OP-148 [Bee Health]
Environmental DNA as a detection approach for Tropilaelaps mites

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Mites in the genus Tropilaelaps parasitize the western honey bee (Apis mellifera), causing severe damage. As these mites have spread far beyond their native range (especially Tropilaelaps mercedesae), they potentially threaten wild and managed Apis mellifera populations worldwide. Early detection of invasive species is key to effective management, and time-efficient and scalable techniques are needed to complement current physical monitoring methods which have limited scalability as they are time consuming and depend on trained personnel. Environmental DNA (eDNA) techniques are used for detection of other invasive species and could be applied to detect Tropilaelaps in samples from honey bee colonies. In this study we aimed to determine which sample types are appropriate for eDNA testing, and how colony infestation levels influence DNA detection. To do so, we collected samples from 106 A. melliferacolonies in Thailand and South Korea, where Tropilaelaps mercedesae mites are present. Sample types included adult bees, brood comb, bottom board debris, and honey – as well as swabs of bottom boards, individual brood cells, and brood comb surfaces. These samples were tested for Tropilaelaps DNA using a qPCR assay. We determined the Tropilaelaps infestation rate of the colonies by uncapping worker cells. For each sample type, we determined a detection threshold – the infestation of Tropilaelaps required to provide 50% probability of detection in the qPCR assay. We found that bottom board swabs collected through the entrance performed consistently well across both countries. This method detected Tropilaelaps in all tested hives in Thailand regardless of infestation levels and had a detection threshold of 2.6% Tropilaelaps brood infestation (infested cells per 100 cells) in Korea. The second-best method was samples of adult bees, with estimated detection thresholds of 0.42% and 3.9% Tropilaelaps brood infestation for Thailand and Korea, respectively. Based on these results, we conclude that eDNA methods are appropriate for Tropilaelaps detection. Bottom-board swabs and adult bee samples are simple and quick to collect. While processing costs are a consideration, the efficiency of eDNA sample collection may make it a good addition to monitoring programs in areas at risk of Tropilaelaps incursions.

Environmental DNA to detect *Tropilaelaps* mites

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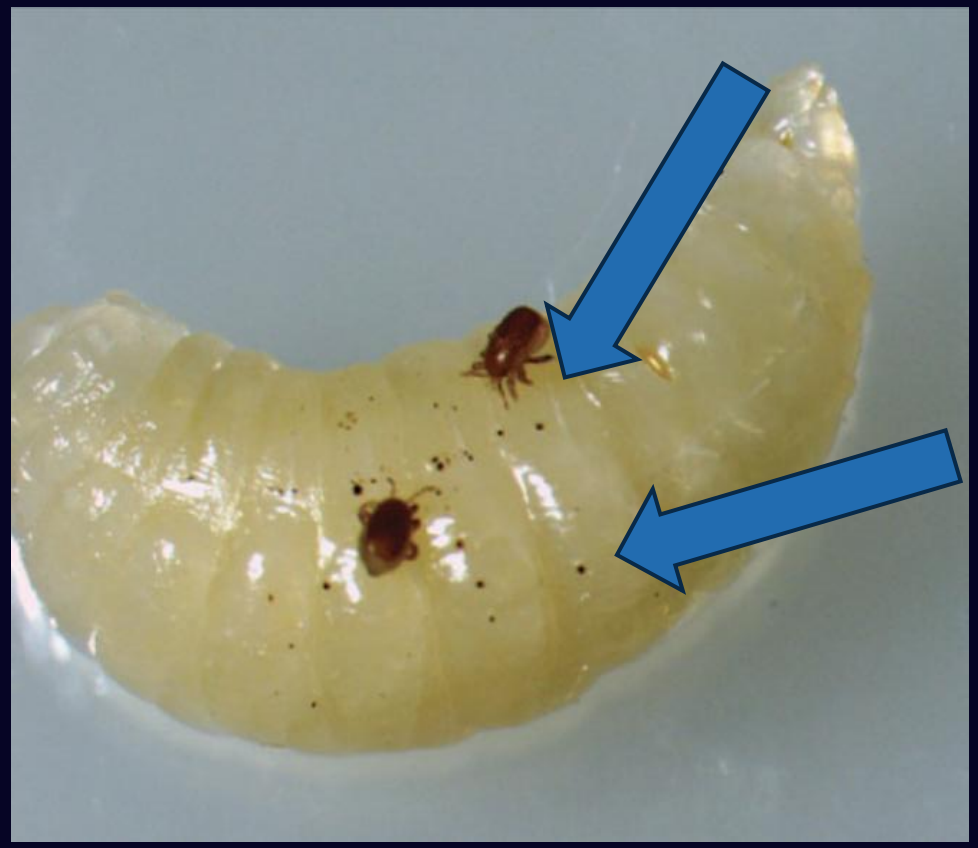
Introduction

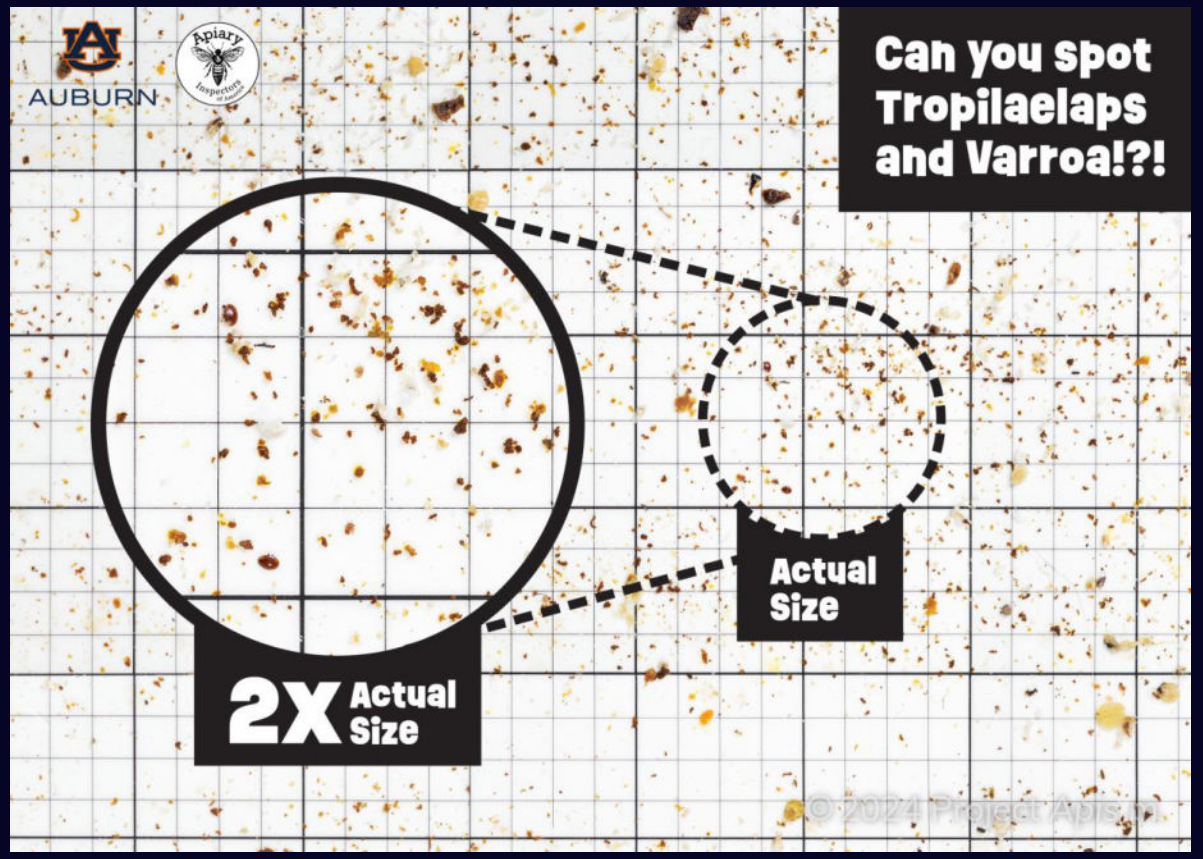
Challenges with *Tropilaelaps* mites

Devastating parasite of *A. mellifera*

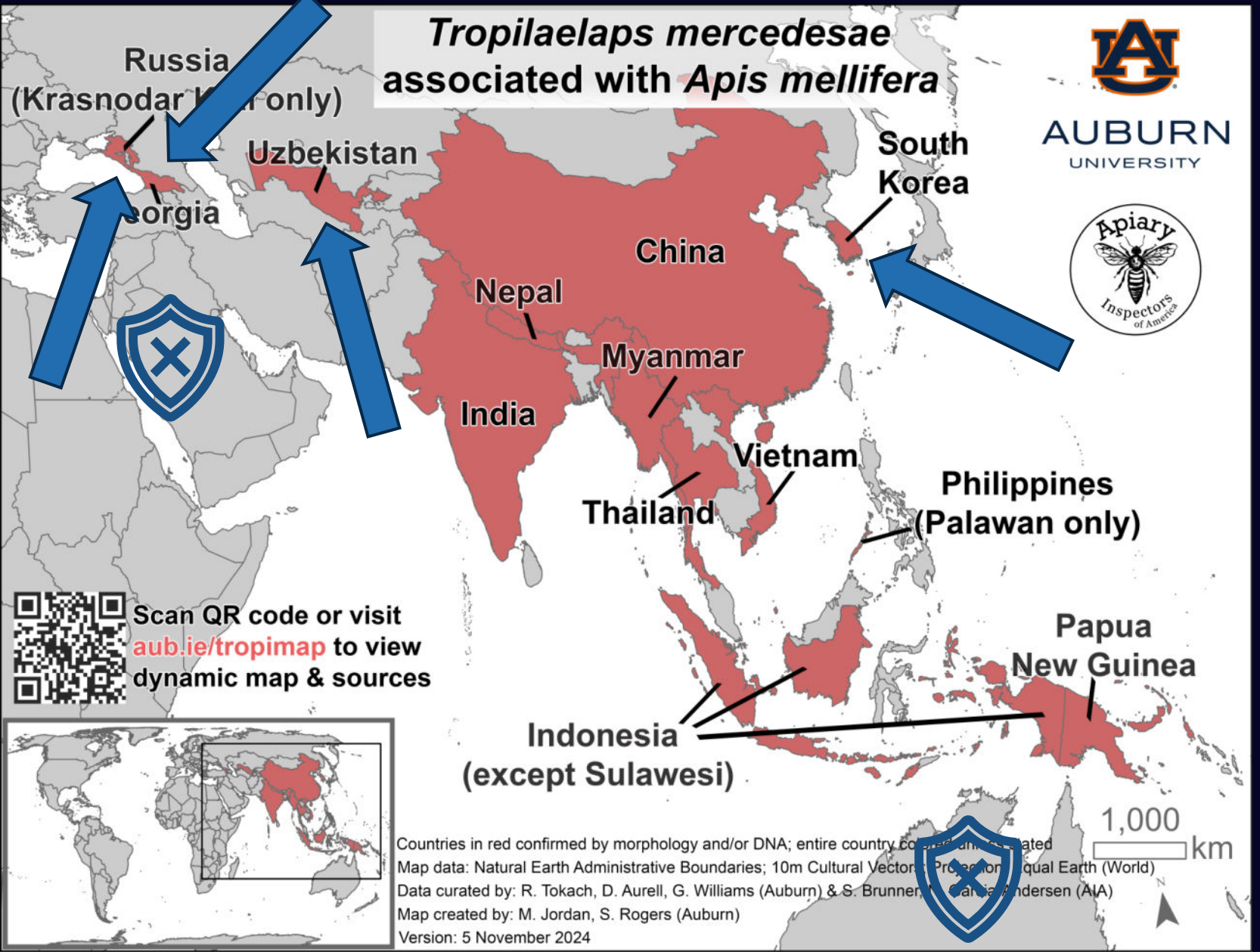


Dongwon / de Guzman et al. 2017

Difficult to detect



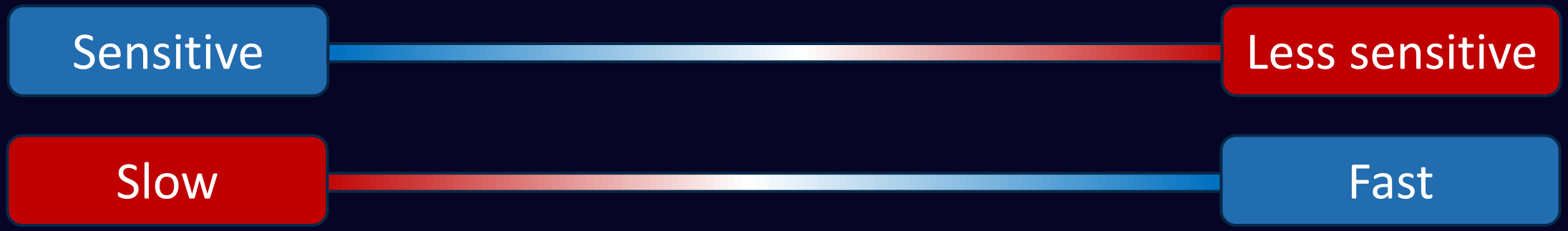
T. mercedesae is expanding its range



Auburn: Tokach/Rogers/Williams
Mohamadzade Namin et al. (2024)
Brandorf et al. (2024)
Janashia et al. (2024)

