Development of air-sampling and molecular detection methods for *Coccidioides* species

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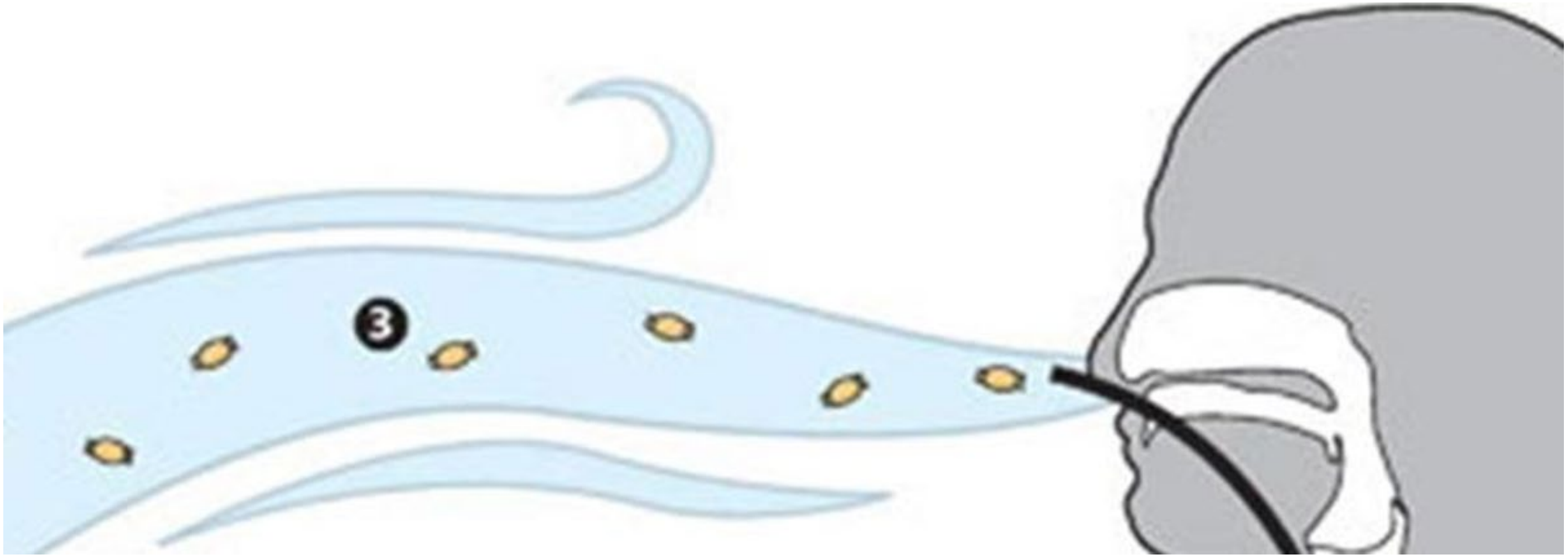
Most people are infected by *Coccidioides* spp. from inhaling arthroconidia



No prevention aside from mitigating occupational risks

Utility of air-surveillance

- To better understand the geographic distribution
- To better understand the epidemiology:
  - Seasonality
  - Severe weather
  - Climate
  - Human factors
- To inform risk prediction models
- Generate “Cocci risk” forecasts
- Establish “Cocci warning” system

Technical challenges

- Identifying efficient air-sampling method
- Developing effective air-sampling strategy
- Developing sensitive and specific molecular detection method

Original Article

Molecular detection of airborne *Coccidioides* in Tucson, Arizona

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Chow et al, 2017, Medical Mycology

https://academic.oup.com/mmy/article/54/6/584/2579270

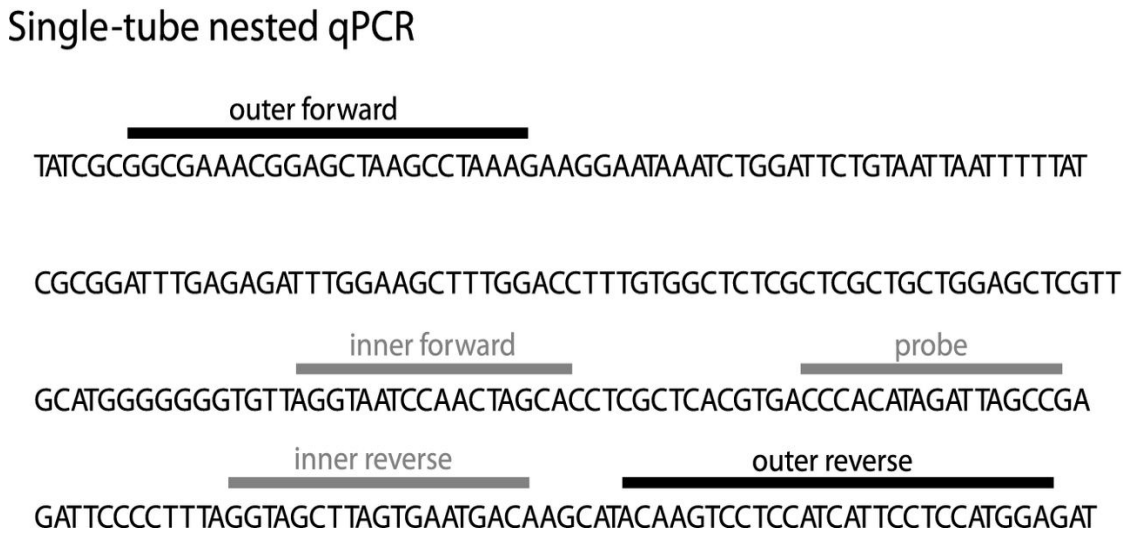
Air samplers

High volume sampler (15 ft^3/min)

