OP-170 [Bee Health]
Pros and Cons of Diagnostic Tests Used for Early Detection and Risk Assessment of American Foulbrood in Small and Large Beekeeping Operations

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American foulbrood (AFB), caused by Paenibacillus larvae, is a devastating contagious disease that is often controlled by antimicrobials in North America. Destruction of affected colonies is mandatory in many jurisdictions. Therefore, early detection of AFB is crucial. However, the efficacy of diagnostic tests and risk assessment strategies for a beekeeping operation is influenced by sample types, timing of sample collection and size of operation.

We conducted multiple clinical investigations of AFB outbreaks and assessed levels of P. larvae spores (hereafter, AFB spores) in various sample types using quantitative bacterial culture. The number of AFB spores in bees, bottom board debris, brood-chamber honey, and extracted honey was comparatively analyzed to evaluate their suitability for AFB risk assessment.

A series of case studies involving AFB outbreaks will be presented, and the validity and feasibility of various sample types for risk assessment will be discussed based on the results of our ongoing clinical investigations. In general, for both small and large beekeeping operations, the most feasible AFB risk assessment method is based on the number of AFB spores in extracted honey at the end of the honey harvesting season (late summer). Additionally, the number of AFB spores in nurse bees during the brood-rearing season, and potentially in bottom board debris during early spring, may also be useful for AFB risk assessment in small beekeeping operations. The number of AFB spores in brood chamber honey could be used for surveillance of colony exposure to AFB over time, similar to the use of serology tests in “herd health" management.

The selection of an appropriate AFB risk assessment strategy depends on the size of the beekeeping operation, the season, and the availability and accuracy of diagnostic thresholds. Future research should focus on refining these diagnostic thresholds to enhance evidence-based AFB control, ultimately reducing reliance on antimicrobials and mitigating the development of antimicrobial resistance.

UNIVERSITY OF SASKATCHEWAN

Western College of Veterinary Medicine

Pros and Cons of AFB Tests

for early detection and risk assessment of AFB in small and large beekeeping operations

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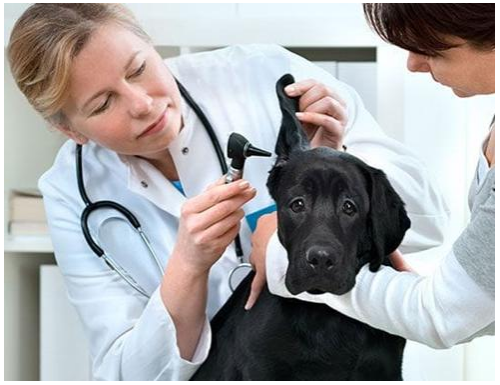
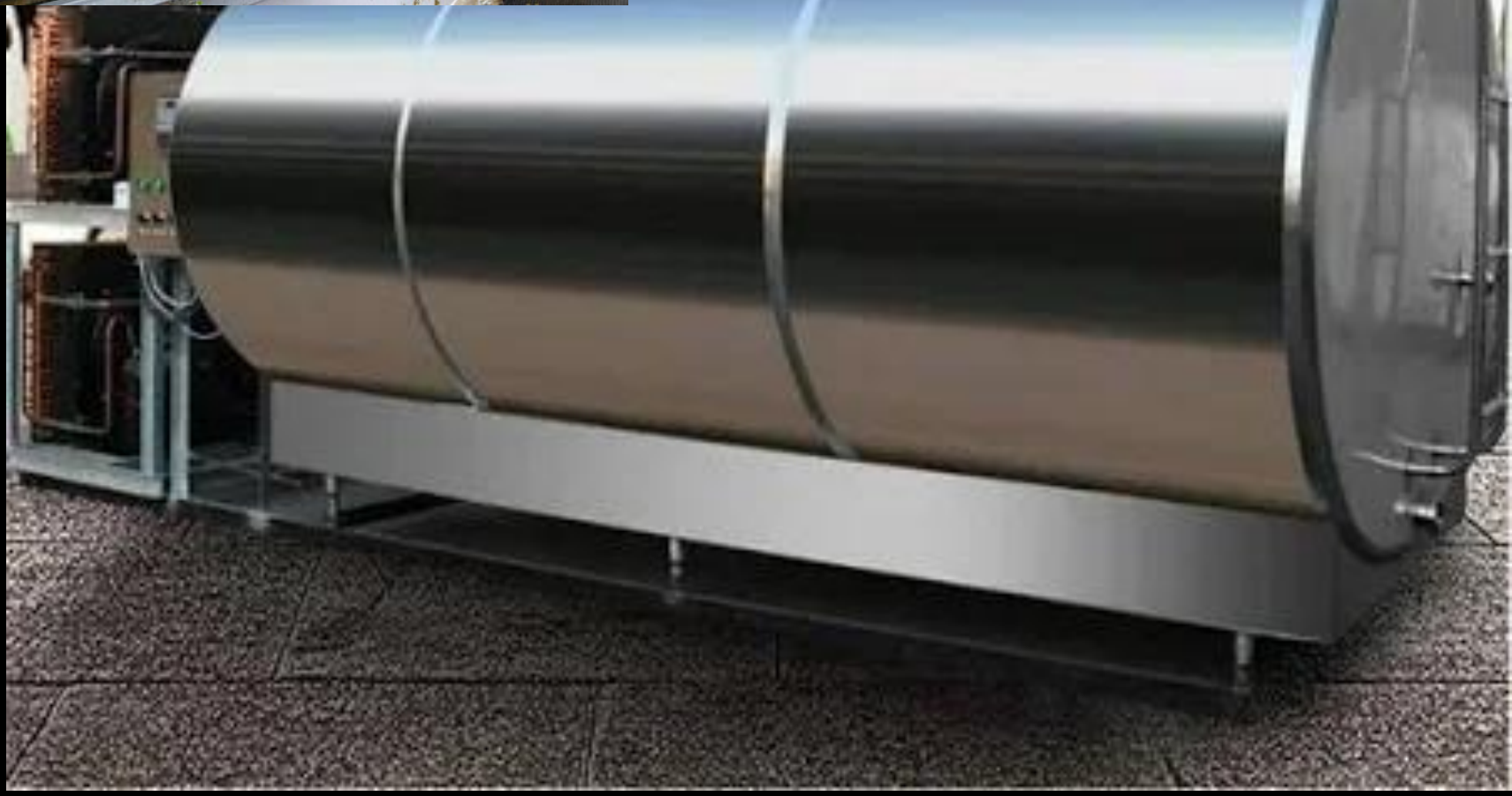
WCVM Honey Bee Health Research Lab

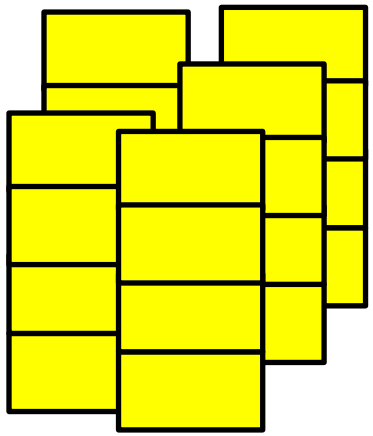
2024 WCVM Honey Bee Health Lab

Paradigm shift in AFB risk assessment: from “pet health” to “herd health”

