

**Be-eRNA: Using next-generation sequencing to characterize the community of honey bee viruses from hive environmental samples**

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The western honey bee (*Apis mellifera* L.) plays a critical role in agricultural pollination services globally, yet is threatened by many pests and pathogens, particularly RNA viruses. Monitoring for viruses is challenging as diagnosis relies on destructively sampling adult bees and screening for a standard virus panel, thus overlooking novel, uncommon, or emerging viral pathogens. Here, we investigated the utility of next-generation sequencing of environmental RNA (eRNA) samples collected from hive substrates to characterize the virus communities of honey bee colonies. We swabbed the following substrates from test hives: hive entrances, hive bottom boards, frame top bars, sugar feeders, honey, and hive tools. RNA was extracted from all swabs and screened for seven viruses using conventional PCR techniques. For two additional samples (swabs of a hive tool and hive entrance), libraries were prepared with and without viral enrichment, and RNA-seq was subsequently performed on an Illumina NovaSeq instrument. Overall, we found that over 85% of eRNA samples were positive for at least one virus. From NGS data, we found the enrichment step nearly doubled the viral transcripts detected in our samples. Shotgun sequencing results from our hive tool eRNA sample detected viruses present in conventional PCRs as well as less common viruses not included in standard viral panels. We were able to characterize the virus community within honey bee colonies from eRNA samples, but there was great variation in viruses detected among hive sampling locations. Thus, our next step will be to determine the hive substrates that will give us the most informative results for characterizing known and emerging viruses. We show that non-destructive environmental sampling combined with high-throughput sequencing can be used to monitor emergent viral threats to honey bee health. This study demonstrates the untapped potential of eRNA as a tool for global viral monitoring in honey bee colonies.

# Be-eRNA: characterizing the community of honey bee viruses from environmental RNA

**Kaitlin R. Deutsch<sup>1</sup>, Rafael Valentin<sup>2</sup>, James D. Ellis<sup>1</sup>, Leigh Boardman<sup>3</sup>**

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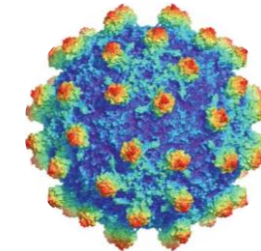
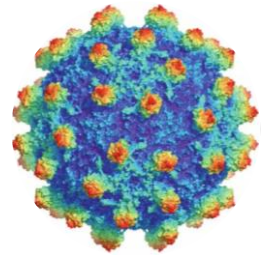
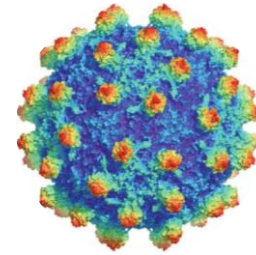
<sup>3</sup> Department of Biological Sciences, University of Memphis, TN, USA



# Introduction:

## *RNA viruses threaten bee health*

One of the key threats to bee health is infectious disease - particularly **single-stranded RNA viruses**.



# Introduction:

## *RNA viruses threaten bee health*

Challenges of monitoring viruses include:

- Viruses do not always elicit clinical signs of infection
  - Monitoring viral pathogens requires destructive sampling of adult bees for molecular screening.
- Novel or emerging viral pathogens may be missed by conventional screening techniques.



# Introduction:

## *RNA viruses threaten bee health*

Challenges of monitoring viruses include:

- Viruses do not always elicit clinical signs of infection.
  - Monitoring viral pathogens requires destructive sampling of adult bees for molecular screening.
- Novel or emerging viral pathogens may be missed by conventional screening techniques.

**Can we develop rapid, nonlethal sampling methods for comprehensive viral monitoring?**



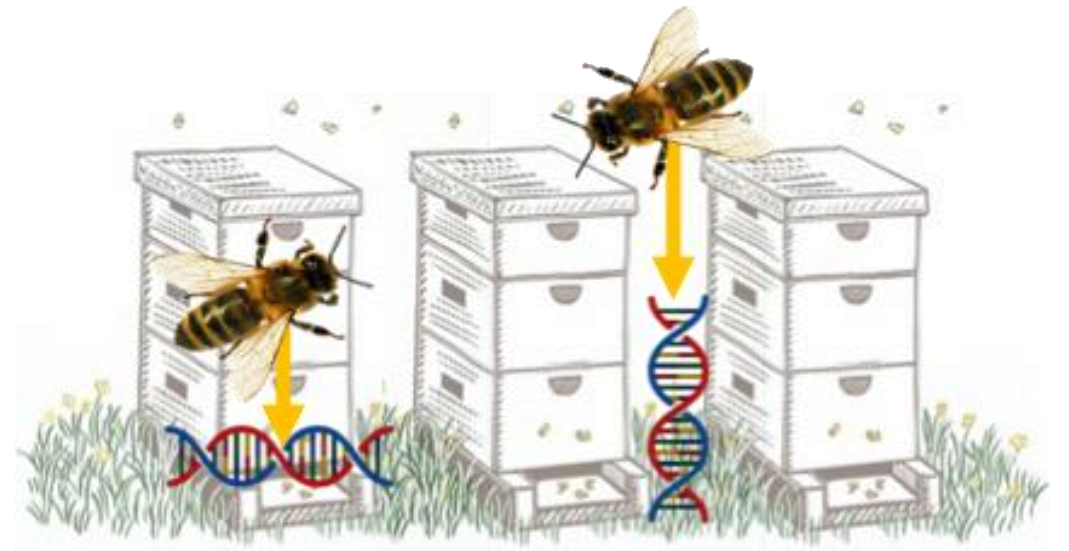
# Introduction:

## *Detecting genetic material from environmental samples*

The use of **environmental DNA (eDNA)** has become widespread.

eDNA refers to genetic material that is collected indirectly from the environment, not from an organism itself.

The use of eDNA has become a powerful tool to monitor biodiversity without relying on visual observations.



# Introduction:

## *Detecting genetic material from environmental samples*

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SPECIAL ISSUE ARTICLE

Environmental DNA  
WILEY

### Novel eDNA approaches to monitor Western honey bee (*Apis mellifera* L.) microbial and arthropod communities

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Grant/Award Number: 1019945

#### Abstract

Pests and pathogens are a continuous threat to the health of Western honey bee *Apis mellifera* L. Monitoring honey bee colonies for arthropod pests, disease-causing bacteria and fungi, and early detection of new invasions is essential to maintain the pollination services provided by honey bees. We investigated the feasibility of using eDNA metabarcoding to detect pests and pathogens in bee hives and across their foraging environment. We sampled 13 sources for eDNA within and outside hives from our test apiary to determine where the most informative eDNA could be obtained, with most sources sampled thrice. This resulted in 61 samples, 20 negative controls, and three positive controls. Furthermore, we compared two eDNA collection techniques—wiping surfaces with moistened forensic swabs and using a spray/wash technique that aggregated surface eDNA into a container, before collecting the eDNA on a filter. We used DNA metabarcoding with universal primer sets to target arthropod, bacterial, and fungal communities. Our results showed that most sources yielded sufficient eDNA and that results of the swab and spray/wash methods were similar when they could be applied to the same surface. We detected DNA from honey bee bacterial symbionts, mycotoxin-producing fungi and *Brachymyrmex* sp. rover ants. Common pests and pathogens of concern to honey bees (i.e., [small hive beetle (*Aethina tumida*), *Varroa destructor*, and *Melissococcus plutonius*]) were detected. This matched our visual observations of clinical signs of these pests and pathogens in the hives we tested. DNA from some species was source specific, which has implications for using eDNA as a monitoring tool. Collectively, our data show that eDNA metabarcoding can accurately detect DNA from arthropods and microbes honey bees contact and can be used as a comprehensive molecular predictor tool for colony health surveys.

#### KEYWORDS

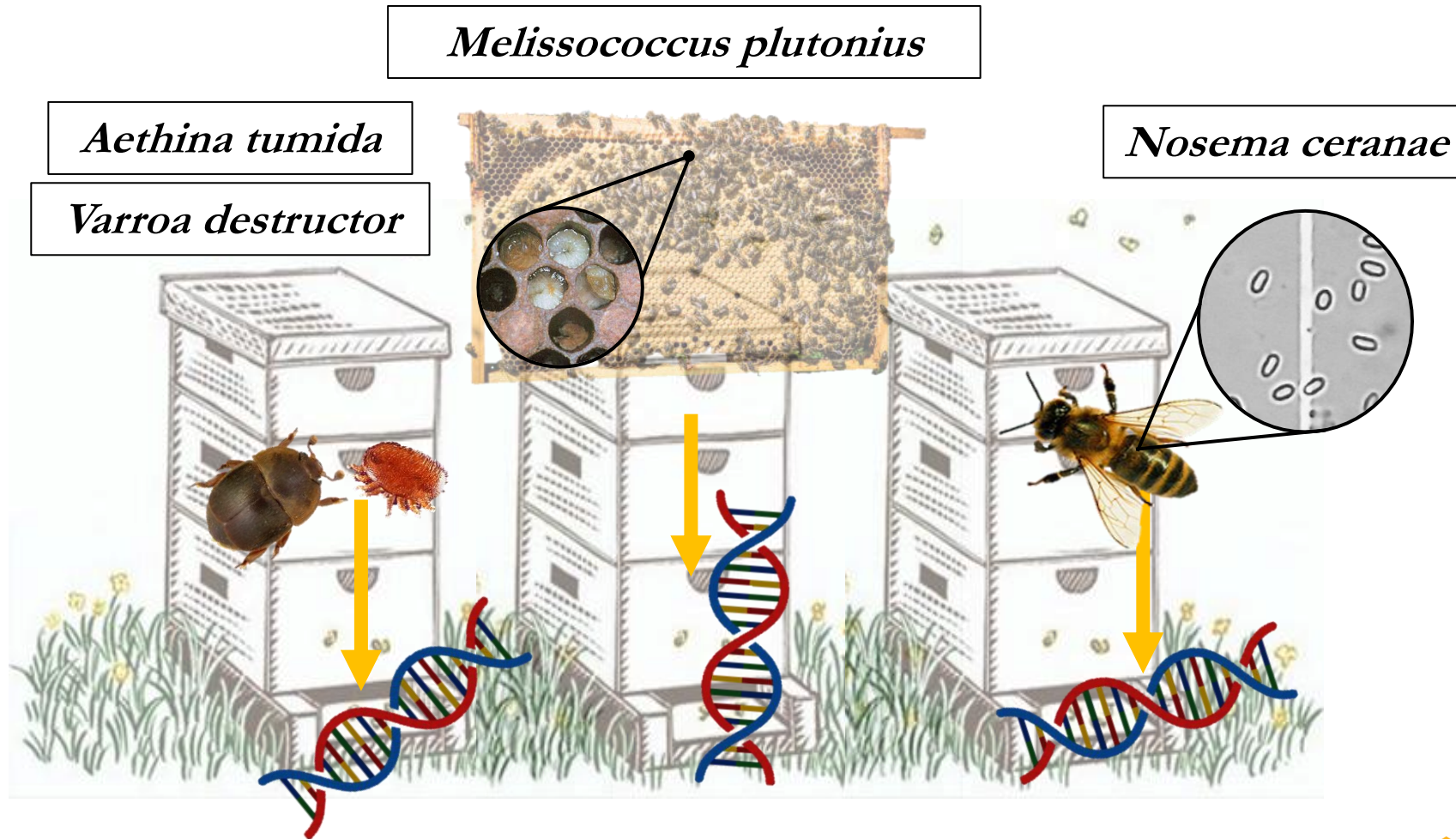
apiary, *Apis mellifera*, arthropod pests, environmental DNA, metabarcoding, pathogens

Previous work from University of Florida has demonstrated the feasibility of eDNA monitoring for honey bee pests and pathogens.



# Introduction:

## *Detecting genetic material from environmental samples*



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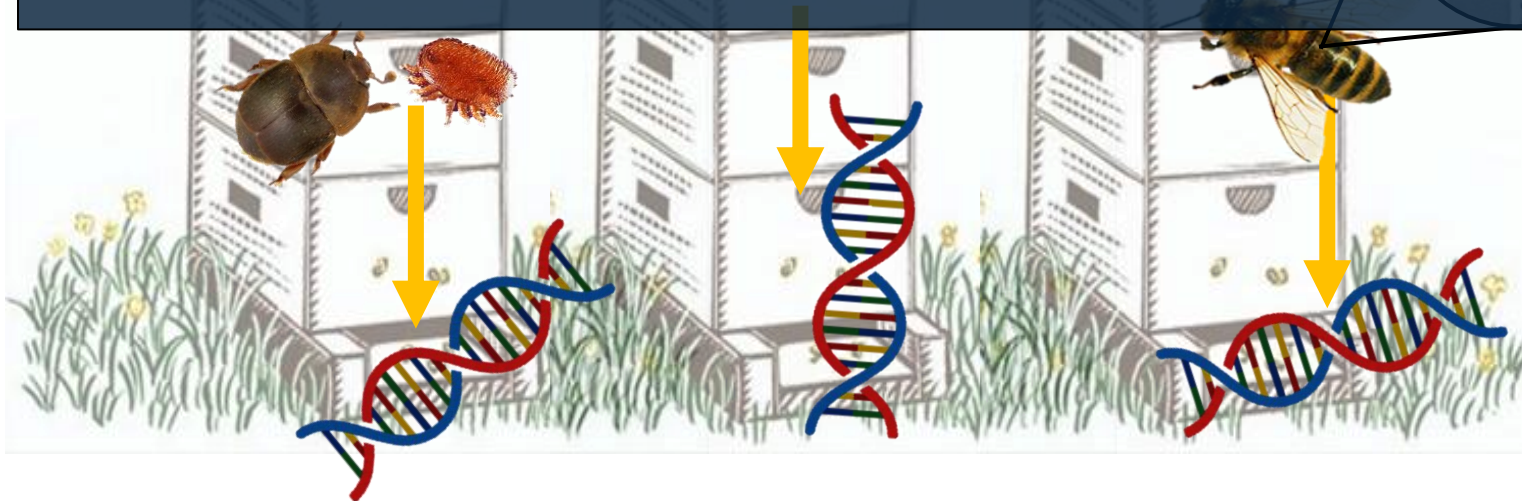
*Melissococcus plutonius*

*Aethina tumida*

*Nosema ceranae*

*Varroa destructor*

Can we use these same methods to monitor viral pathogens?

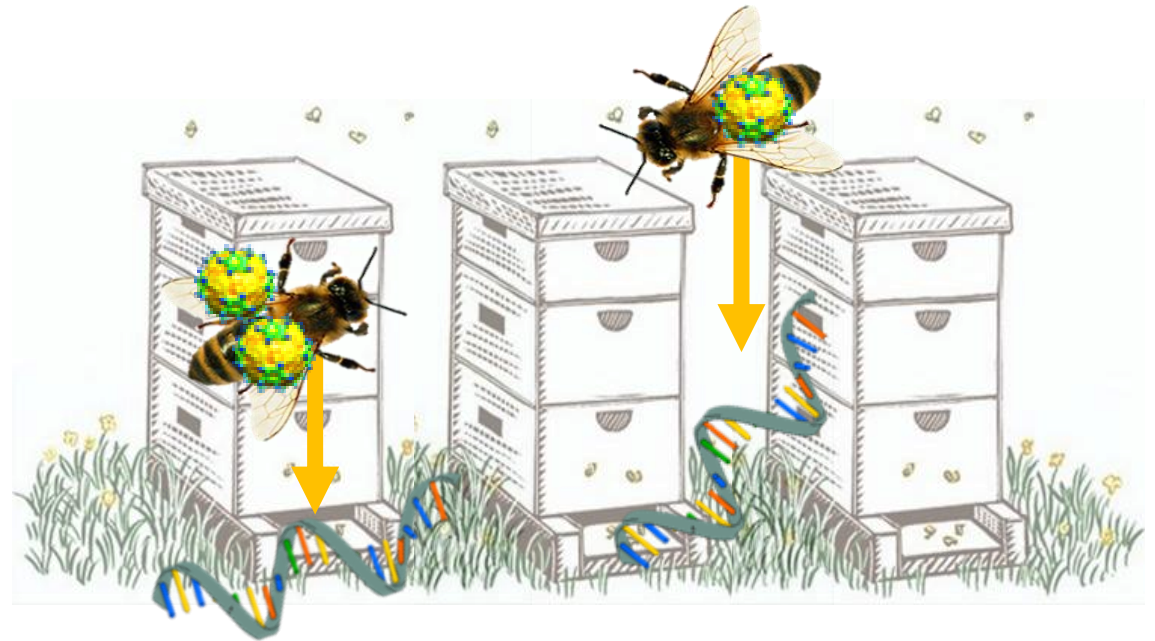


# Introduction:

## *Detecting genetic material from environmental samples*

The main challenge of using environmental RNA is the rapid degradation of RNA compared to DNA and lack of persistence in the environment.

- However, the RNA of many viruses is packaged in a protective capsid.
- In a human health context, eRNA methodologies have been developed to monitor viruses.





**Research Question:** Can we develop eRNA tools to monitor honey bee viruses?

# Methods:

*Can we develop eRNA tools for virus monitoring?*

- Q1:** Can we detect viral RNA from hive environmental samples? If so, what hive substrates most reliably yield virus RNA?
- Q2:** Can next generation sequencing of eRNA samples advance our ability to monitor the full community of bee viruses?

