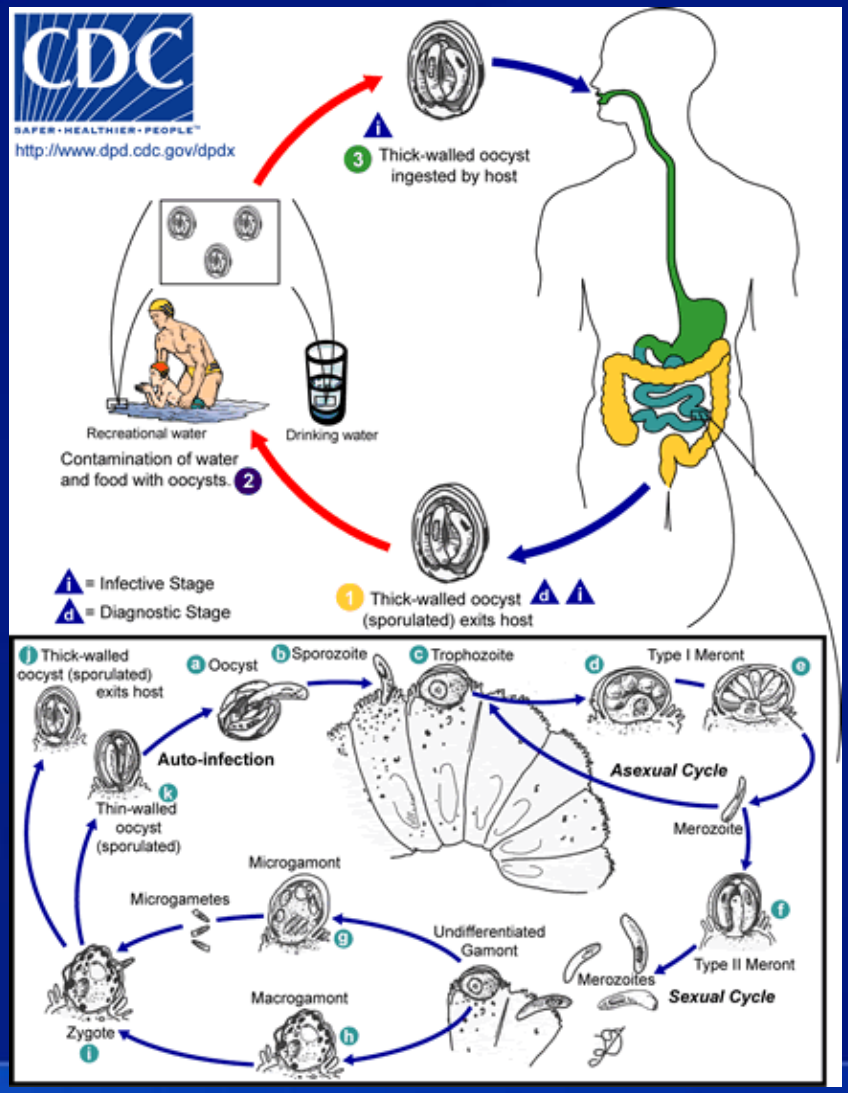
- **Environmentally-resistant, chlorine-tolerant protozoan parasite**
  - Can survive 2-6 months in a moist environment
- **Primary site of infection is within the distal region of the SI and proximal colon**
- **Transmission routes include:**
  - Contact with infected persons or animals
  - Contaminated 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



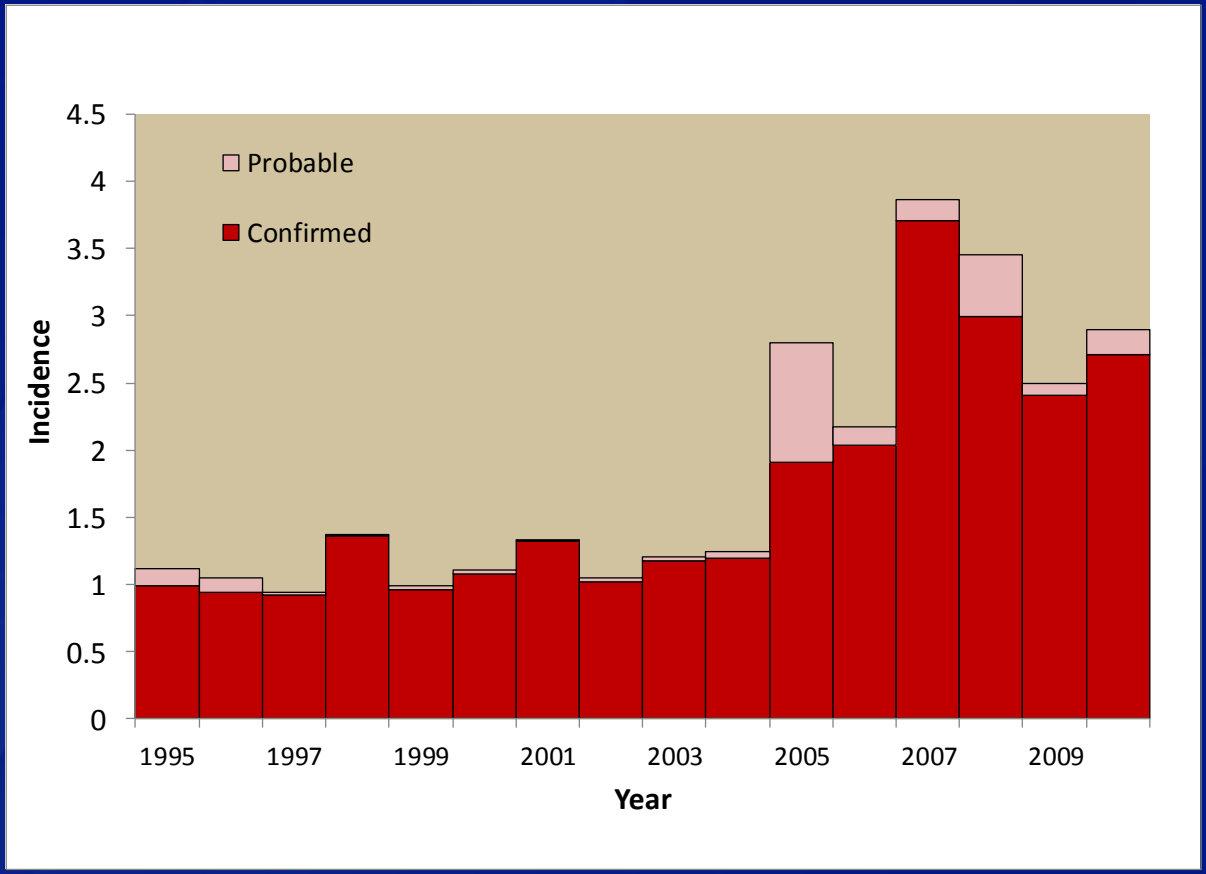
Cryptosporidiosis

- **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

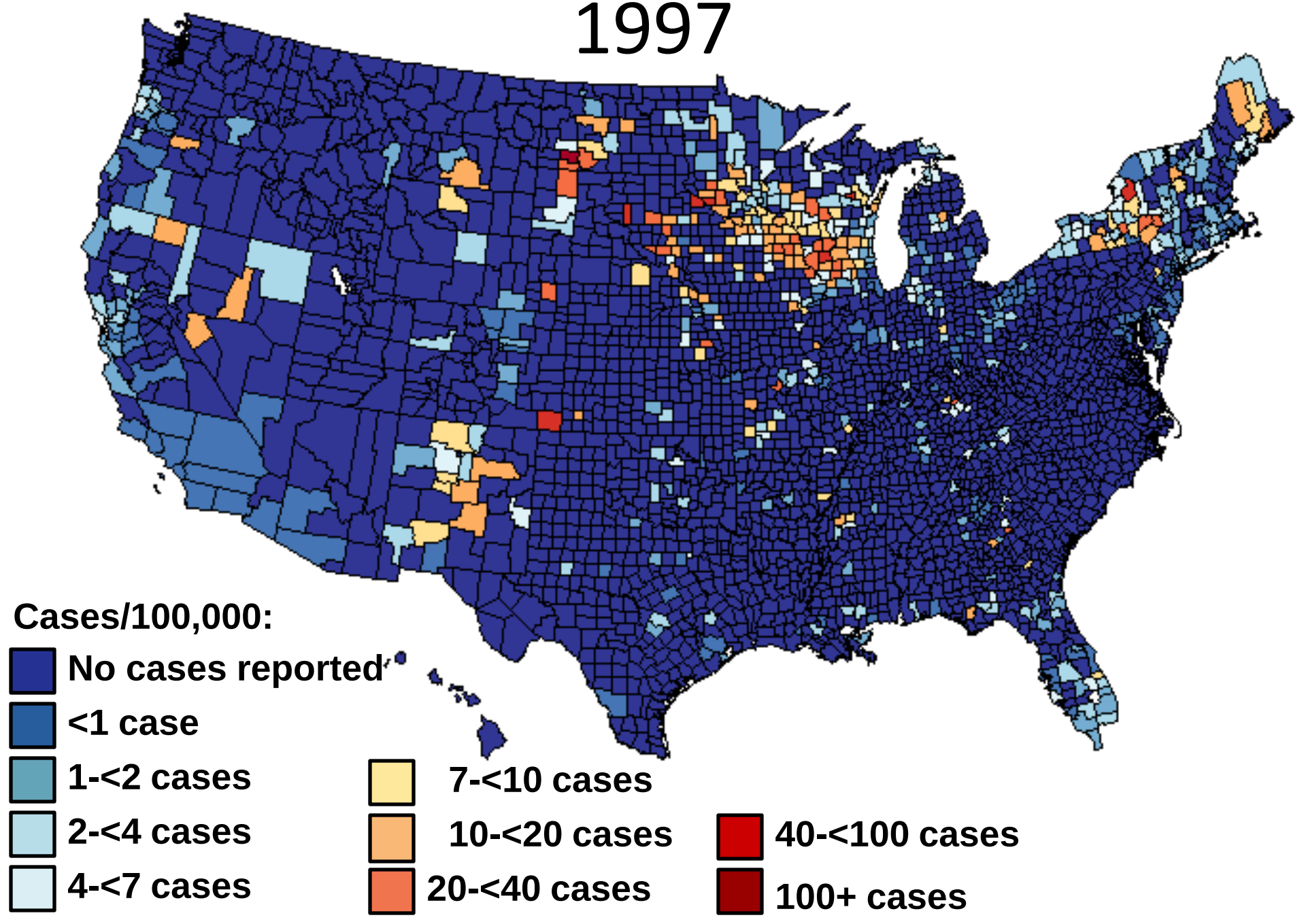


*Per 100,000 population.

†N=85,514.

§First full year of national reporting.

1997


Cases/100,000:
No cases reported
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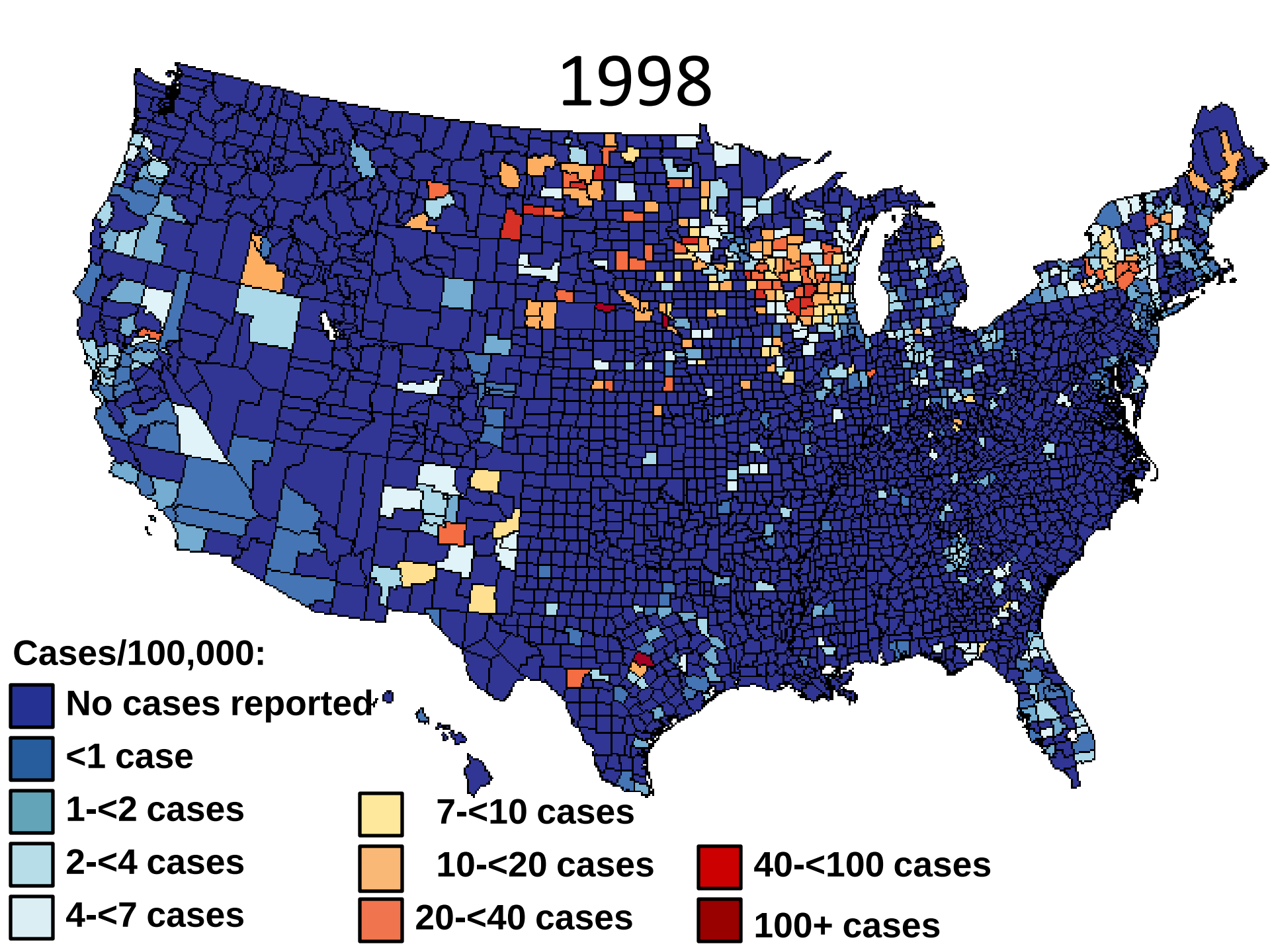
10-<20 cases
20-<40 cases
40-<100 cases
100+ cases