Monitoring to protect beekeeping

Challenges with physical monitoring techniques



- Need for trained experts
- Scalability
- Turnaround time

[1]Pettis et al. (2013)

eDNA-based monitoring

Environmental DNA (eDNA)

DNA released from an organism into the environment[1]

Potential benefits:

- Taxon-specific
- Quick to collect
- Scalable

[1]USGS (2025)

Objectives

1. Compare sensitivity between sample types

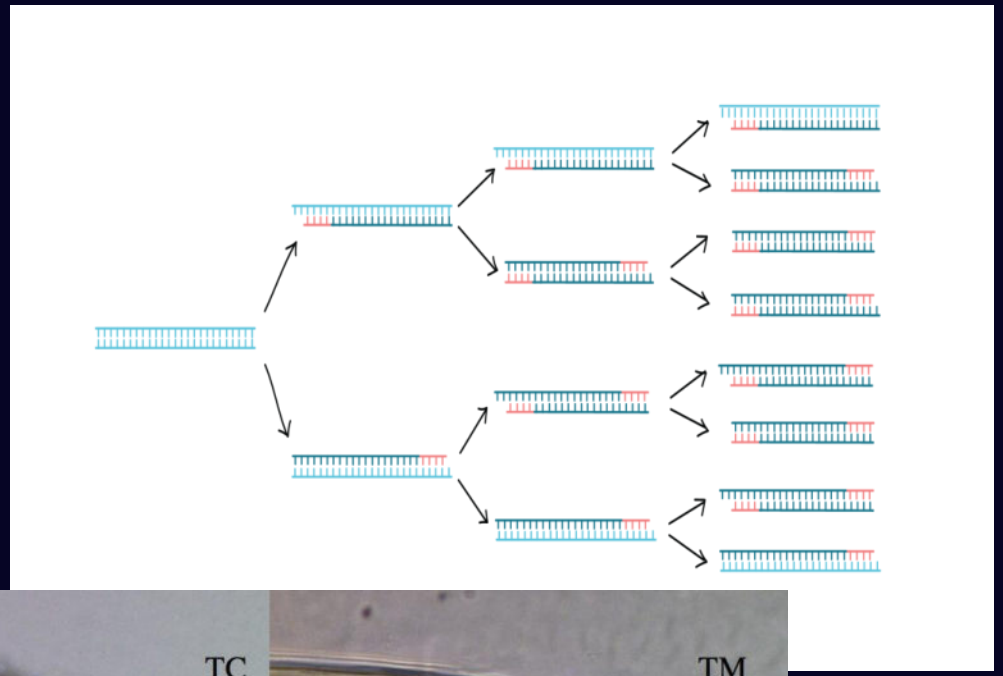
- To guide choice of eDNA sample types to collect in monitoring efforts

2. Assess influence of *Tropilaelaps* infestation on eDNA detection

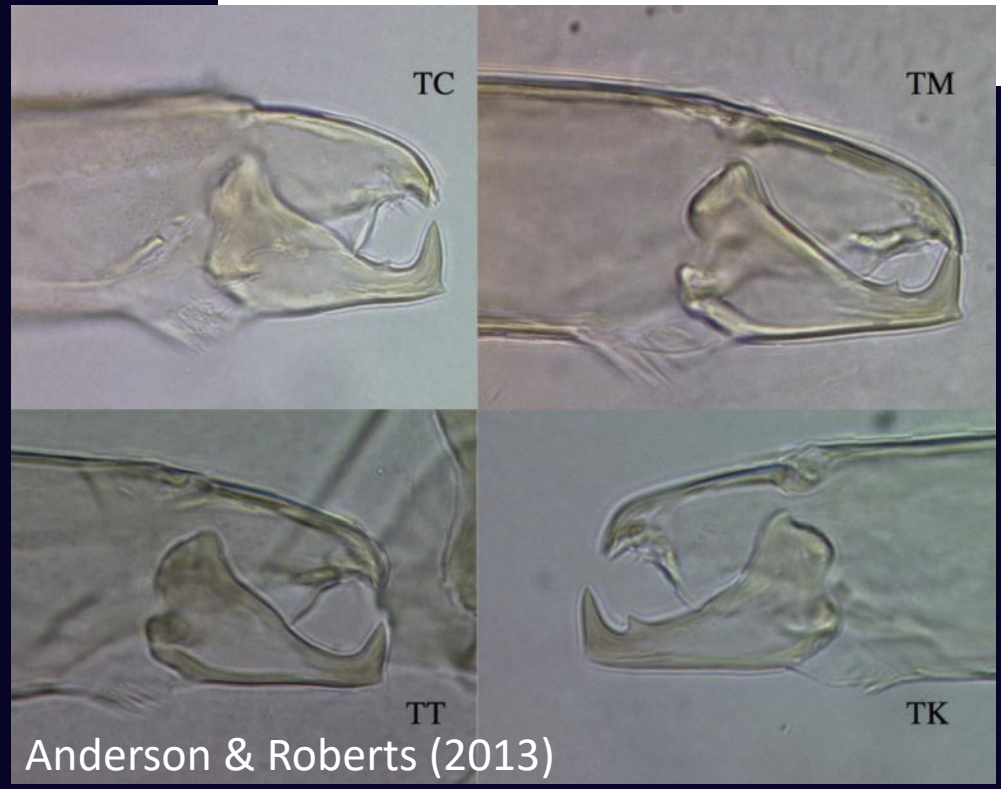
- To understand how high *Tropilaelaps* infestations must be before eDNA detection becomes reliable

Methods

Tropilaelaps eDNA assay

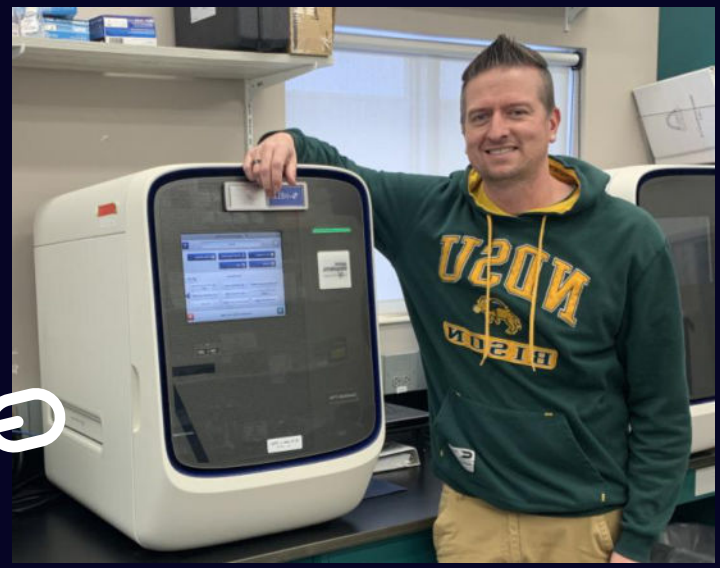


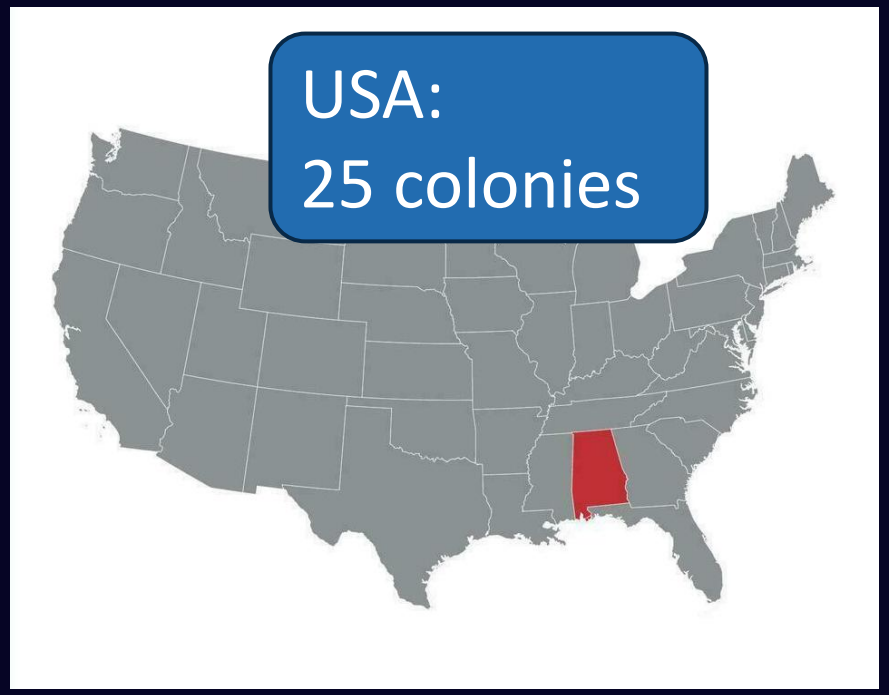
- qPCR assay
- ITS region of rDNA
- Target sequence shared among *Tropilaelaps* species
- Validated using synthetic DNA

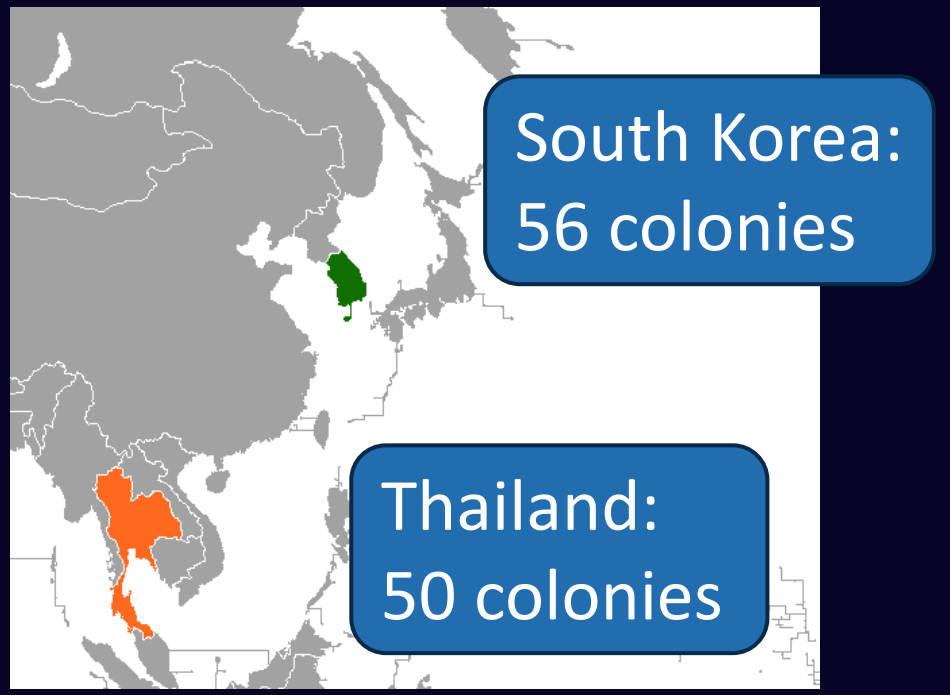


Anderson & Roberts (2013)