# Q1 Methods:

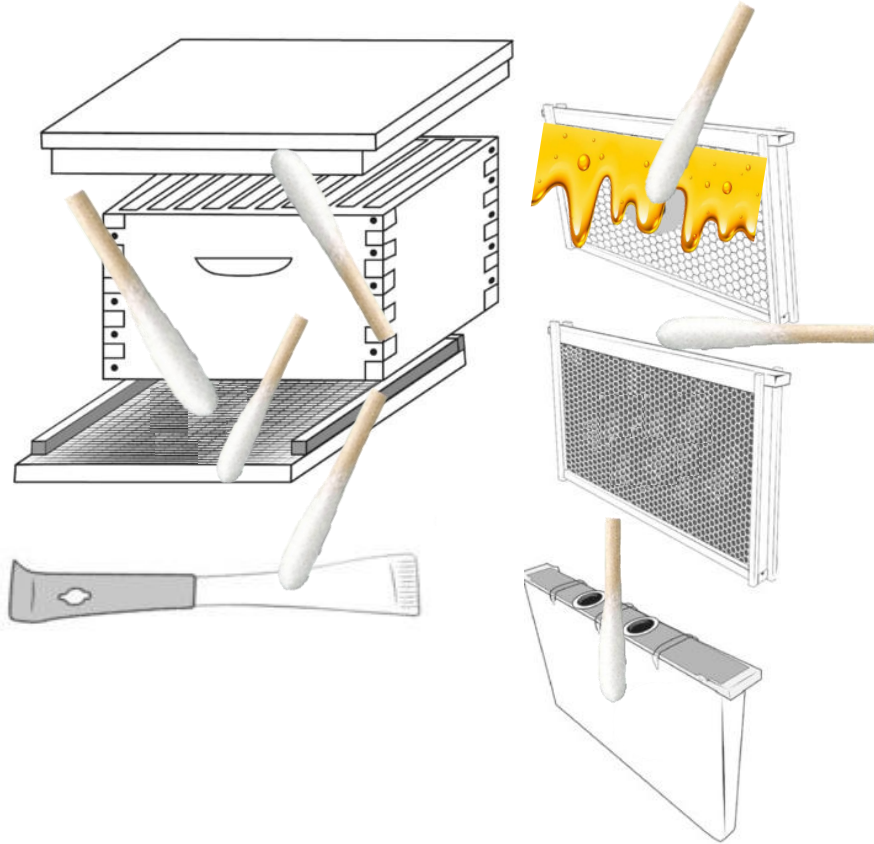
*Can we detect viral RNA from hive environmental samples?*

- We systematically collected eRNA samples from four honey bee colonies, focusing on seven hive substrates (total  $n = 28$ ).



# Q1 Methods:

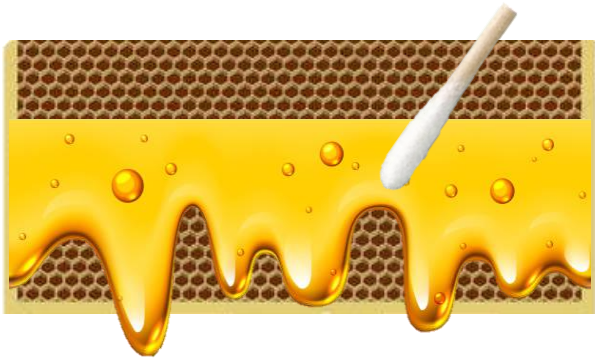
*Can we detect viral RNA from hive environmental samples?*



1. Hive entrance
2. Inside of hive lid
3. In-hive sugar feeder
4. Bottom board
5. Top bar of frame
6. Honey
7. Hive tool

# Q1 Methods:

*Can we detect viral RNA from hive environmental samples?*

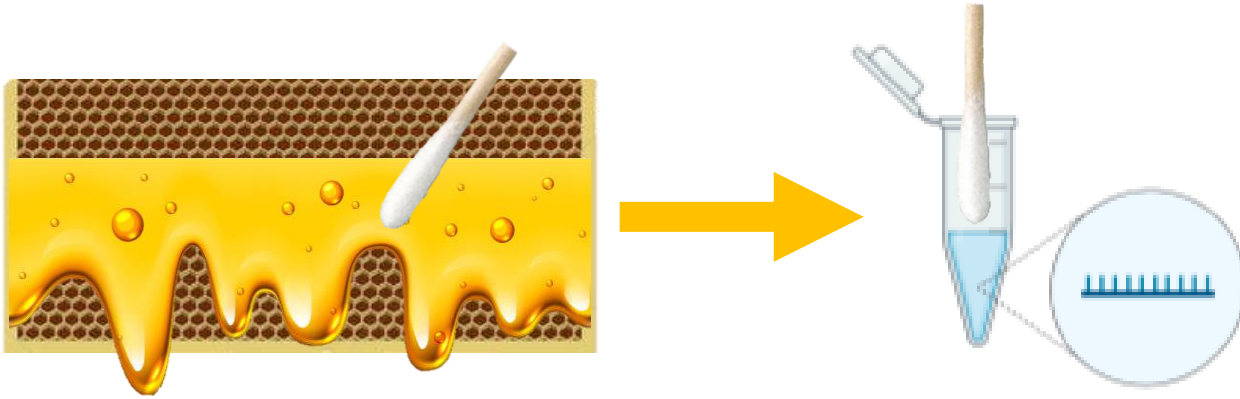


## Sample collection

- A moistened, sterile swab was passed over a target substrate 15-30 seconds to collect RNA.

# Q1 Methods:

*Can we detect viral RNA from hive environmental samples?*



## Sample collection

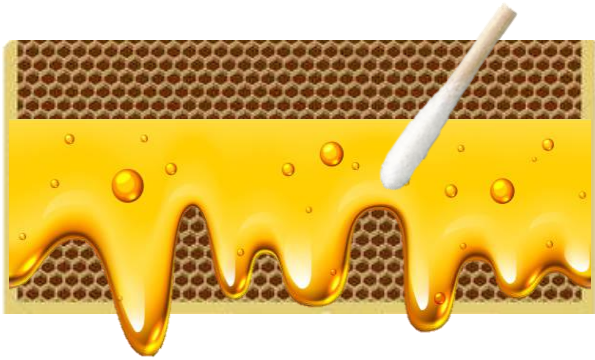
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## RNA extraction

- Using molecular techniques, RNA was extracted and isolated from each swab.

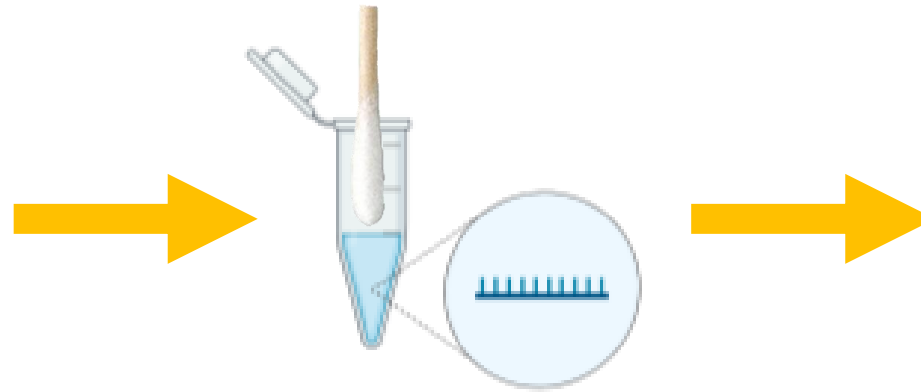
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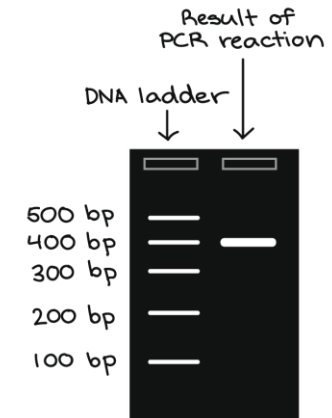
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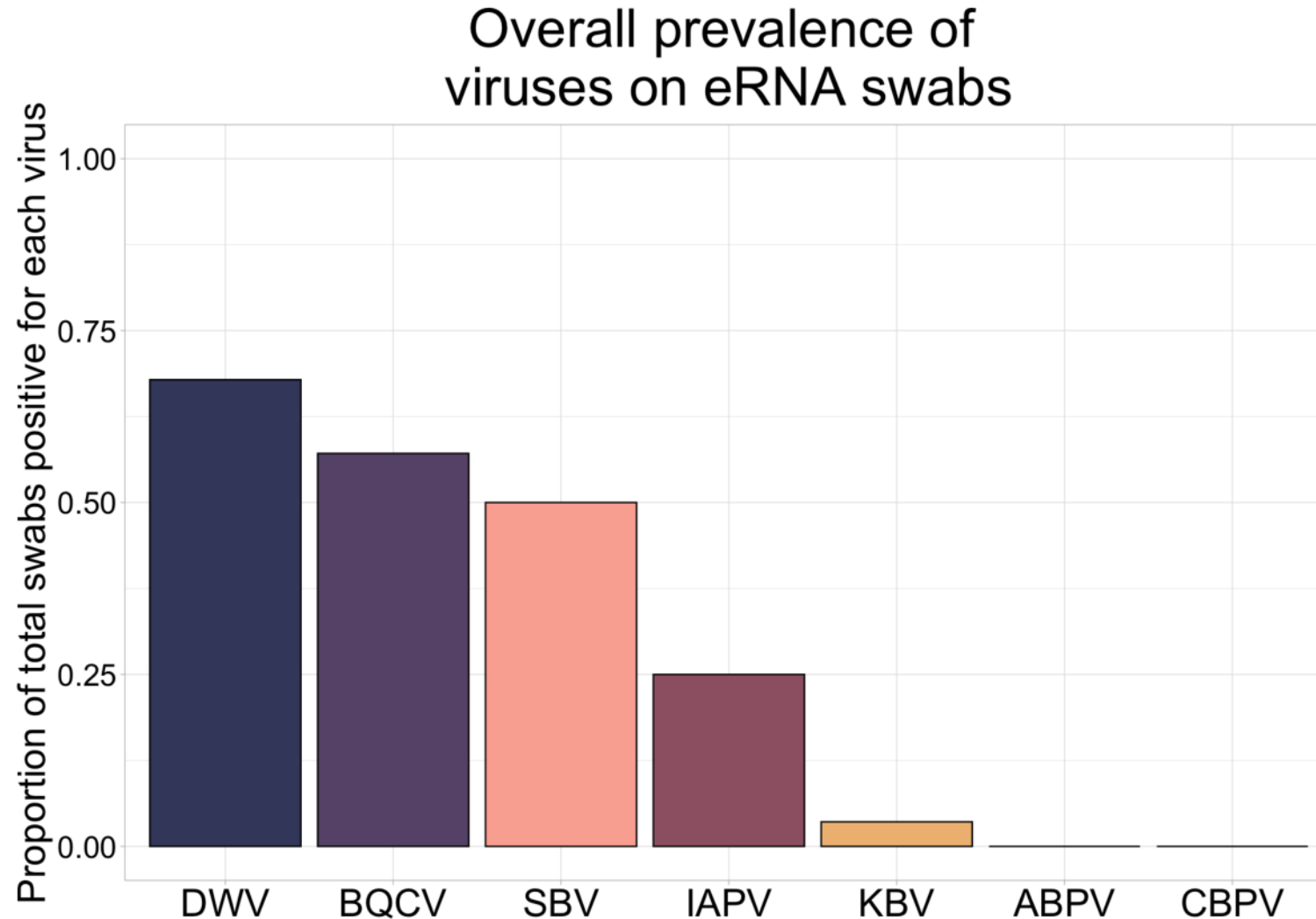


## Multiplex PCRs

- Polymerase chain reactions (PCRs) were used to determine if 7 viruses were present in each RNA sample.

# Q1 Results:

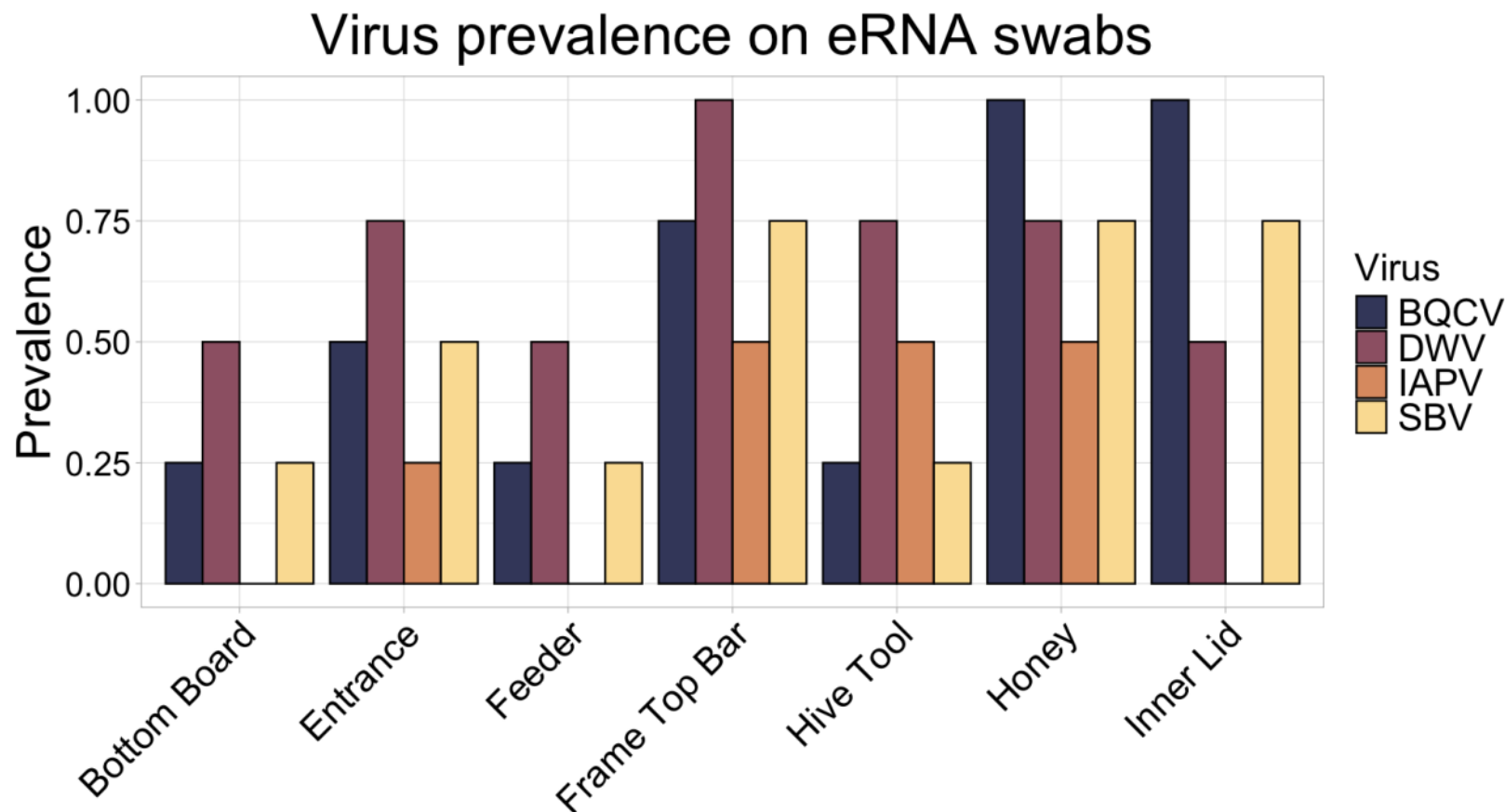
*Can we detect viral RNA from hive environmental samples?*



86% of all swabs were positive for at least 1 virus.