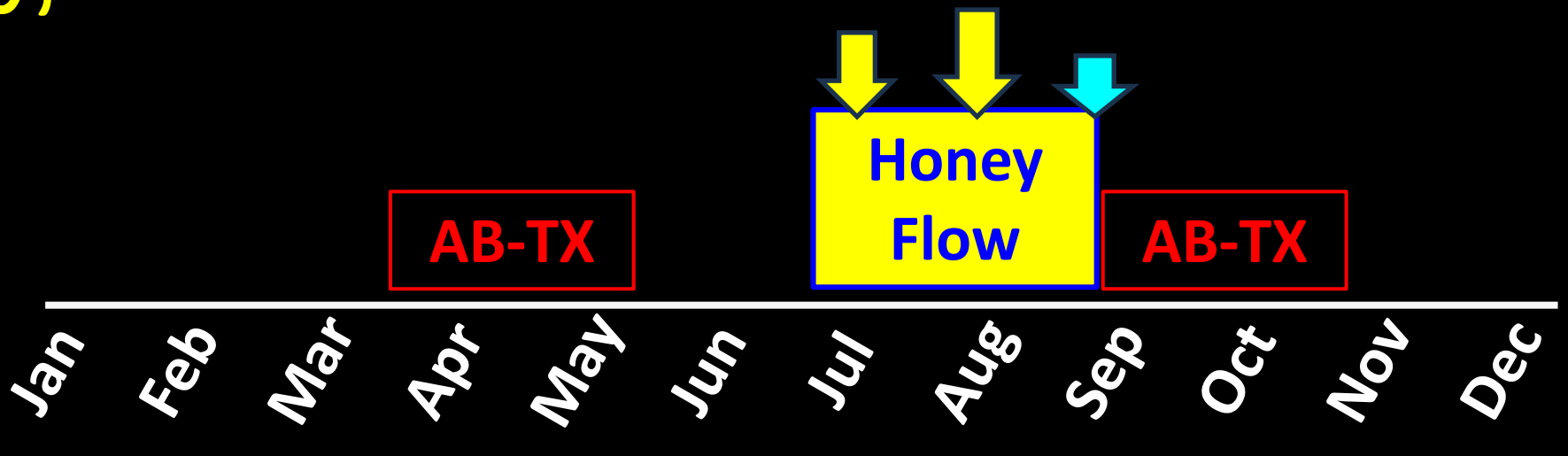


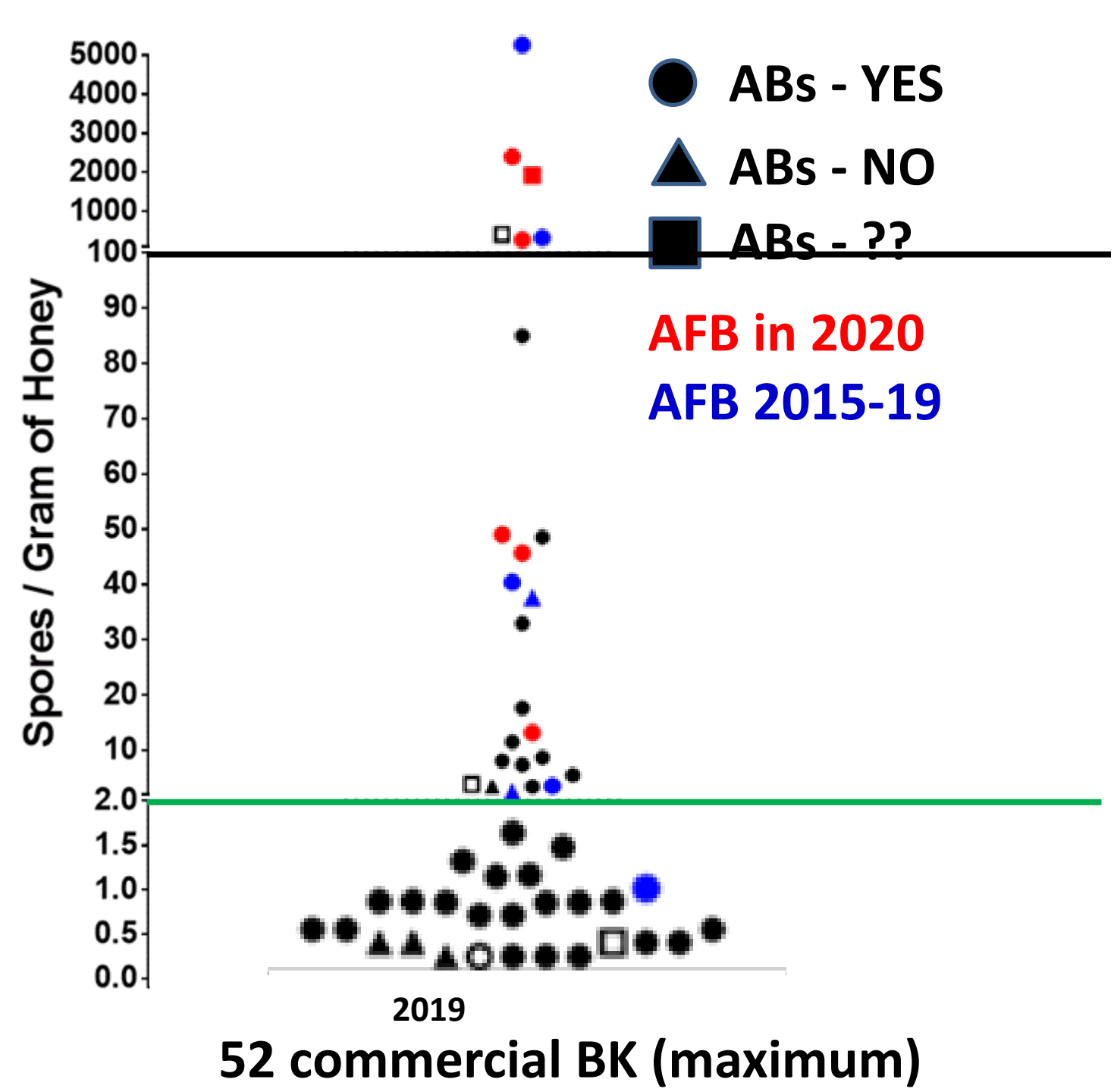


Mixing and sedimentation of honey



Sampling of honey




ABs - YES
ABs - NO
ABs - ??
AFB in 2020
AFB 2015-19
Spores / Gram of Honey
5000
4000
3000
2000
1000
100
90
80
70
60
50
40
30
20
10
2.0
1.5
1.0
0.5
0.0
2019
52 commercial BK (maximum)

Please, meet the following beekeeper

Ben

500
hives

American foulbrood spores and antimicrobial susceptibility test results

Ben's Honey
benhoney@gmail.com
306-883-2010 (Terry)
POBox 0001, Saskatchewan, Canada

Sample	Barrel/Lot Number	Year	Spores/g of honey	AST result
1	1	2023	6200	**S**
2	2	2023	5300	**S**

Antimicrobial susceptibility of isolates was determined by Minimum Inhibitory Concentration (MIC) using broth microdilution technique according to CLSI Vet 06 1 Ed. "Antimicrobial susceptibility testing for fastidious bacteria" 2019.
MIC is the minimal concentration of antibiotic that inhibit growth of bacteria.
There are two possible outcomes of these results:
S -- Susceptible: a microorganism is inhibited by oxytetracycline, tylosin and lincomycin.
R -- Resistant: a microorganism is not inhibited by oxytetracycline, tylosin or lincomycin.