Burkard mutli-stage liquid impinger (0.46 ft^3/min)

Molecular detection

- Target unique transposable element in *Coccidioides* genome
- Approximately 70 copies per genome
- Not found in other fungal pathogens and related species
- Several variations of this assay, all target the same region



Chow et al, 2017

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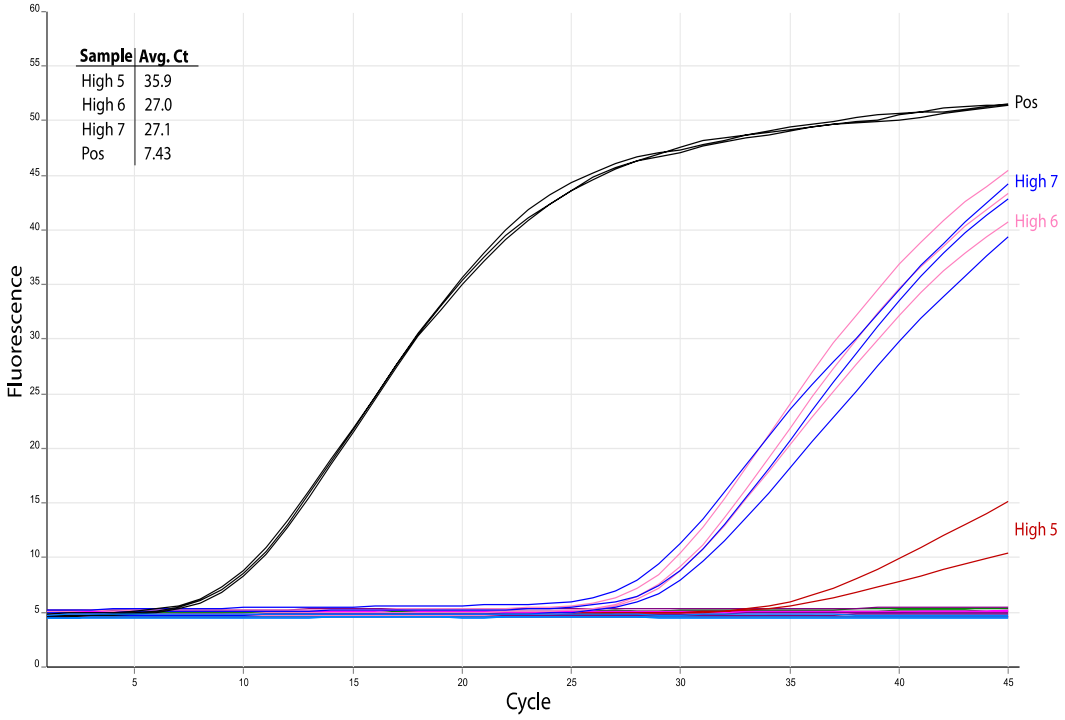
Air-samples were collected above known positive soils

Sampling ambient air

Sampling disturbed dust

Coccidioides DNA was detected in dust samples

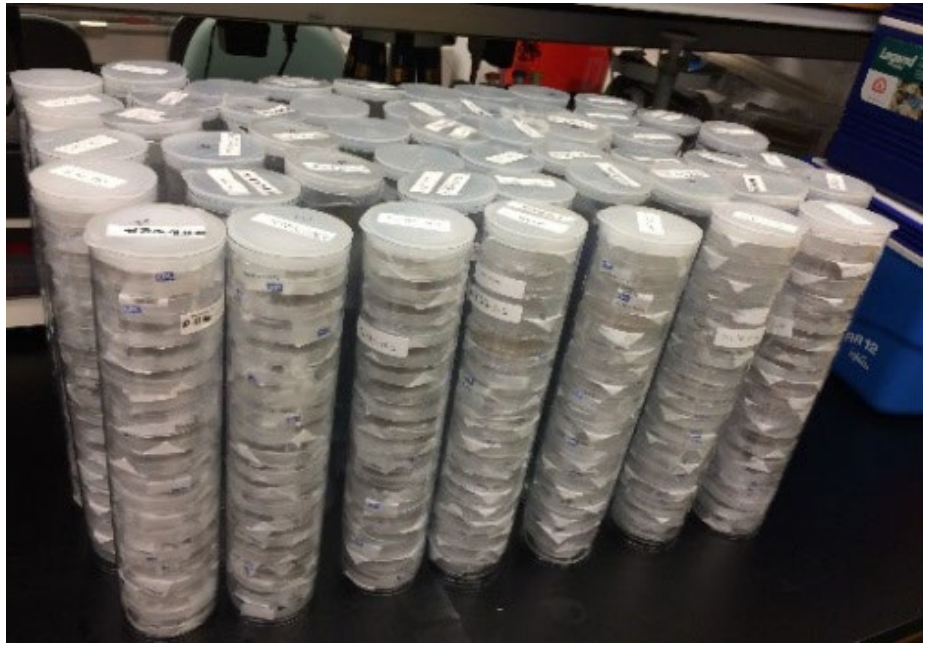


Real-time PCR results from dust samples collected in the study

- *Coccidioides* DNA can be detected in disturbed dust samples using molecular methods
- Only high-volume samplers were successful
- Insufficient sampling time or methodology for detection in ambient air