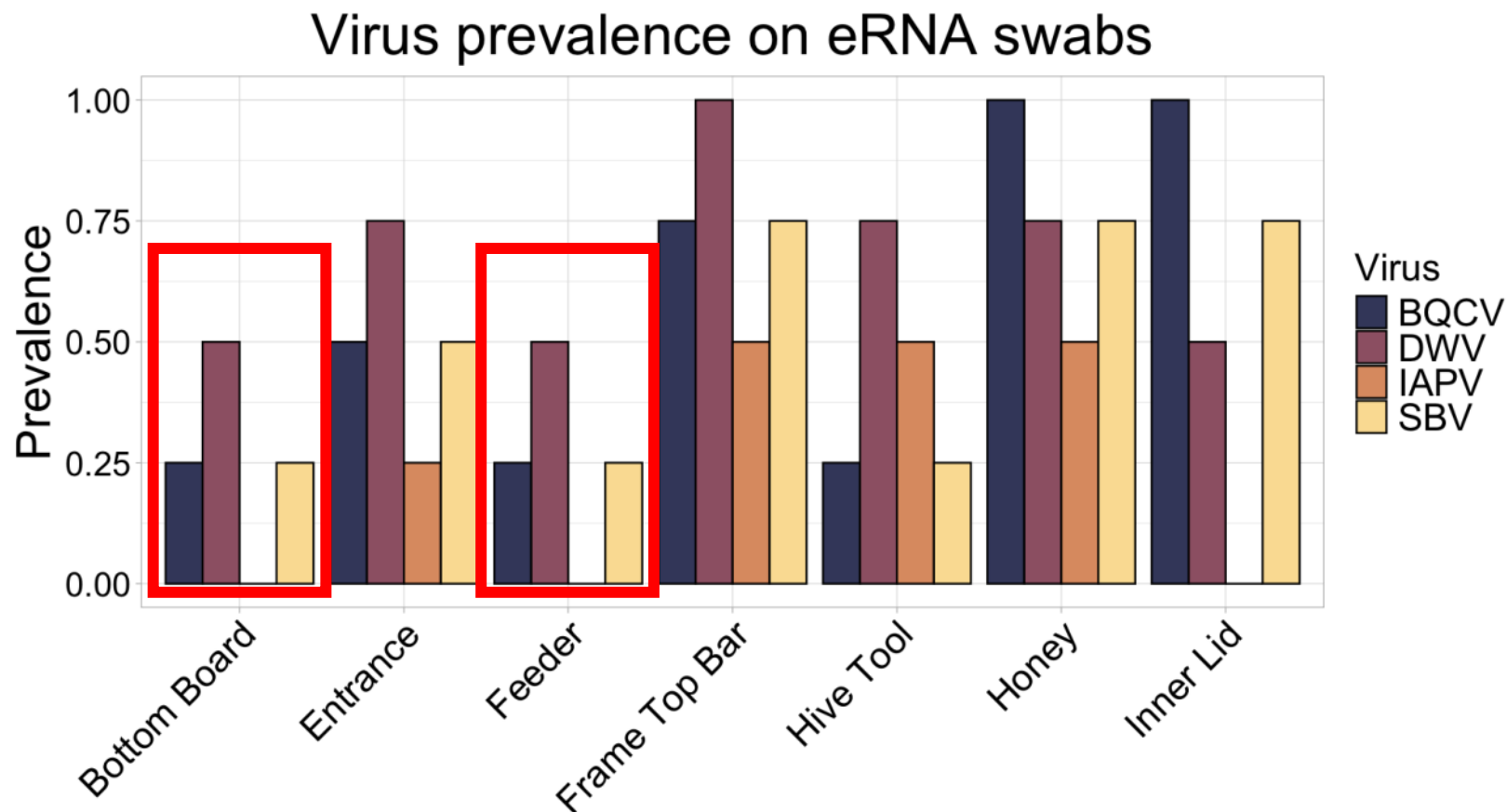
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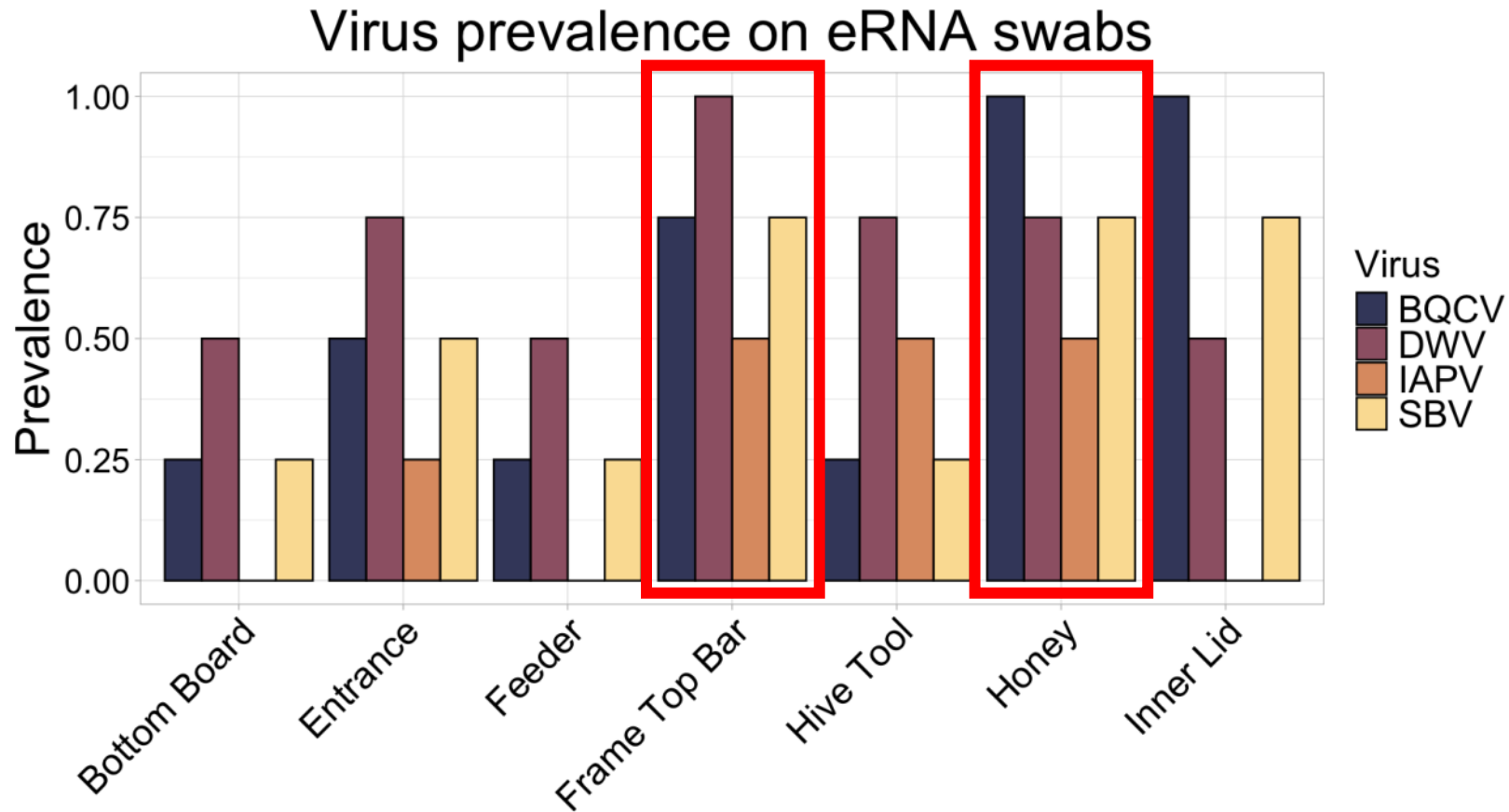
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# Q1 Conclusions

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**Yes!** We detected multiple viruses on the majority of eRNA samples.

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**Yes!** We detected multiple viruses on the majority of eRNA samples. While data are still preliminary (based on four colonies), current trends suggest that **there are areas in the hive that are best for collecting virus RNA.**

# Q1 Conclusions

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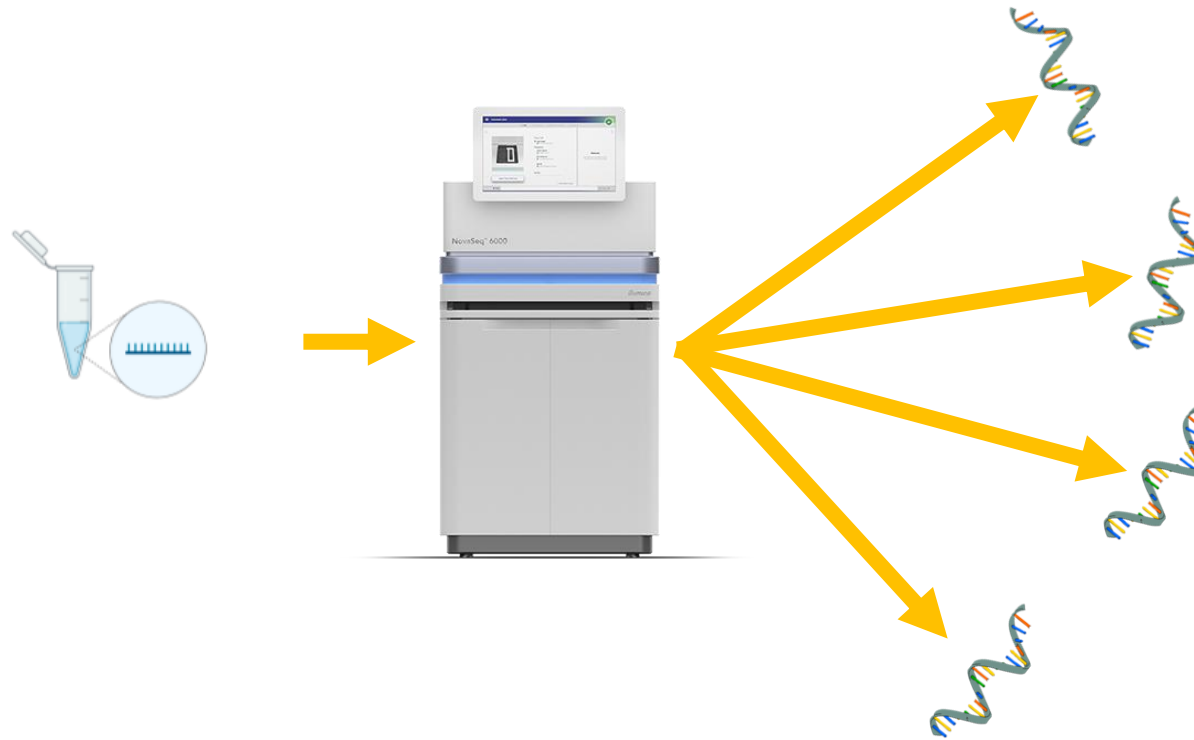
**Yes!** We detected multiple viruses on the majority of eRNA samples. While data are still preliminary (based on four colonies), current trends suggest that **there are areas in the hive that are best for collecting virus RNA.**

*... Q2: can we characterize the full community of honey bee viruses from eRNA samples using next generation sequencing?*

## Q2 Methods:

*Can next generation sequencing characterize full virus community using eRNA?*

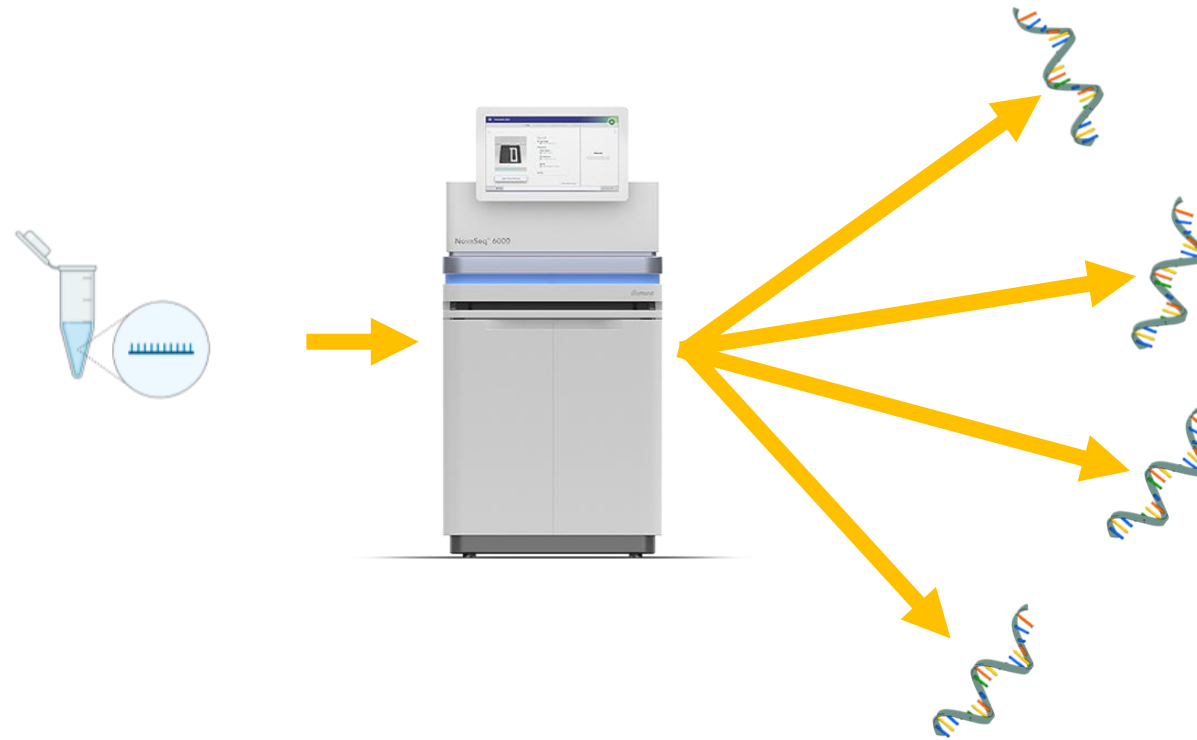
**Next-generation sequencing (NGS)** will detect and “read” the genetic code for all genetic material in a sample – meaning you can detect species you may not know to look for.



## Q2 Methods:

*Can next generation sequencing characterize full virus community using eRNA?*

When applied to eRNA samples, **NGS tools** could facilitate rapid monitoring for emerging viral pathogens, which is particularly important given the global trade in honey bees & bee products.



## Q2 Methods:

*Can next generation sequencing characterize full virus community using eRNA?*

We selected two pilot eRNA samples:

**Hive Tool Swab**



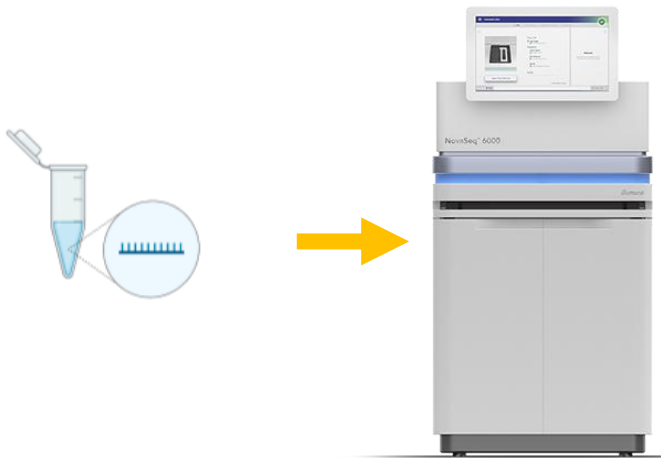
**Entrance Swab**



## Q2 Methods:

*Can next generation sequencing characterize full virus community using eRNA?*

We performed shotgun RNA sequencing on these two samples.

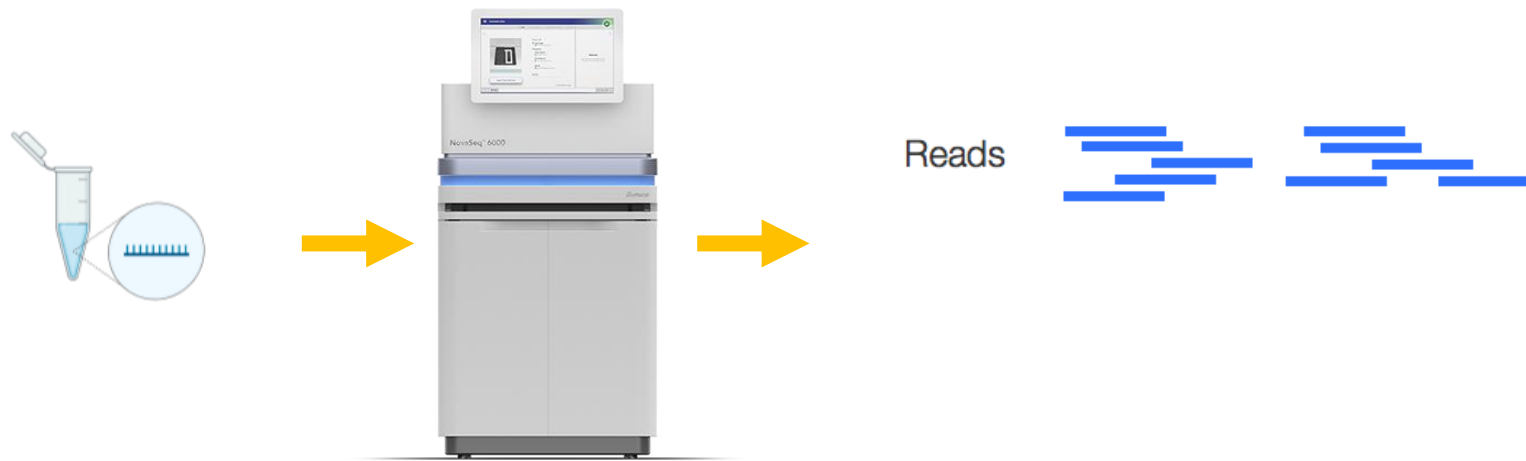


Samples were run on Illumina NovaSeq with 150bp paired end reads.