1998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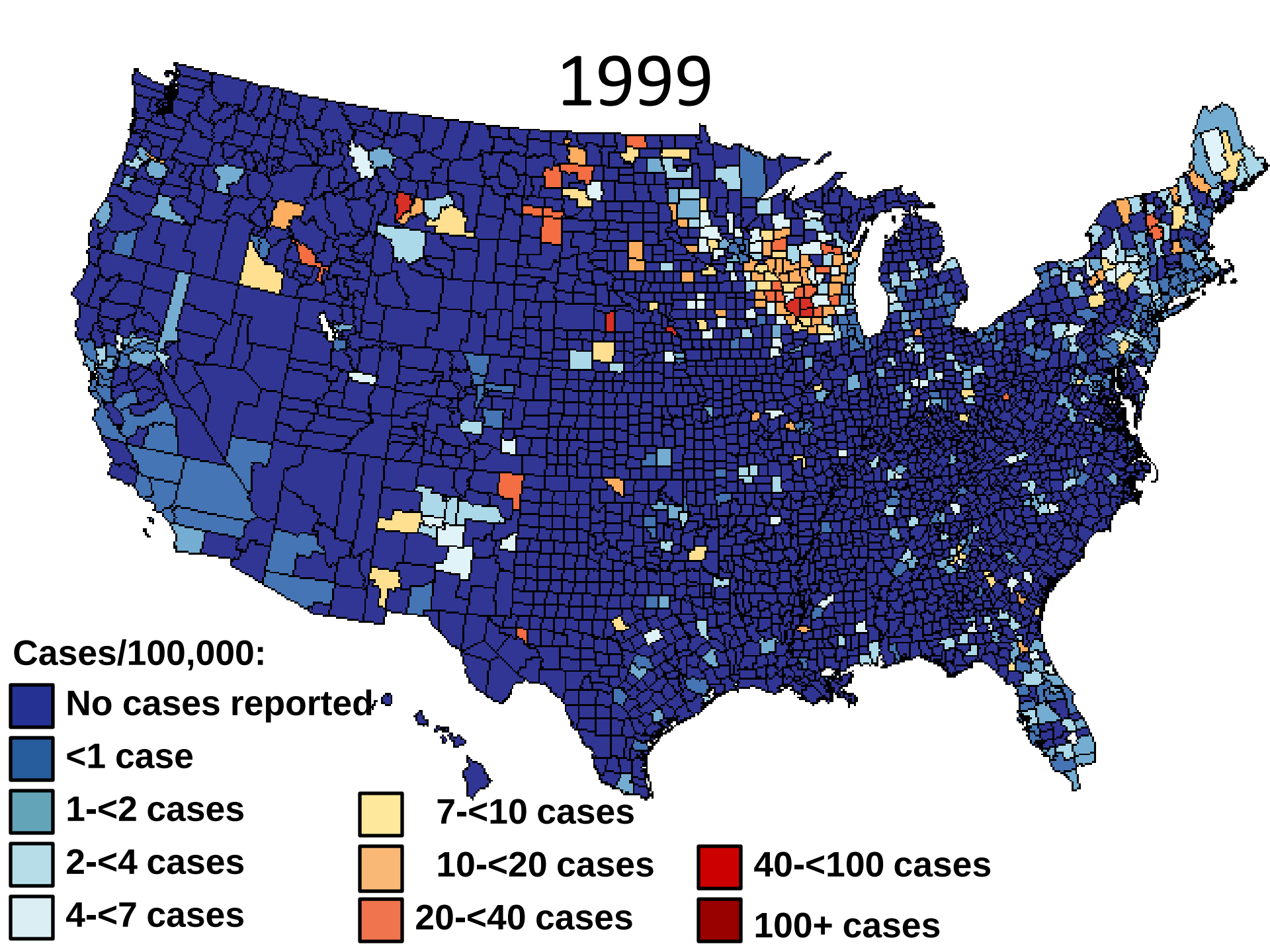
1999
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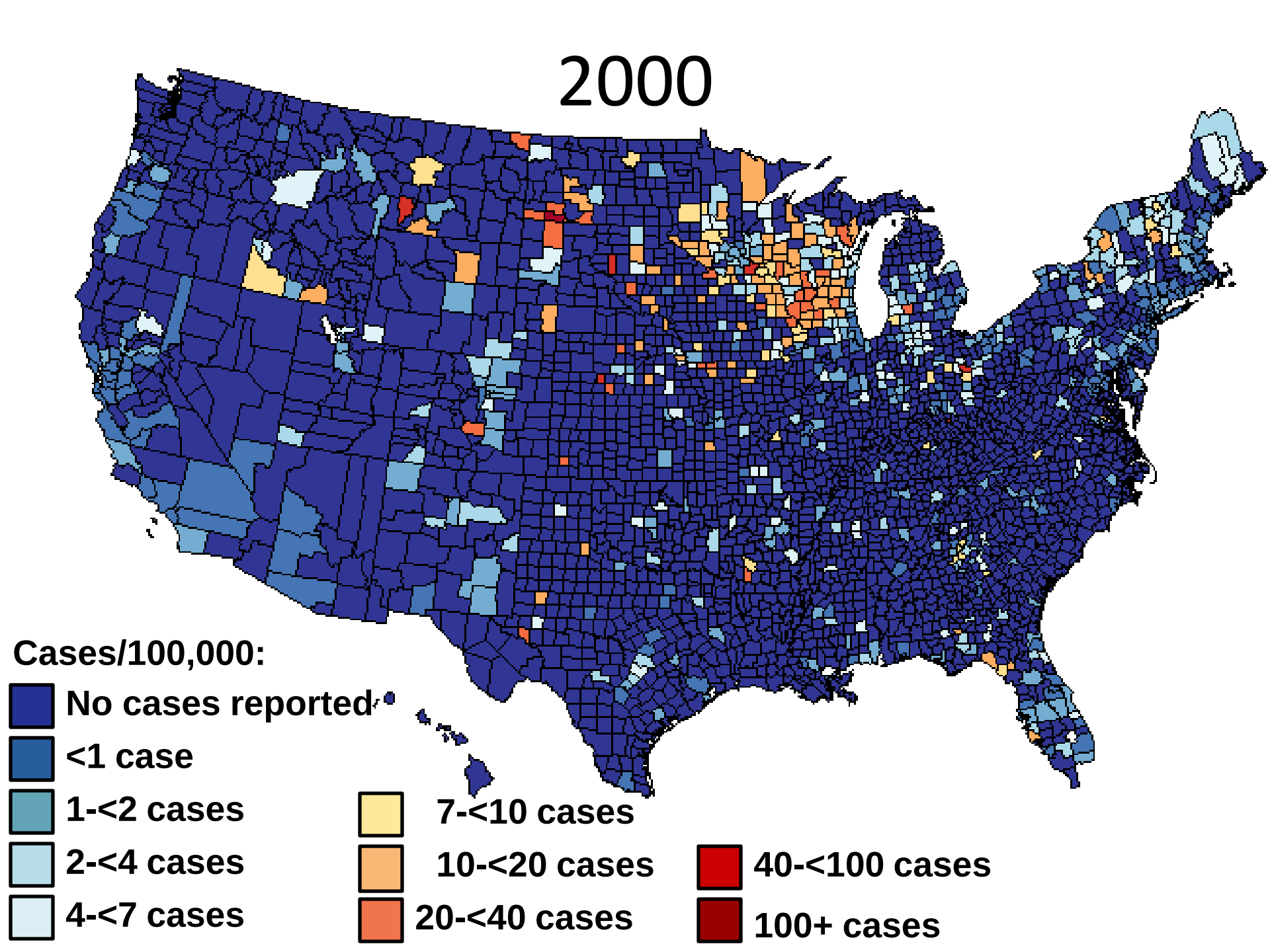
2000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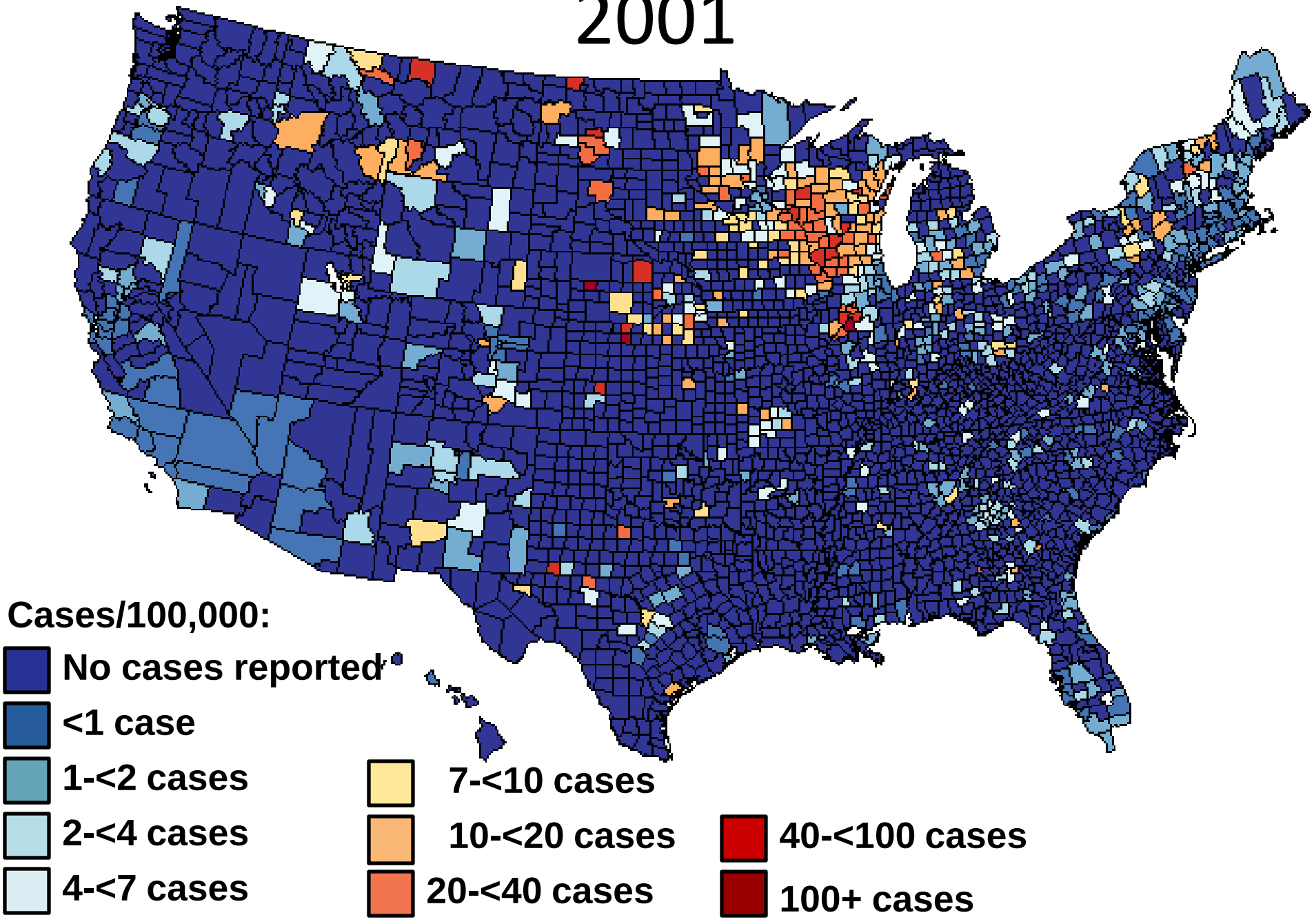
2001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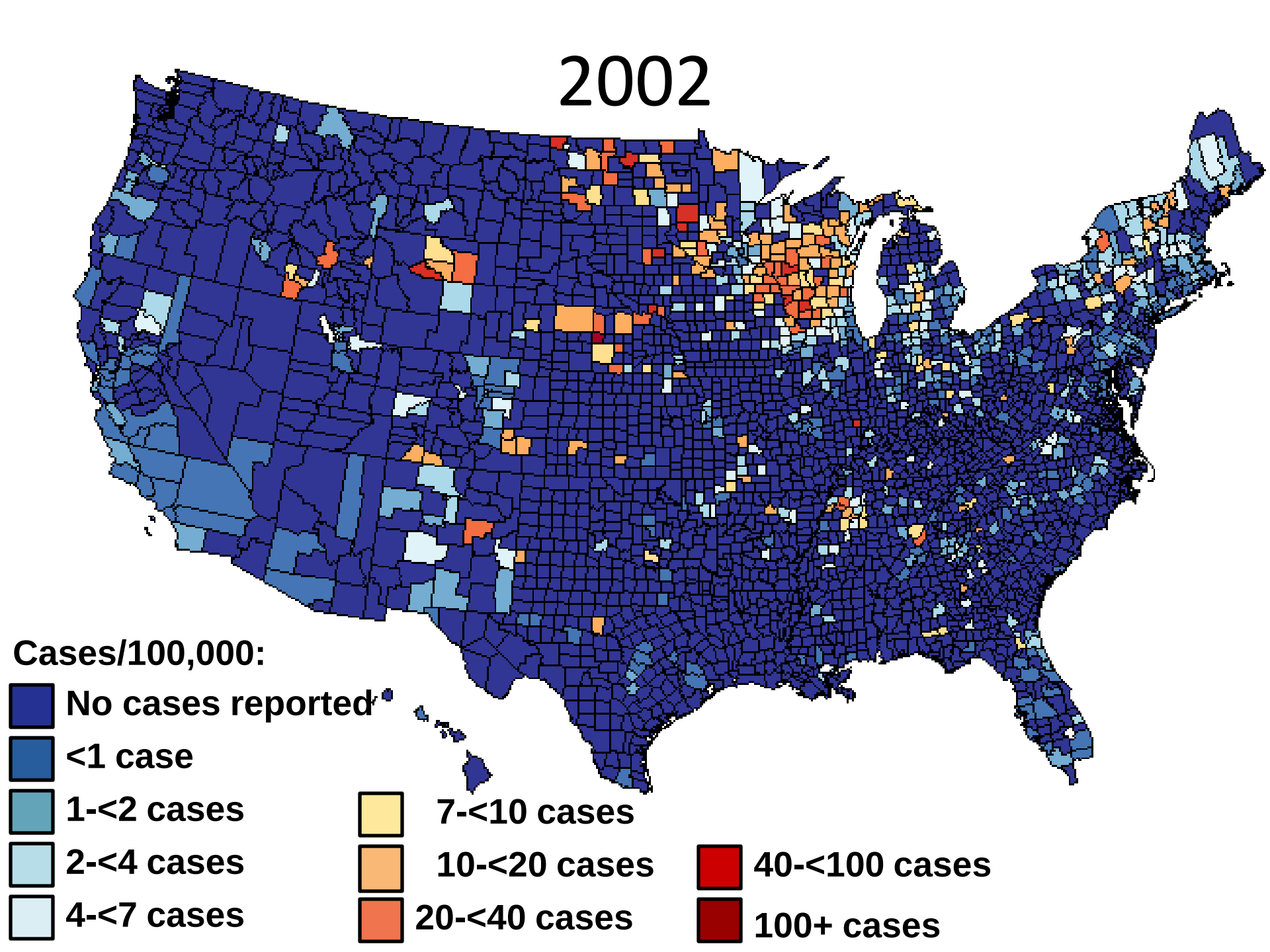
2002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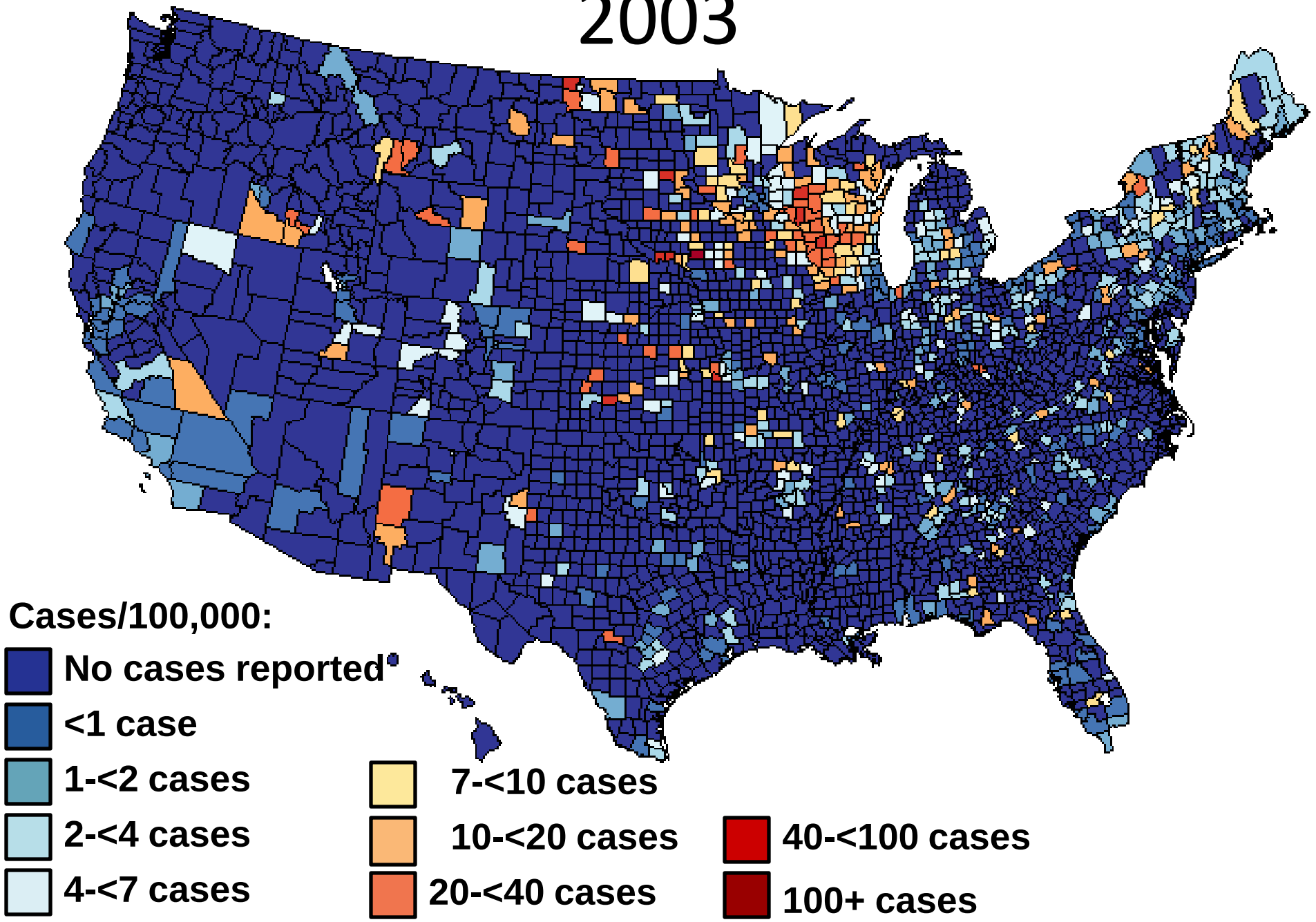
2003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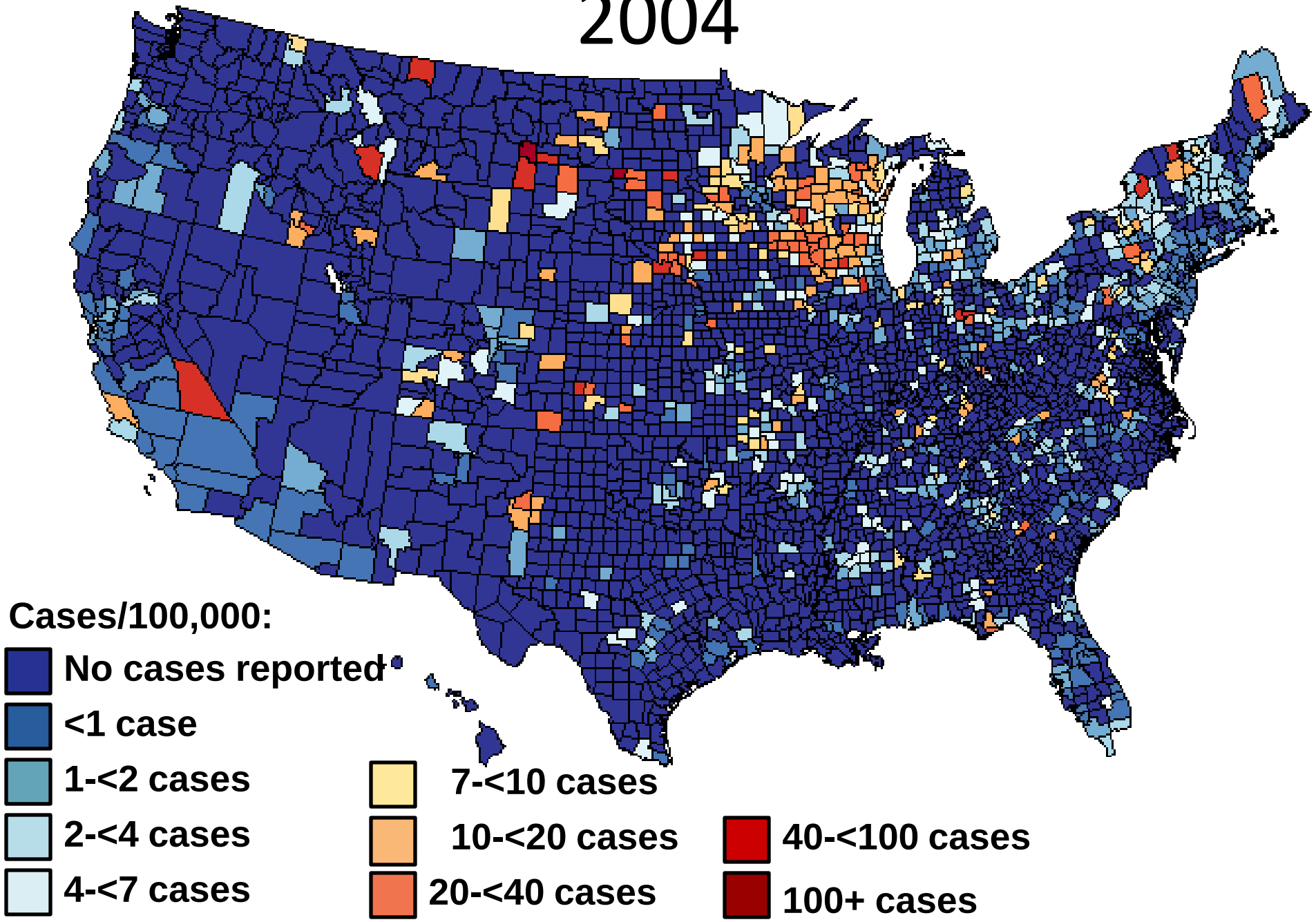
100+ cases