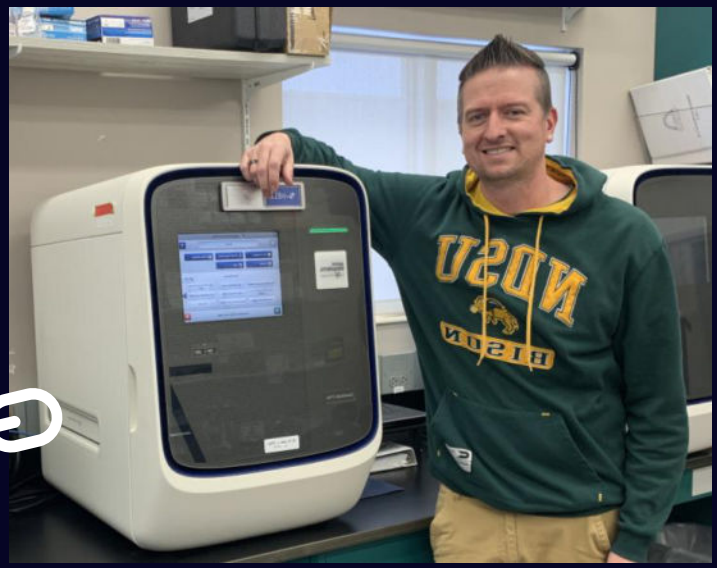
Study design







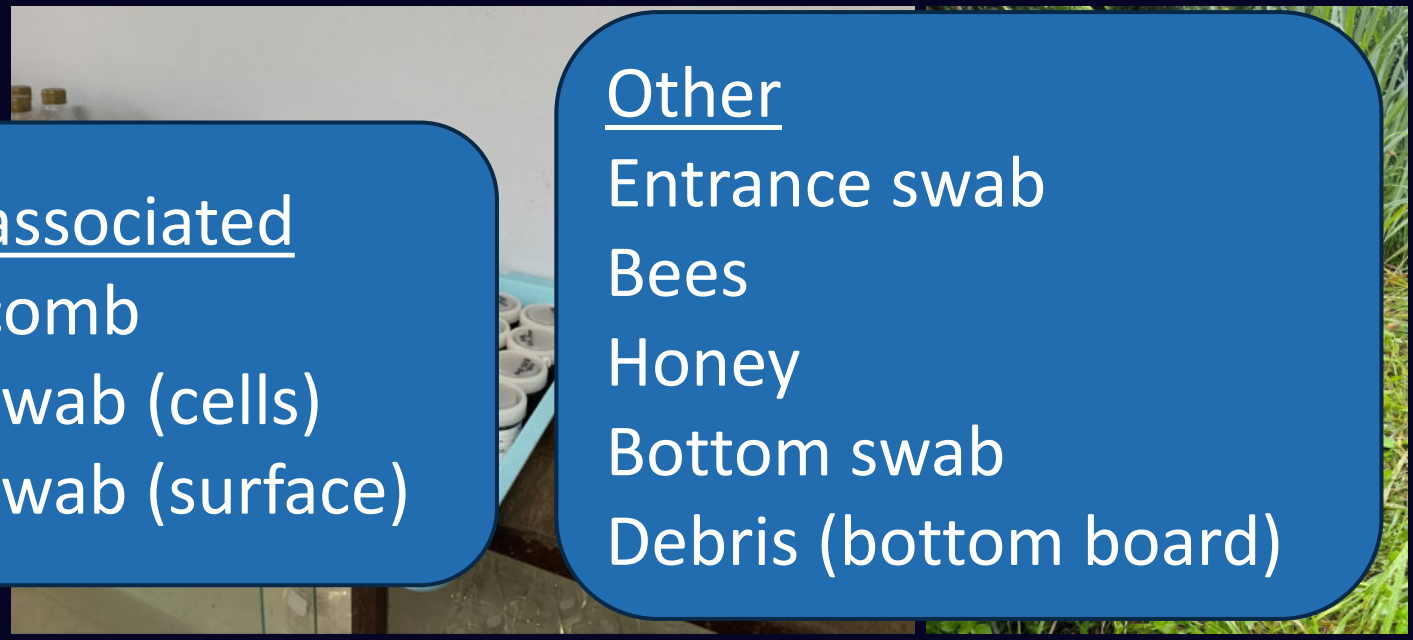
Data collected



Brood infestation

- Uncap 200 worker brood cells – inspect for *Tropilaelaps*[1]

eDNA detection in various sample types



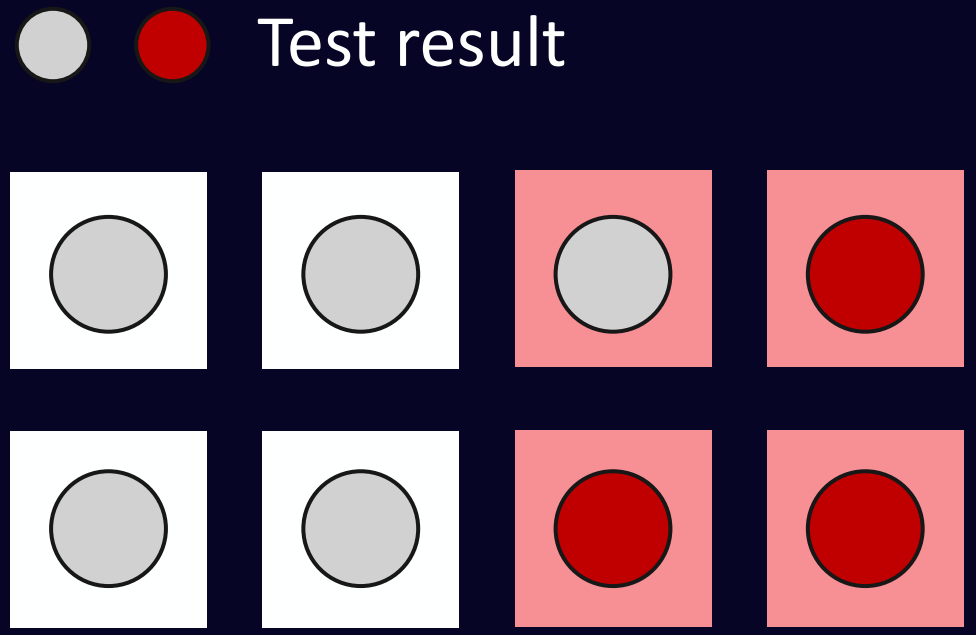
<u>Brood-associated</u>
Brood comb
Brood swab (cells)
Brood swab (surface)

<u>Other</u>
Entrance swab
Bees
Honey
Bottom swab
Debris (bottom board)