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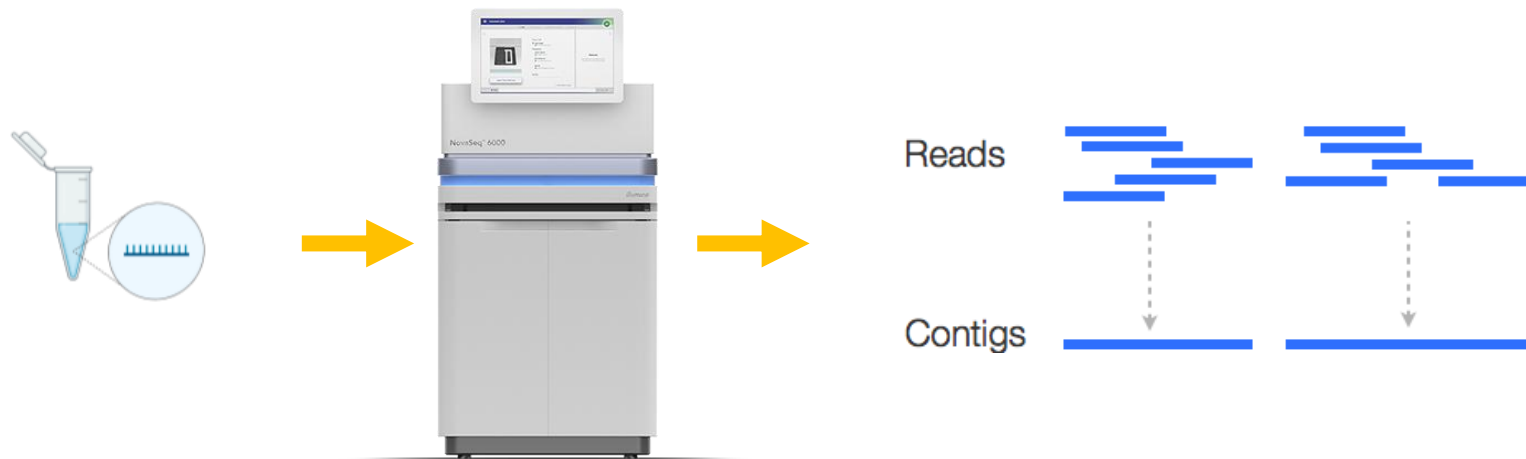


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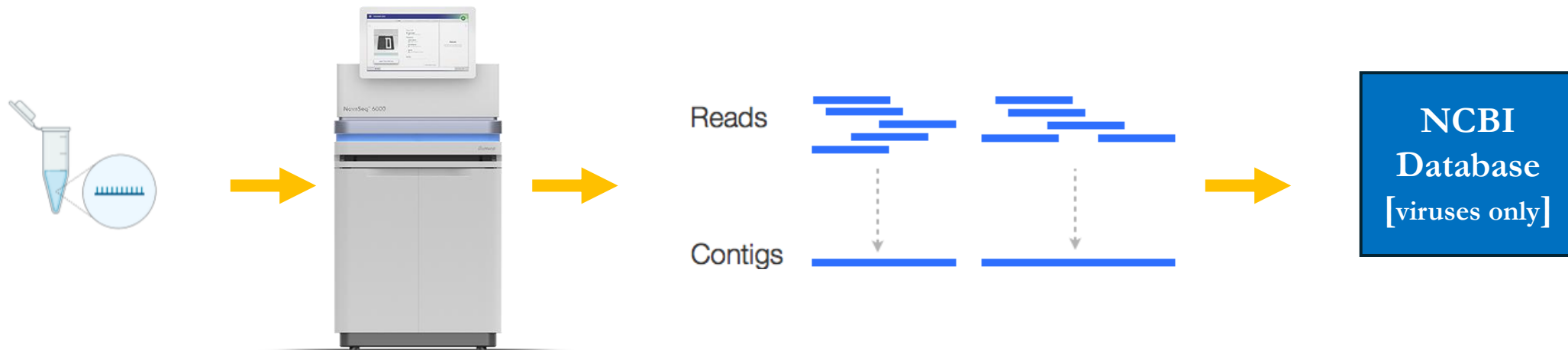


Short reads were assembled into longer, contiguous sequences called “contigs.”

## Q2 Methods:

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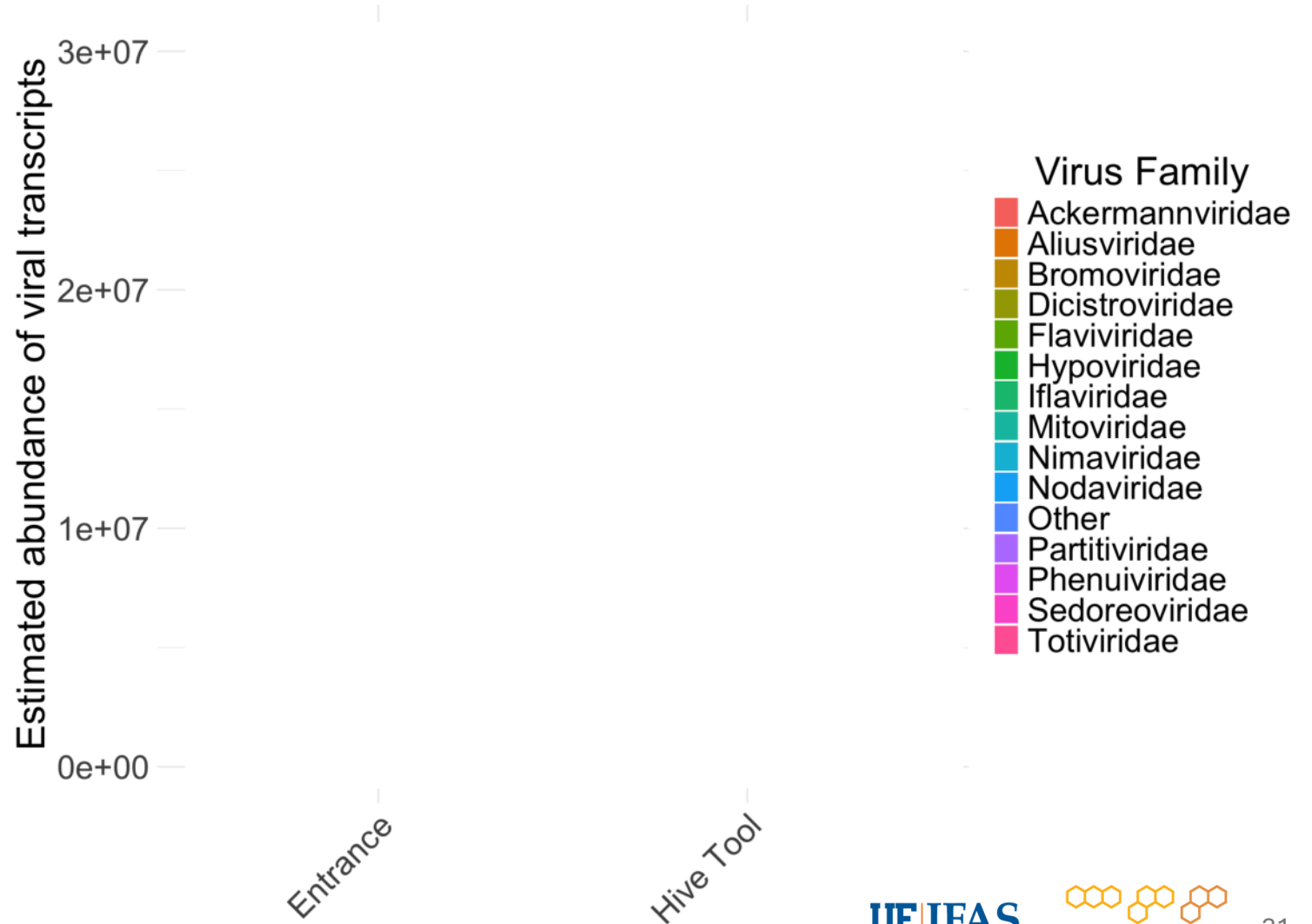
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Contig sequences were assigned taxonomic identifications based the full NCBI nucleotide database for viruses.

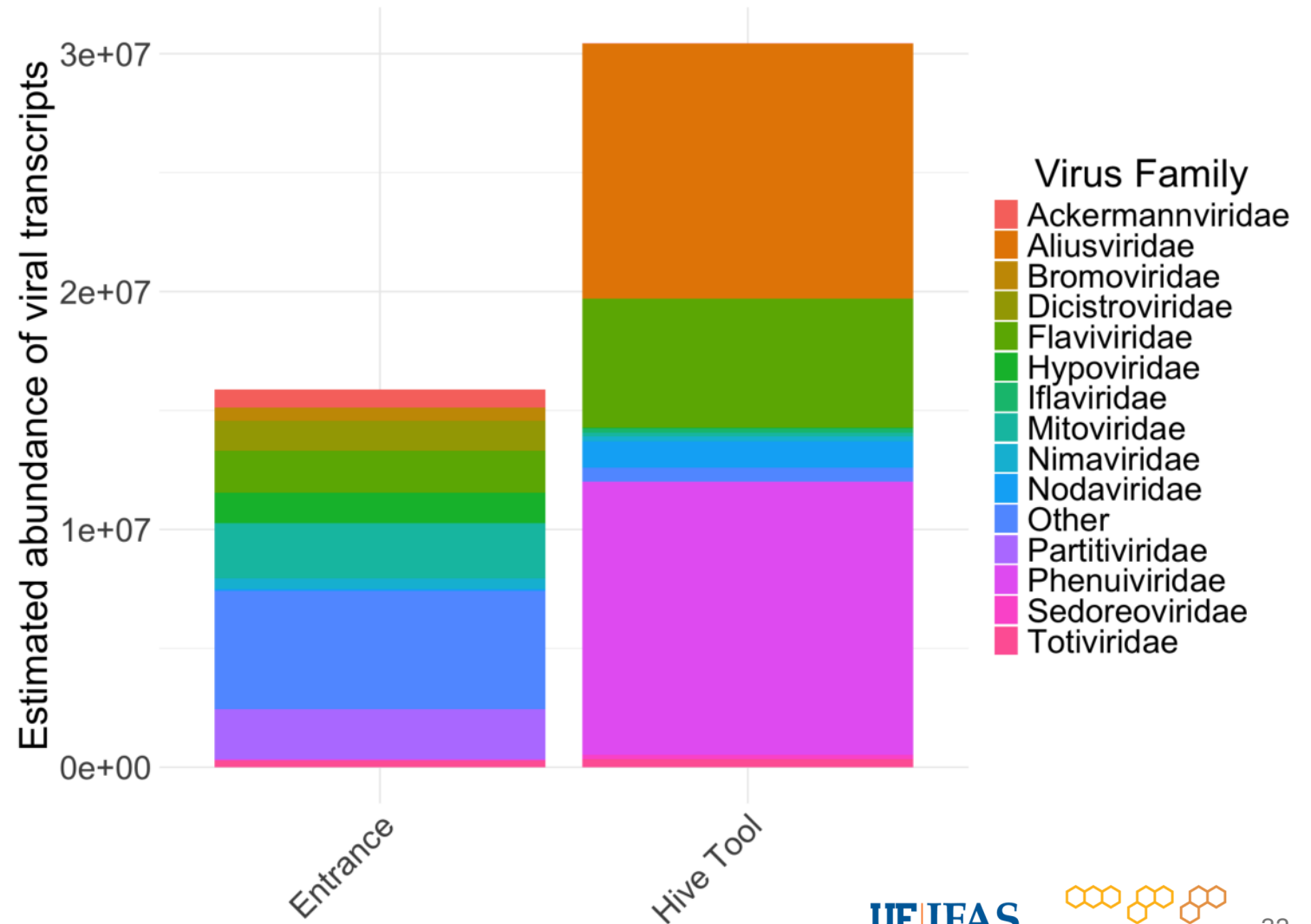
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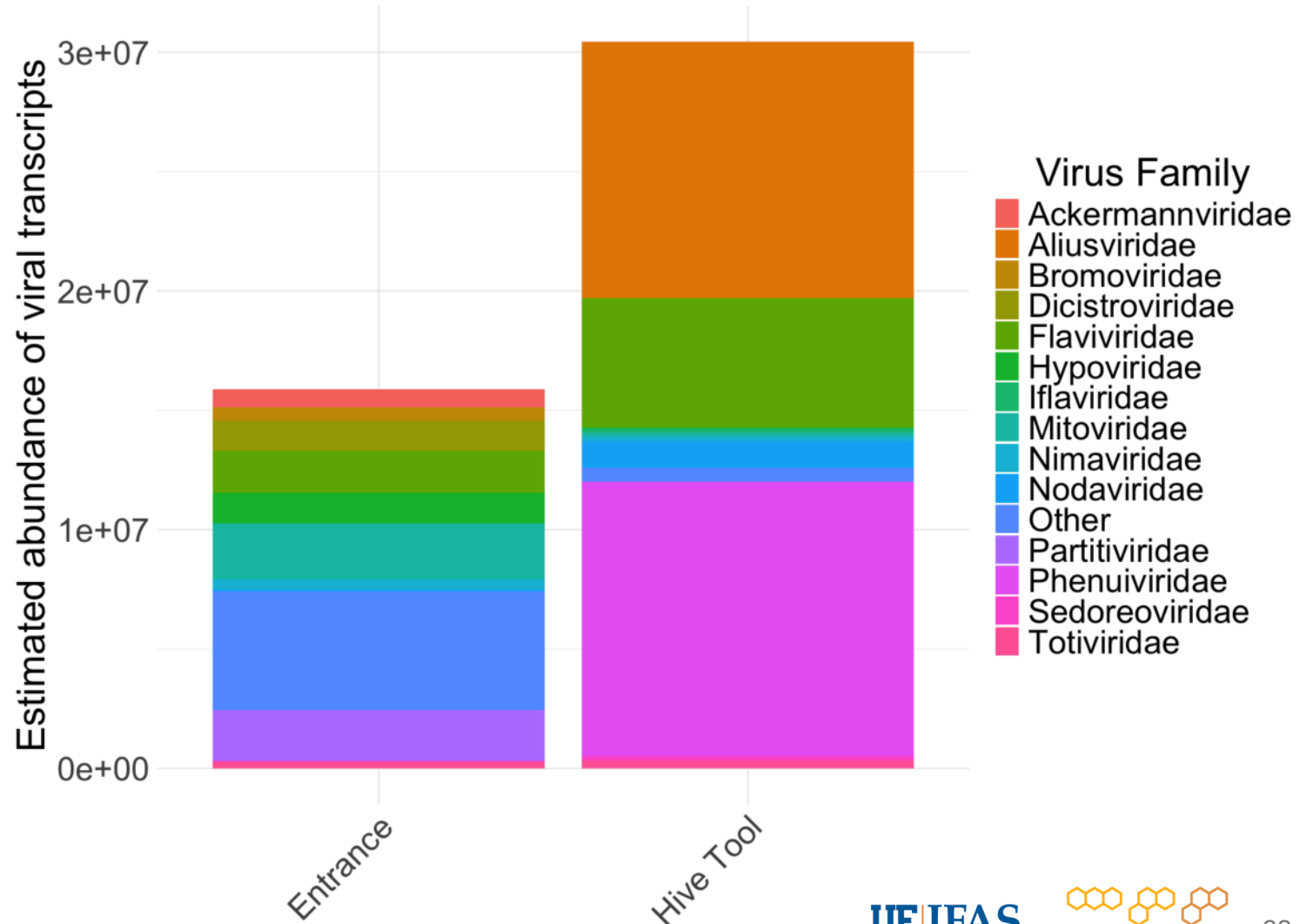
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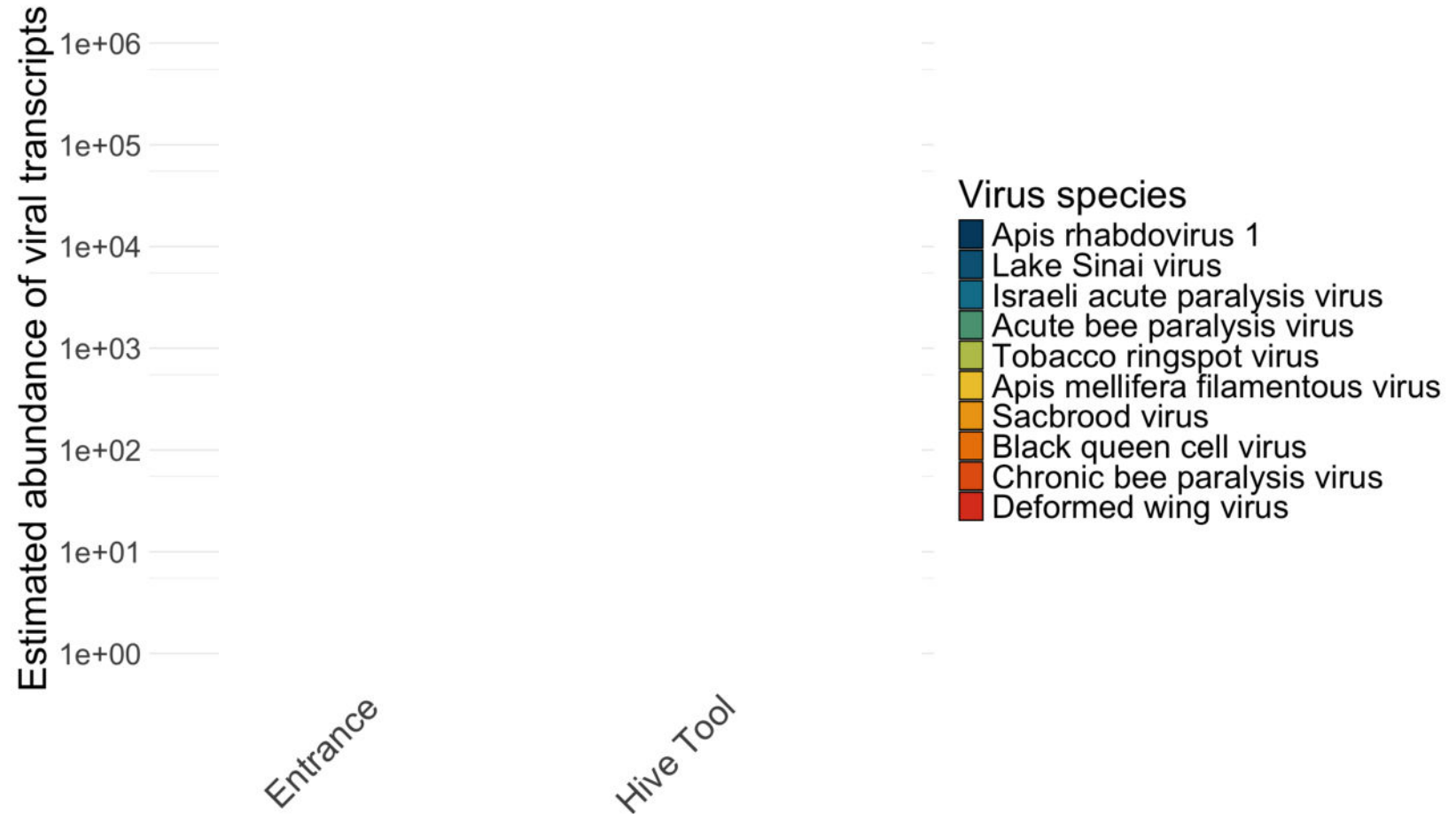
*Can next generation sequencing characterize full virus community using eRNA?*

- Hive tool harbors more virus eRNA than hive entrance.
- Many different viruses present in each sample – more than just bee viruses.