Ambient Air Surveillance, 2016 Pilot in Arizona

- Daily collection of air-samples using high volume samplers
- Twenty-one sites around Phoenix, AZ
- Residual air filters shared from Sept 25-Nov 11, 2016



Air-filters collected in the pilot study

tgen NORTH

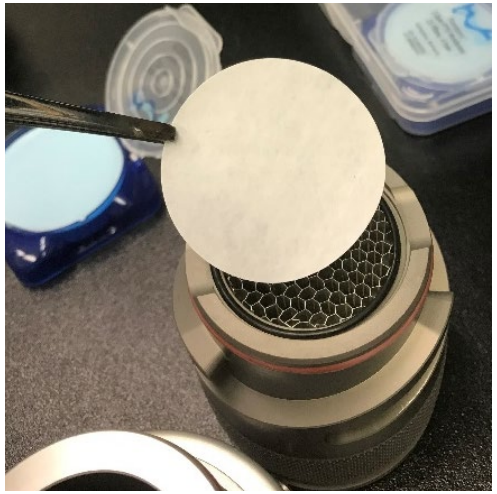
Maricopa County Public Health

ARIZONA DEPARTMENT OF HEALTH SERVICES

High-volume samplers

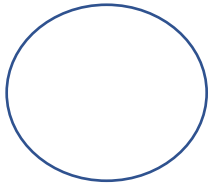
Air sampler

- Air-flow rate 100 L/min
- Collection time 24 h = 144,000 L of air sampled
- For comparison, a human inhales 8,000-9,000 L/day