Analyses

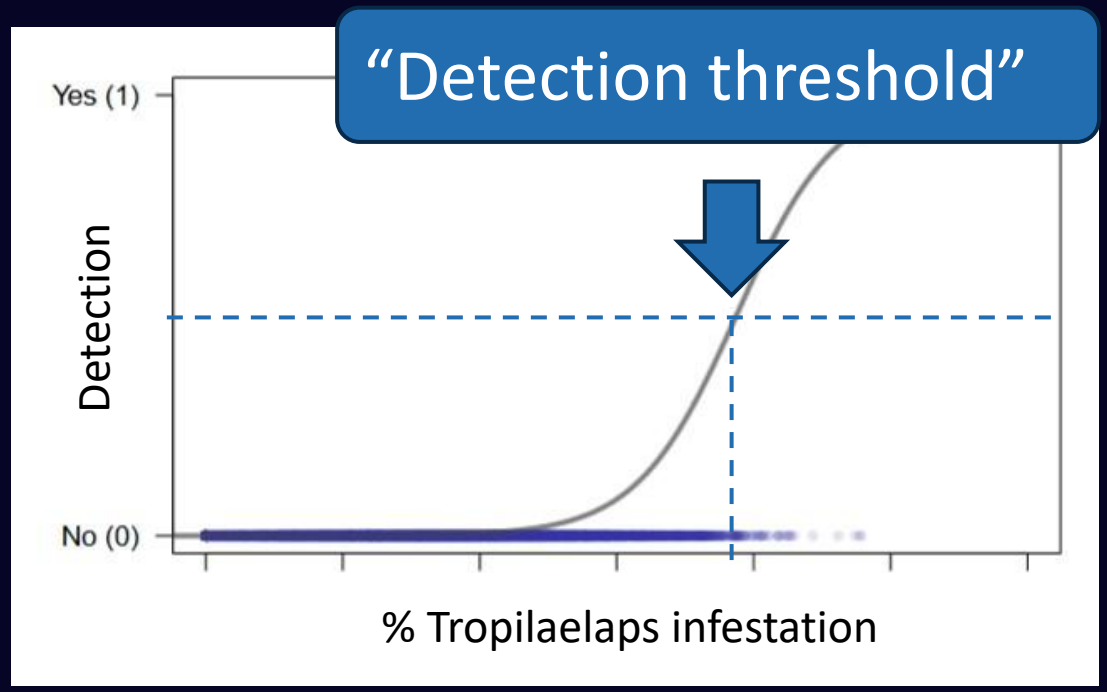
Sensitivity

Proportion of truly infected individuals that are identified as infected



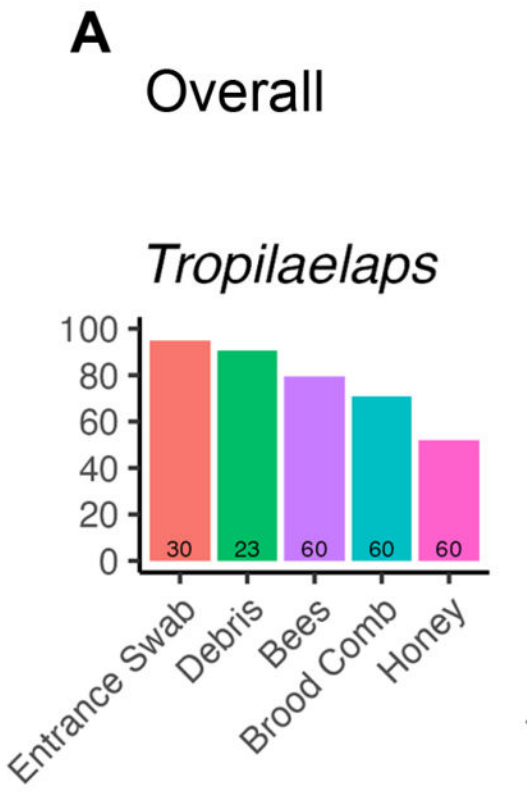
Detection threshold

Tropilaelaps infestation needed for 50% detection probability with eDNA

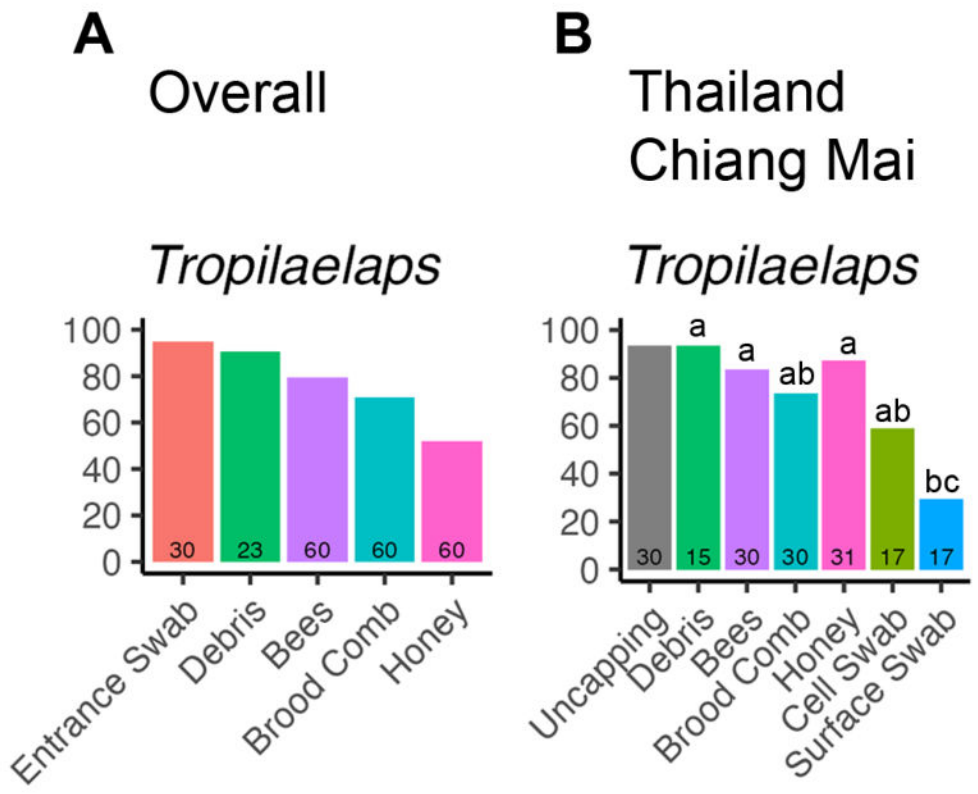


Results

Several sample types performed well



Several sample types performed well



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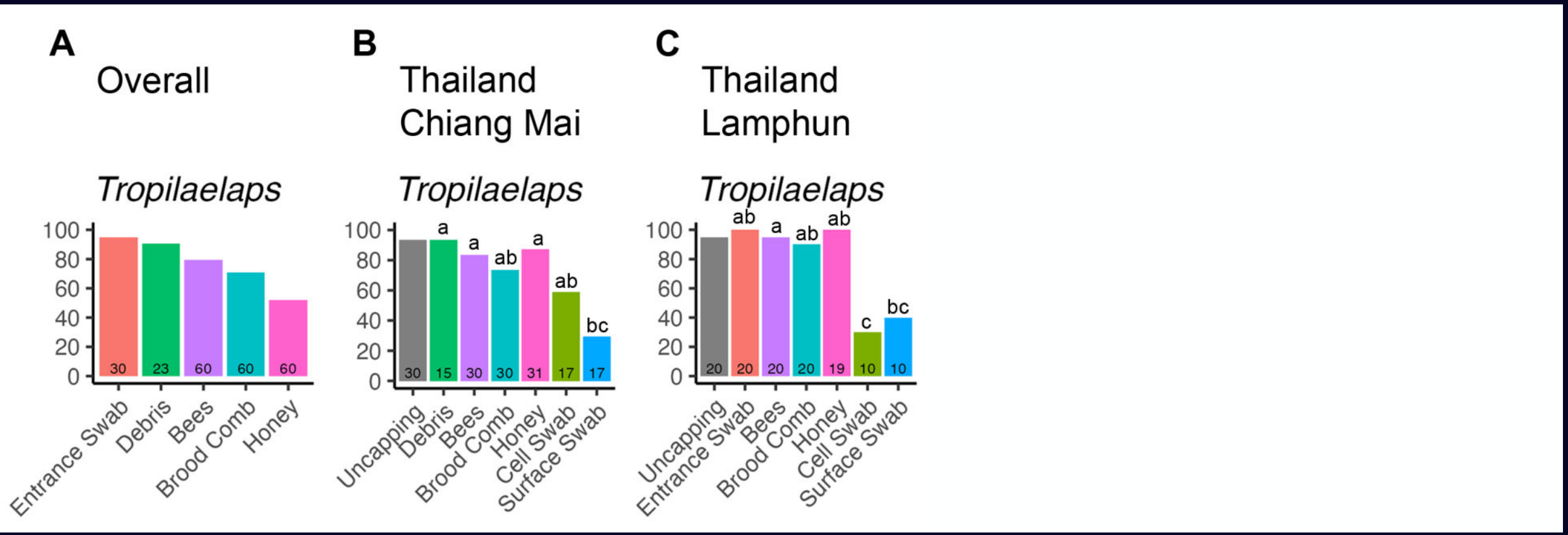

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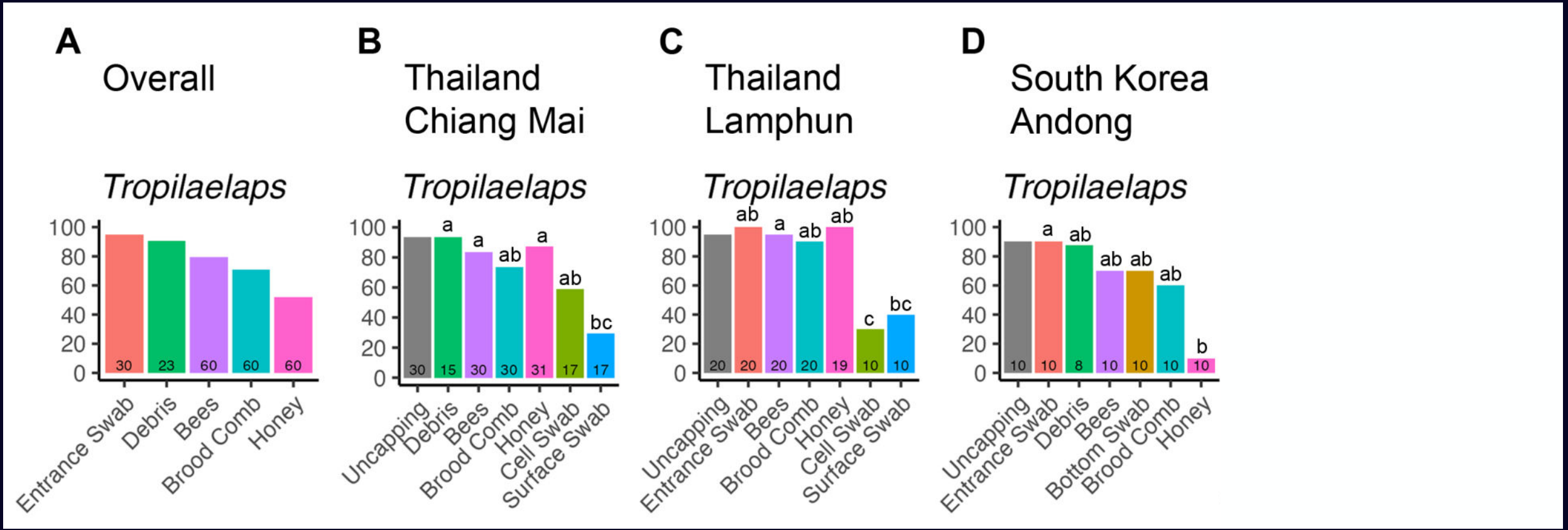
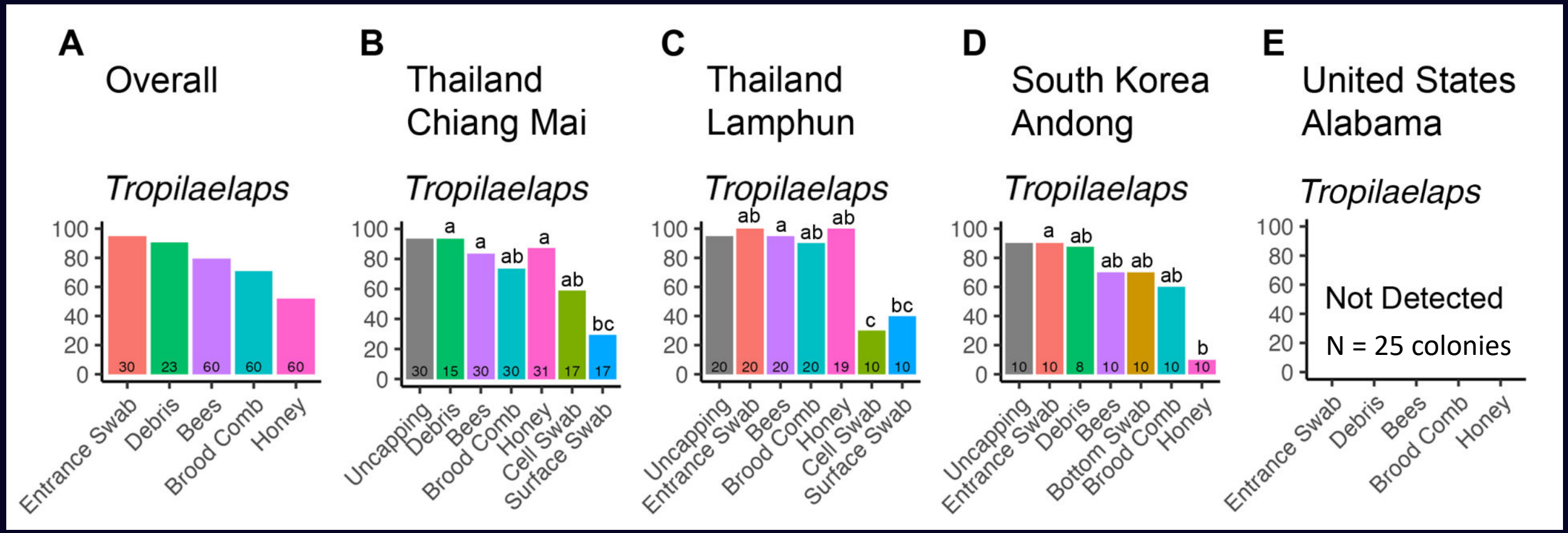
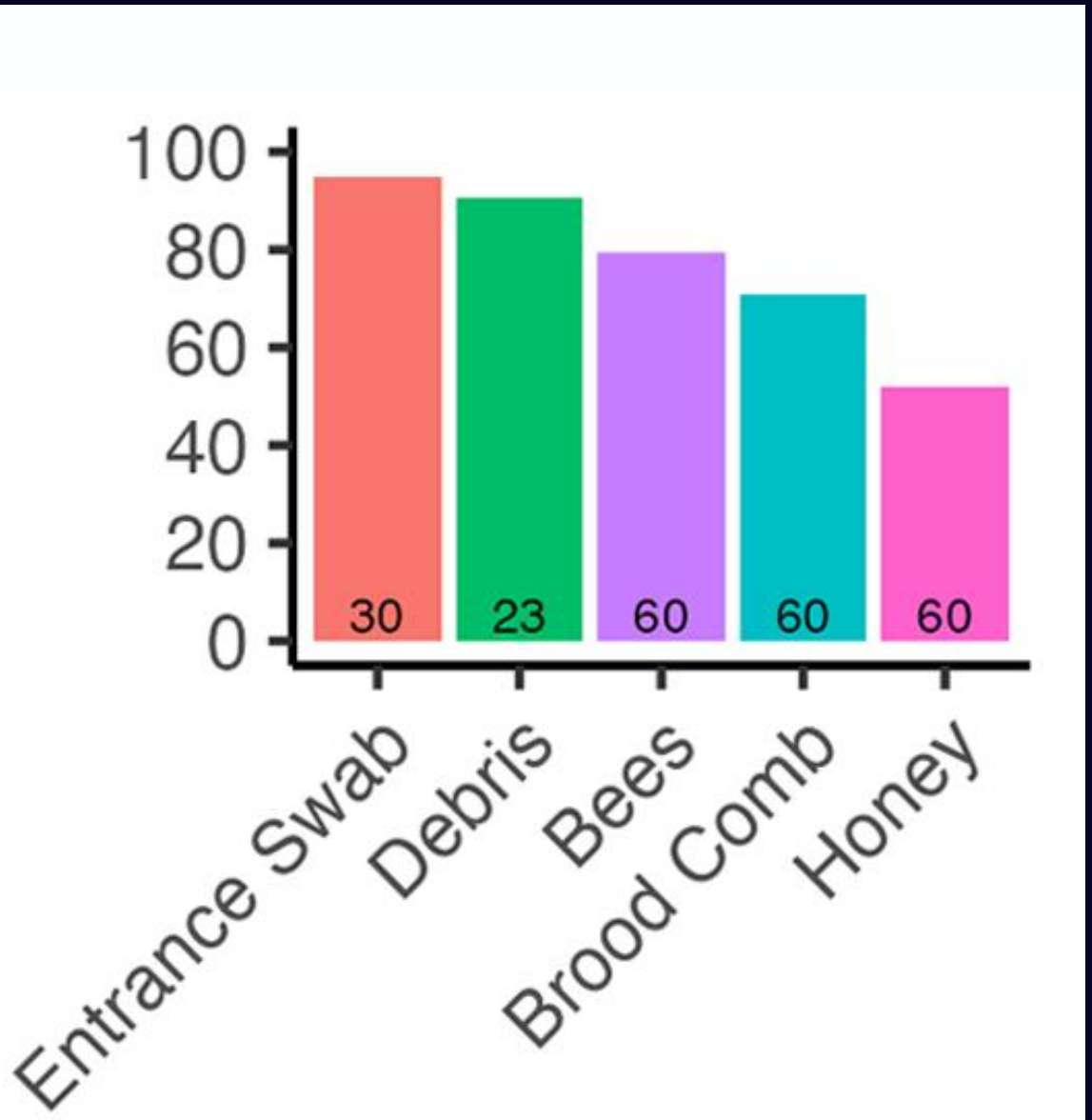