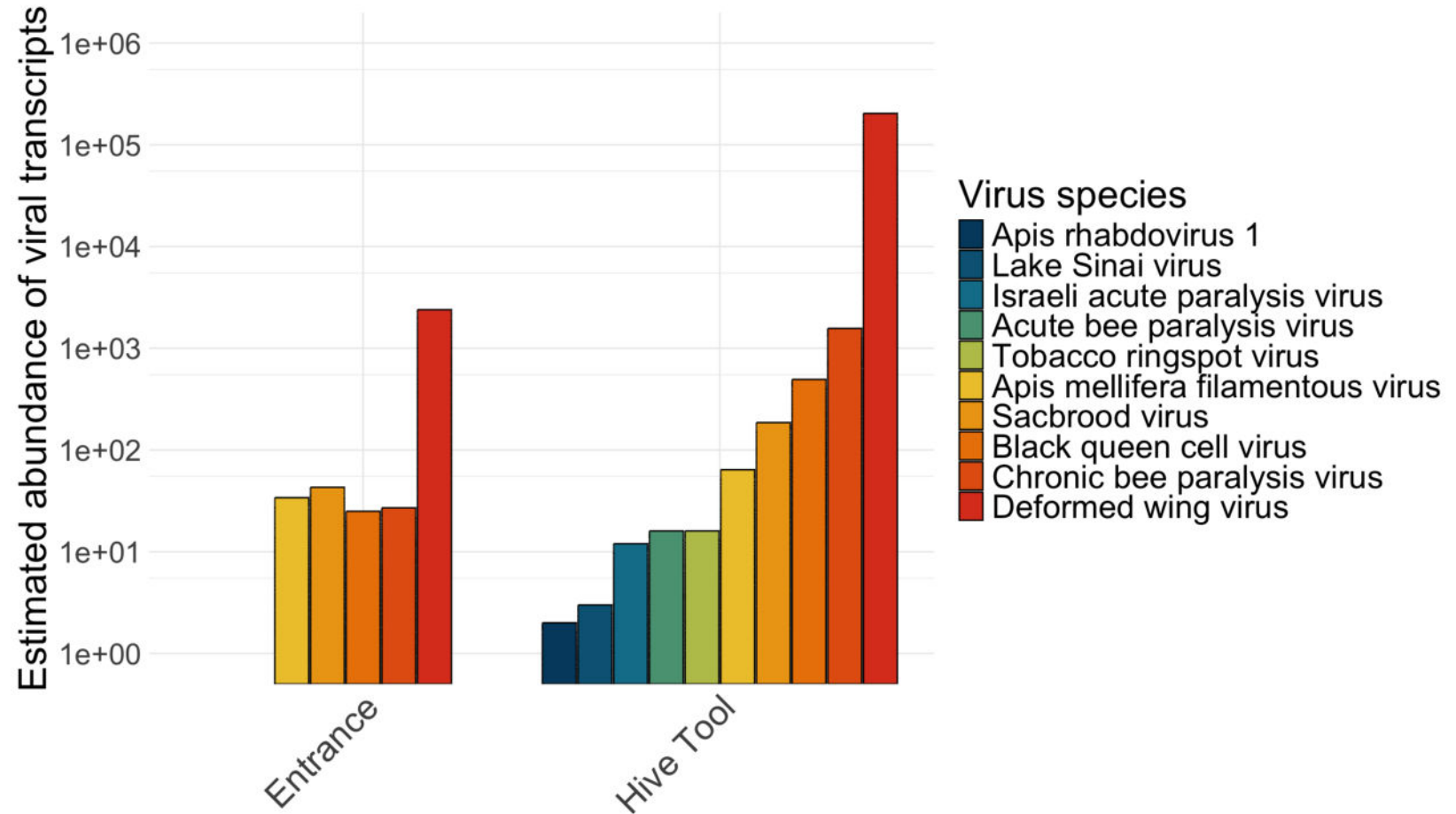
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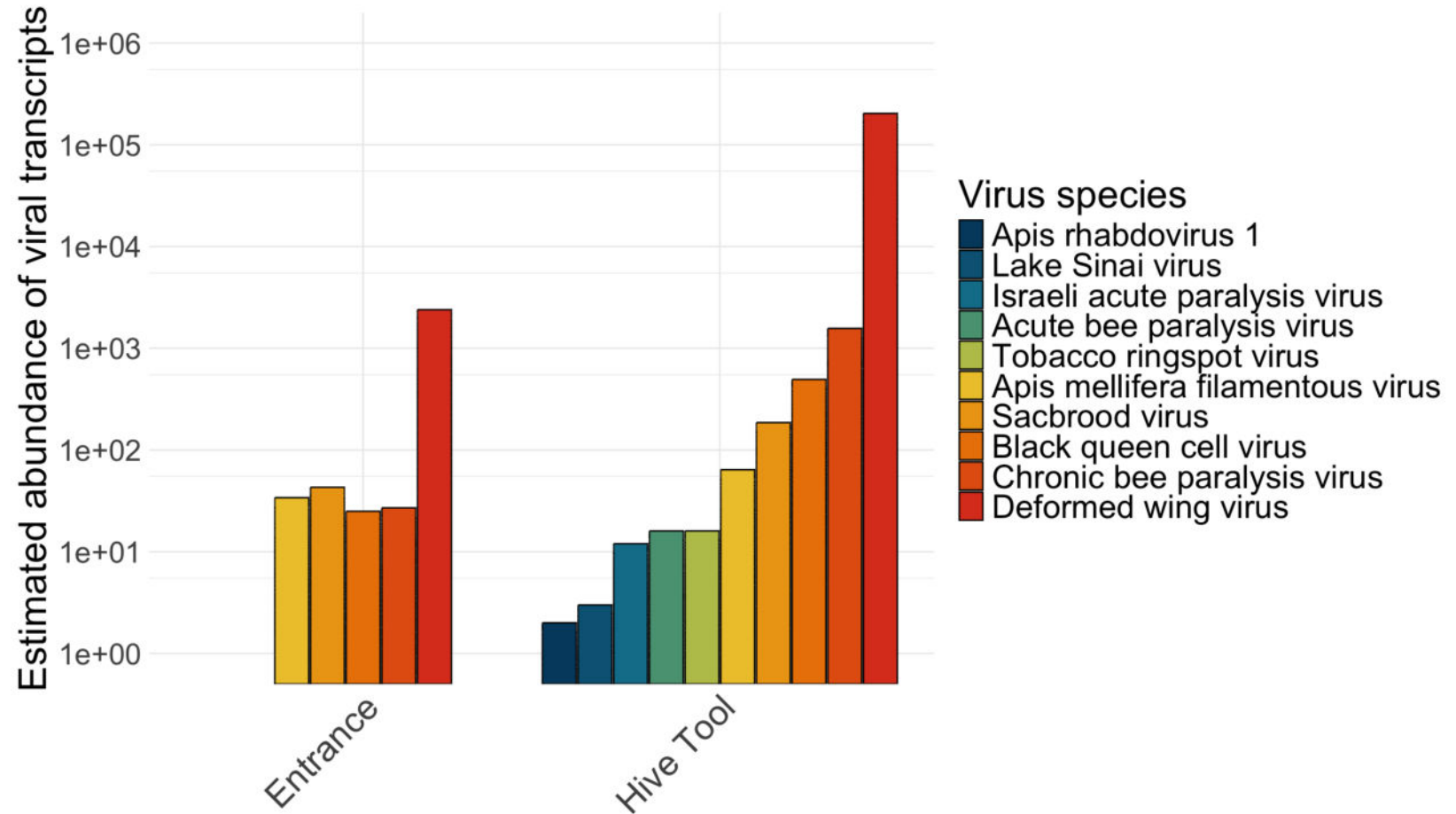
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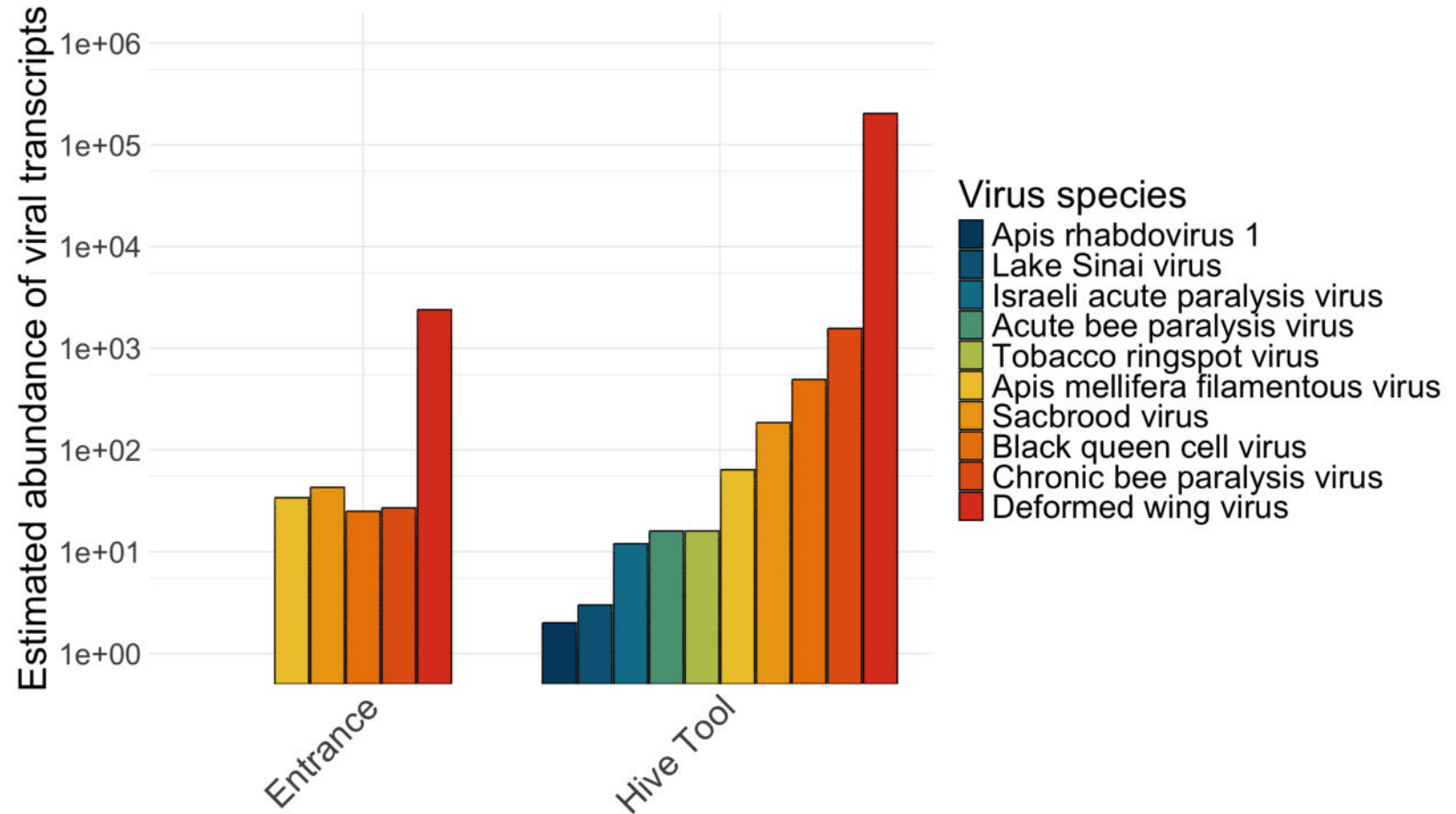
- Many viruses detected not commonly included in conventional viral screening.



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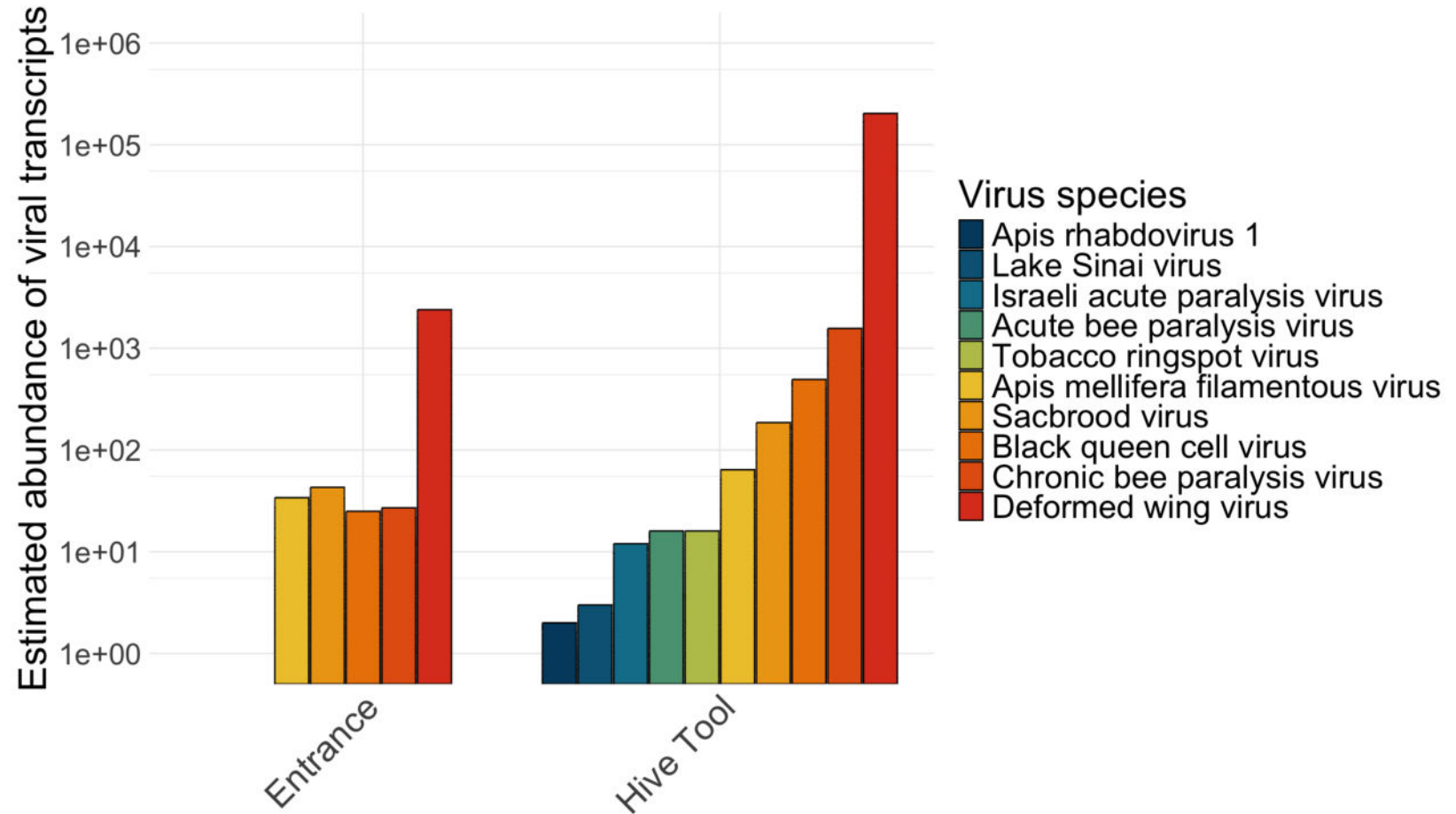
- Many viruses detected not commonly included in conventional viral screening.
- We detected RNA from the DNA-based virus ***Apis mellifera* filamentous virus**.



## Q2 Results:

*Can next generation sequencing characterize full virus community using eRNA?*

- Many viruses detected not commonly included in conventional viral screening.
- *Is there potential to screen for other DNA based pests and pathogens using RNA (arthropods, bacteria, fungi)?*





**Research Question:** Can we develop eRNA tools to monitor honey bee viruses?

# Key Take-Aways

- 1) We frequently detect many honey bee viruses on eRNA swab samples, offering an alternative to destructive sampling of bees.
- 2) Untargeted, NGS approaches to virus analyses enhance our detection of uncommon, new, and/or emerging viruses.
- 3) Some places in the hive are more informative than others for reliably detecting viruses.

# Key Take-Aways

- 1) We frequently detect many honey bee viruses on eRNA swab samples, offering an alternative to destructive sampling of bees.
- 2) Untargeted, NGS approaches to virus analyses enhance our detection of uncommon, new, and/or emerging viruses.
- 3) Some places in the hive are more informative than others for reliably detecting viruses.

**These results demonstrate the untapped potential of eRNA as a tool for expansive viral monitoring in honey bee colonies.**

# Acknowledgements

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United States Department of Agriculture  
National Institute of Food and Agriculture

## Special thanks to:

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Packard, Chris Oster, and  
Devan Rawn).