Interpretation of your results: 2 out of 2 honey samples from your operation were positive for AFB spores. Based on our previous studies (Zabrodski et. al. 202200), AFB risk thresholds of less than 2, 2-100 and more than 100 spores per gram of tested honey corresponds to low, medium and high risk of AFB outbreak, respectively. All of the positive samples were susceptible to oxytetracycline, tylosin and lincomycin.

If you have any question about these results please contact Dr. Elemir Simko (306) 966-7307 or e-mail: els920@mail.usask.ca. If you were not aware of AFB resistance in your operation we strongly suggest to contact Provincial Apiculturist or your veterinarian.

Please, meet the following beekeeper

Ben

500
hives

2023/24 -> 45%

What happened?

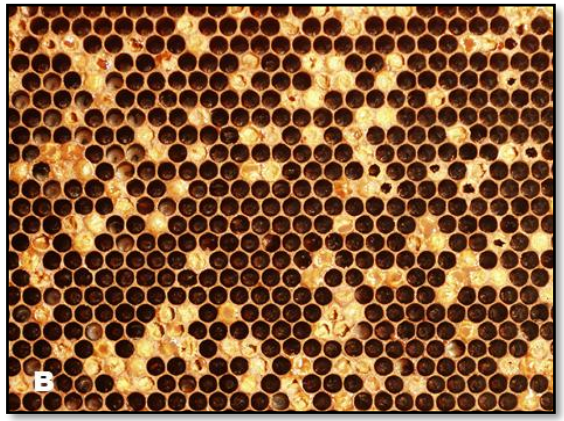
America foulbrood AFB

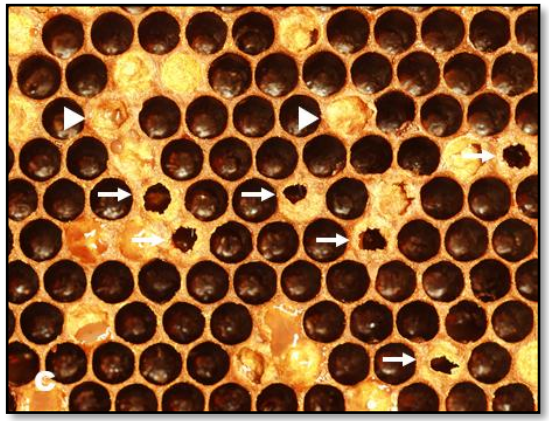
AFB – confirmatory diagnosis

1. Presence of AFB disease

- Patchy, “shotgun” brood pattern
- Abnormal cell cappings

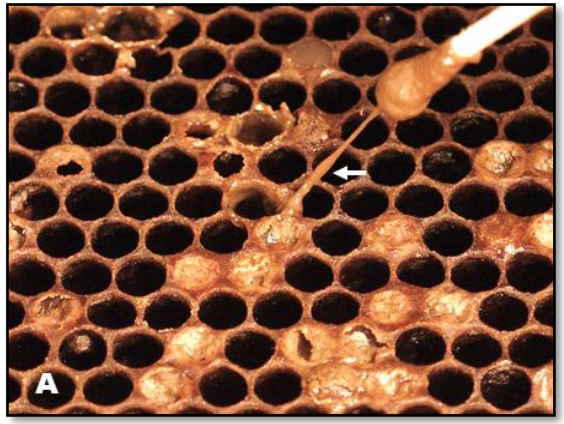
Non-specific

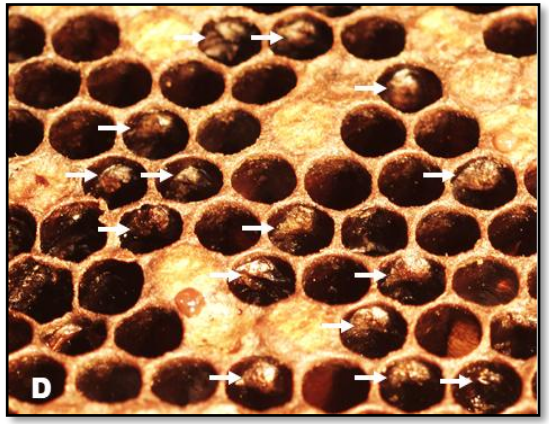




- **Larval ropiness**
- **Larval scale**

Specific





2. Demonstration of pathogen

AFB – confirmatory diagnosis

1. **Presence of AFB disease**
   - **Ropiness, Scales…**

2. **Presence of *P. larvae* spores**
   - **Bacterial culture -> 4+ (thousands to millions of bacteria)**
   - **Real Time PCR (low CT value -> the lower CT the higher number of bacteria)**

Ben's Yards

Yard ID	# hives	# dead	% mortality
X	32	15	47
Y	48	21	44
Z	24	13	54
Q	36	16	44

Sampled:

1. **Bottom board debris**
2. **Brood chamber honey**

22 dead hives with AFB+

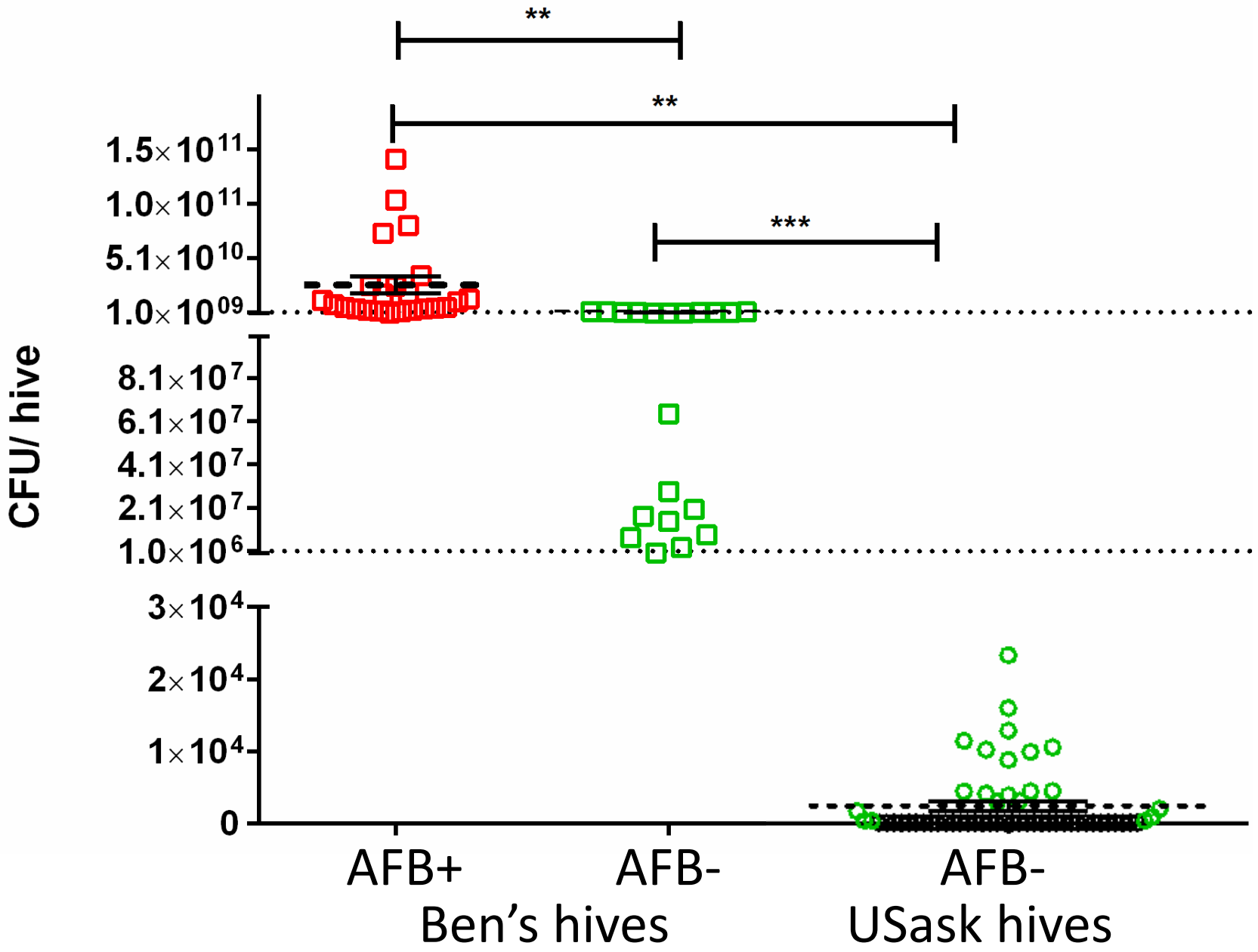
23 dead hives no AFB-

Control USask Yards

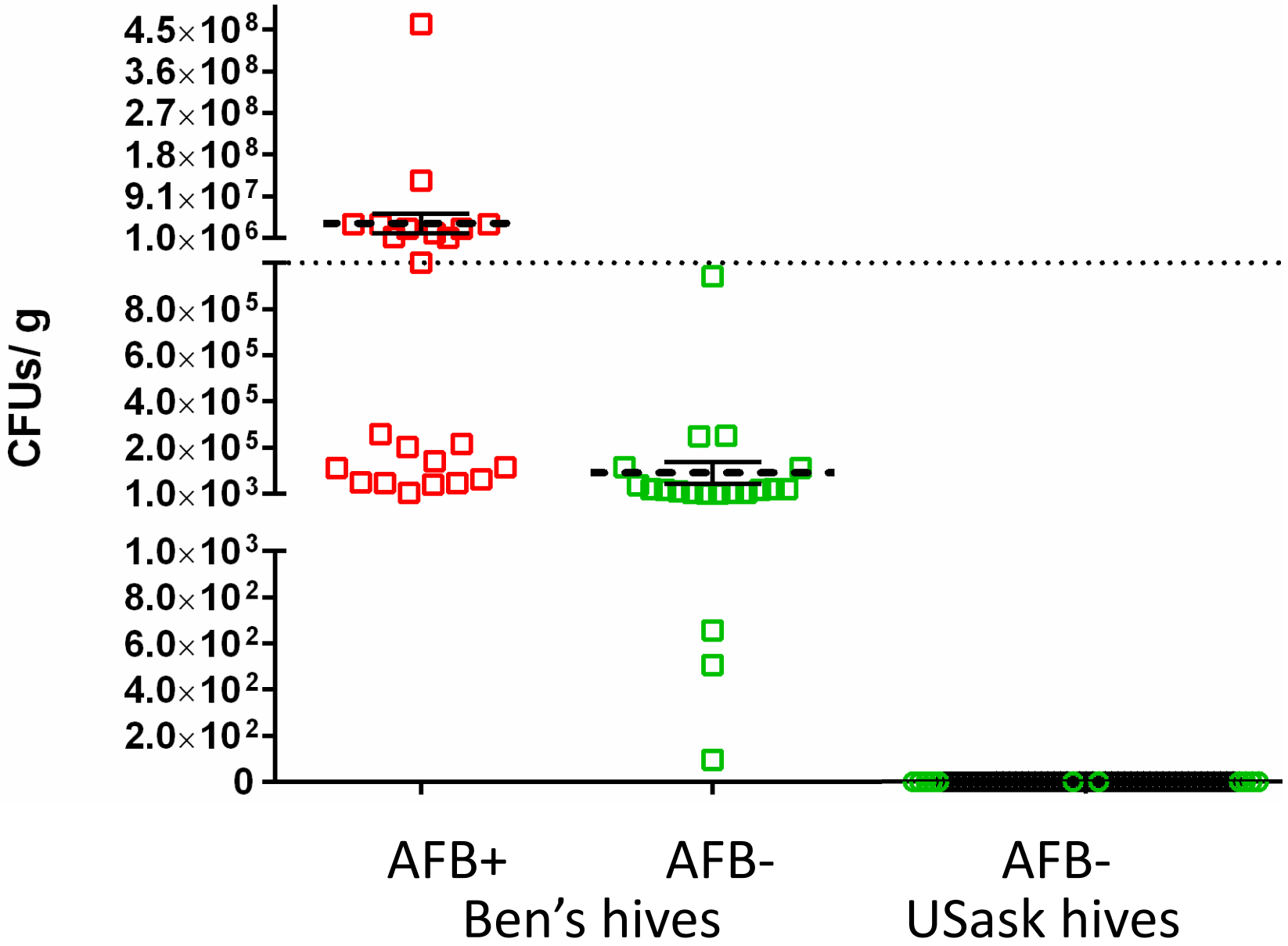
Yard ID	# hives	# dead	% mortality
USask 1	40	4	10
USask 2	16	3	19

56 live&dead hives no AFB-

AFB spores in bottom board debris



AFB spores in brood chamber honey



Actions???

Ben

500

hives

BK500

2023 -> 45%

AFB

Actions: All dead colonies destroyed

Ben

Live colonies

↓

Tylosin Tx

Concerns!!!