Hydrophobic polytetrafluoroethylene (PTFE) filters

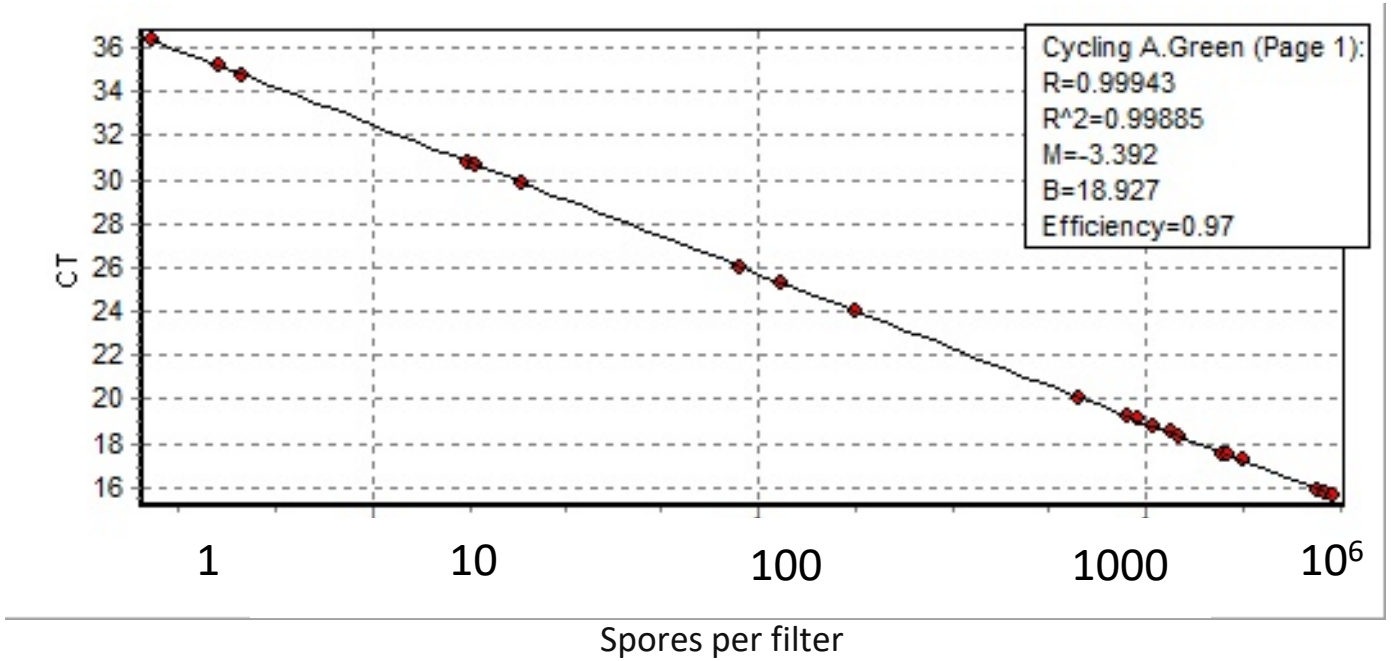
Modified DNA extraction



qPCR testing

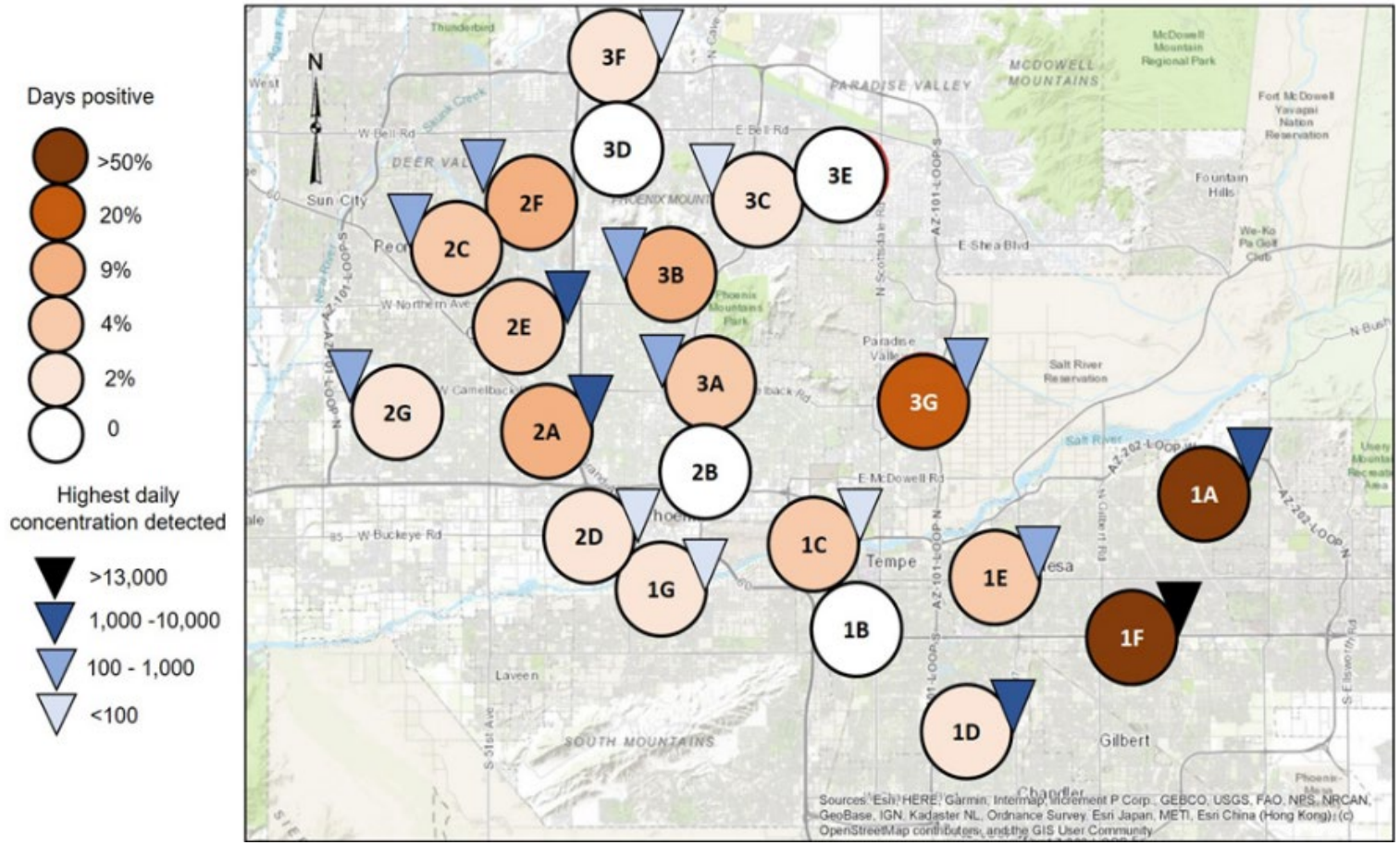
STnested qPCR Limit of Detection

- Blank PTFE filters (without dust and other background) spiked with arthroconidia
- 10^6 - 1 arthroconidia per filter
- Able to detect one arthroconidium per filter