2004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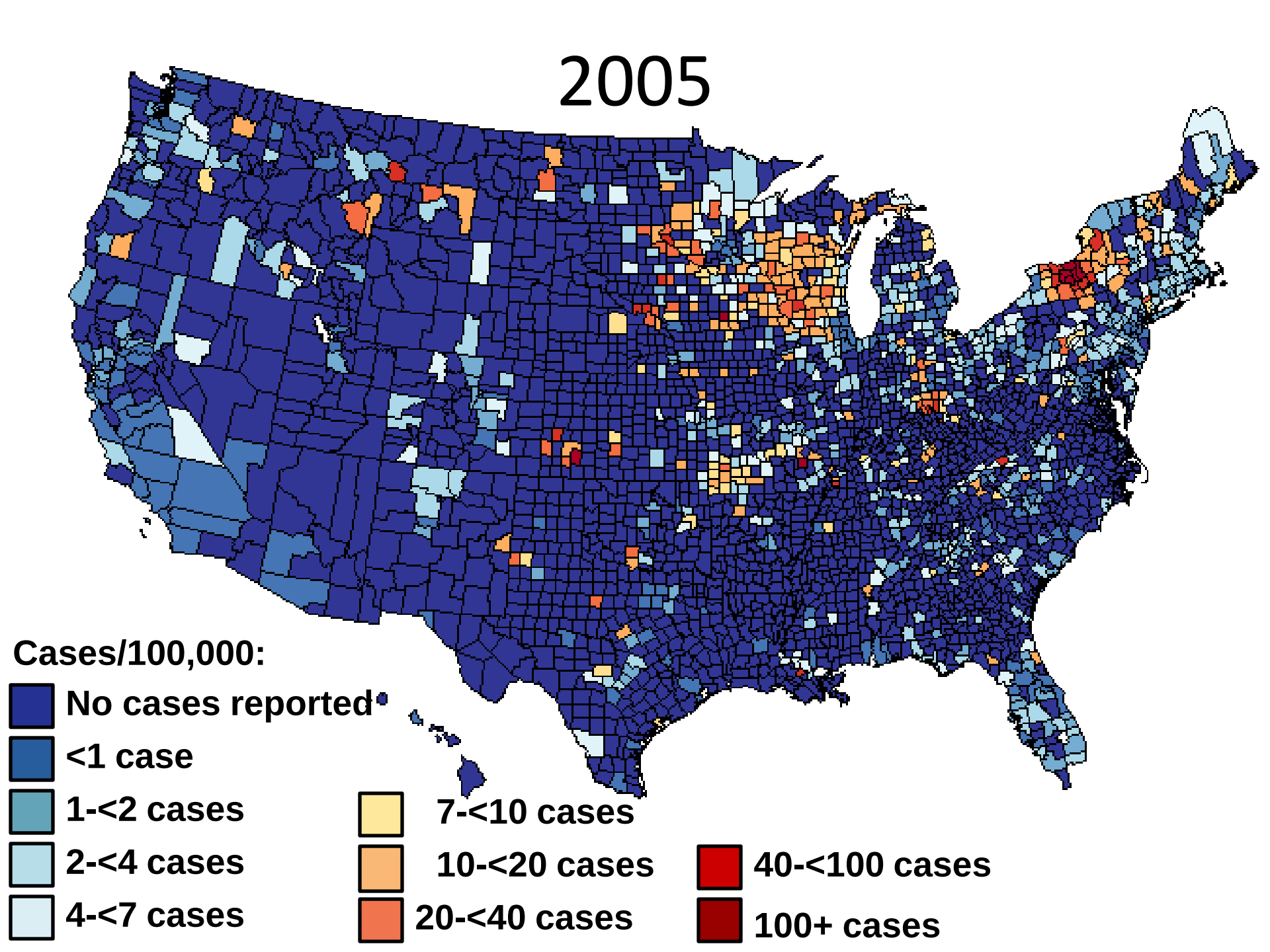
2005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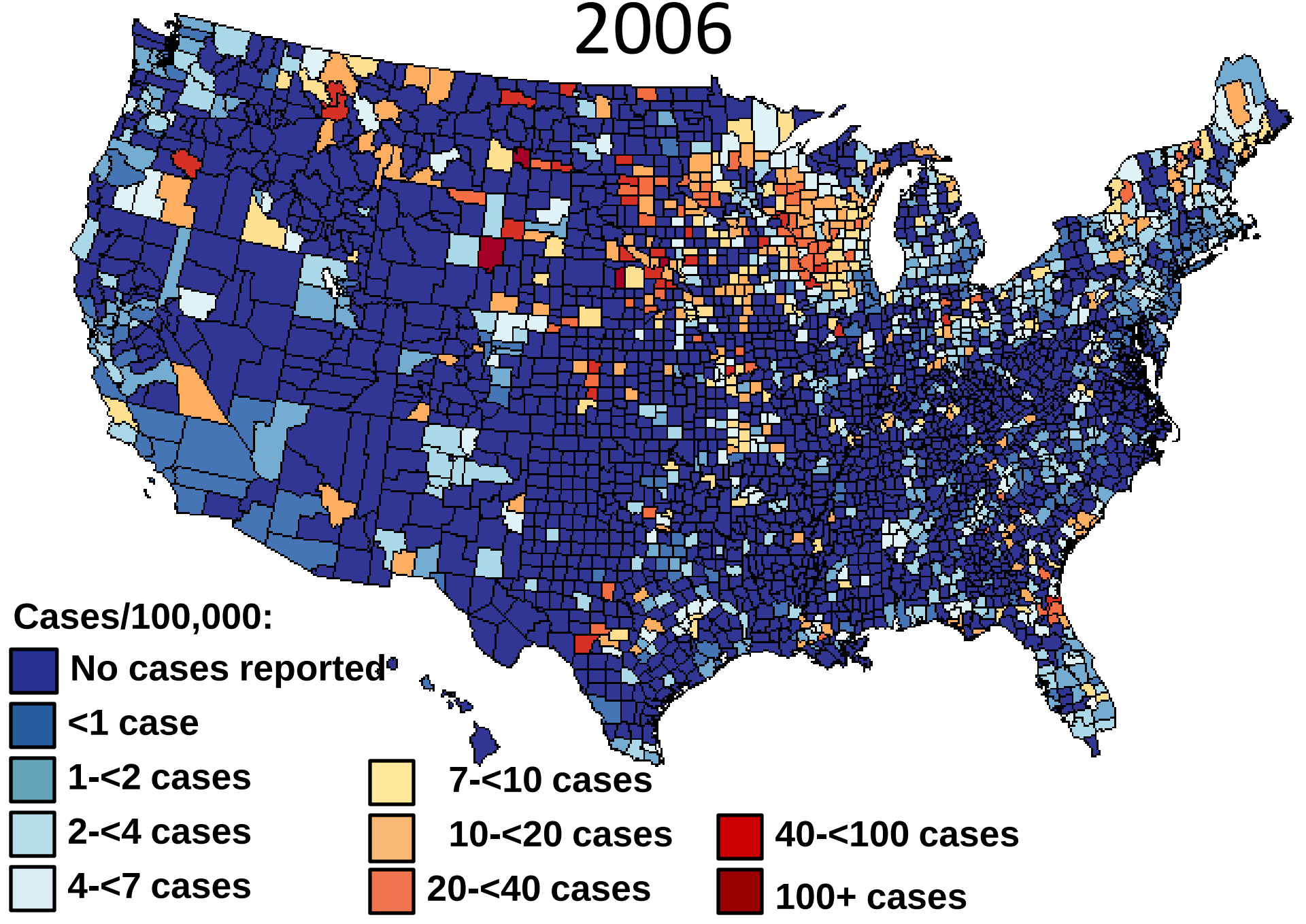
2006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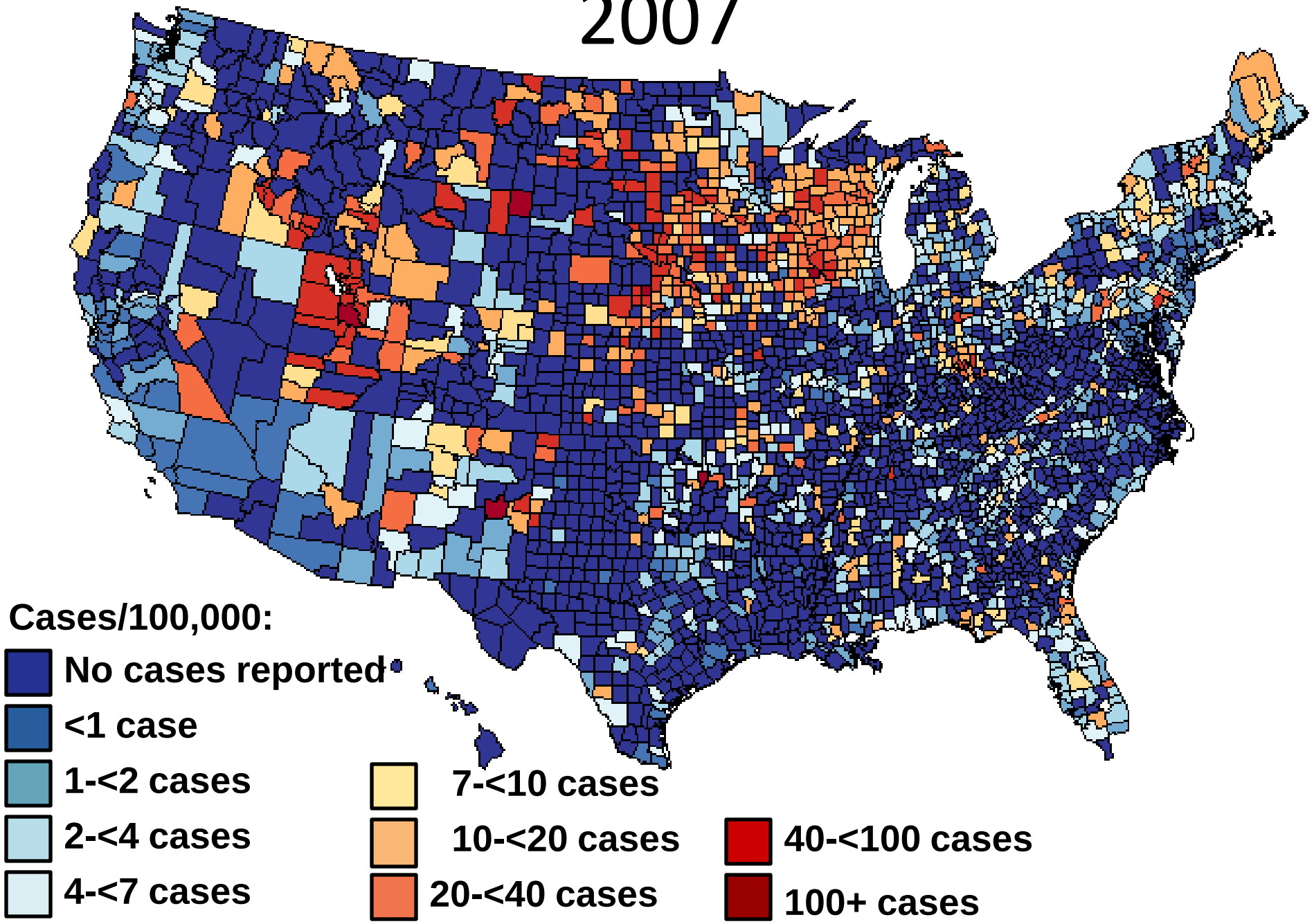
2007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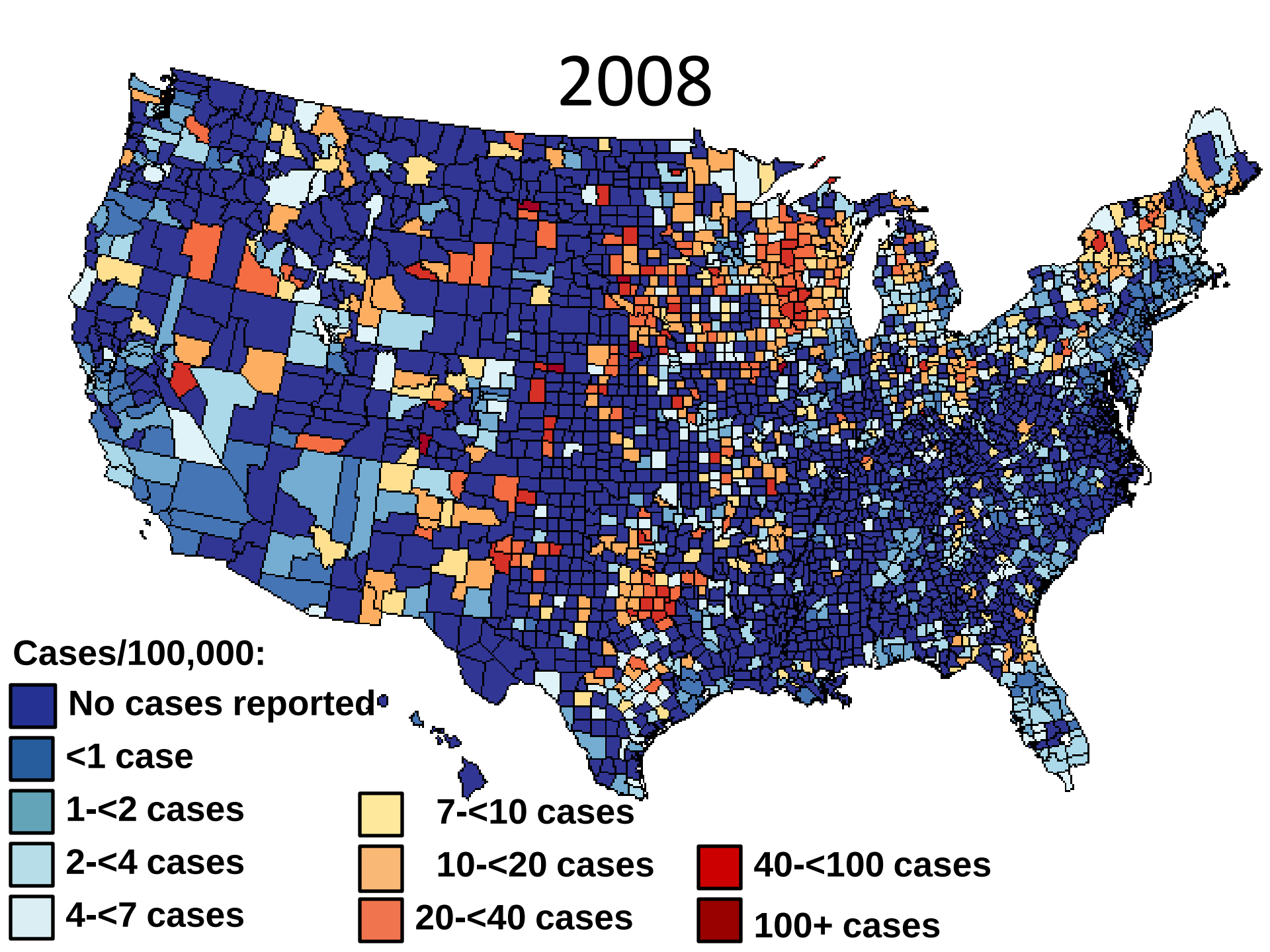