Several sample types performed well



Several sample types performed well

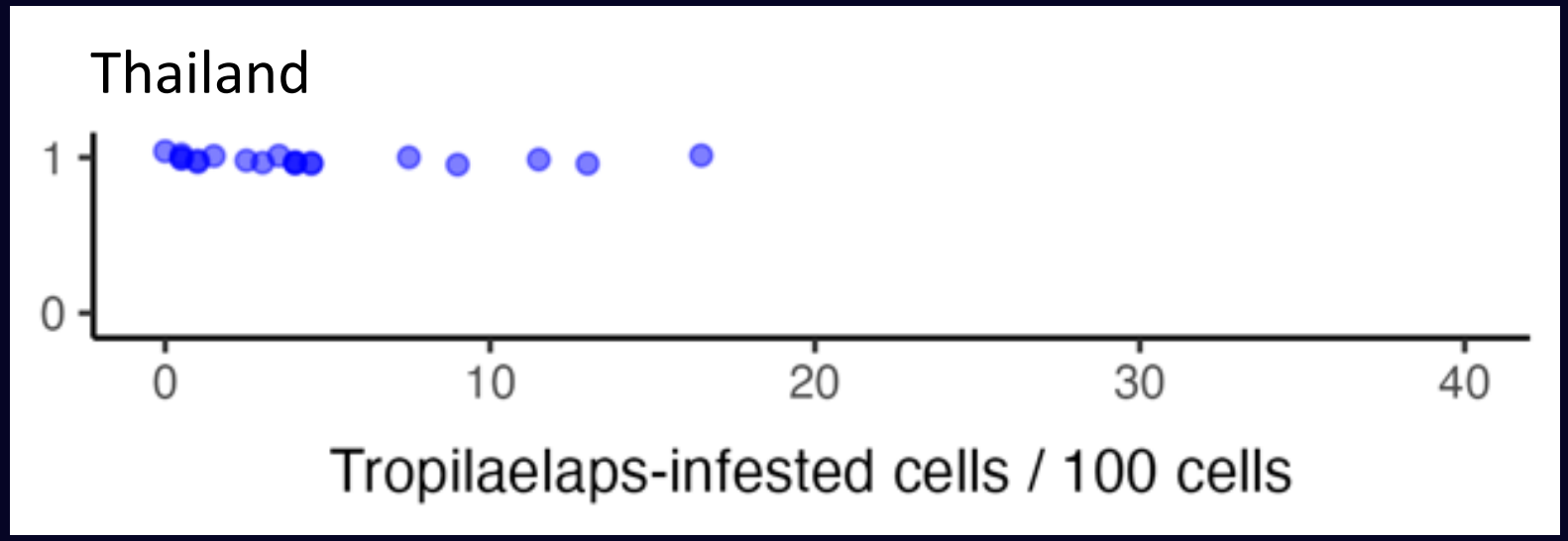


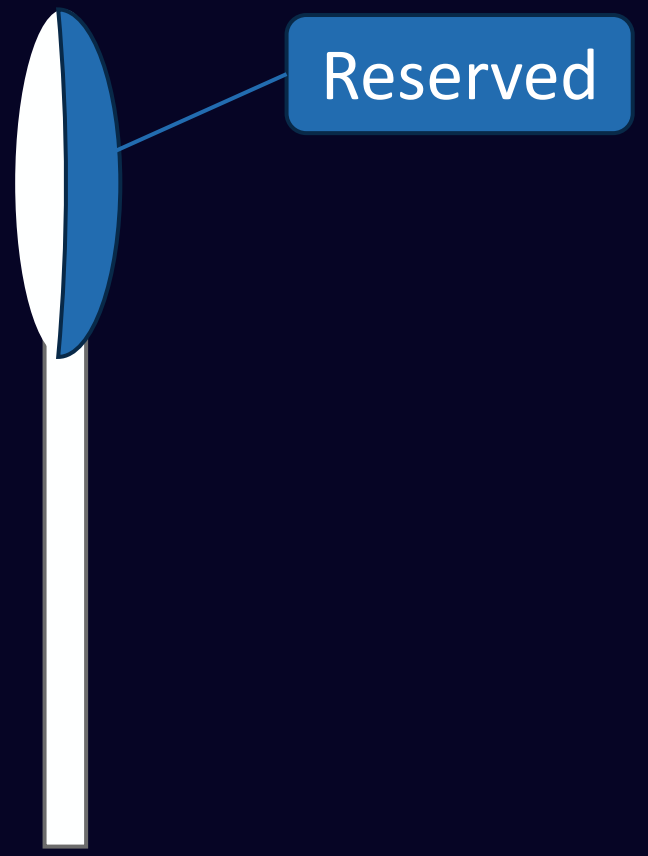
Sensitivity:

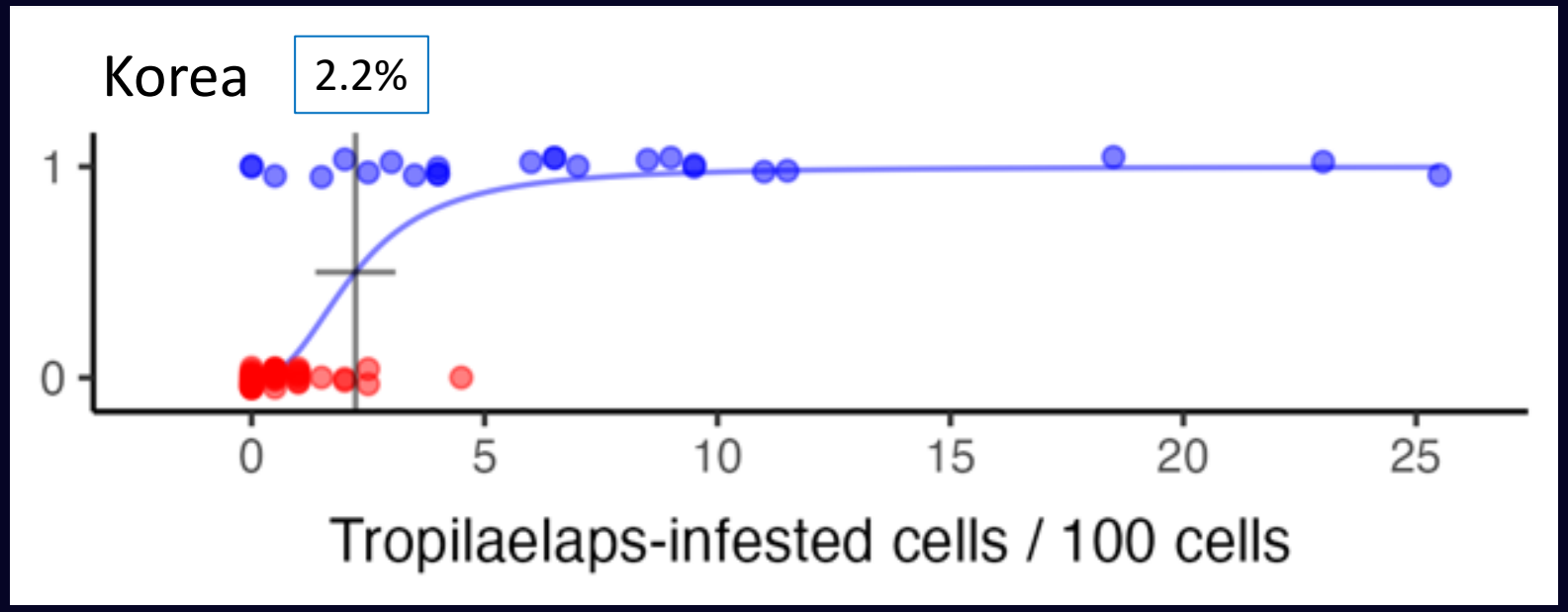
- Entrance swab – 95%
- Debris – 90%
- Bees – 80%