- Done in triplicates



Real-time PCR standard curve showing the correlation between added arthroconidia and Ct value

Daily prevalence of arthroconidia at 21 sites around Phoenix, AZ, September 25 - November 11, 2016



Gade et al 2019

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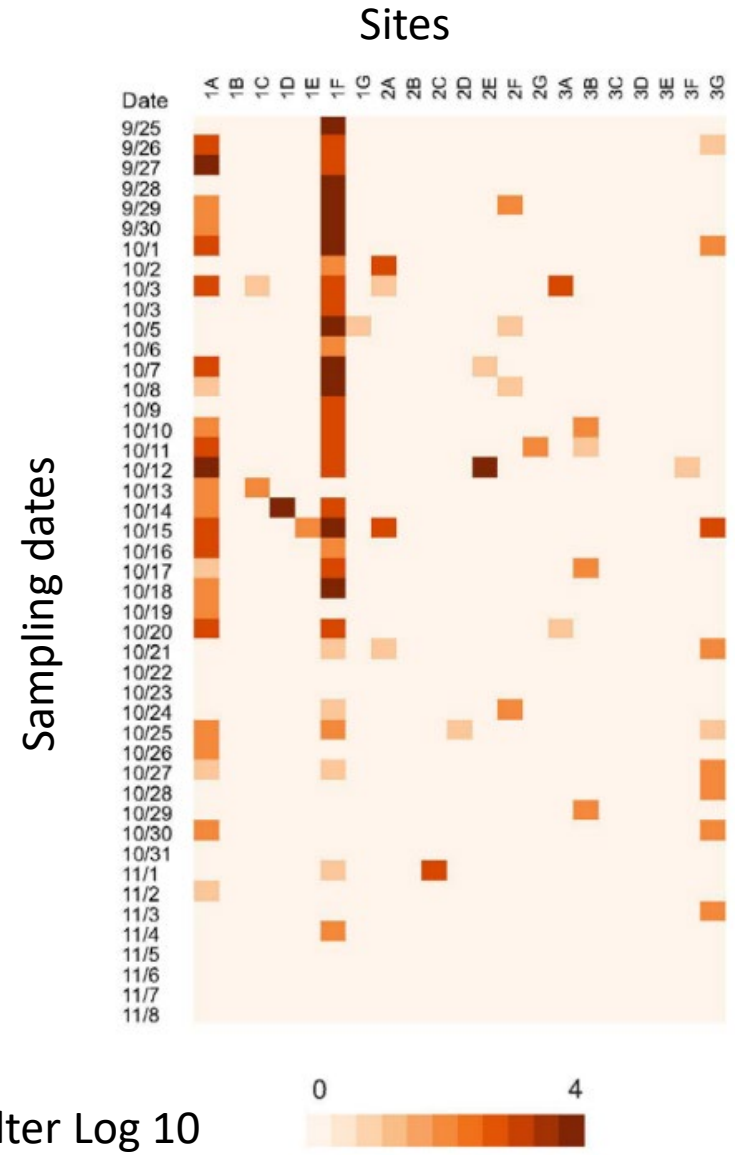
Temporal distribution of arthroconidia among 21 sites