2008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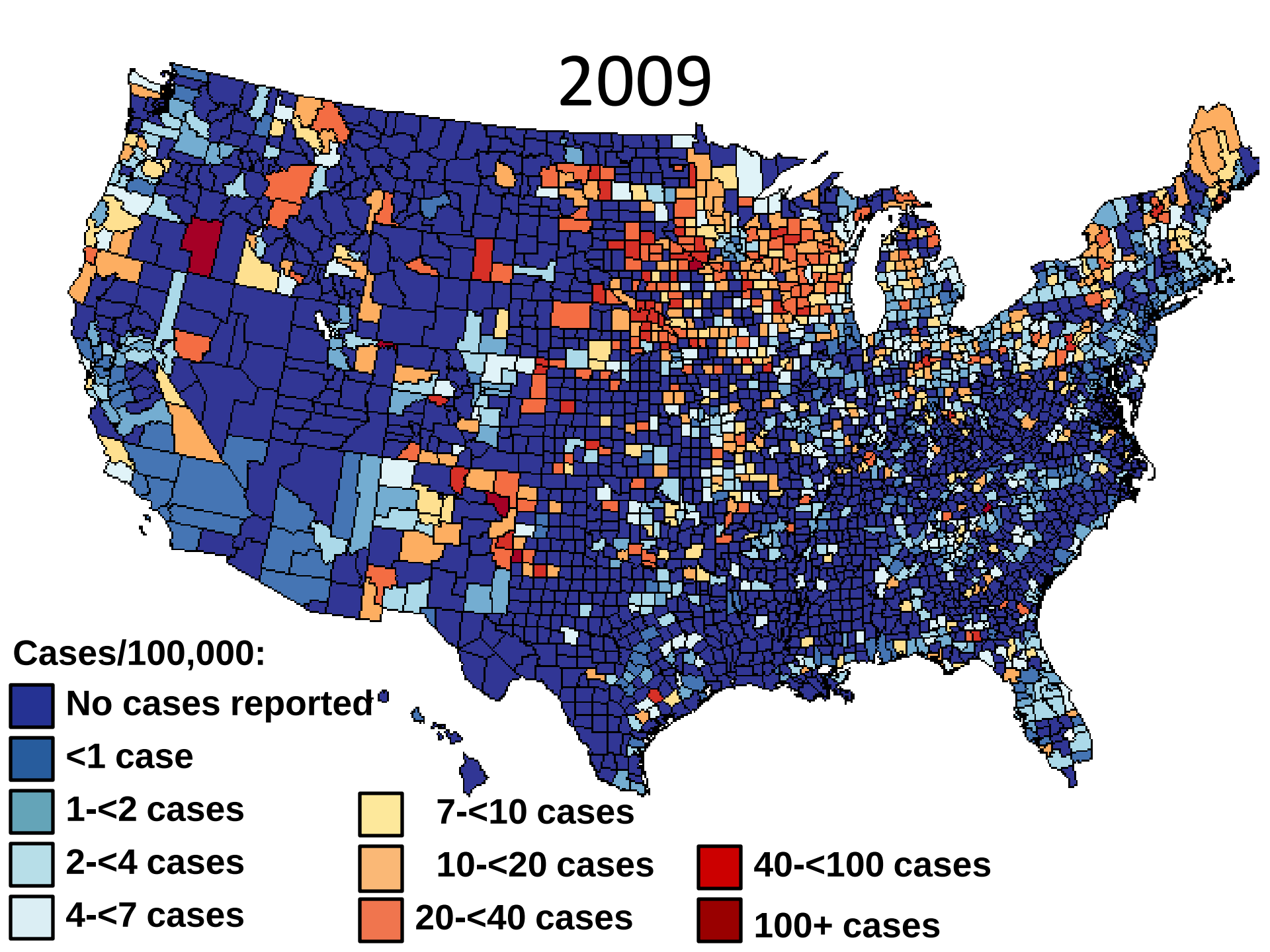
2009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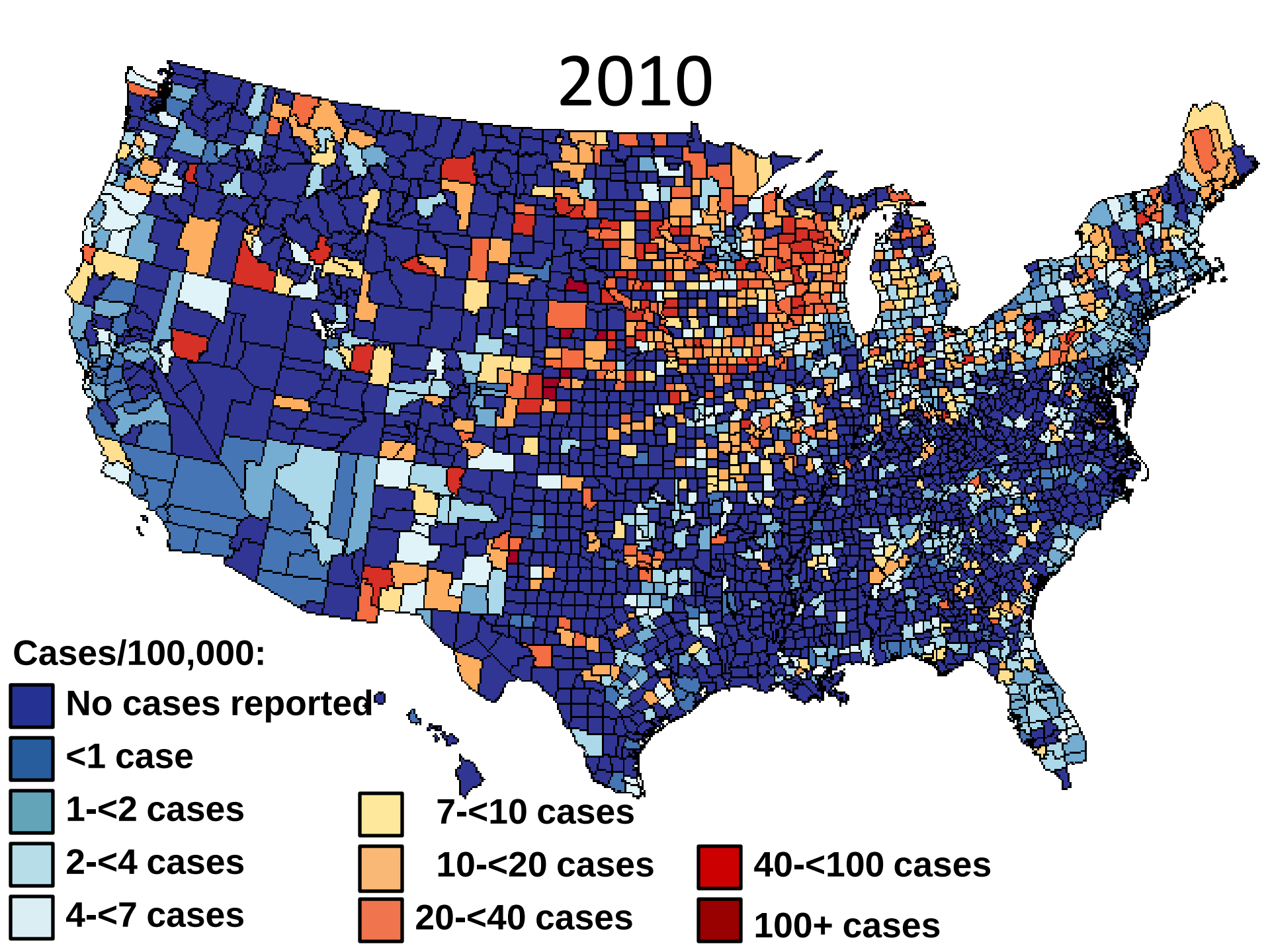
2010
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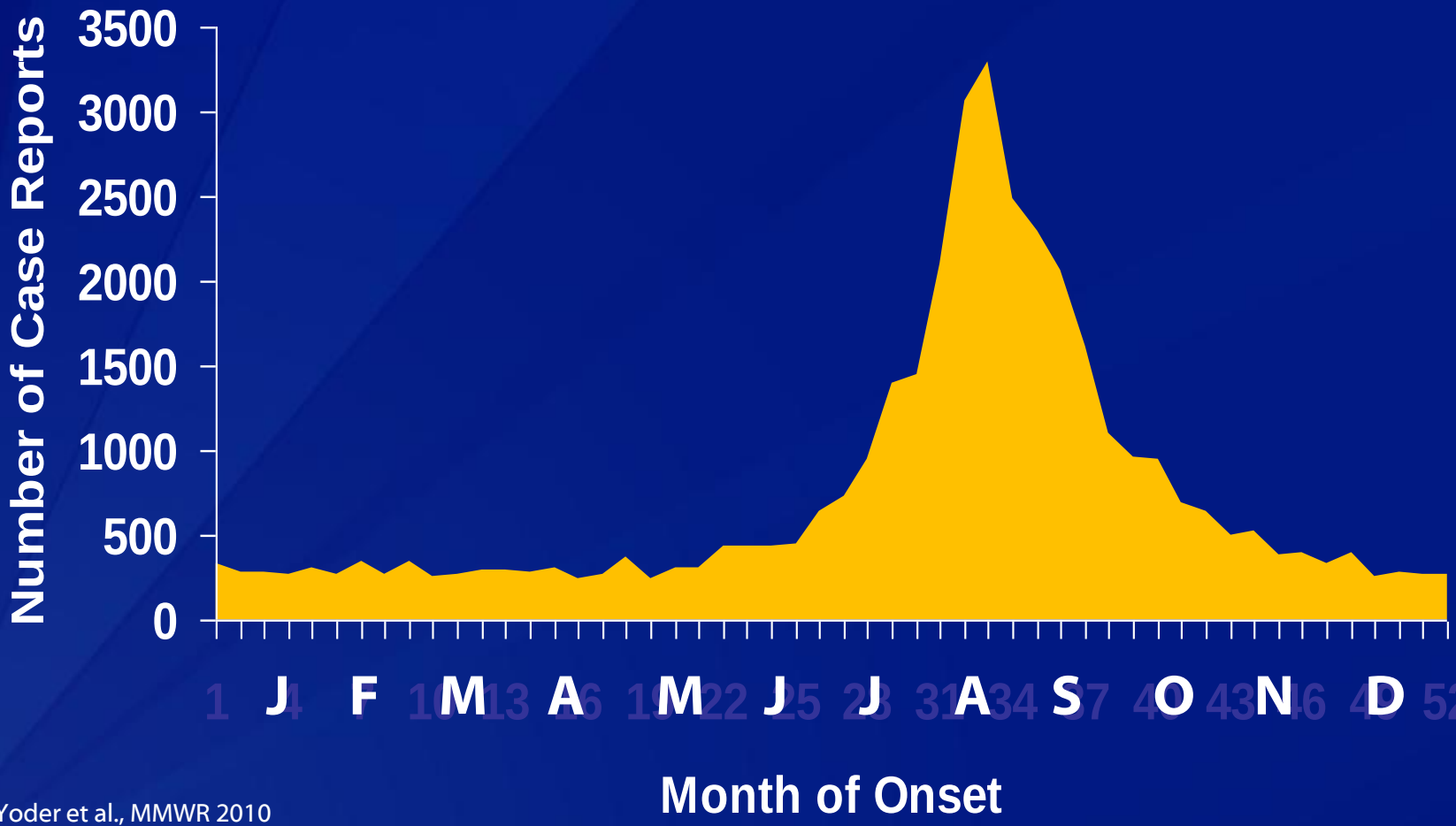
100+ cases