Locations, then countries averaged together

Entrance swab: performed well







Entrance swab: performed well

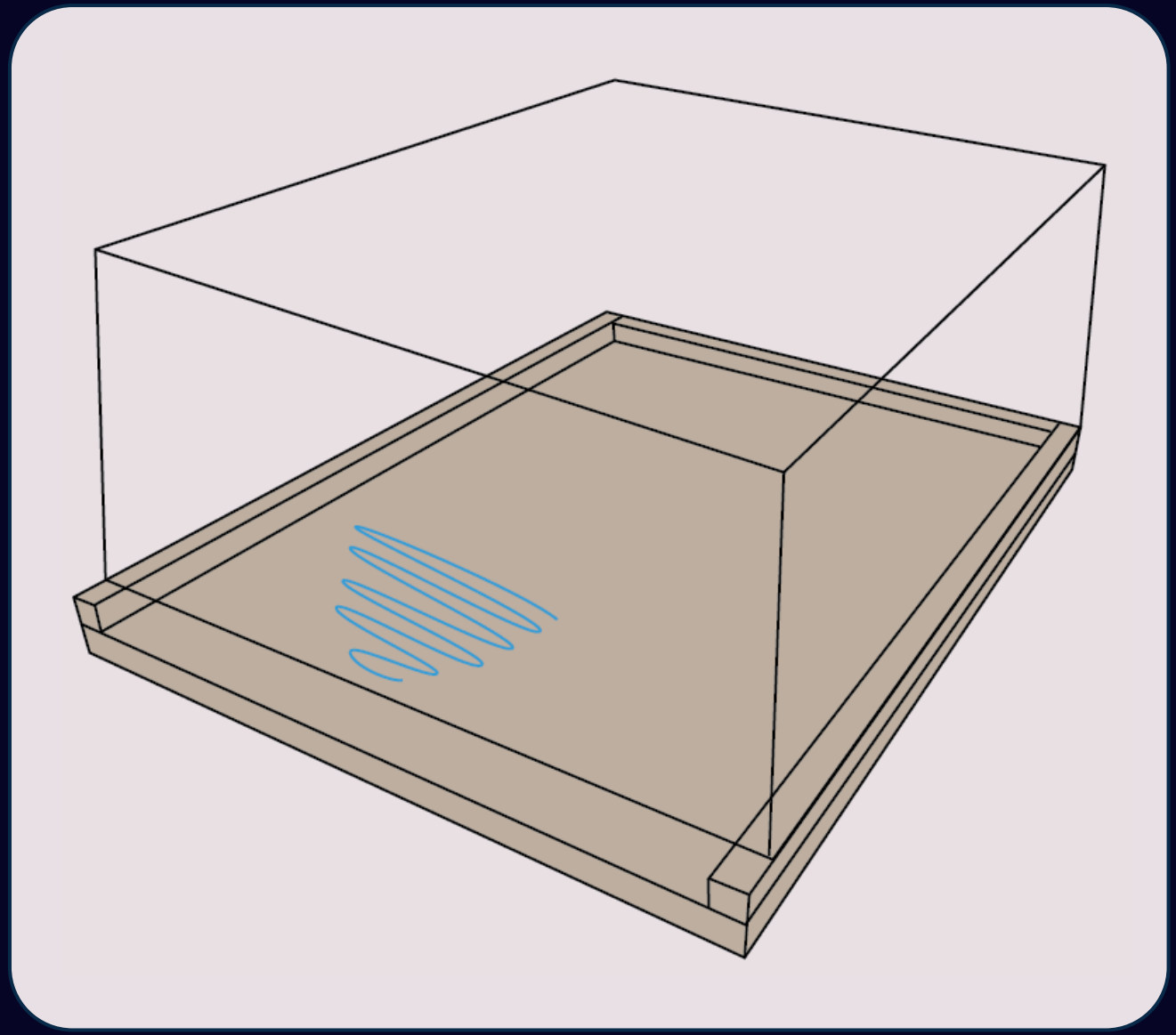


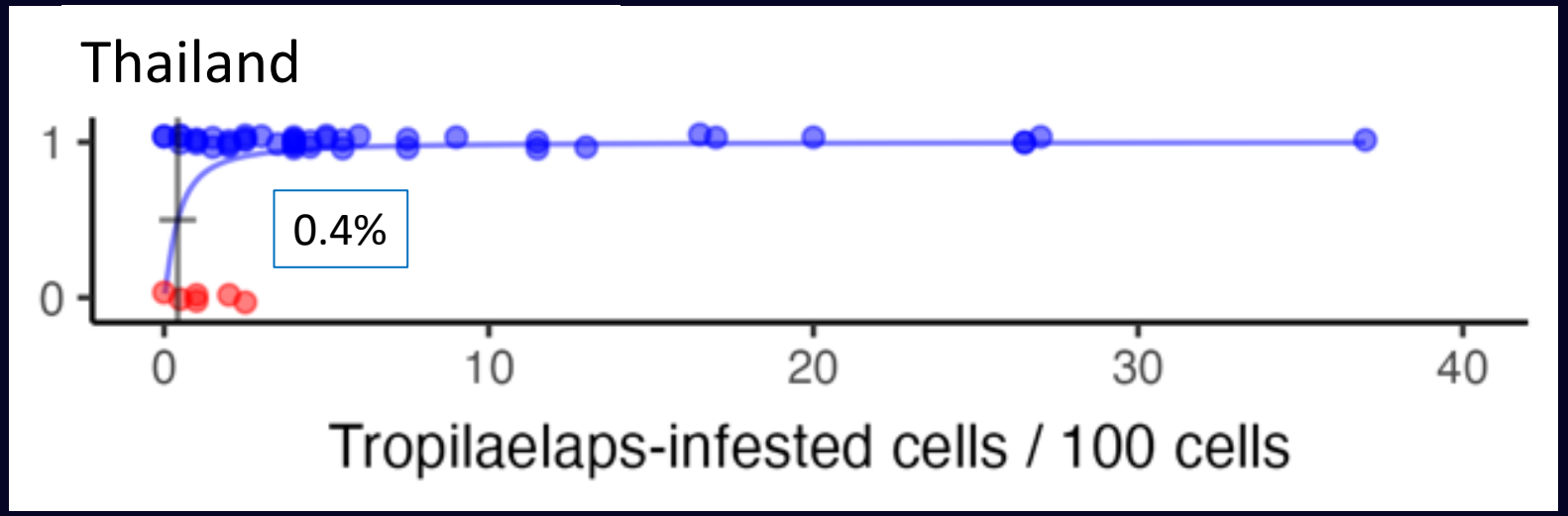
Image: Dan Aurell

Bottom board debris

Mixed results:

- High sensitivity
- High DNA yield (when positive)
- Several false negatives from highly infested colonies

Adult bees: performed well



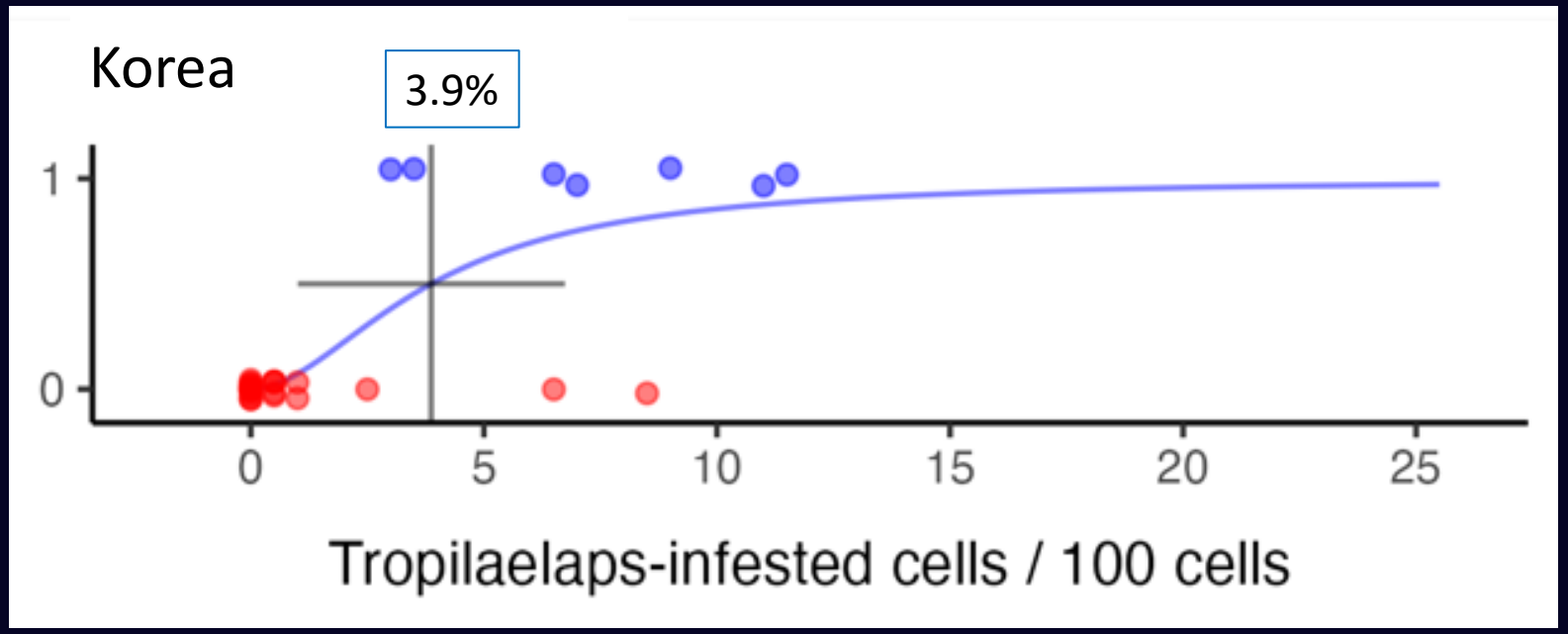


Image: S. Bruckner

Brood-associated

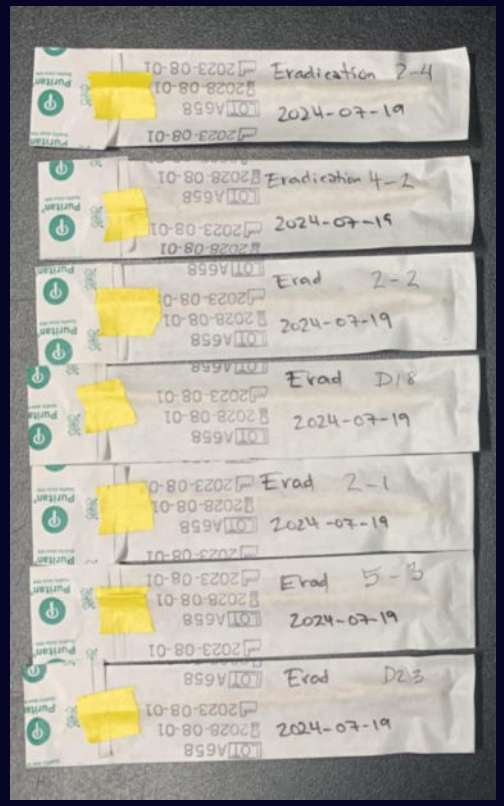
- Brood comb
- Brood swab (surface)
- Brood swab (cells)

Other

- Entrance swab
- Bottom swab
- Bees
- Debris (bottom board)
- Honey

Why did entrance swabs perform well?

 # 1 - Entrance swab

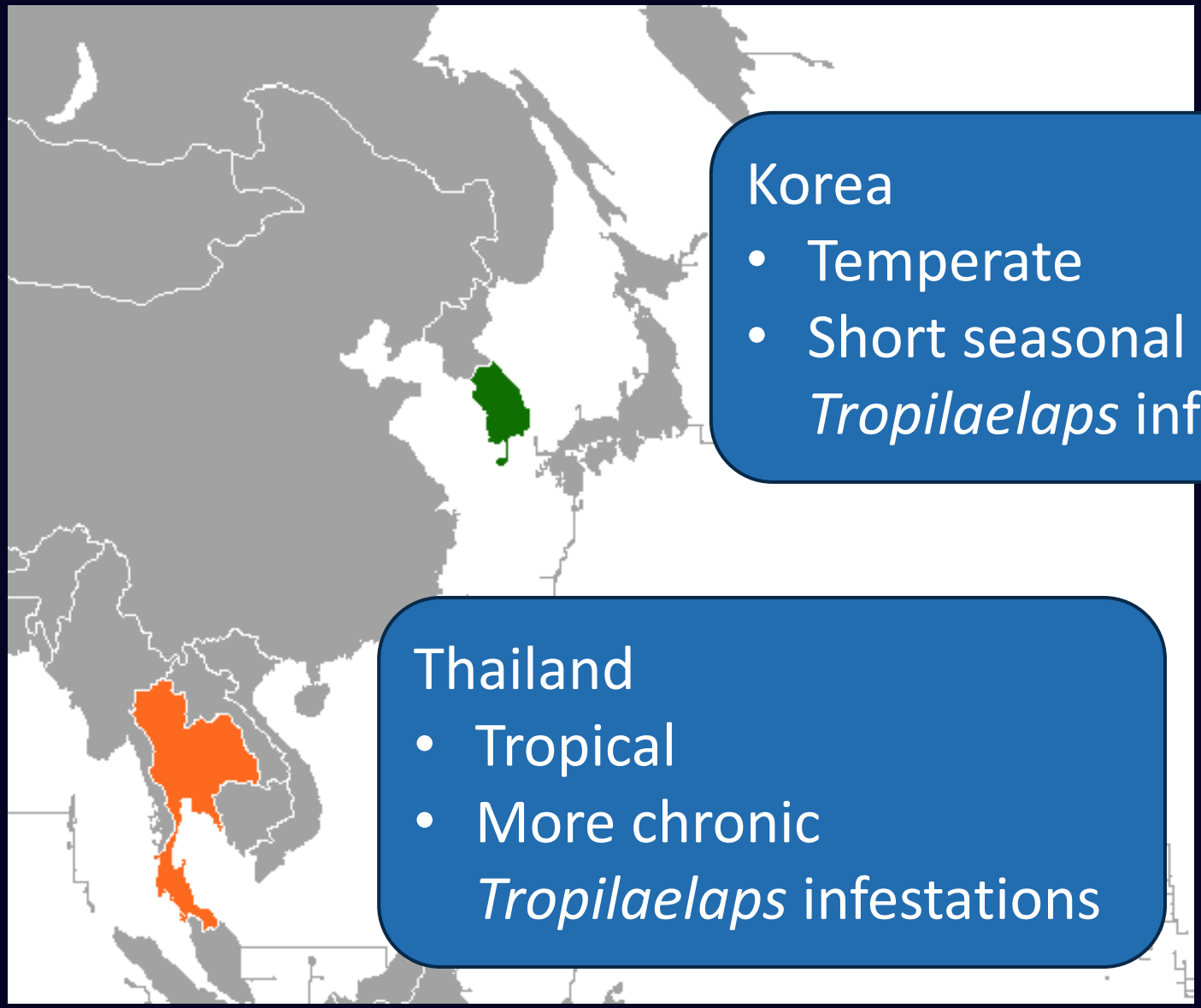


Probable route of accumulation:

- Mites fall to bottom board[1]
- DNA accumulates

[1]Pettis et al. (2013)

Why was eDNA more sensitive in Thailand?