Gade et al 2019

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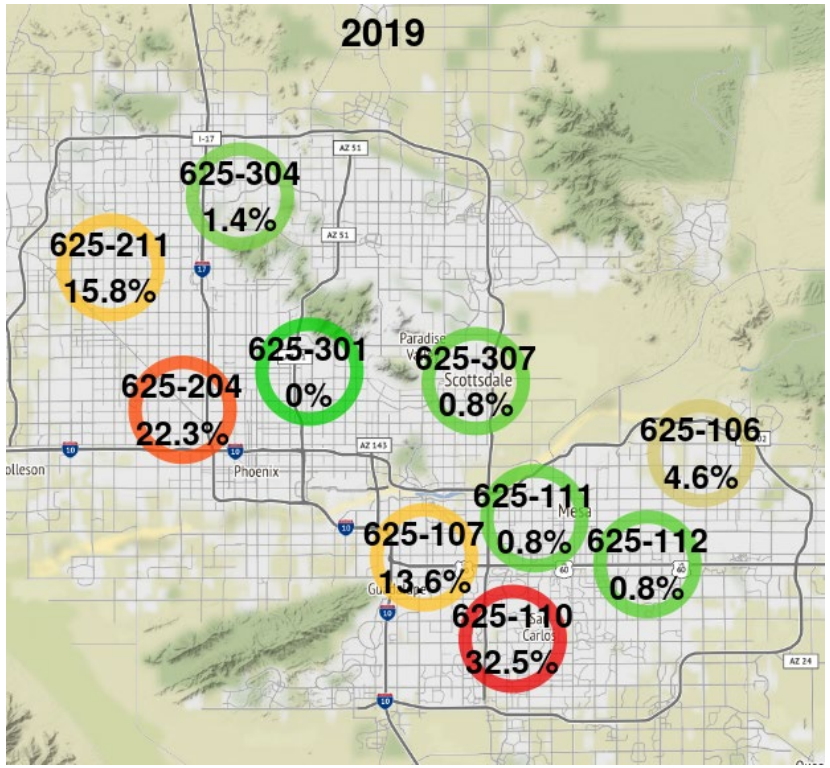
Conclusions from the pilot

- *Coccidioides* can be successfully detected on PTFE air filters from ambient air
- Limit of detection: up to one spore on a filter in the laboratory
- Pilot testing results suggest uneven geographic prevalence and possible temporal signal

CocciWatch –Longitudinal air surveillance study



Same sites, 2017, 2018, 2019

Current datasets:

- Reported cases (ADHS)
- Air filters from Phoenix area (ADHS, DHS)
- Processed air filters (TGen, CDC)
- Weather data (TGen, NOAA)

Additional datasets we are working to include:

- Clinical testing data (positive/negative tests) (SQL)
- Valley fever cases in dogs
- Veterinarian testing data

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Sonora Quest Laboratories™ A Subsidiary of Laboratory Sciences of Arizona

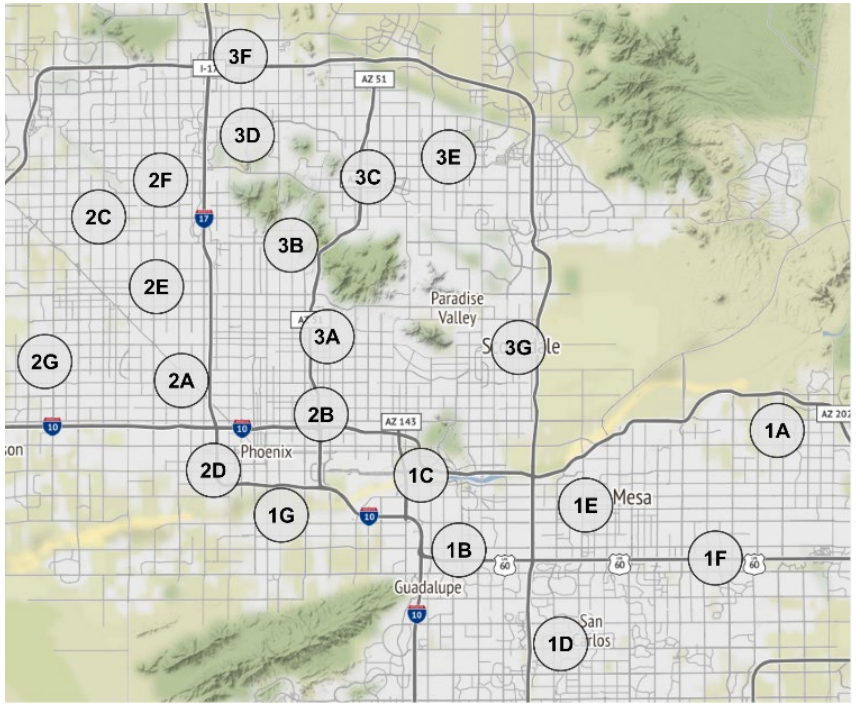
NOAA NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION U.S. DEPARTMENT OF COMMERCE