Jen

2000 hives

BK2000

Ben

500 hives

BK500

Jake

10 hives

BK10

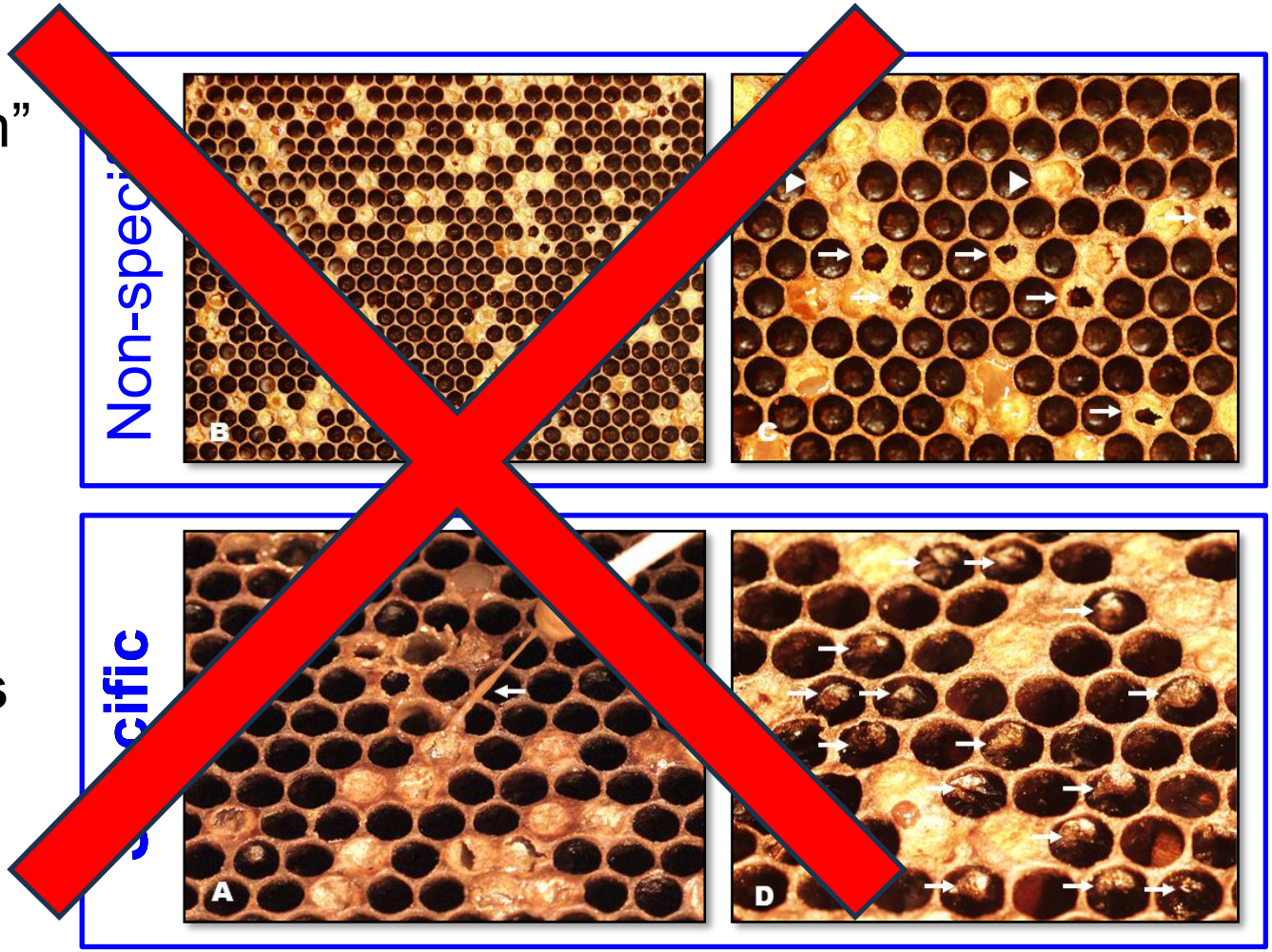
2023 -> 45%

AFB

AFB Clinical Symptoms – ERIC I

- Patchy, “shotgun” brood pattern
- Abnormal cell cappings

- **Larval ropiness**
- **Larval scale**



Zabrodski et al, Can Vet J 2020:61;1055-1059

AFB risk assessment: **Visual inspection**

- Highly diagnostic for clinical infection when scales and ropiness are present
- Labor intensive
- False negatives: early AFB
- Cannot predict risk -> multiple inspections needed

AFB risk assessment: **Feasibility**

Test	BK 2000	BK 500	BK 10
Multiple visual inspection	X	?	√

Risk!!!