Korea
- Temperate
- Short seasonal spike in *Tropilaelaps* infestations[1]

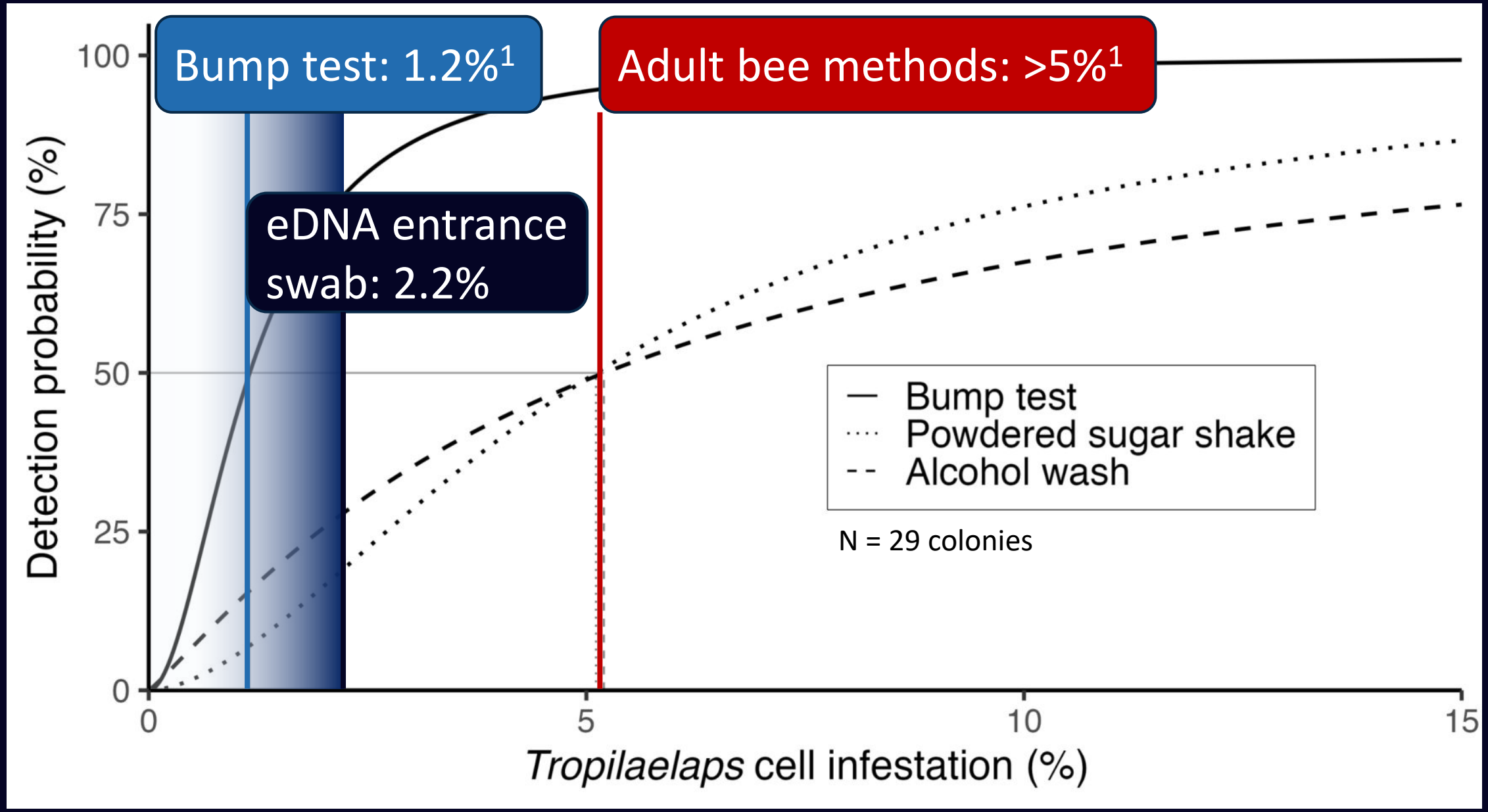
Thailand
- Tropical
- More chronic *Tropilaelaps* infestations

[1]Dongwon (2016)

Bump test

[1]NHBDS Protocol

Images: Project Apis m.



[1]Tokach, Aurell et al. (in prep)

Conclusions

eDNA entrance swab sampling addresses many “cons”

- <1 minute per sample
- Sensitivity similar to bump method
- Scalable

Pooled testing

- Processing cost can be brought down
- Starting to understand tradeoffs

Stay tuned for a paper!

References

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USGS, 2025. https://www.usgs.gov/special-topics/water-science-school/science/environmental-dna-edna

Thank you

Image: Edna Fernandez-Figueroa

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NORTH DAKOTA DEPARTMENT of AGRICULTURE

Alabama Agricultural Experiment Station
AT AUBURN UNIVERSITY

USDA Agricultural Research Service
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Limitation - processing costs

Honey Bee Testing

Full HB-Q Panel

1-10 samples $360
11+ samples $180

Triplex Pathogen Group

1-10 samples $150
11+ samples $60

Single Test

1-10 samples $90
11+ samples $50

Pooled processing

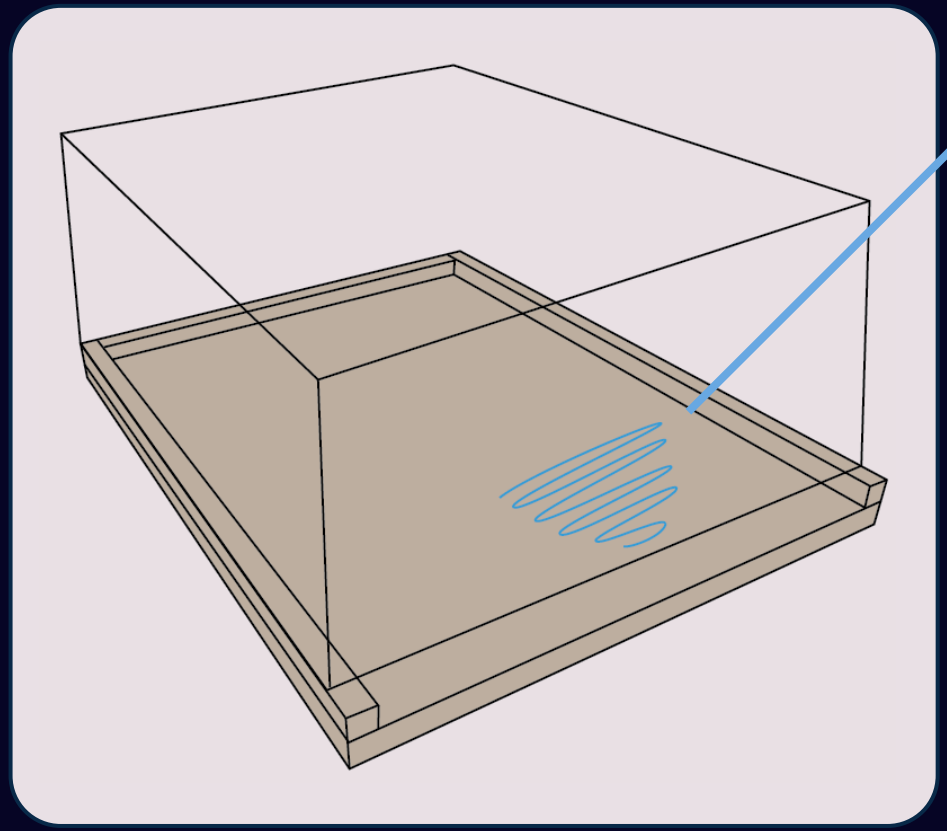
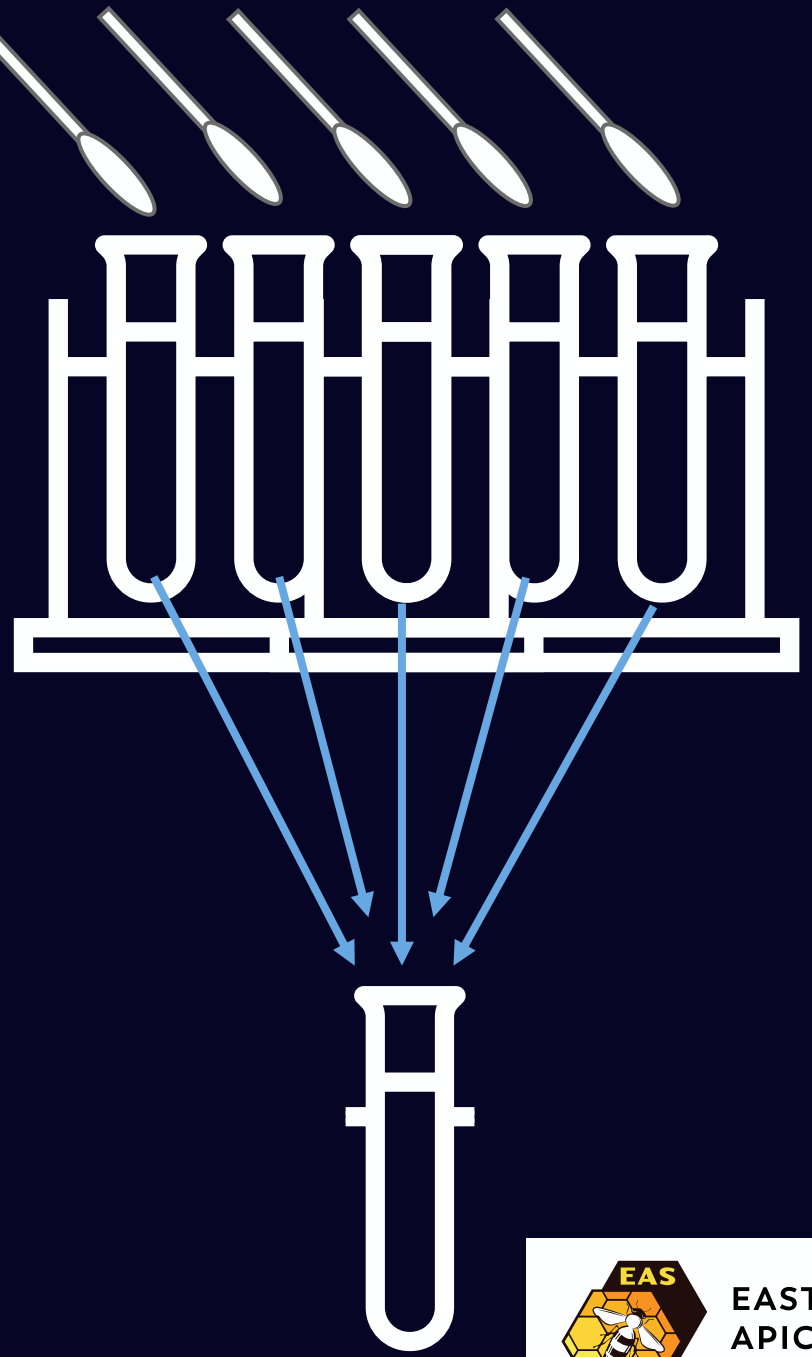