# Thank You!

## Questions?

## Contact

Email: [krdeutsch@ufl.edu](mailto:krdeutsch@ufl.edu)



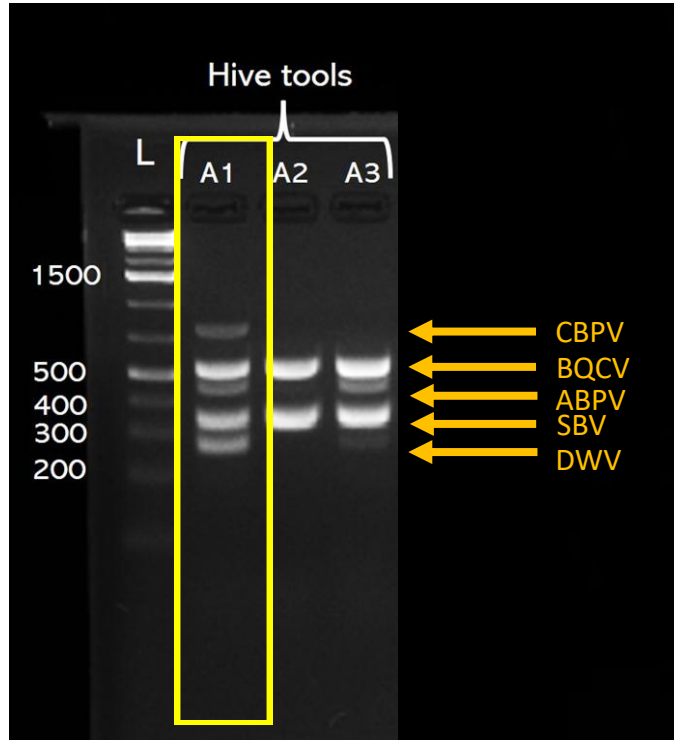
**HONEY BEE RESEARCH &  
EXTENSION LABORATORY**



## Q2 Methods:

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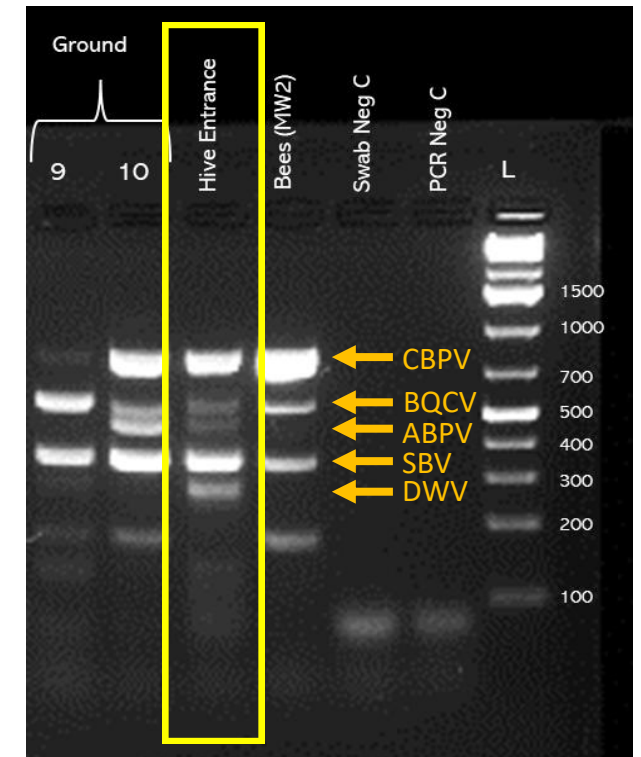
### 1) Hive tool swab (HVTL)



We selected two pilot eRNA samples that were positive for 5 viruses simultaneously:

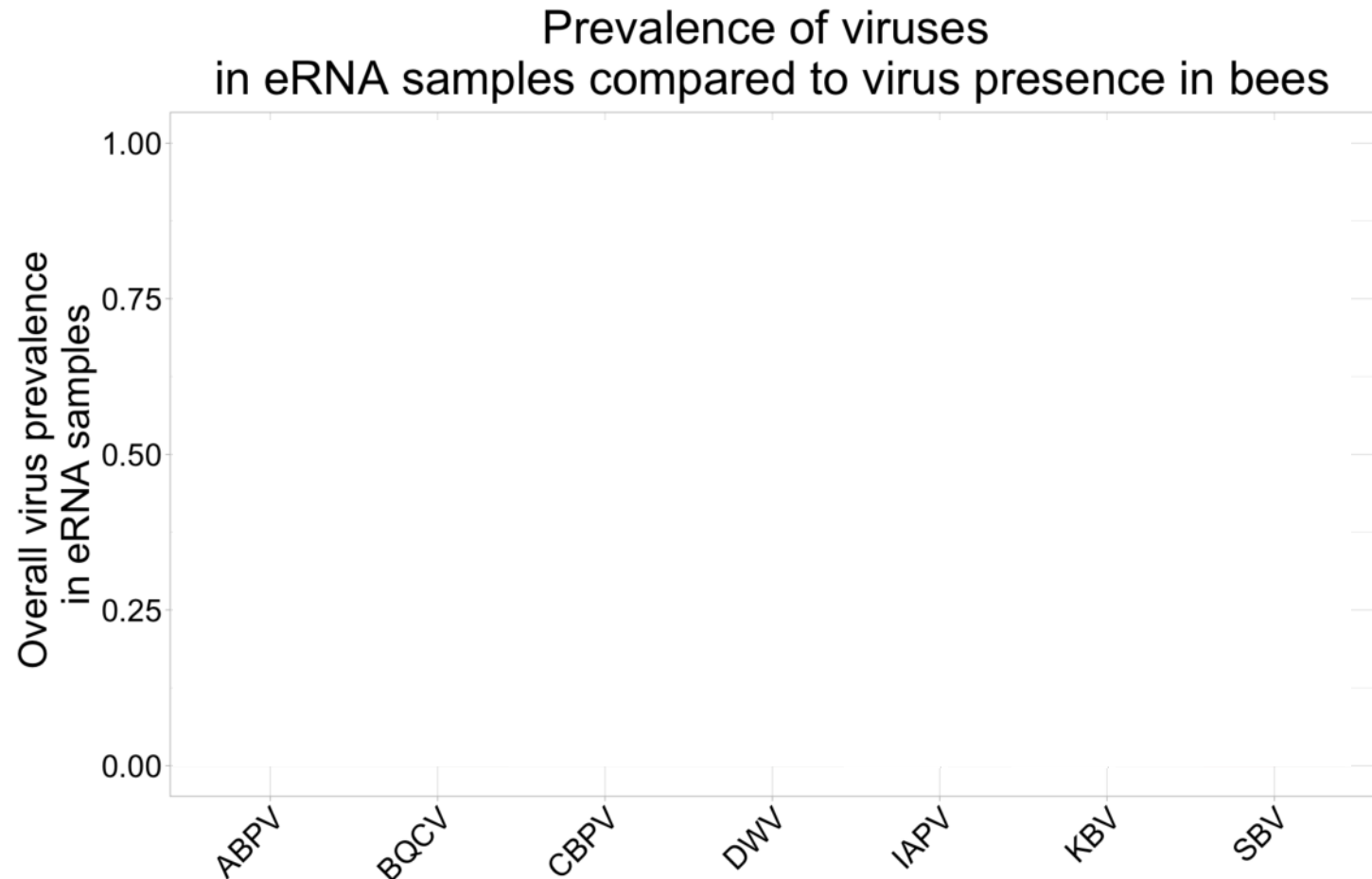
**CBPV, BQCV, ABPV, SBV, and DWV**

### 2) Hive entrance swab



# Q1B Results:

*Do viral eRNA samples accurately reflect current viral infections in bees?*

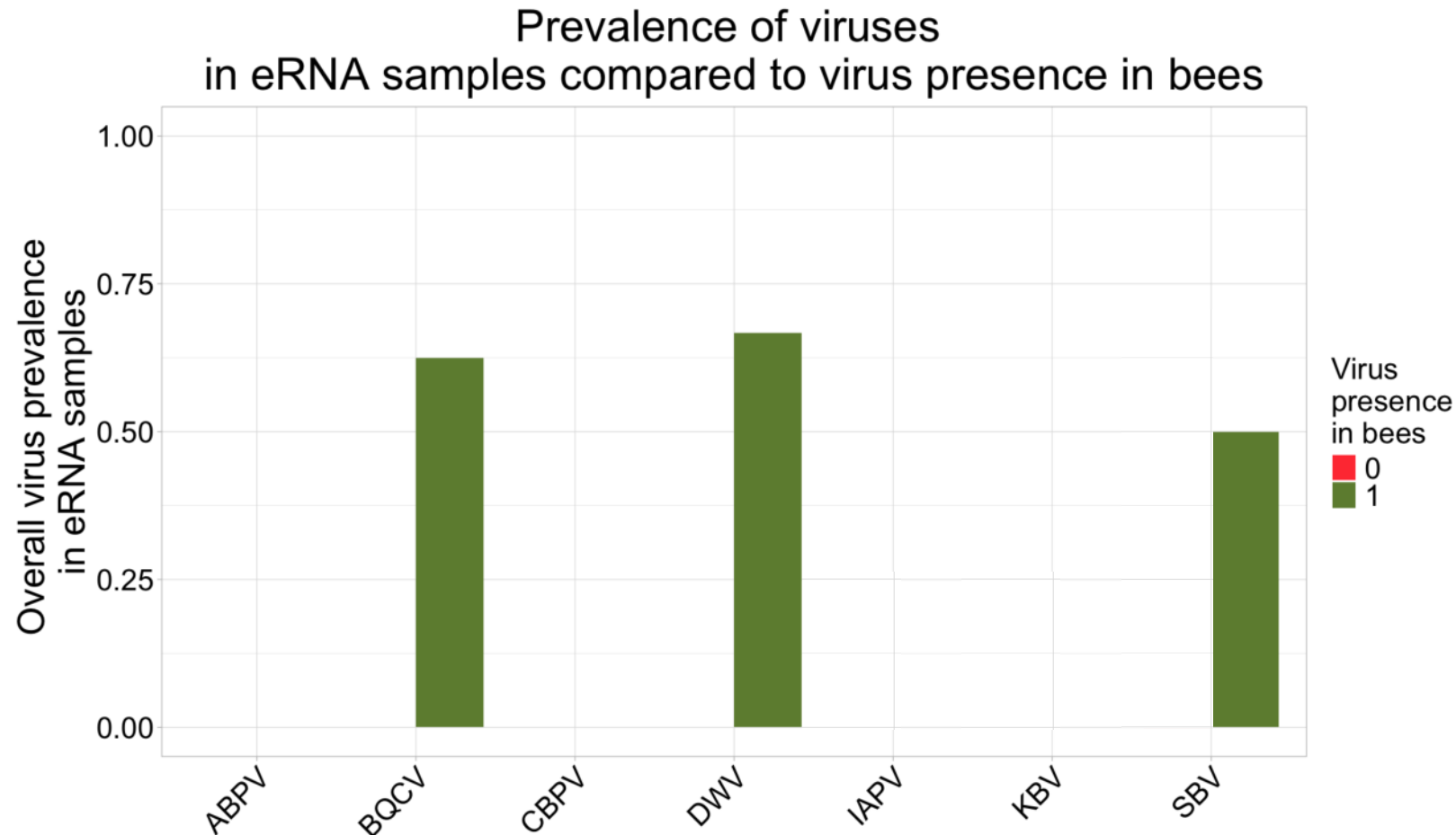


Virus  
presence  
in bees  
0  
1



## Q1B Results:

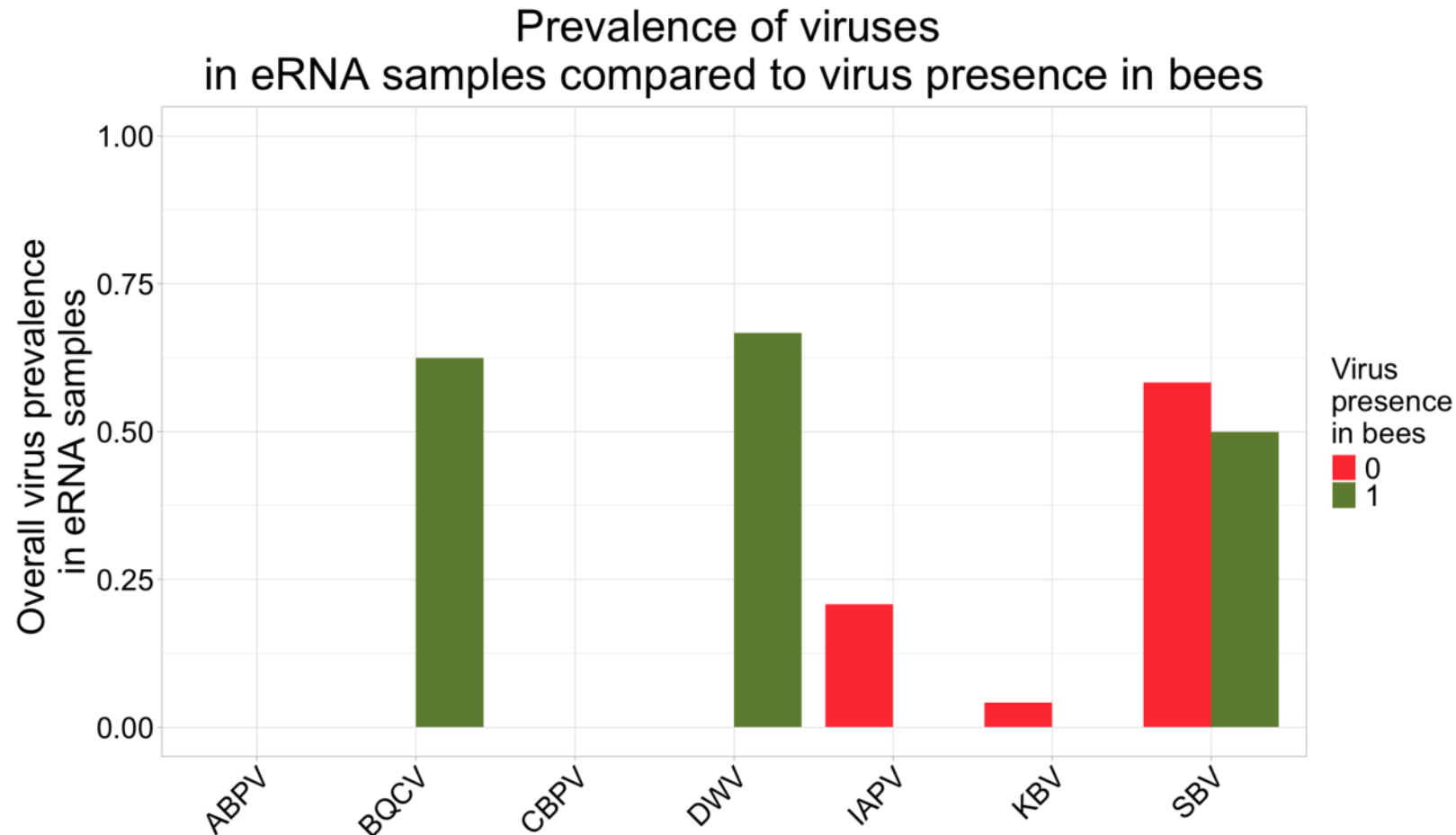
*Do viral eRNA samples accurately reflect current viral infections in bees?*



When viruses are present in honey bees, those viruses are also detected in 50-65% of eRNA samples.

## Q1B Results:

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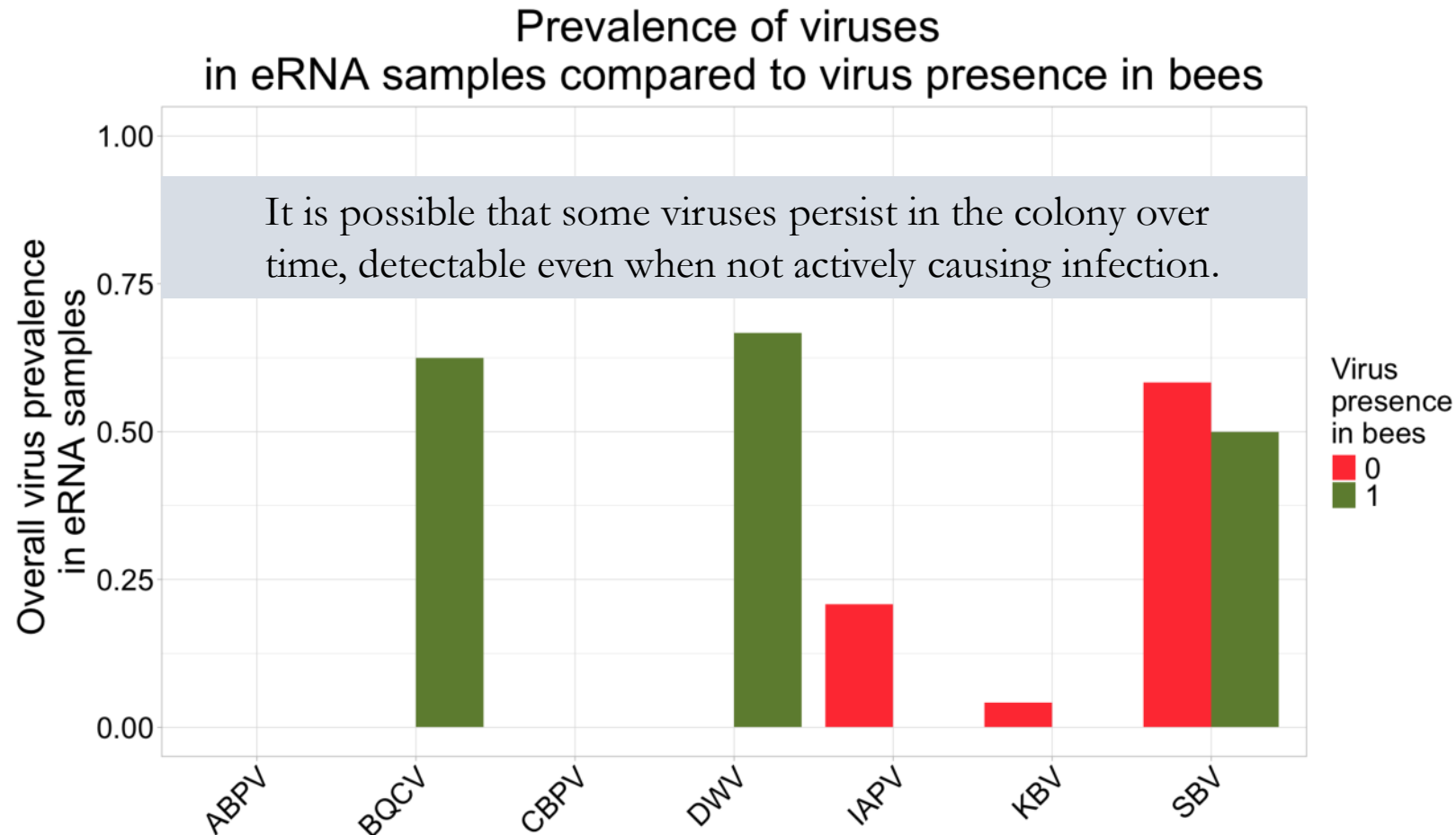


When viruses are present in honey bees, those viruses are also detected in 50-65% of eRNA samples.