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

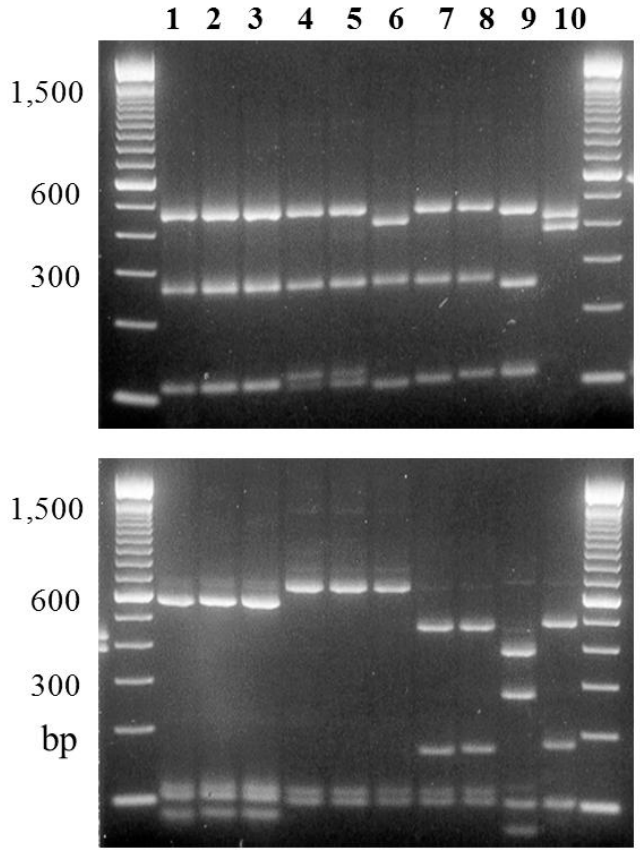
- Detection of *Cryptosporidium* organisms in stool samples
- Detection of *Cryptosporidium* antigen by immunodiagnostics (EIA, DFA)
- Detection by rapid cartridge assay kits
- **Detection of *Cryptosporidium* DNA in stool samples**

GENOTYPING OF *CRYPTOSPORIDIUM* SPP.

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
C. hominis	Ia
	Ib
	Id
	Ie
	If
	Ig
C. parvum	IIa
	IIb
	IIc
	IId
	IIe
	IIf
	IIg
	IIh
	IIi
	IIk
	IIl

Xiao, Experimental Parasitology 2010

Resources and costs

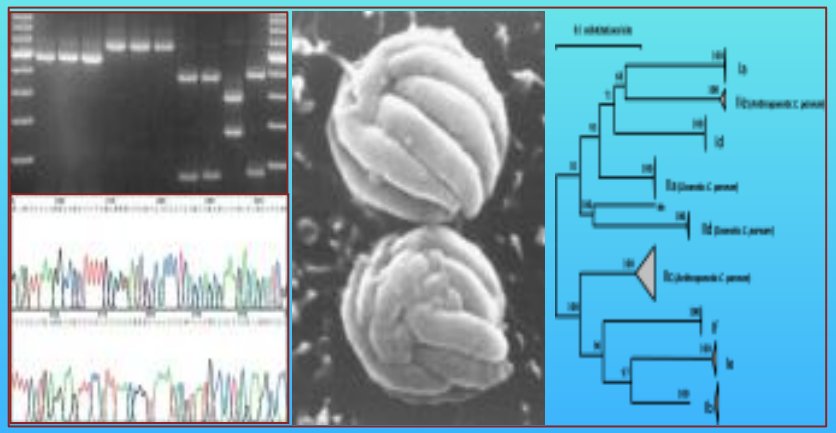
- **Equipment**
  - Thermal cycler
  - Water bath
  - Electrophoresis system
  - Microcentrifuge
  - ABI 3130xl Genetic Analyzer
- **Estimated cost: ~$9.50/sample**