Image: Dan Aurell



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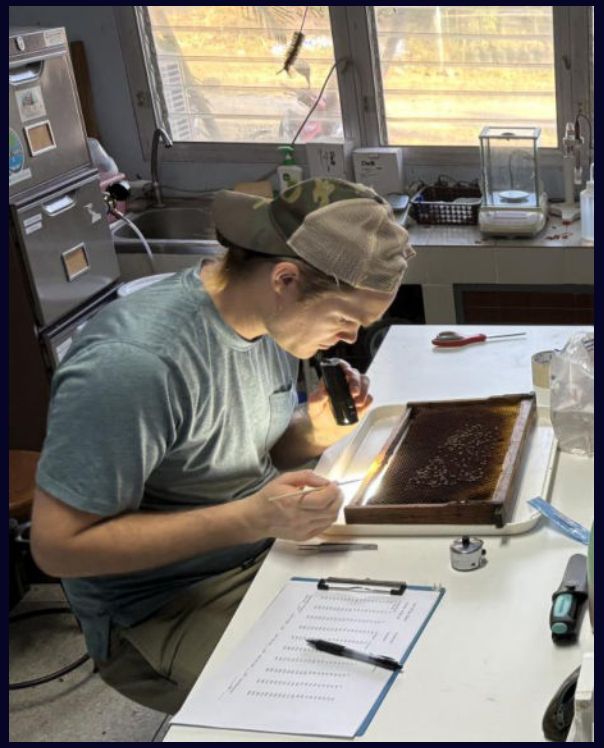
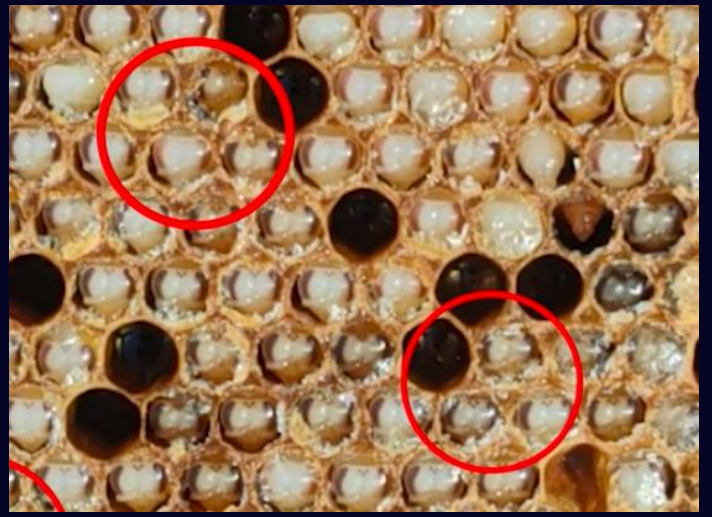
Further comparisons of physical monitoring

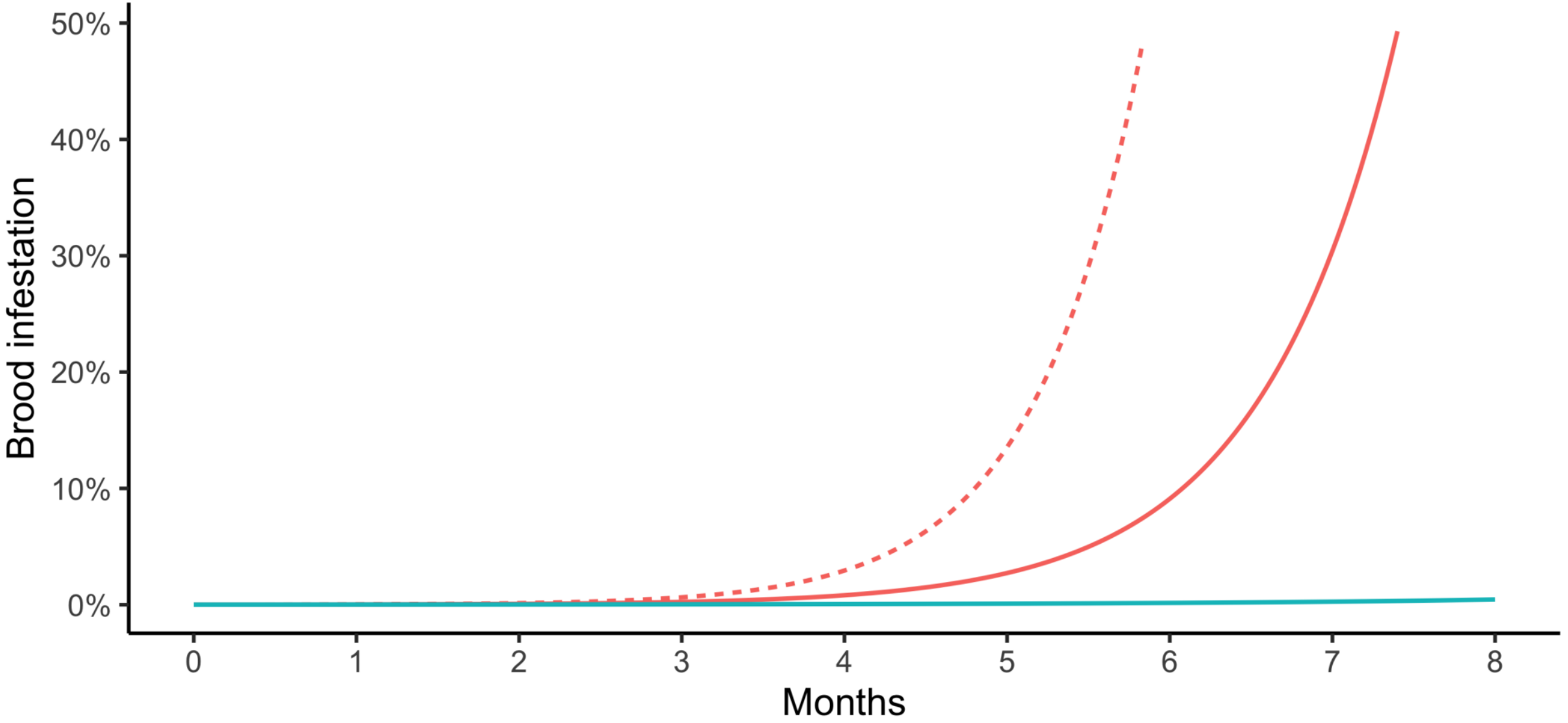




And pooled testing

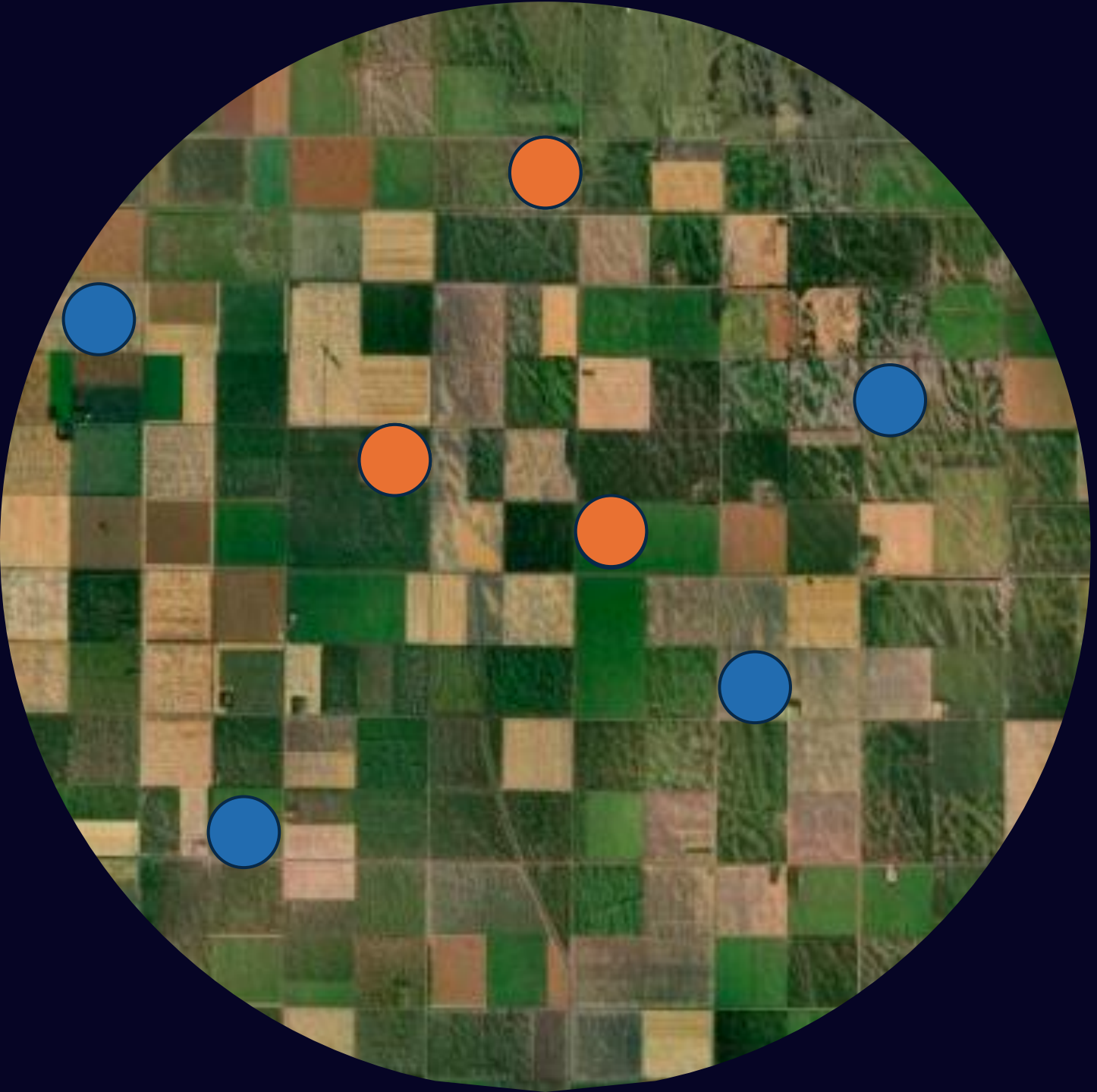
Uzunov et al.

Assess eDNA accumulation



Population growth

Natural dispersal



Apiculture and Social Insects

A Rapid Survey Technique for *Tropilaelaps* Mite (Mesostigmata: Laelapidae) Detection

JEFFERY S. PETTIS,[1] ROBYN ROSE,[2] ELINOR M. LICHTENBERG,[3] PANUWAN CHANTAWANNAKUL,[4] NINAT BUAWANGPONG,[5] WEERAYA SOMANA,[4] PRACHAVAL SUKUMALANAND,[5] AND DENNIS VANENGELSDORP[3,6]

[1]Pettis et al. (2013)

Detection test	Test sensitivity		Total time (min.)
	Infestation verified (% ± SE)*	Universal infestation assumed (% ± SE)*	
Tropilaelaps mite			
Drop	81.3 ± 4.9(A)	54.2 ± 5.1(E)	29
Wash	7.8 ± 3.4(B)	5.2 ± 2.3(F)	15–20
Bump	50.0 ± 4.1(CD)	36.3 ± 3.4(GH)	15
Worker Brood	75.7 ± 3.3(A)	56.7 ± 3.3(E)	20
Drone Brood	32.0 ± 5.4(D)	24.7 ± 4.4(H)	10
Brood Removal + Bump	61.5 ± 4.2(AC)	49.1 ± 3.5(EC)	17
All tests combined	N/A	74.6 ± 2.8	

Sensitivity of current *Tropilaelaps mercedesae* monitoring methods in *Apis mellifera* colonies

Rogan Tokach[1,*], Dan Aurell[1], Bajaree Chuttong[2], Geoffrey R. Williams[1,*]

Colony #	Tropilaelaps cell infestation (%)	Number of Tropilaelaps mites recovered by: Sticky board	Bump	Alcohol wash	Sugar shake
19	0	11	0	0	0
20	0	4	0	0	0
30	1	2	0	0	0
5	1	3	2	0	0
10	1	3	1	0	0
14	1	3	0	0	0
9	2	18	2	1	2
1	2	7	0	0	0
6	2	1	1	0	0
21	2	18	2	0	0
29	2	11	0	0	0
3	3	25	5	1	5
13	3	9	3	1	0
31	3	9	0	0	0
18	4	67	5	0	0
24	4	12	4	2	0
27	4	14	1	0	0
4	5	8	4	2	1
16	6	81	8	1	1
28	6	25	3	0	0
2	6	7	3	0	1
12	12	29	2	4	0
25	17	20	18	0	1
22	20	69	15	4	6
11	27	750	99	29	25
23	27	137	6	1	1
Proportion detection	24/26	26/26	19/26	10/26	9/26
Percentage detection	**92%**	**100%**	**73%**	**38%**	**35%**