However, some viruses are present in eRNA samples but are NOT detected in bees collected from the same colony.

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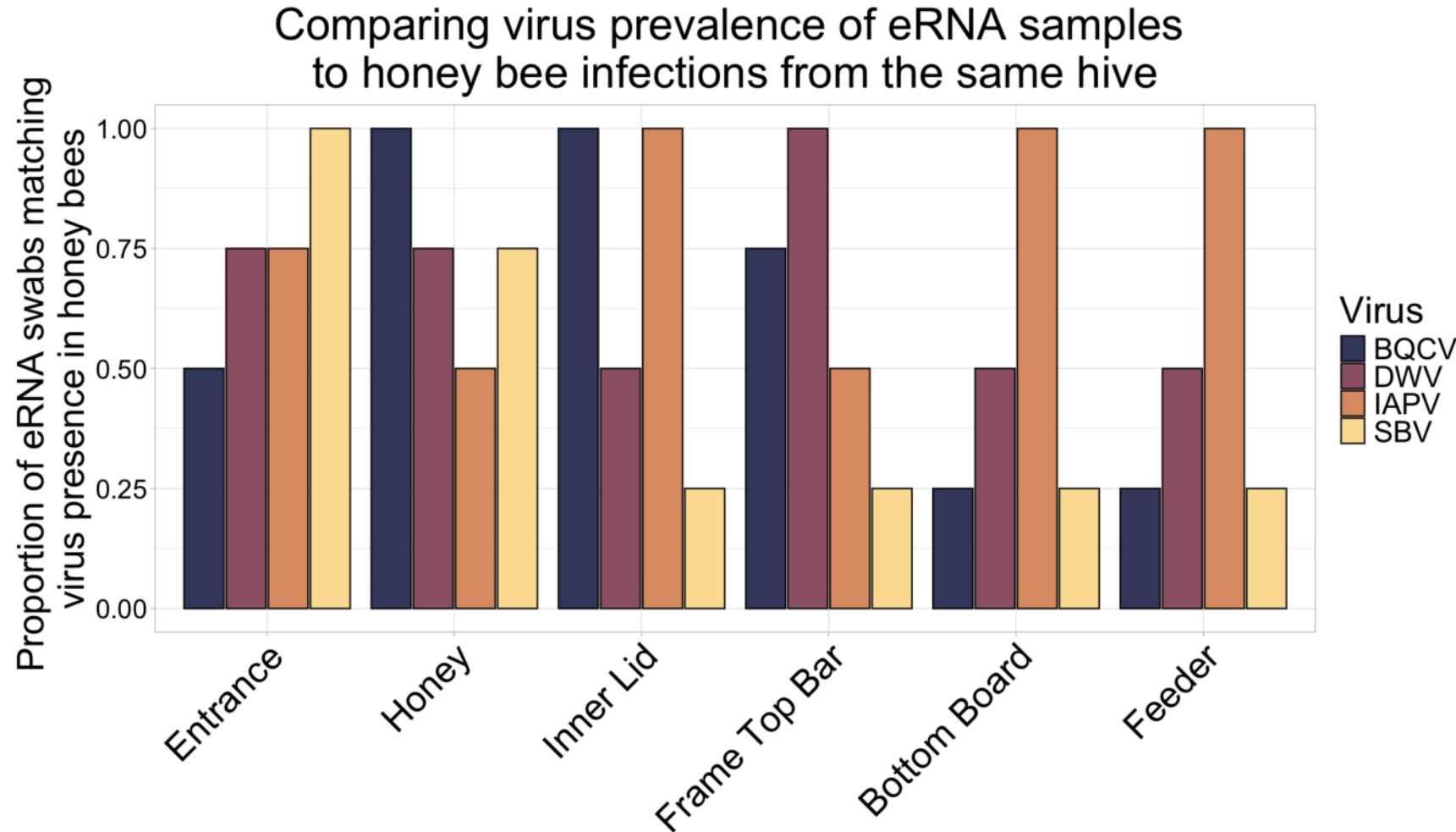


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However, some viruses are present in eRNA samples but are NOT detected in bees collected from the same colony.

## Q2 Results:

*Do viral eRNA samples accurately reflect current viral infections in bees?*



Hive substrates varied in accuracy in reflecting honey bee virus trends.

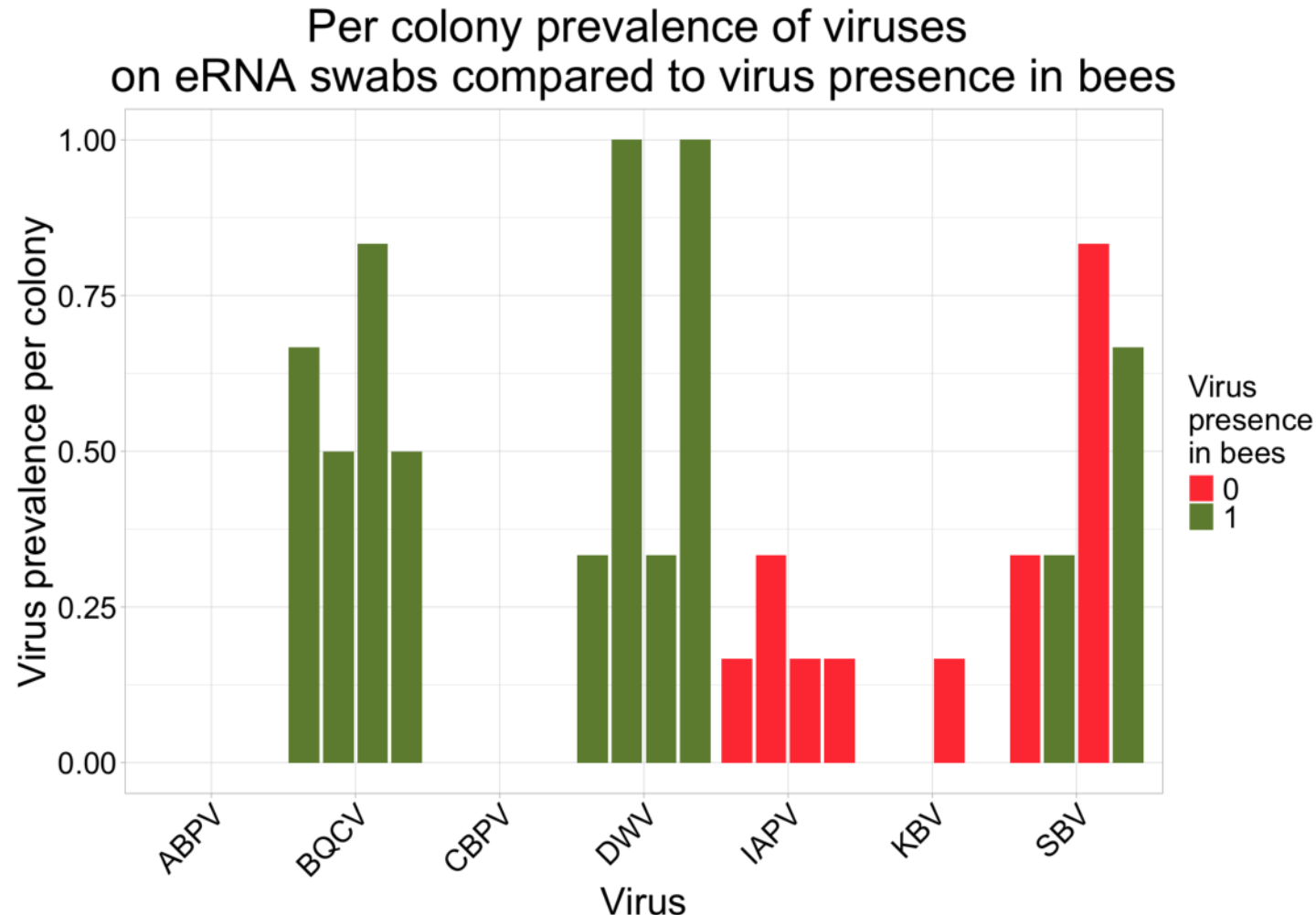
## Q1B Conclusions:

*Do viral eRNA samples accurately reflect current viral infections in bees?*

- For common viruses (DWV and BQCV), eRNA samples reliably reflected honey bee virus presence.
- For less common viruses (IAPV, KBV, and SBV), eRNA samples less reliably reflect virus presence in honey bees.

## Q2 Results:

*Do viral eRNA samples accurately reflect current viral infections in bees?*

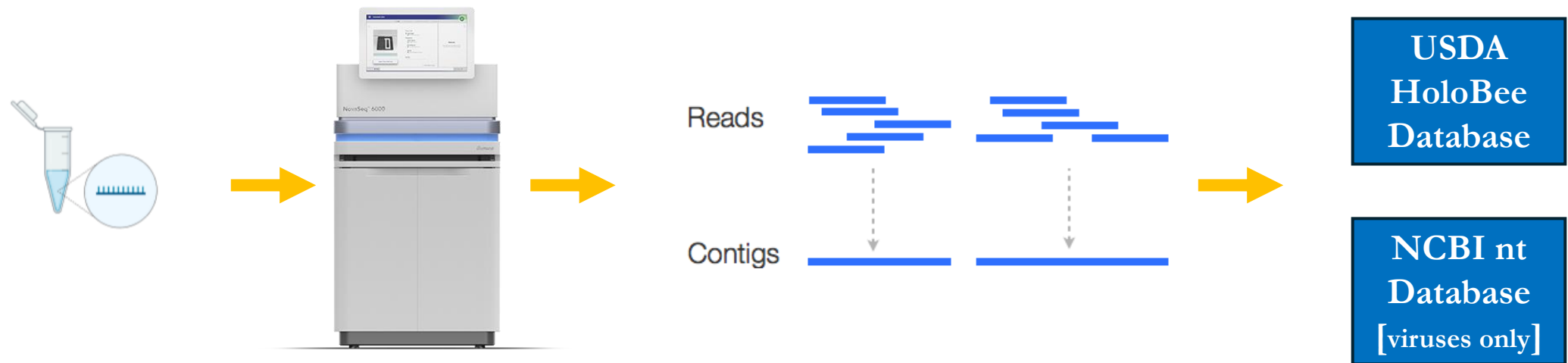


Some viruses are present in swab eRNA samples but NOT in the bees collected from that colony.

## Q2 Methods:

*Can next generation sequencing characterize full virus community using eRNA?*

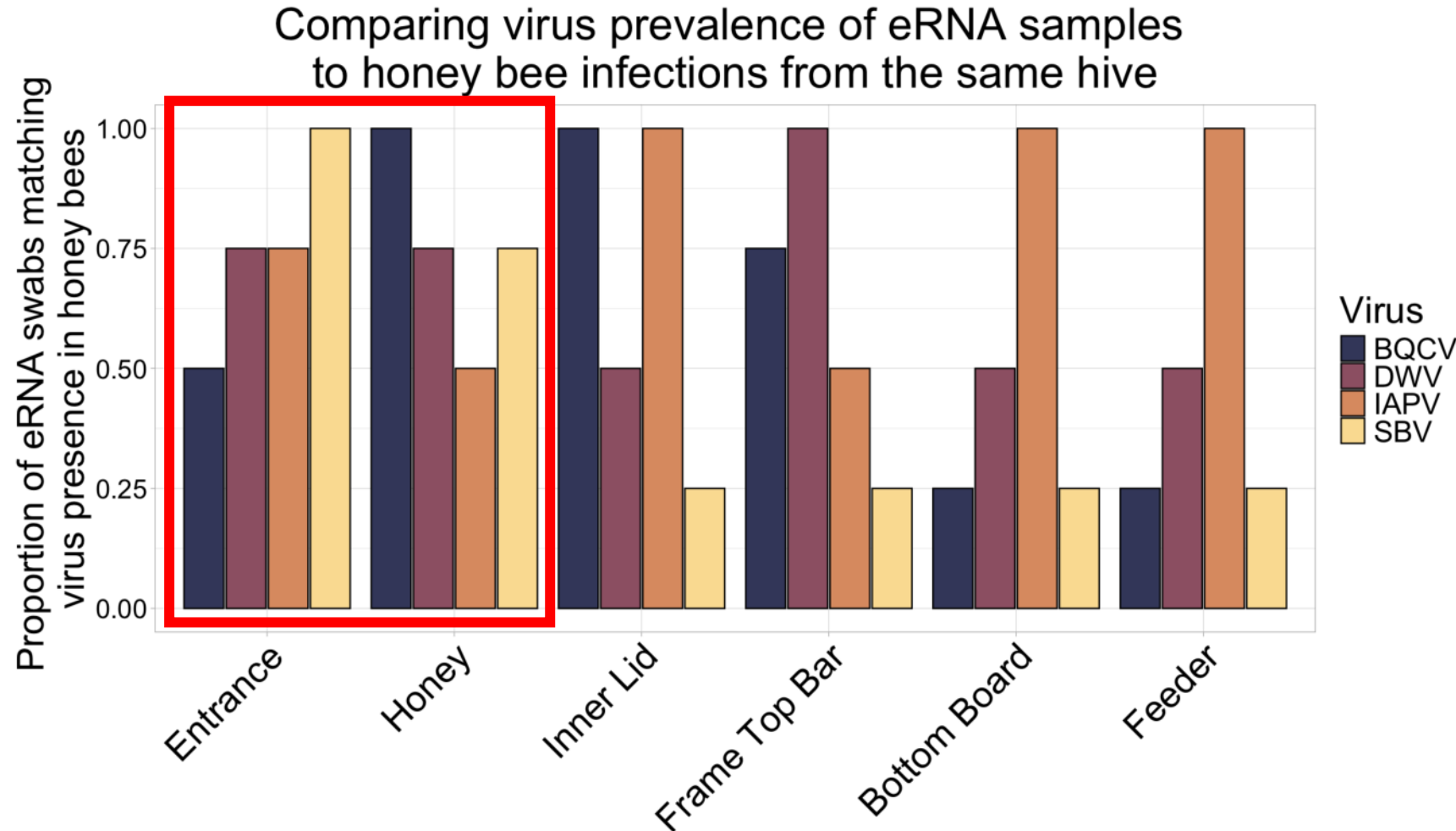
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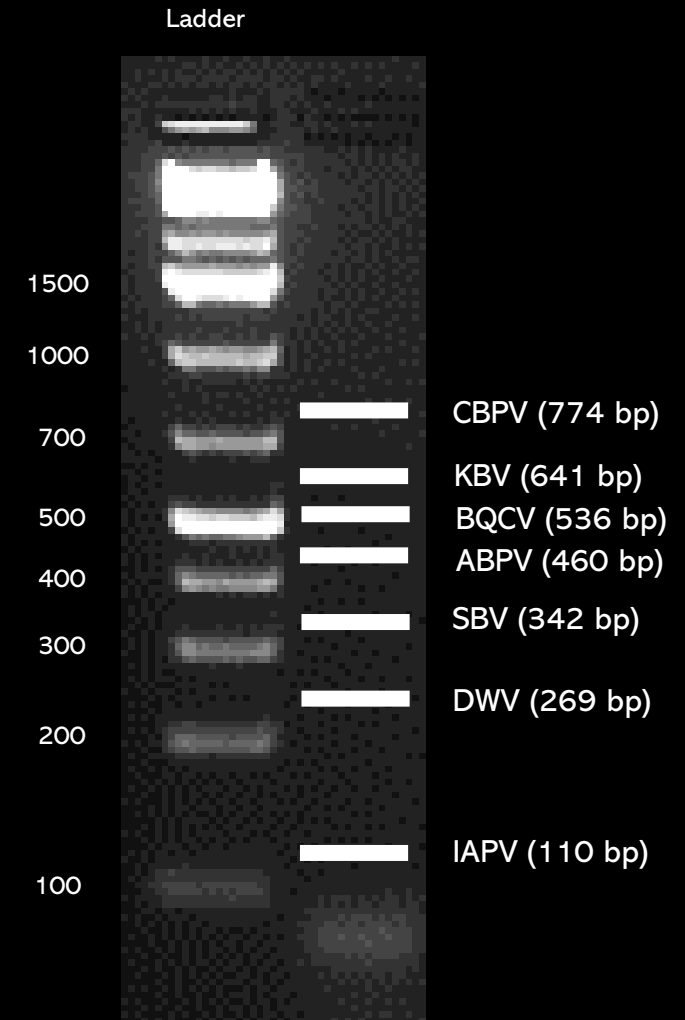


Hive substrates varied in accuracy in reflecting honey bee virus trends.