Jen

2000 hives

BK2000

Ben

500 hives

BK500

Jake

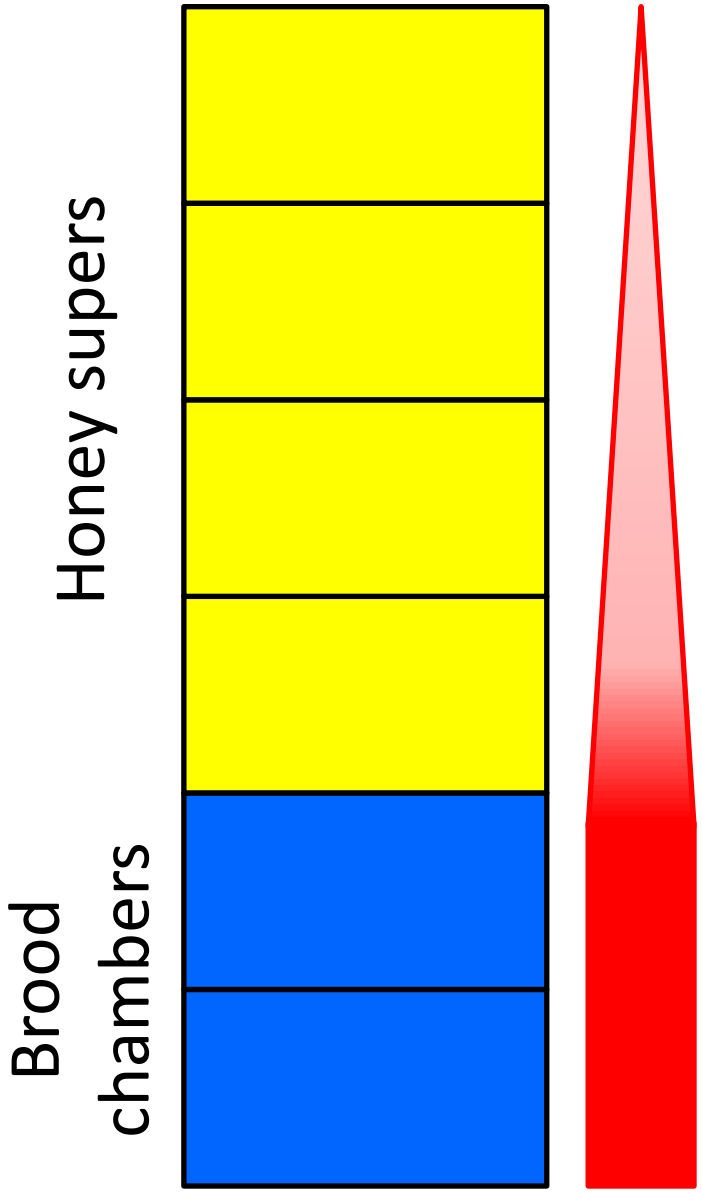
10 hives

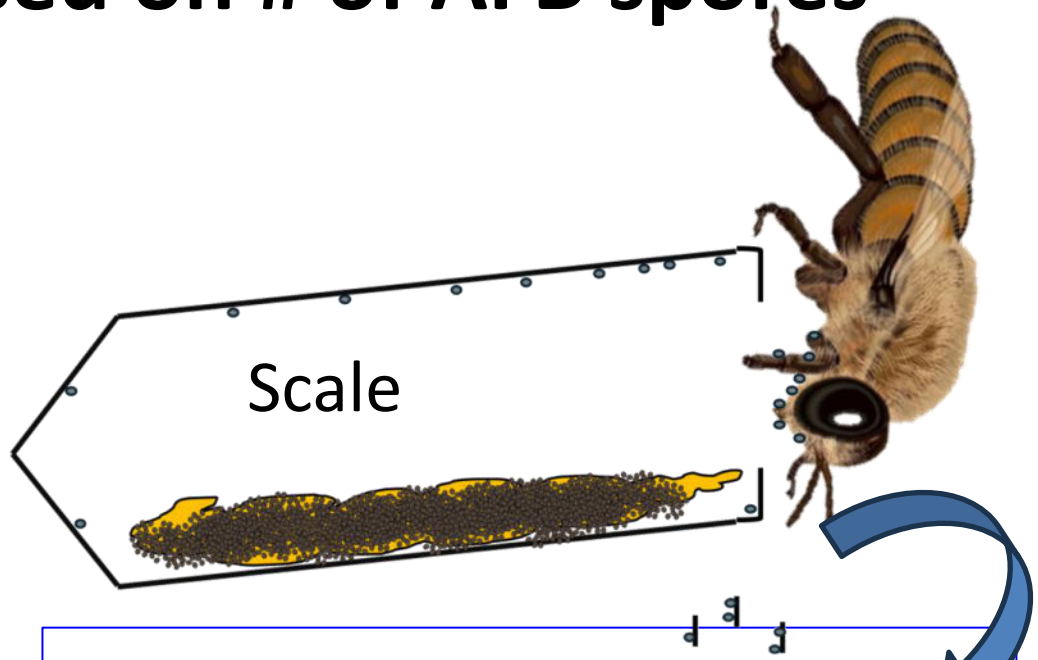
BK10

2023 -> 45%

AFB

AFB risk based on # of AFB spores





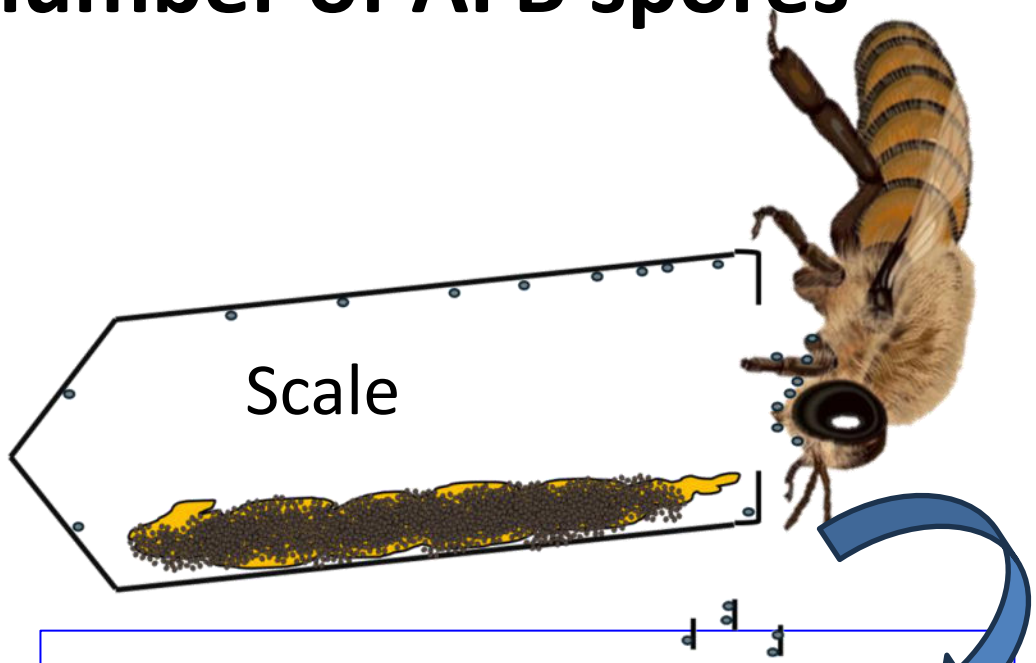
- Bees
- Brood chamber honey
- Bottom board debris
- Honey super honey

? risk based on number of AFB spores

Honey supers

Brood chambers

AFB spore concentration in hive



- Bees
- Brood chamber honey
- Bottom board debris
- Honey super honey

What is the best sample for AFB risk?

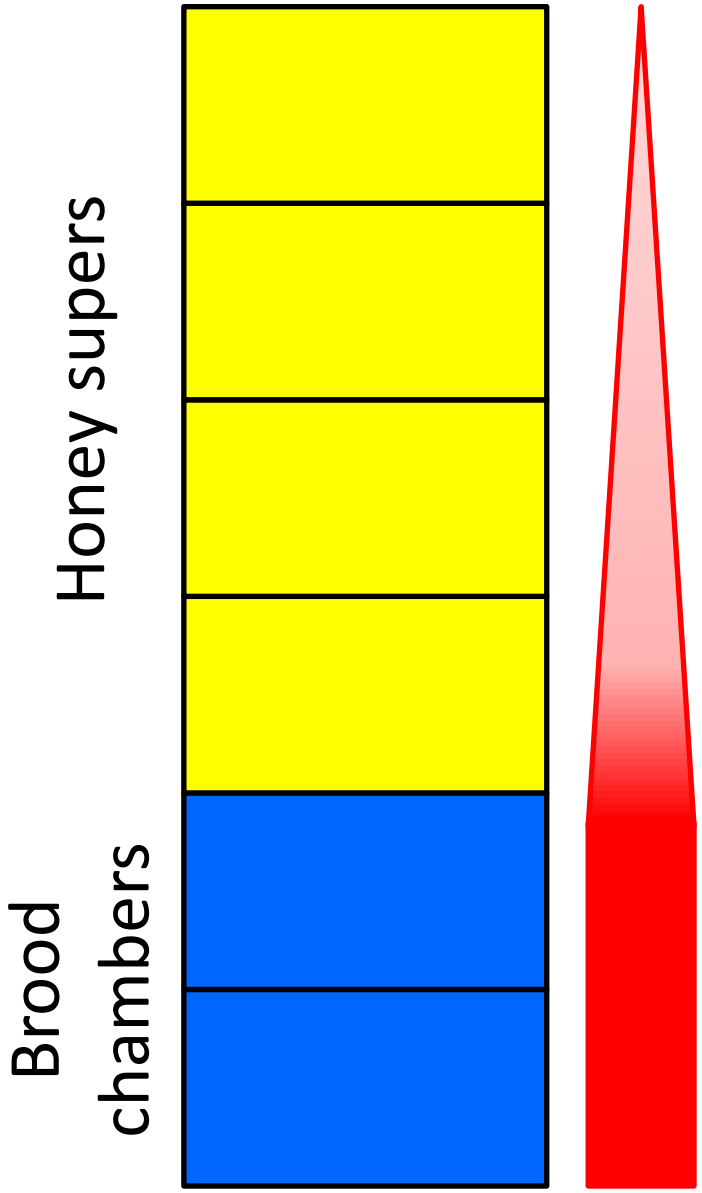
AFB risk assessment: **Bee**

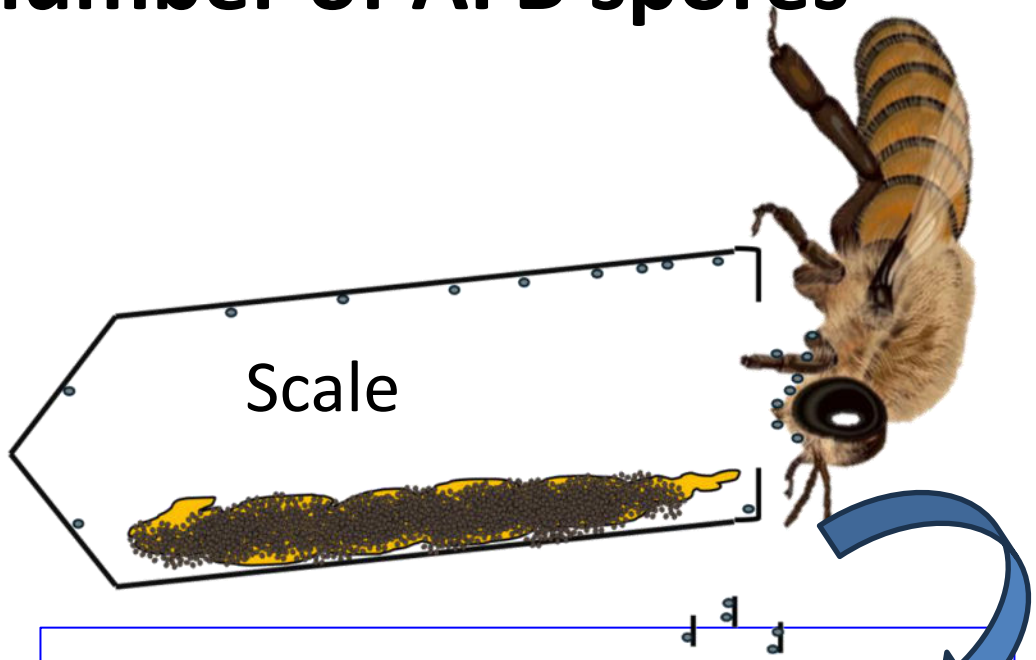
- High spore count per bee correlates well with the extent of clinical symptoms and predicts AFB risk
- Can detect subclinical AFB
- Used for risk assessment by NBDC
  - 1-99 CFU/bee: possible risk
  - 100-999 CFU/bee: moderate risk
  - >1000 CFU/bee: severe risk
- Forager bees are less reliable
- Suitable for small/medium operations as in EU
- Need to sample a lot of colonies in large operations, hence sampling is labor intense and testing expensive