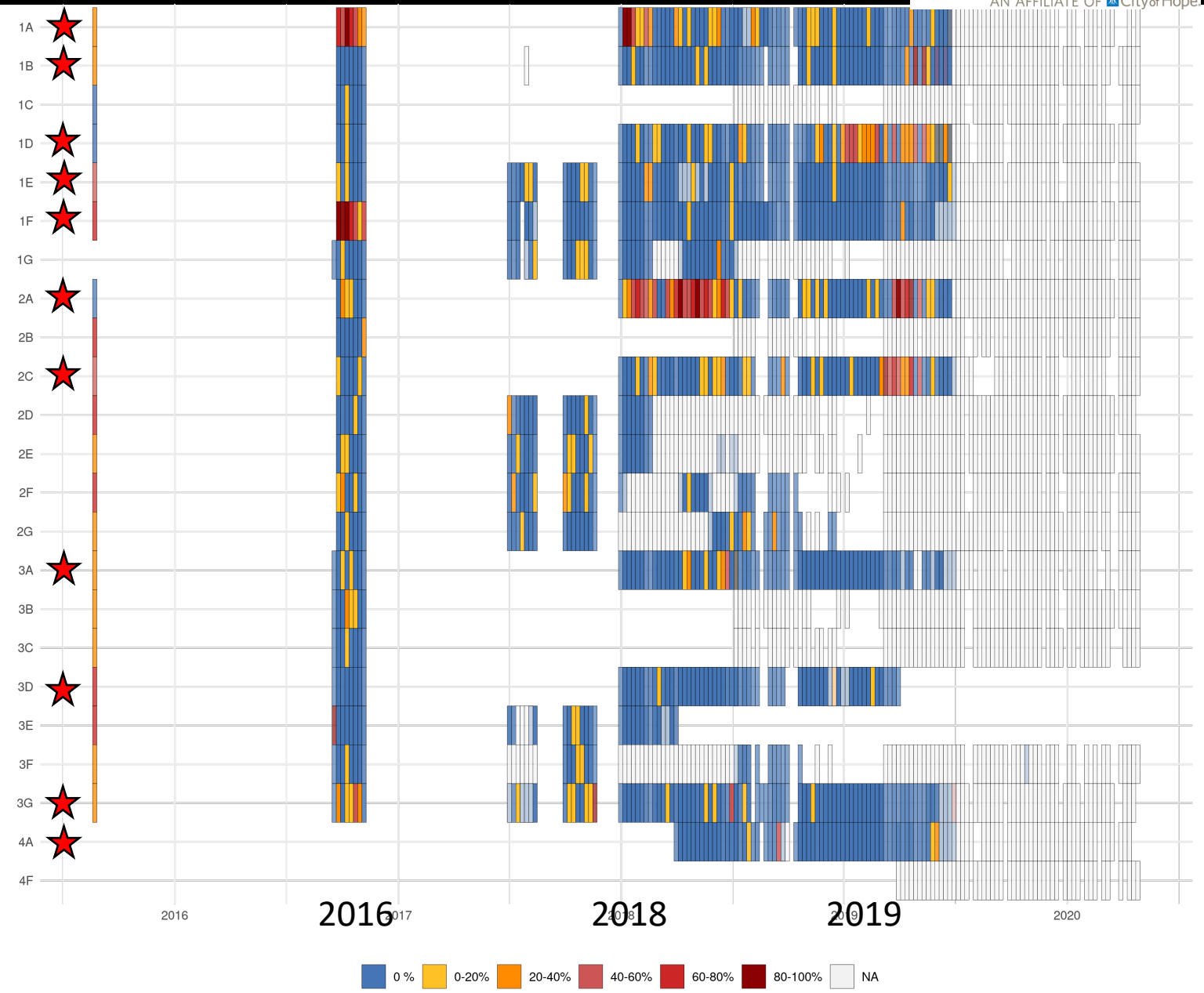
ABRC

NIH NIAID

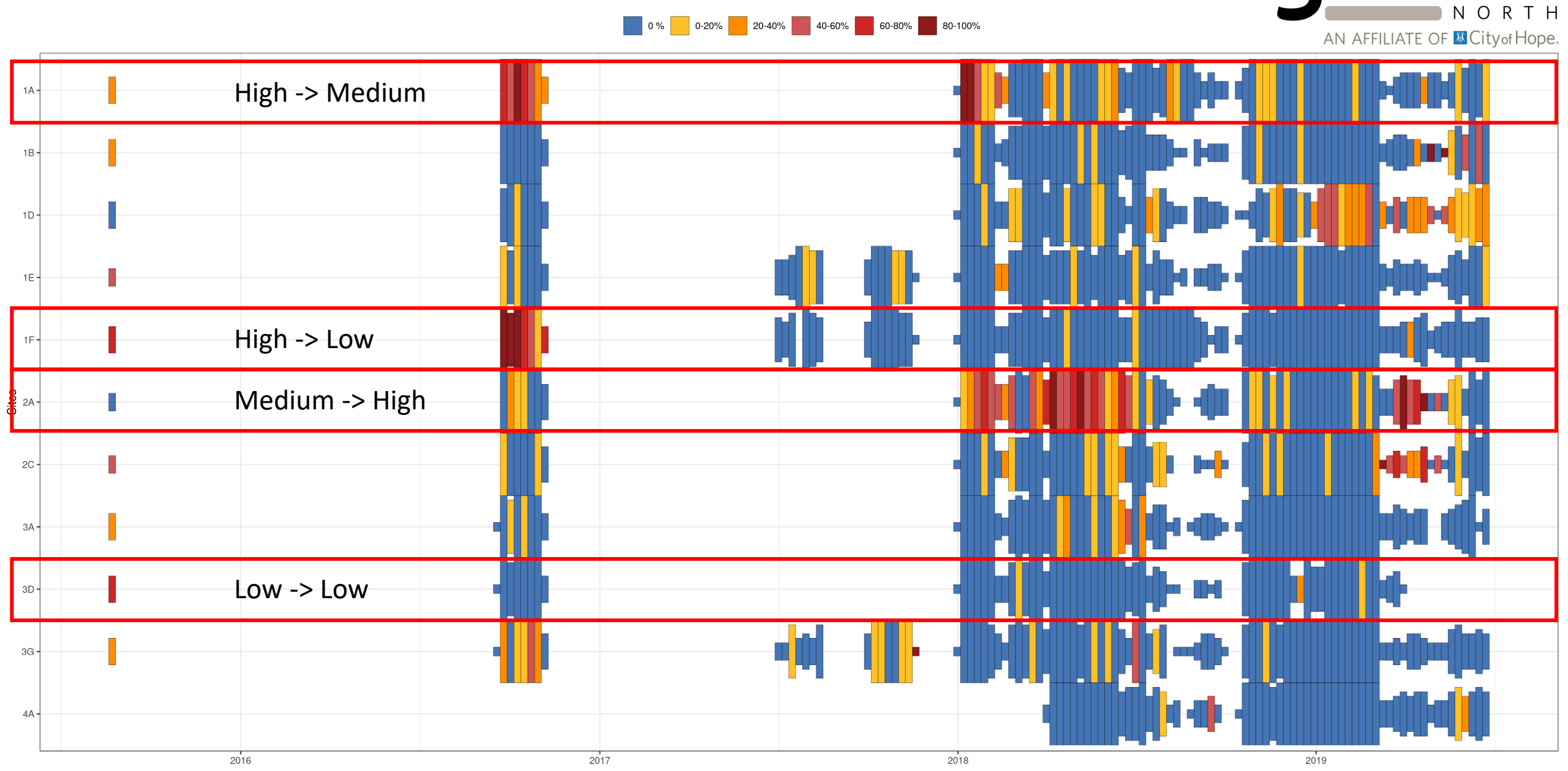
CocciWatch

- Filter collections from 23 sites (n = ~10K).
- Processing has focused on 11 of 23 sites, up to July 2019 (n = 5,055).