# Q1 Methods

*Can we detect viral RNA from hive environmental samples?*

- We looked for seven viruses using an established multiplex PCR:
  - Acute Bee Paralysis Virus (ABPV)
  - Black Queen Cell Virus (BQCV)
  - Chronic Bee Paralysis Virus (CBPV)
  - Deformed Wing Virus (DWV)
  - Israeli Acute Bee Paralysis Virus (IAPV)
  - Kashmir Bee Virus (KBV)
  - Sacbrood Virus (SBV)



# Q1 Results

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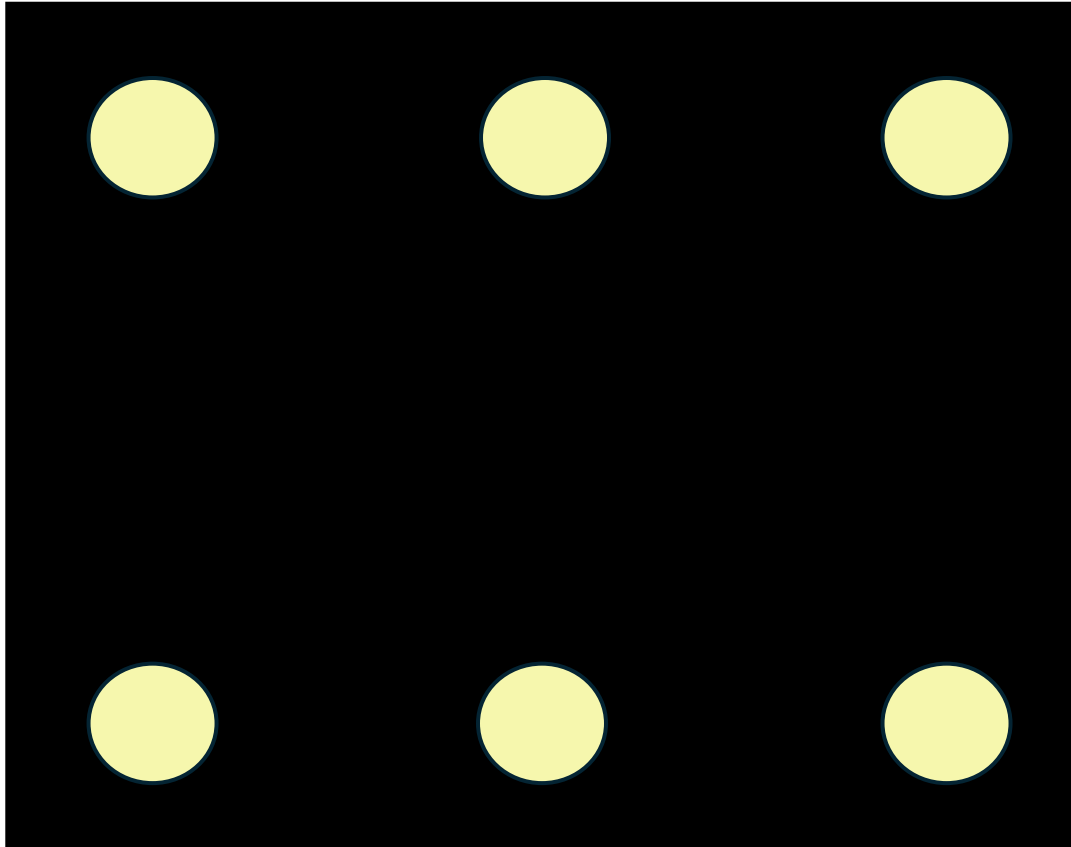


- We were able to find viruses everywhere we looked!
- eRNA samples were positive for up to five viruses simultaneously.

# Methods:

*Can we develop non-lethal eRNA tools for more expansive viral monitoring?*

Q1: Targeted analysis



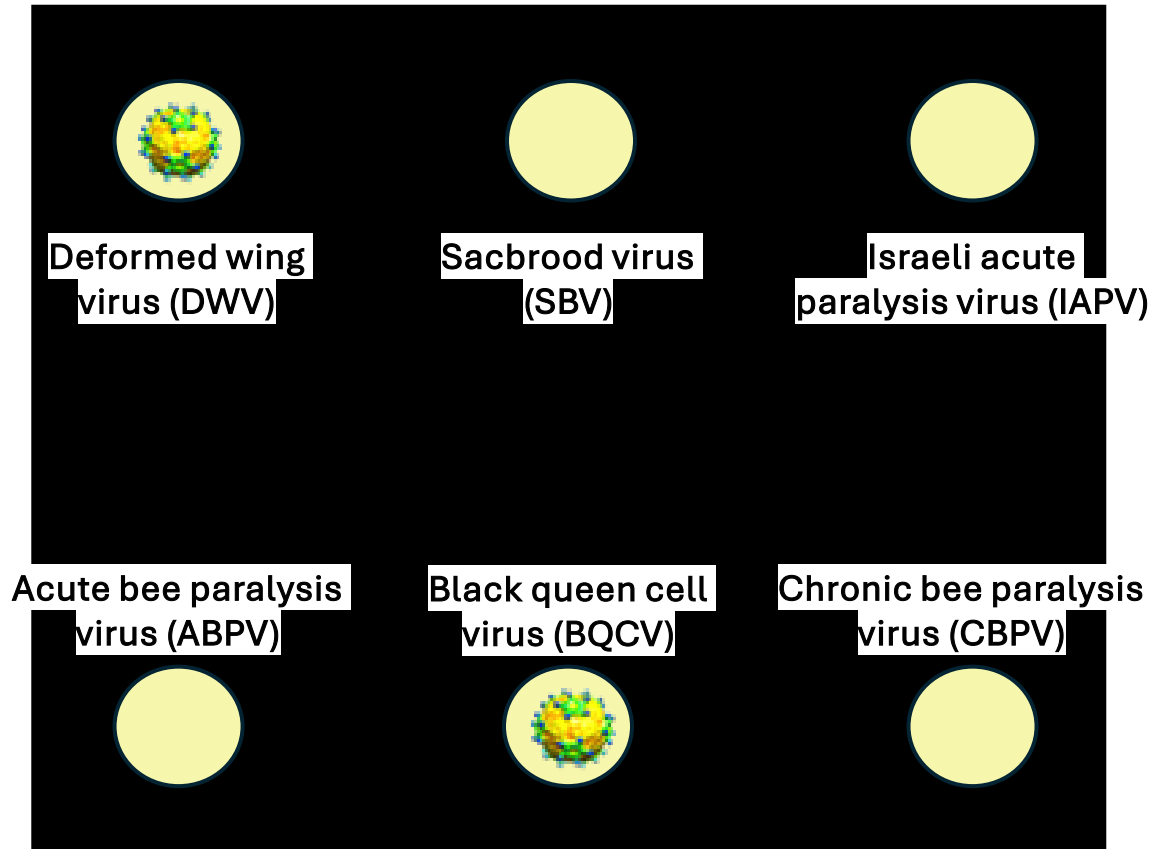
Q2: Untargeted analysis



# Methods:

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## Q1: Targeted analysis



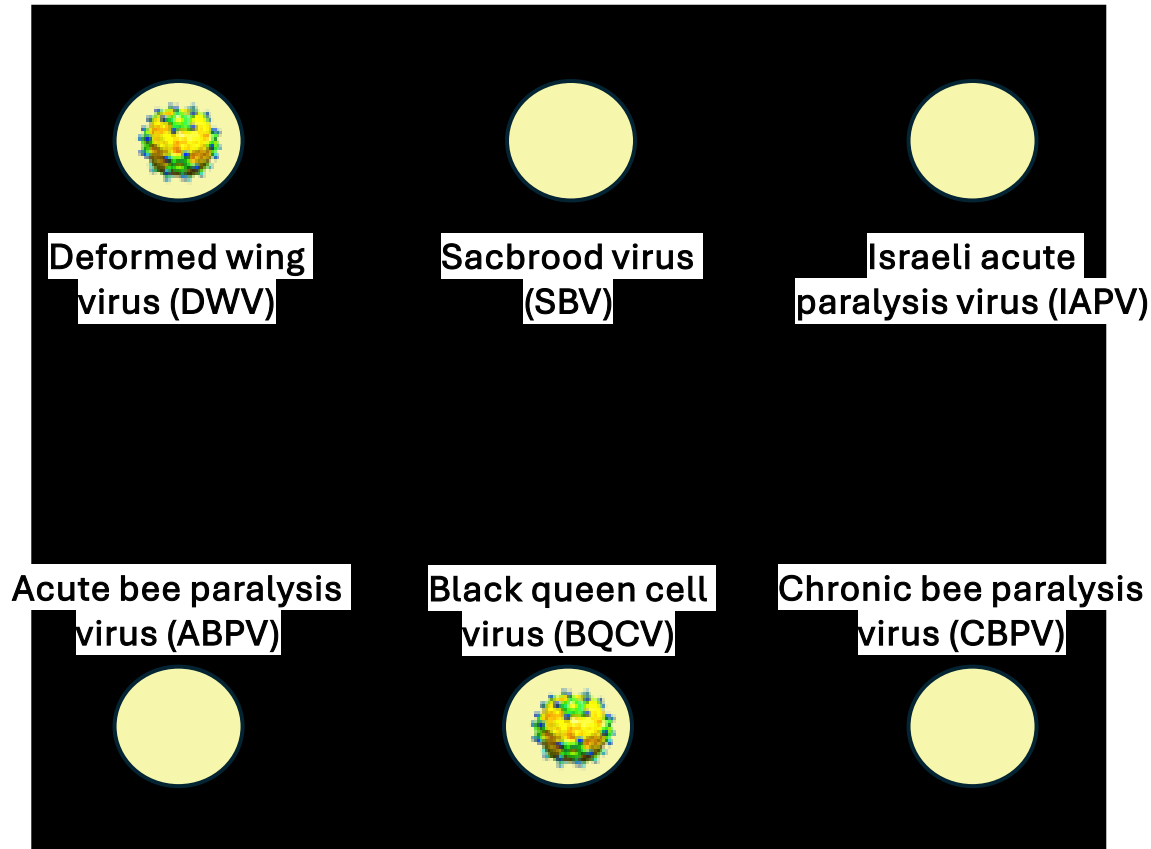
## Q2: Untargeted analysis



# Methods:

*Can we develop non-lethal eRNA tools for more expansive viral monitoring?*

## Q1: Targeted analysis



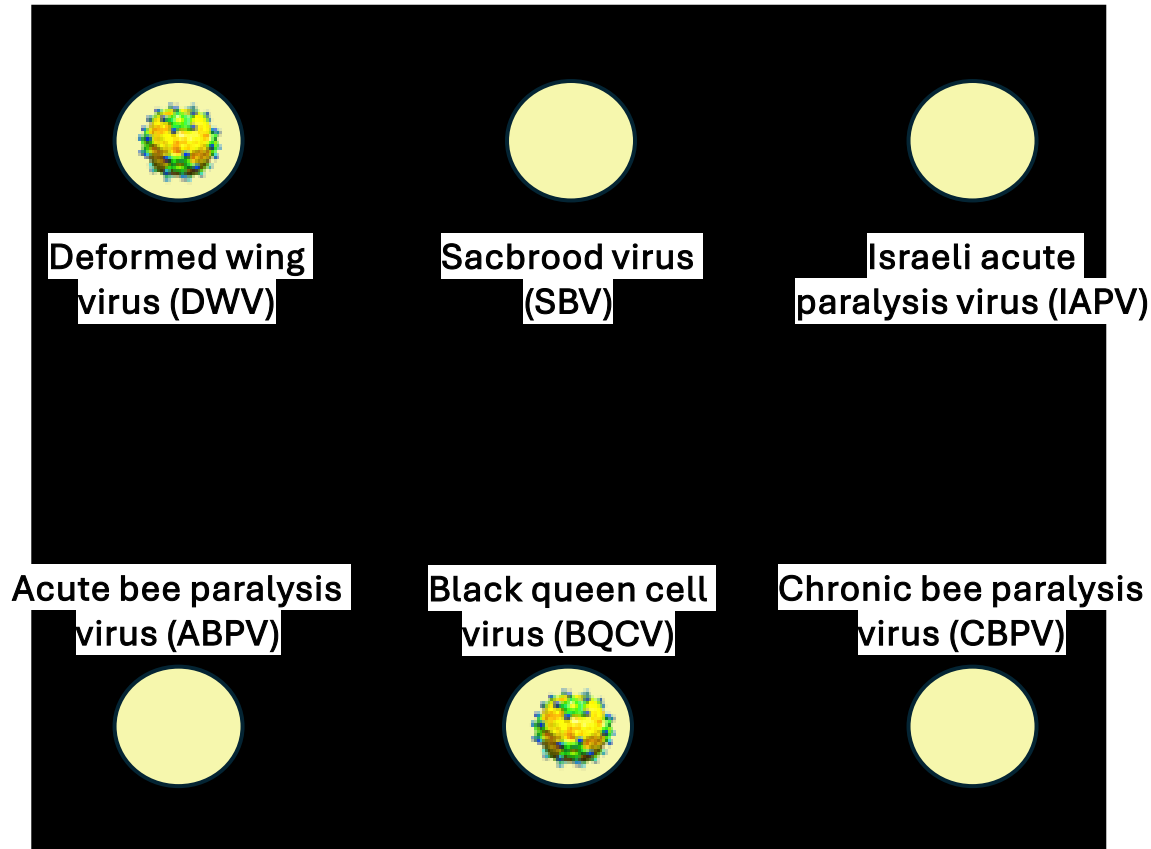
## Q2: Untargeted analysis



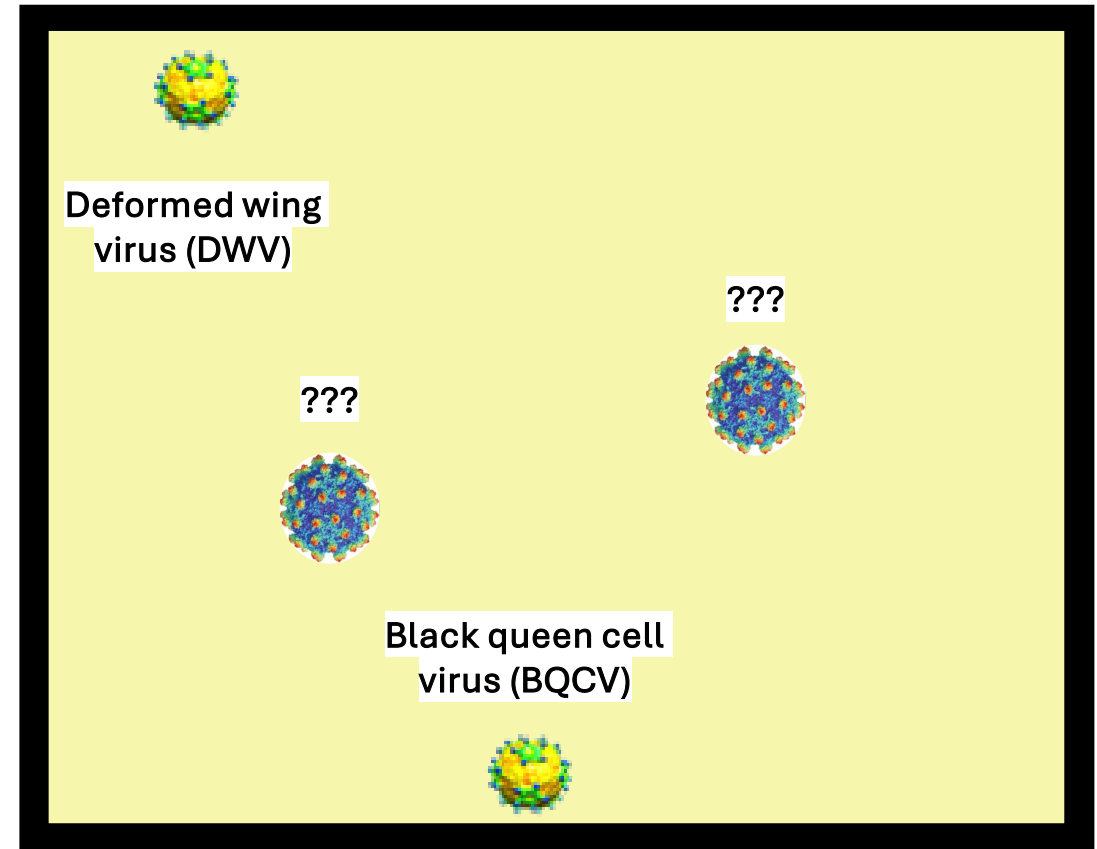
# Methods:

*Can we develop non-lethal eRNA tools for more expansive viral monitoring?*

## Q1: Targeted analysis



## Q2: Untargeted analysis



## Q2 Conclusions:

*Can next generation sequencing characterize full virus community using eRNA?*

- Next generation sequencing results from eRNA samples detected both viruses present in conventional PCRs as well as less common viruses.
- Our ability to accurately characterize the full virus community using NGS techniques is greatly enhanced by removal of ribosomal RNA from DNA-based organisms.
- We should consider the type of hive substrates that will give us the most informative results on both known and new, emerging viruses!

## Q2 Results:

*Can next generation sequencing characterize full virus community using eRNA?*

- Again, ribodepletion increased the number of detectable bee viruses from both samples.
  - Entrance: 2 → 5 viruses
  - Hive tool: 7 → 10 viruses
- The hive tool had a greater diversity of viruses – likely because it is being used in many hives.