AFB risk assessment: **Feasibility**

Test	BK 2000	BK 500	BK 10
Multiple visual inspection	X	?	√
Bees	?	?/√	√

AFB risk based on number of AFB spores

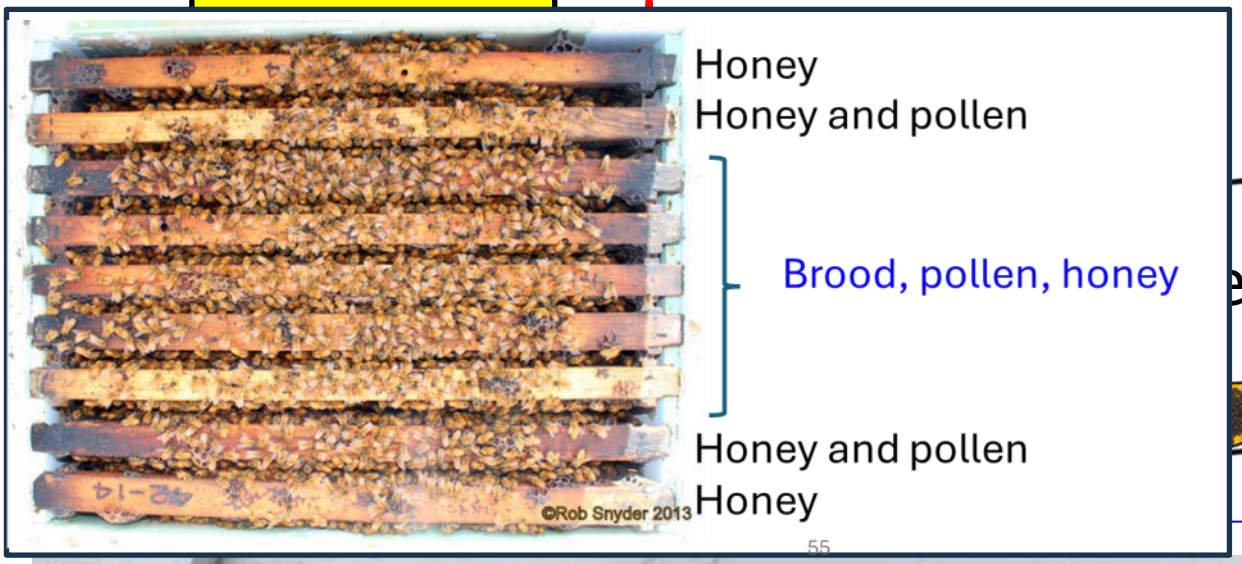




- Bees
- Brood chamber honey
- Bottom board debris
- Honey super honey

What is the best sample for AFB risk?

AFB risk based on number of AFB spores



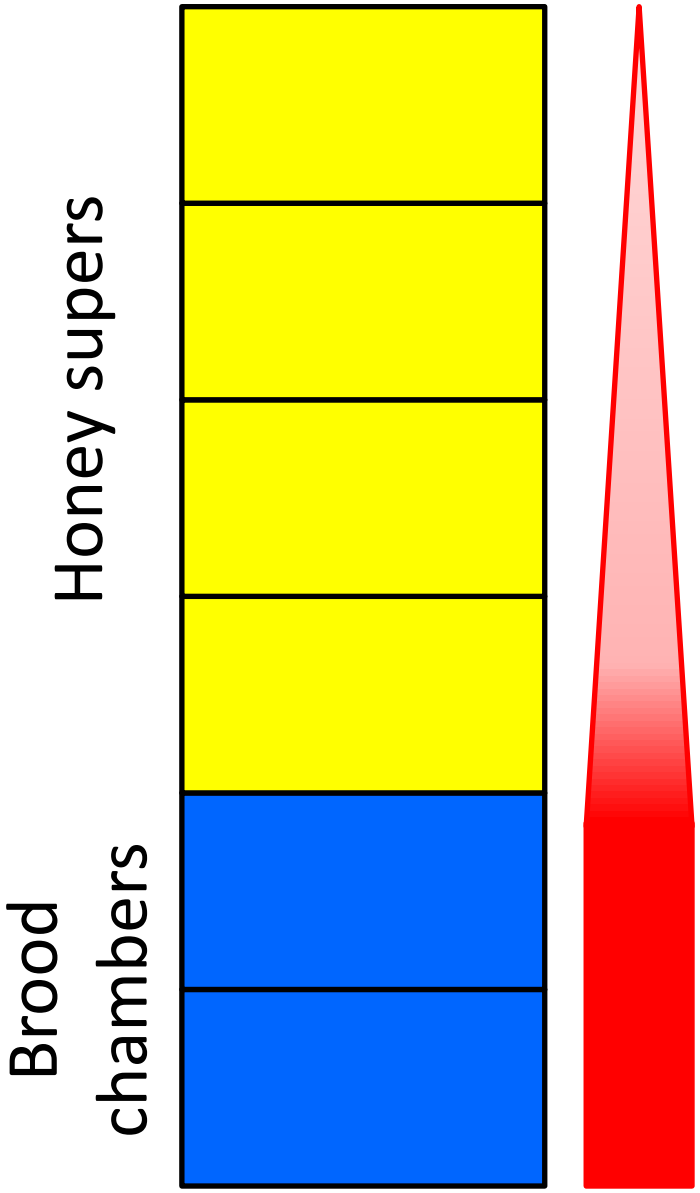
AFB risk assessment:
Brood chamber honey