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**

CryptoNet

CryptoNet USA



National Molecular Surveillance for Cryptosporidium

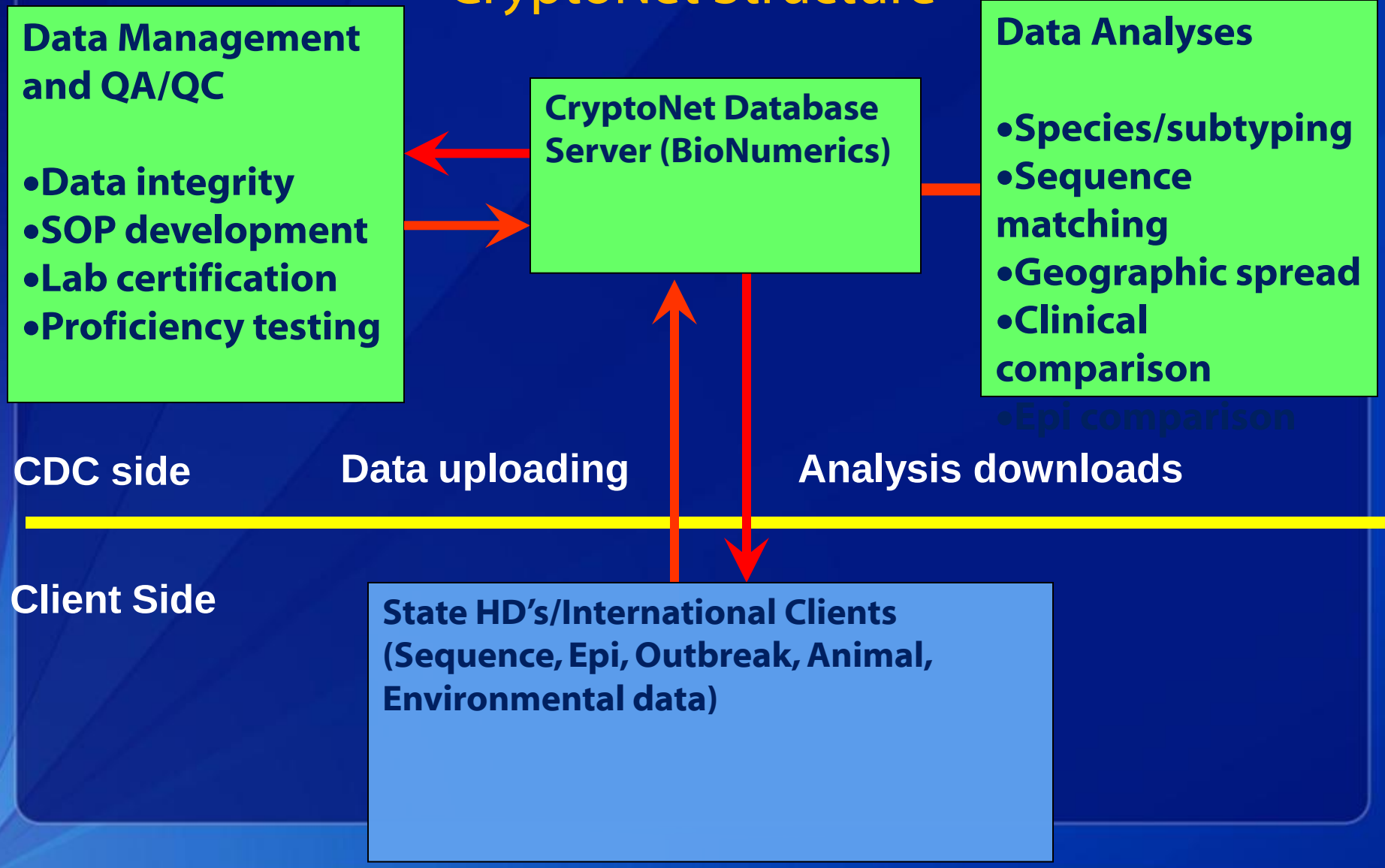
CDC

Centers for Disease Control and Prevention

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

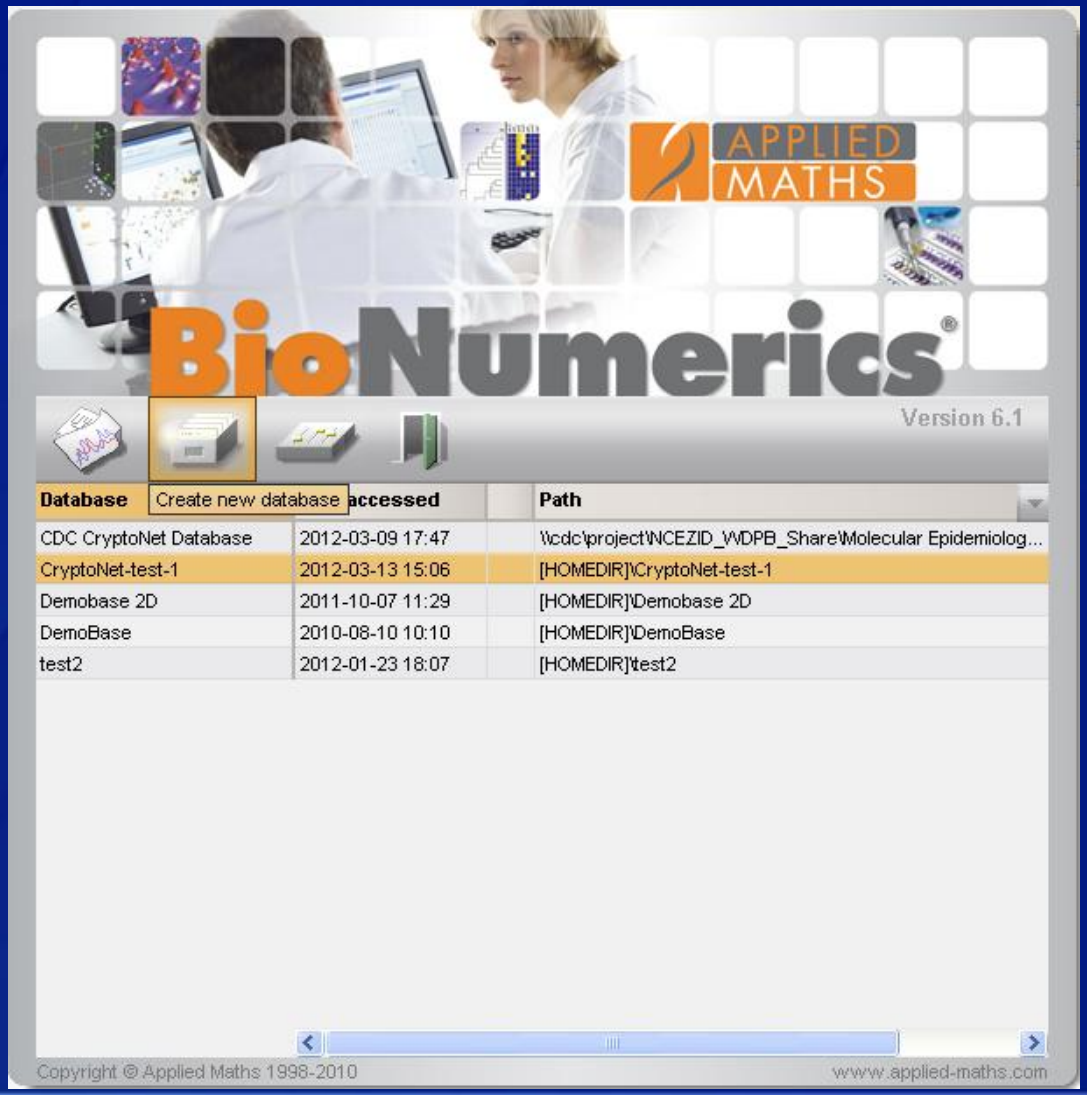
CryptoNet Structure



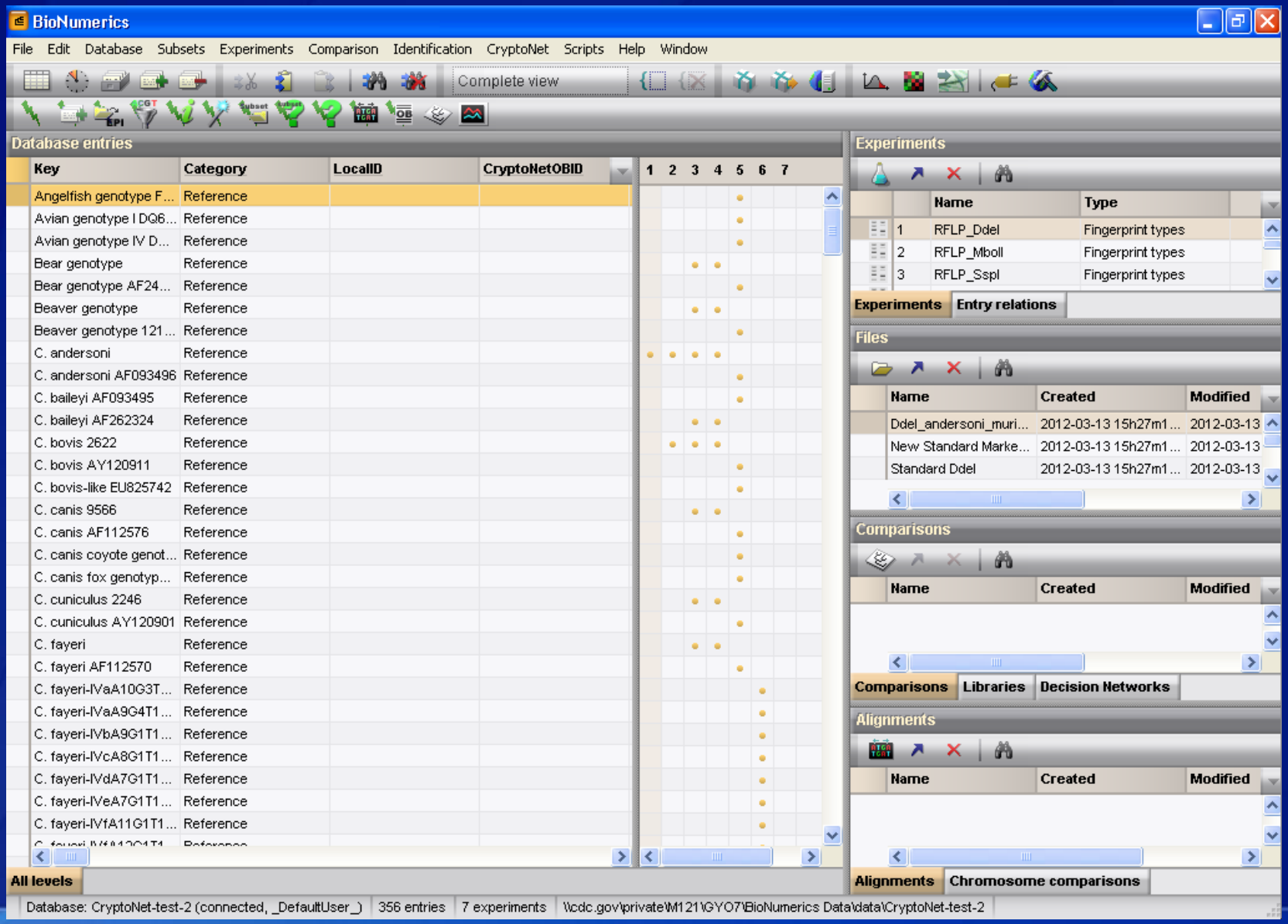
Data Management

- **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

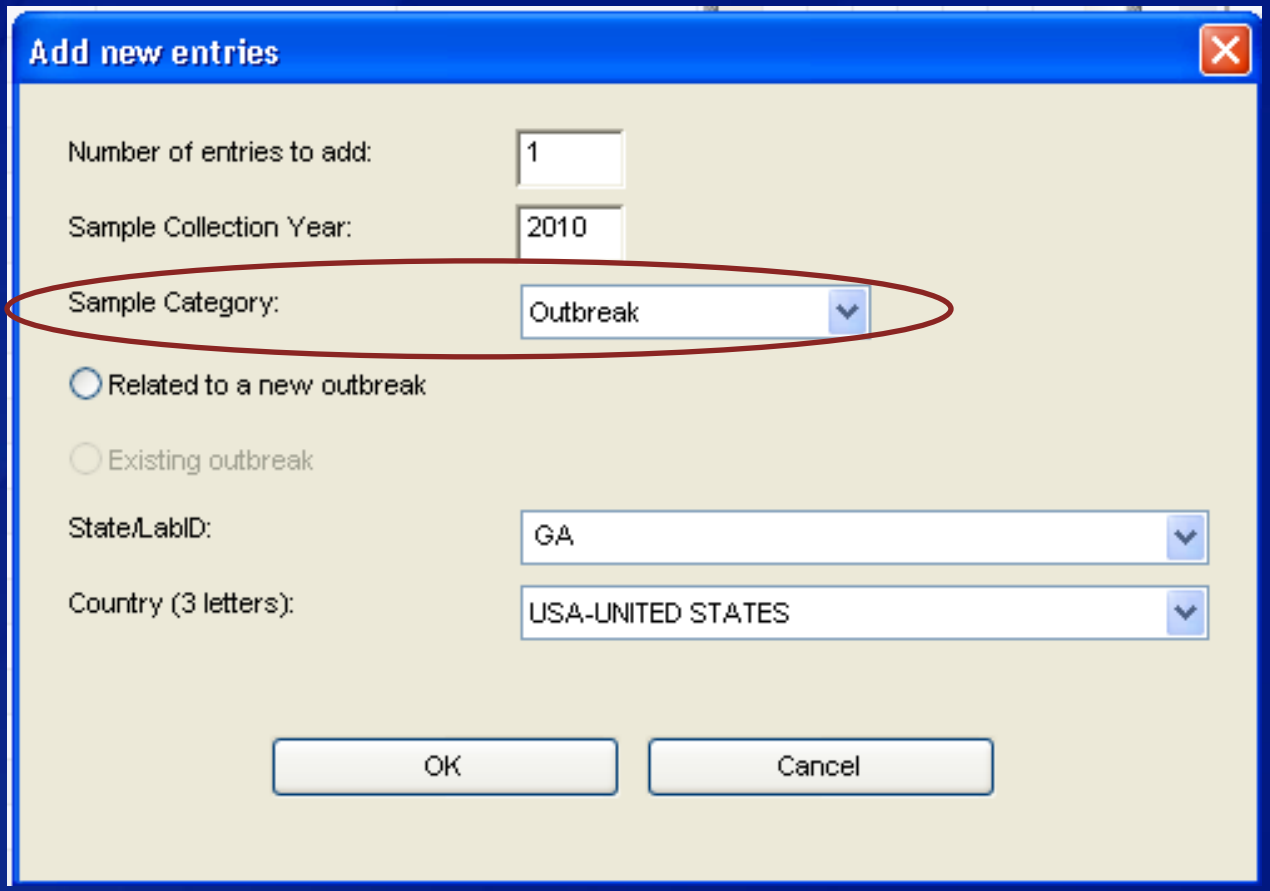


Reference strains

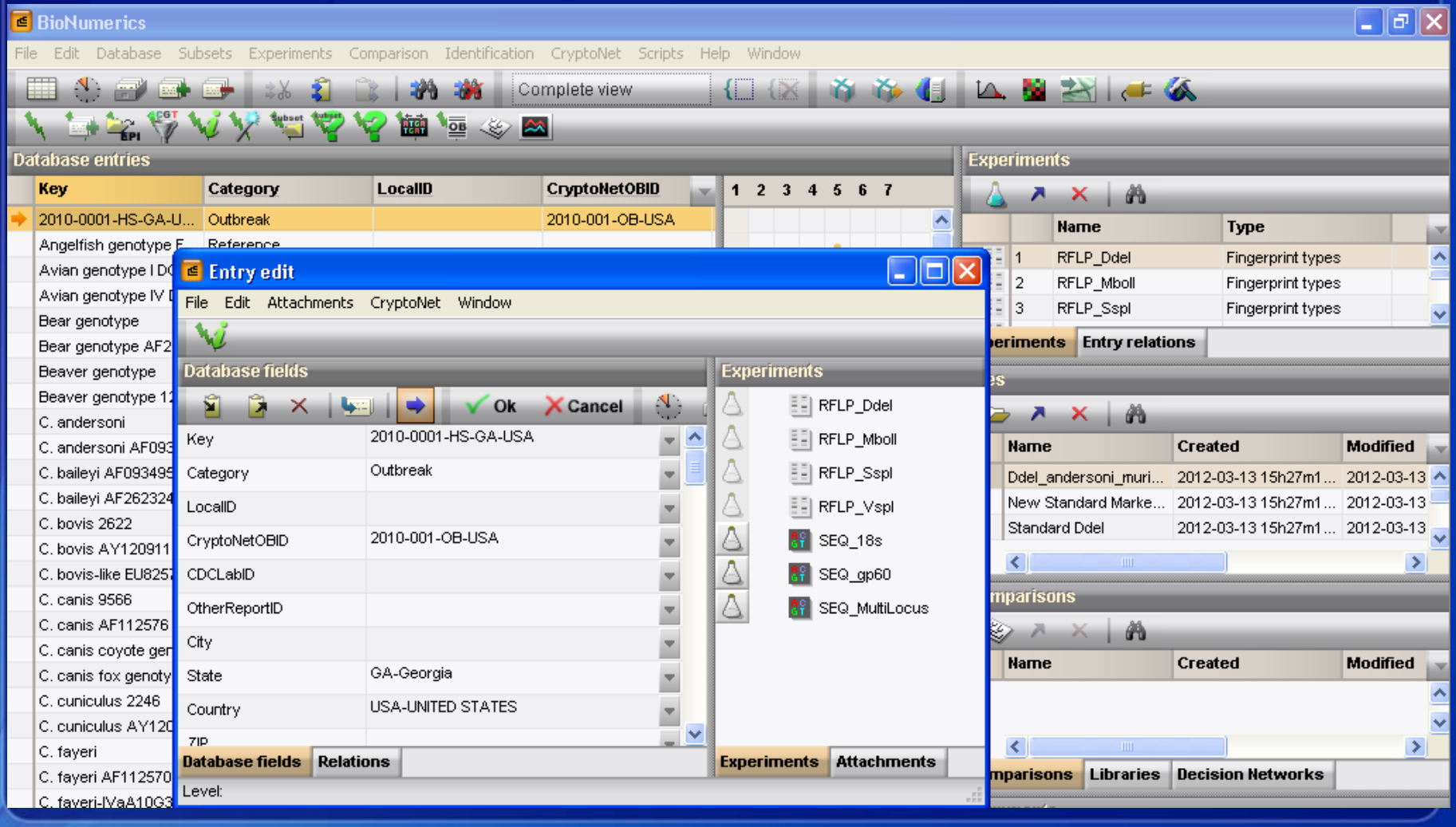


Inputting Data

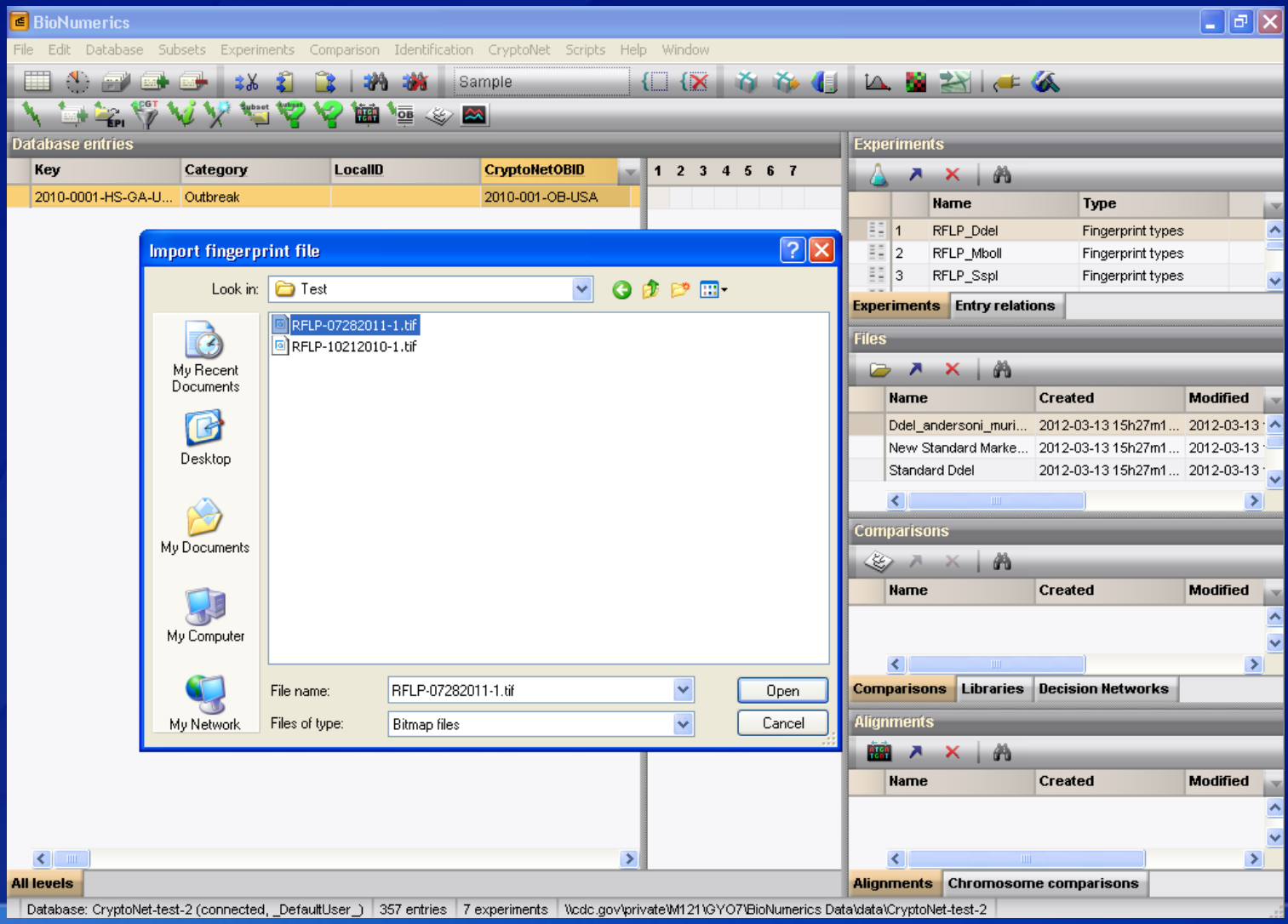
- **Differentiate outbreak clusters from sporadic cases**