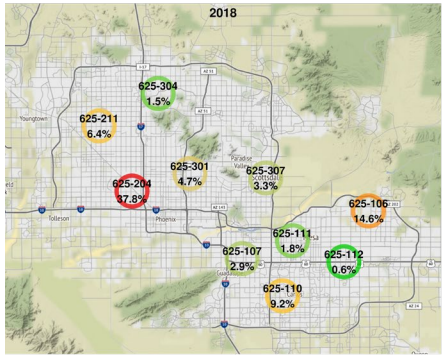


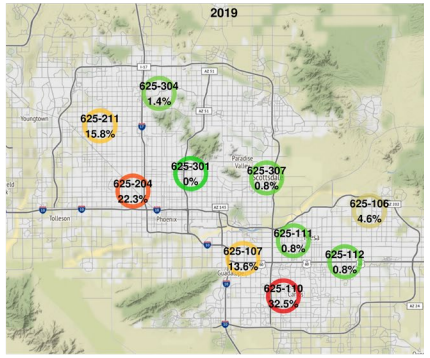
CocciWatch- Current Data

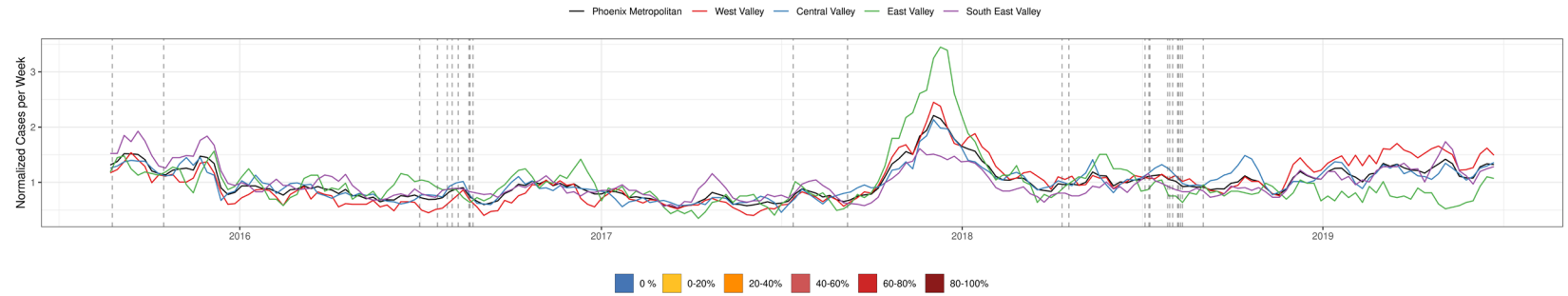


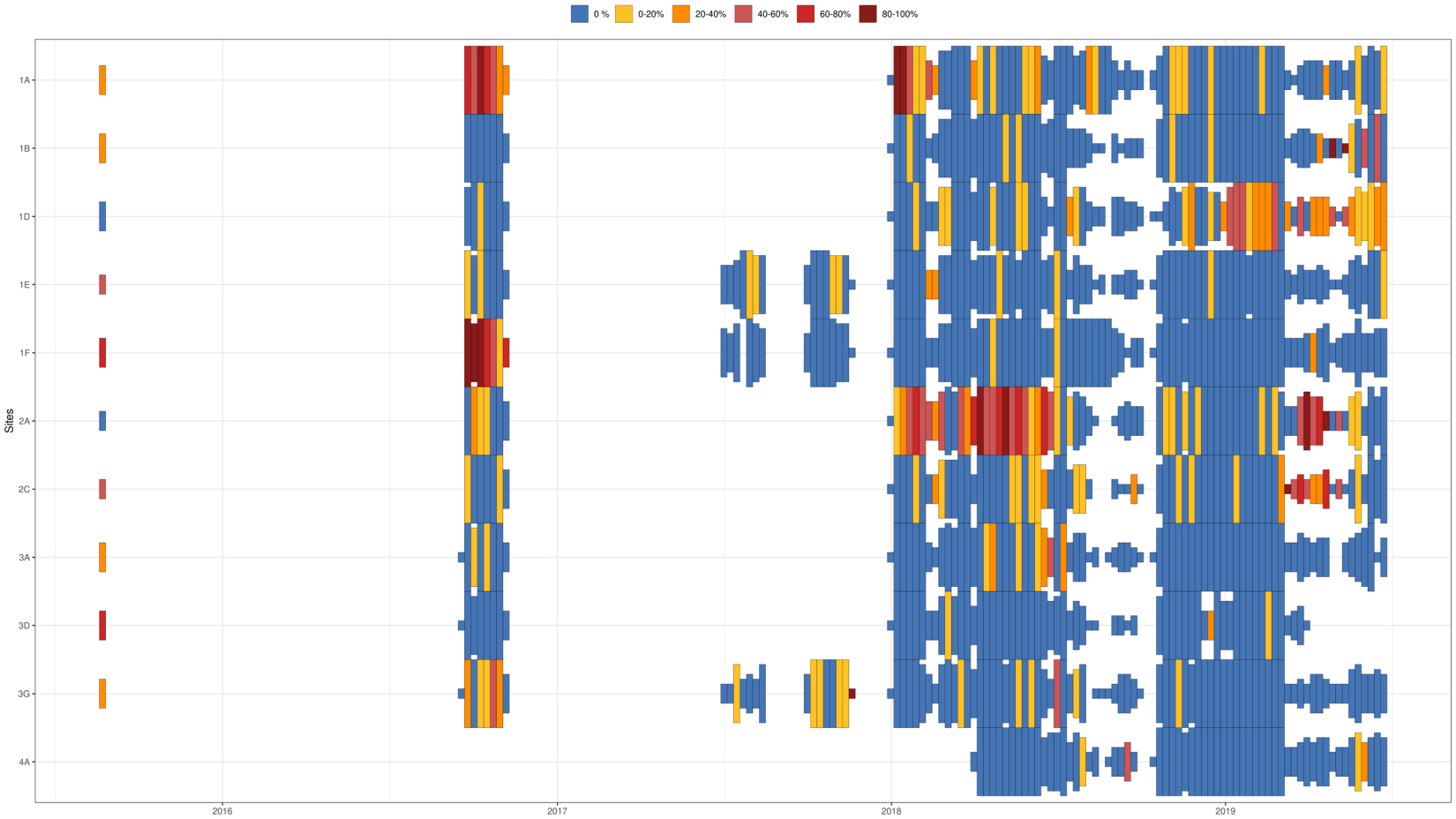
CocciWatch- Surveillance conclusions

- **High variance in prevalence across space and time suggesting that risk is spatially and temporally variable.**
  - Supported by ecology- *Coccidioides* must be introduced into the soil and then aerosolized.
- **Local drivers influence site prevalence.**
  - Specific weather patterns?
  - Soil disturbance?
  - Land cover around sites?









Summary

- Environmental surveillance is informative for understanding the epidemiology of Valley Fever
- “Patchy” distribution of arthroconidia in the air: Local conditions likely control the prevalence of arthroconidia in the air
- Air-monitoring can be informative for addressing specific questions and testing hypotheses; however, the results are site-specific

Possible applications

- To better understanding of the geographic distribution
  - To monitor emergence in new areas due to climate change
- To identify potential “hot spots” (for targeted vaccination)
- To identify human activities that release arthroconidia in the air to better understand and mitigate occupational risks

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Thank you!

For more information, contact CDC
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