Inputting Epidemiologic Data

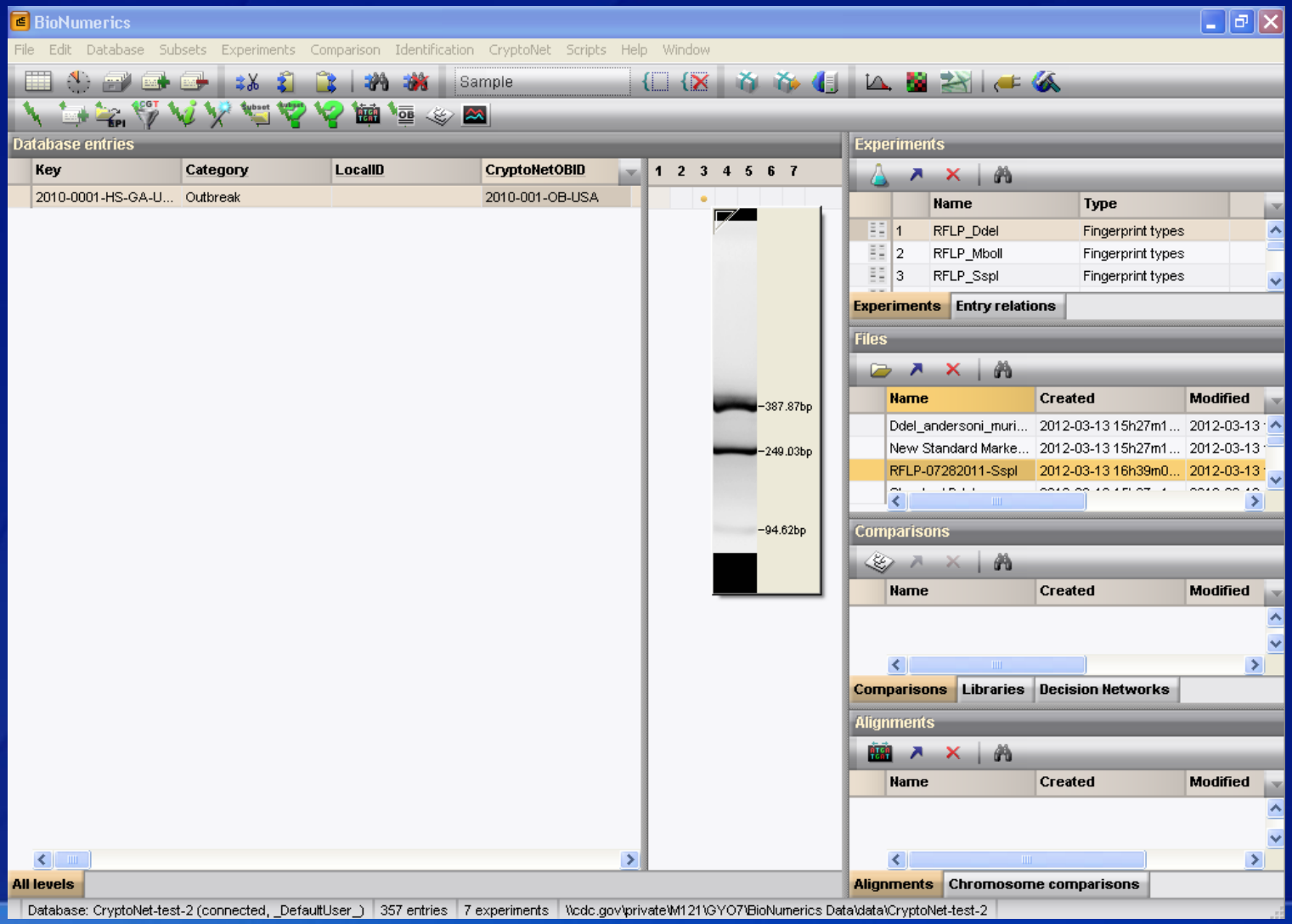


Inputting RFLP Data

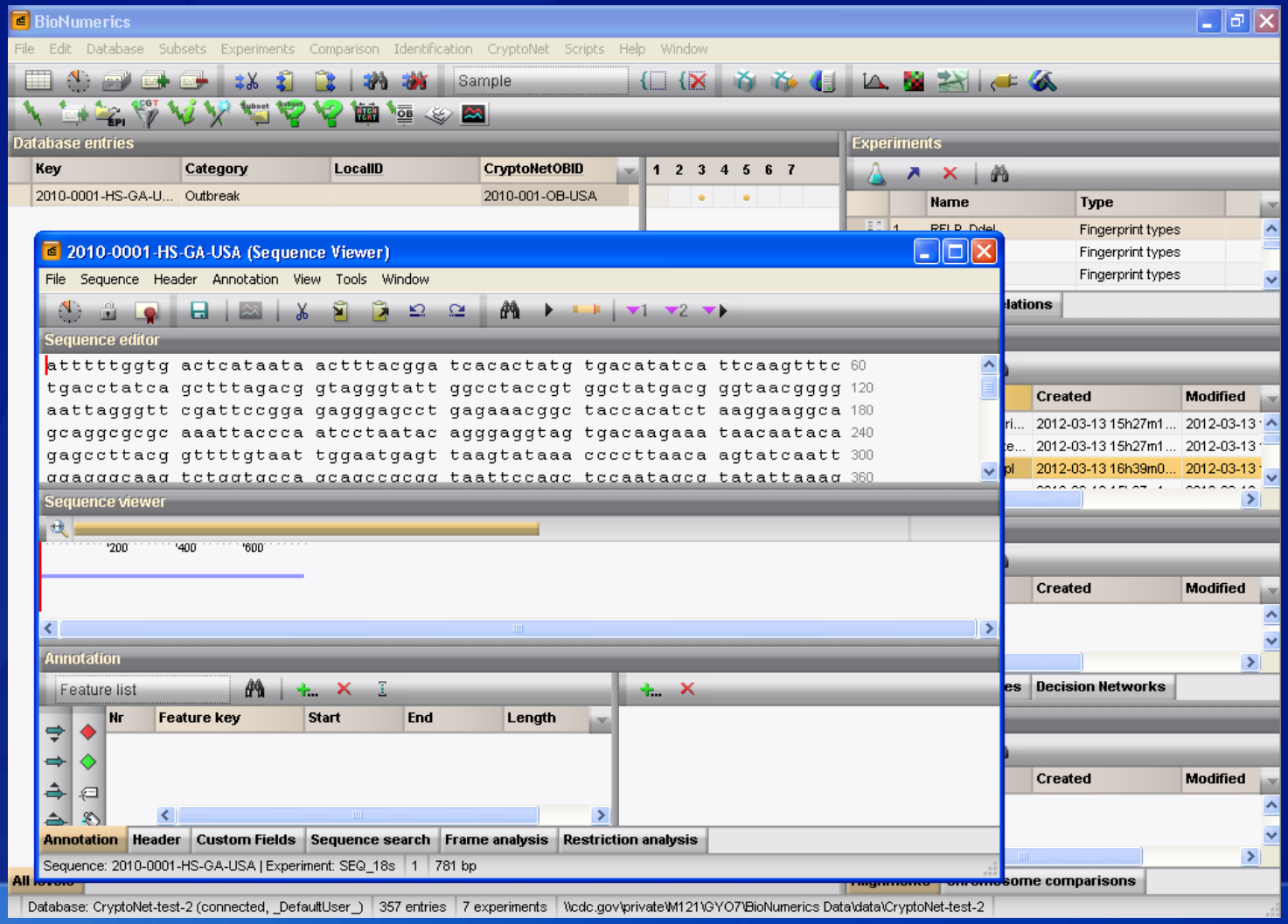


Ability to Edit and Normalize

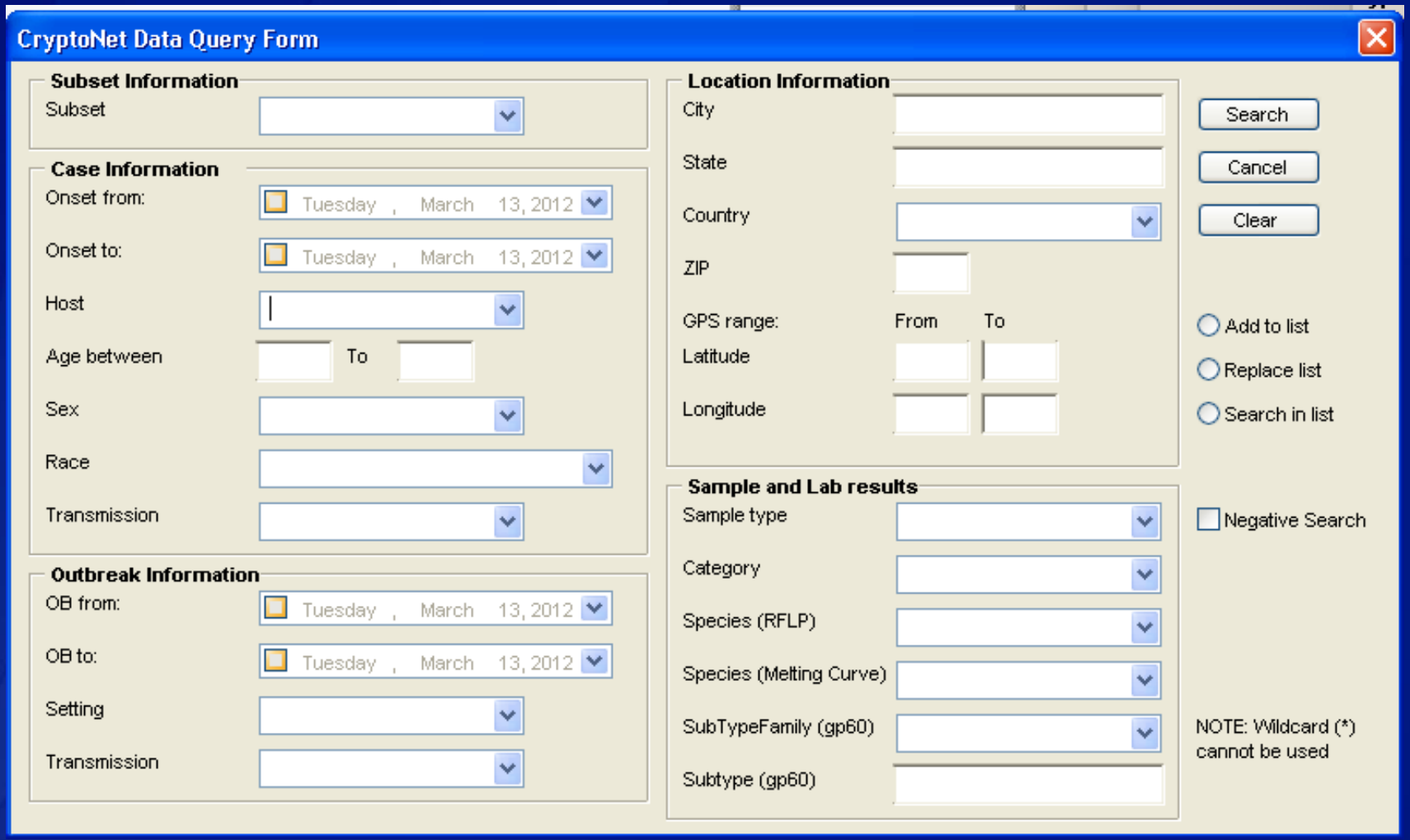
Completed Experiment Input



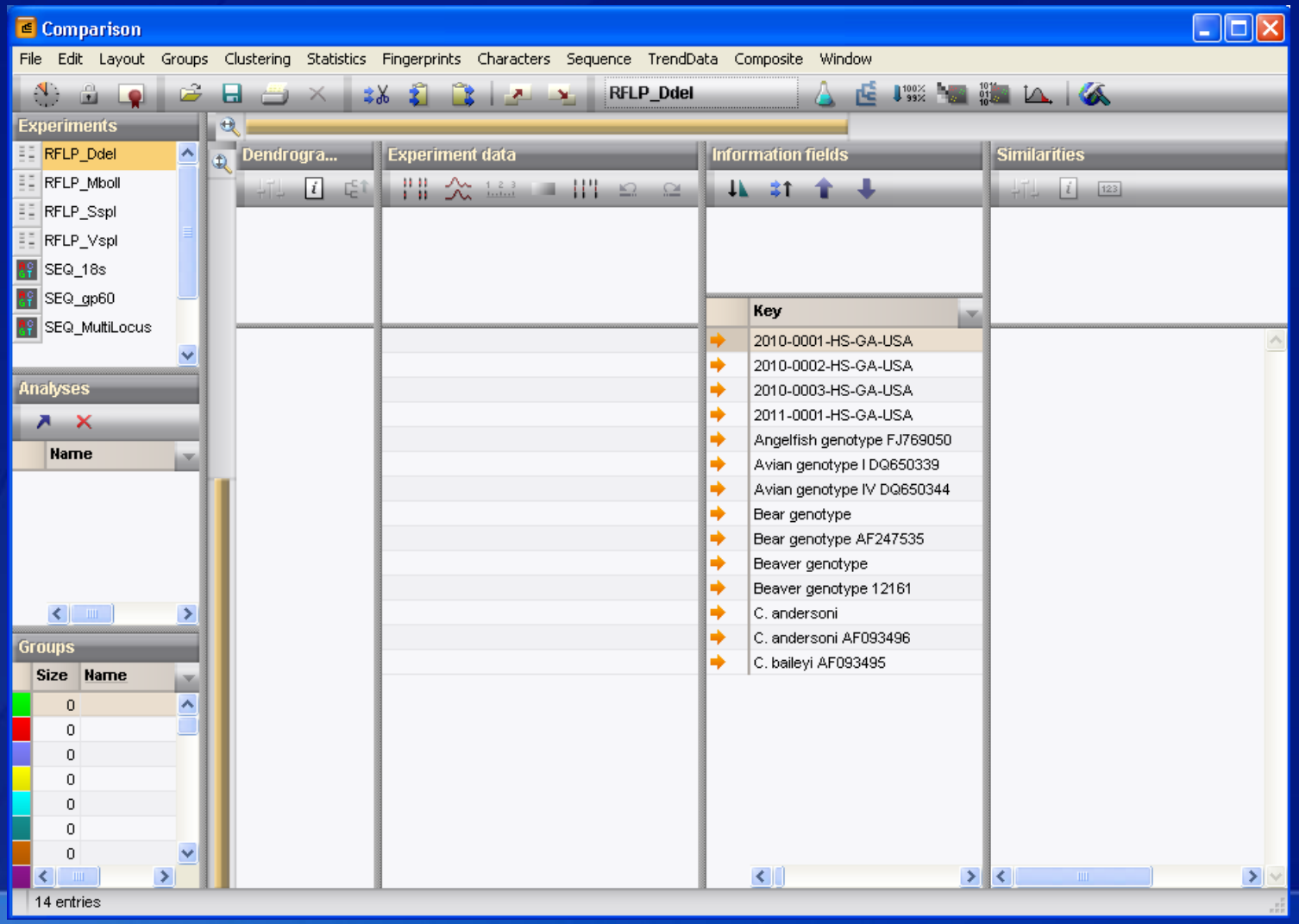
Inputting Sequencing (fasta) Data

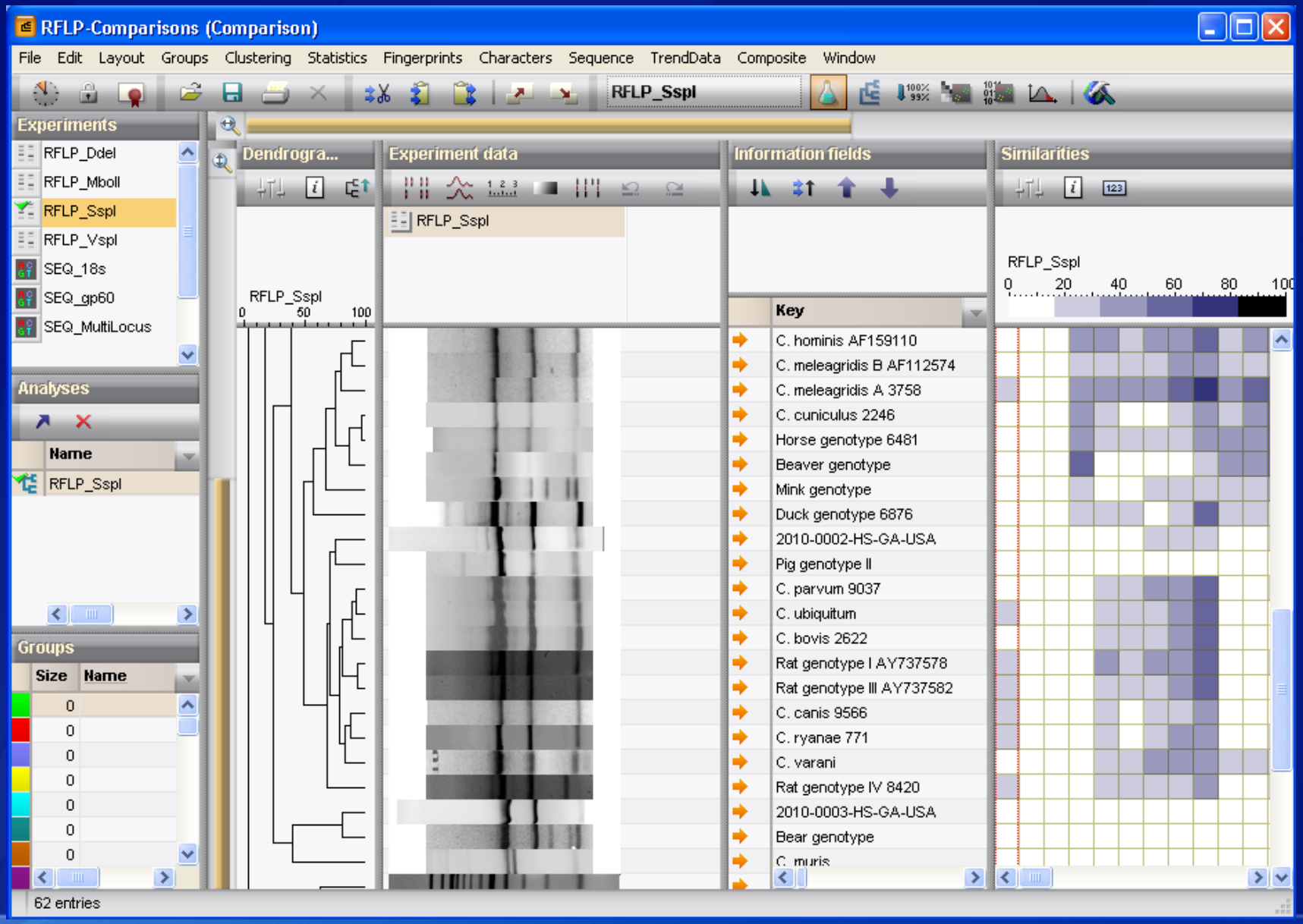


Query


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)
Search
Cancel
Clear
Add to list
Replace list
Search in list
Negative Search
NOTE: Wildcard (*) cannot be used

Data Analysis by Comparison




RFLP-Comparisons (Comparison)
File Edit Layout Groups Clustering Statistics Fingerprints Characters Sequence TrendData Composite Window
RFLP_SspI
Experiments
RFLP_DdeI
RFLP_MboII
RFLP_SspI
RFLP_VspI
SEQ_18s
SEQ_gp60
SEQ_MultiLocus
Analyses
Name
RFLP_SspI
Groups
Size
Name
0
0
0
0
0
0
0
62 entries
Dendrogra...
RFLP_SspI
0
50
100
Experiment data
RFLP_SspI
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
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Pig genotype II
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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
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Bear genotype
C. muris
Similarities
RFLP_SspI
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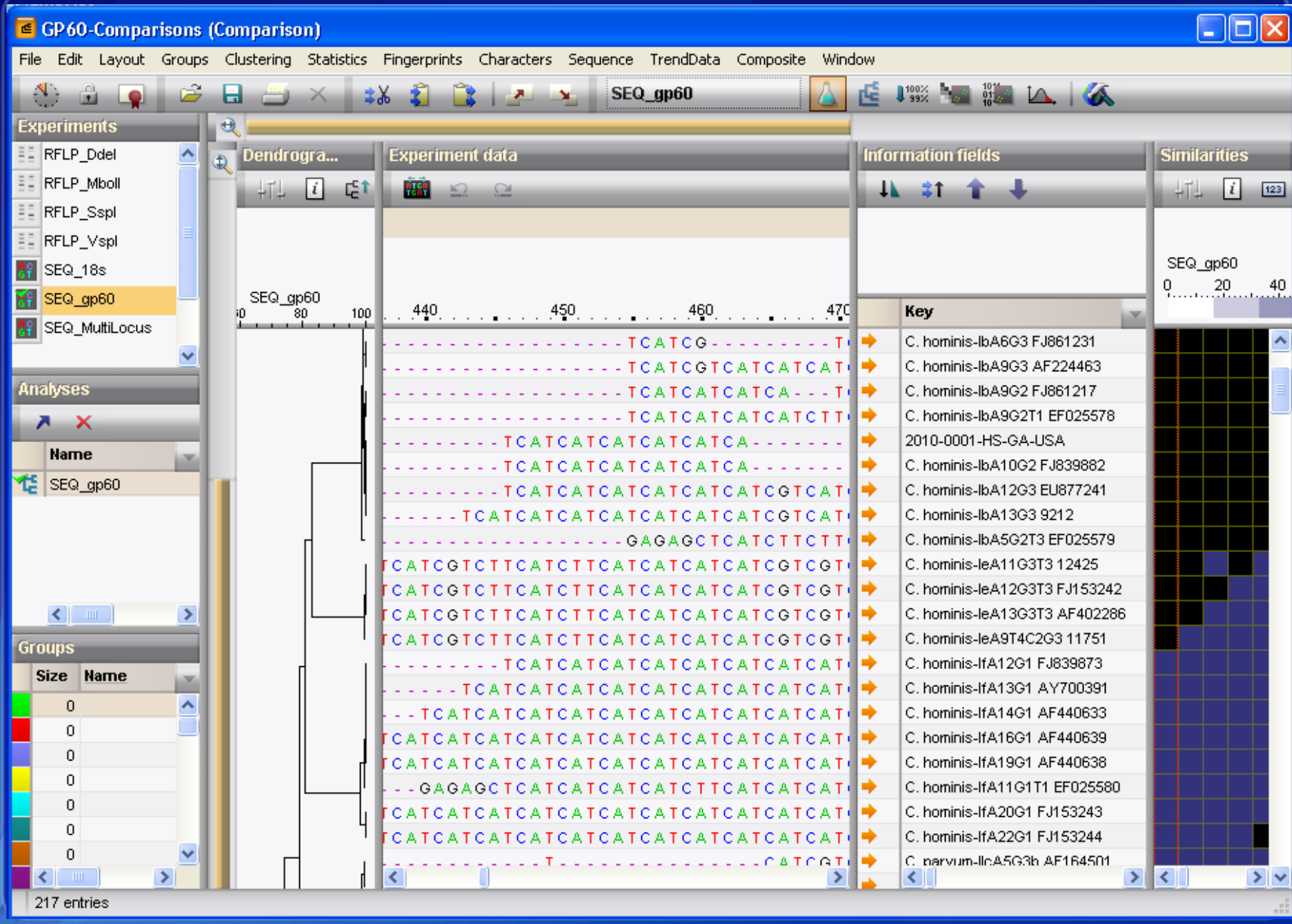
20
40
60
80
100


GP60-Comparisons (Comparison)
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SEQ_gp60
Experiments
RFLP_DdeI
RFLP_MboII
RFLP_SspI
RFLP_VspI
SEQ_18s
SEQ_gp60
SEQ_MultiLocus
Analyses
Name
SEQ_gp60
Groups
Size Name
Dendrogra...
Experiment data
Information fields
Similarities
SEQ_gp60
440 450 460 470
Key
C. hominis-IbA6G3 FJ861231
C. hominis-IbA9G3 AF224463
C. hominis-IbA9G2 FJ861217
C. hominis-IbA9G2T1 EF025578
2010-0001-HS-GA-USA
C. hominis-IbA10G2 FJ839882
C. hominis-IbA12G3 EU877241
C. hominis-IbA13G3 9212
C. hominis-IbA5G2T3 EF025579
C. hominis-IeA11G3T3 12425
C. hominis-IeA12G3T3 FJ153242
C. hominis-IeA13G3T3 AF402286
C. hominis-IeA9T4C2G3 11751
C. hominis-IfA12G1 FJ839873
C. hominis-IfA13G1 AY700391
C. hominis-IfA14G1 AF440633
C. hominis-IfA16G1 AF440639
C. hominis-IfA19G1 AF440638
C. hominis-IfA11G1T1 EF025580
C. hominis-IfA20G1 FJ153243
C. hominis-IfA22G1 FJ153244
217 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

Collier et al., MMWR 2011

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

NC outbreak 2009-Moleculer Epidemiology

	Cryptosporidium parvum	
	#	Subtype
Human	8	IIaA17G2R1 (8)
Calf	3	IIaA17G2R1(2) IIaA15G2R1
Goat kid	1	IIaA17G2R1 (1)
Piglet	1	IIaA17G2R1 (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

Caccio et al., Veterinary Parasitology, 2012

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

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- **CaliciNet**
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  - 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.