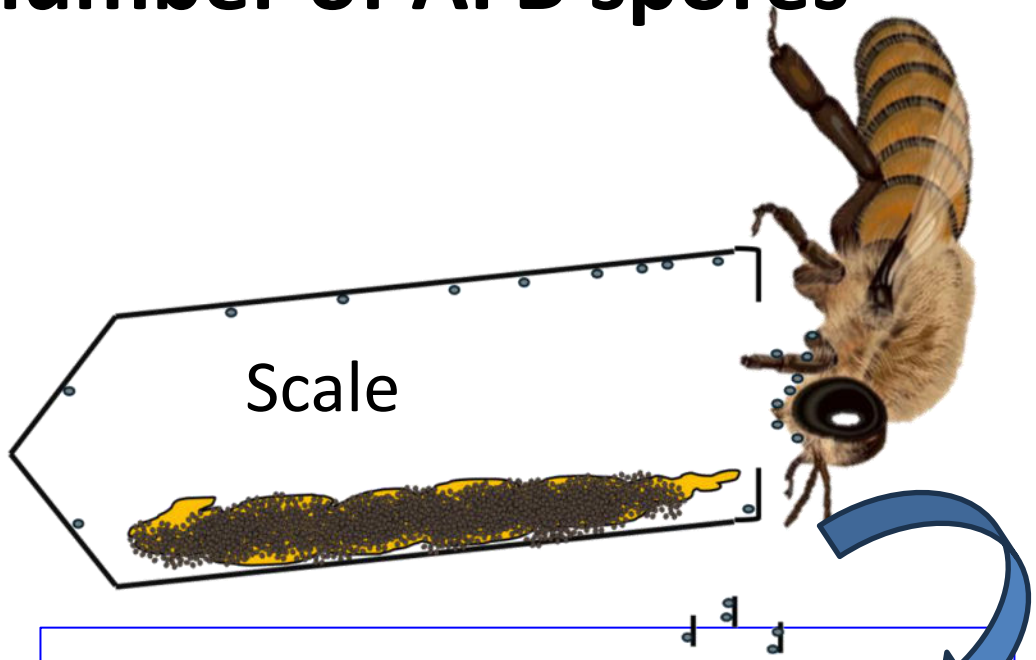
- Does not correlate well with the presence of clinical disease (unless very high count)
- Indicates potential exposure to AFB over past undefined period and predict risk
- May be "equivalent" to serology detection of previous exposure
- Used for risk assessment in some EU countries
- Suitable for small operation
- Need to sample a lot of colonies in large operations, hence sampling is labor intense and testing expensive

AFB risk assessment: **Feasibility**

Test	BK 2000	BK 500	BK 10
Multiple visual inspection	X	?	√
Bees	X	?/√	√
Brood honey	X	?	√

AFB risk based on number of AFB spores





- Bees
- Brood chamber honey
- Bottom board debris
- Honey super honey

What is the best sample for AFB risk?

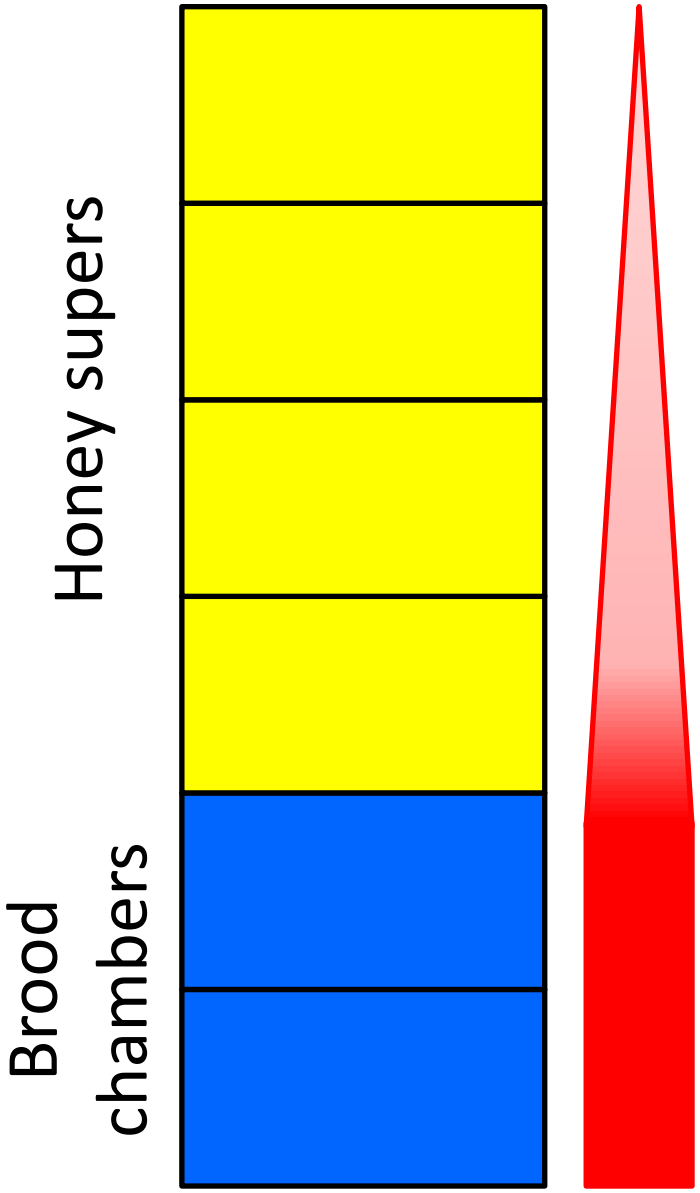
AFB risk assessment:
Bottom boards

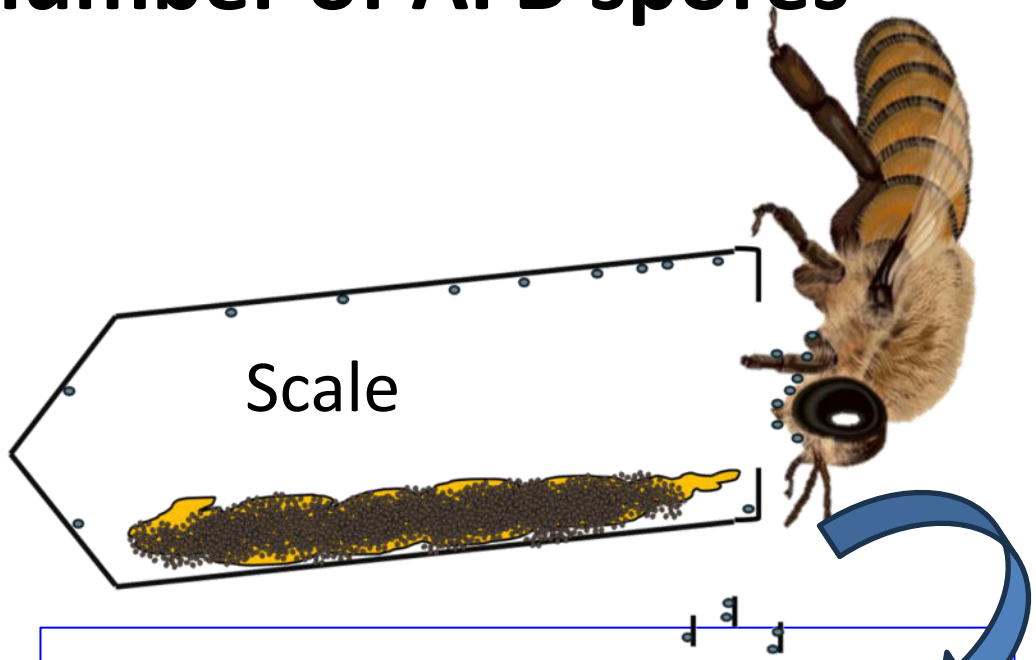
- High spore count correlates well with the extent of clinical symptoms
- Can detect subclinical AFB
- Can predict risk, thresholds poorly fefined
- Suitable for small/medium operation
- Need to sample a lot of colonies in large operations, hence sampling is labor intense
- **Foster method:**
  - Quantitative PCR for swabs of entrance bottom board
  - Can detect subclinical AFB, and predict risk
  - Can pool up to a dozen hives?

AFB risk assessment: **Feasibility**

Test	BK 2000	BK 500	BK 10
Multiple visual inspection	X	?	√
Bees	X	?/√	√
Brood honey	X	?	√
Bottom board debris	X/?	√	√

AFB risk based on number of AFB spores





- Bees
- Brood chamber honey
- Bottom board debris
- Honey super honey

What is the best sample for AFB risk?

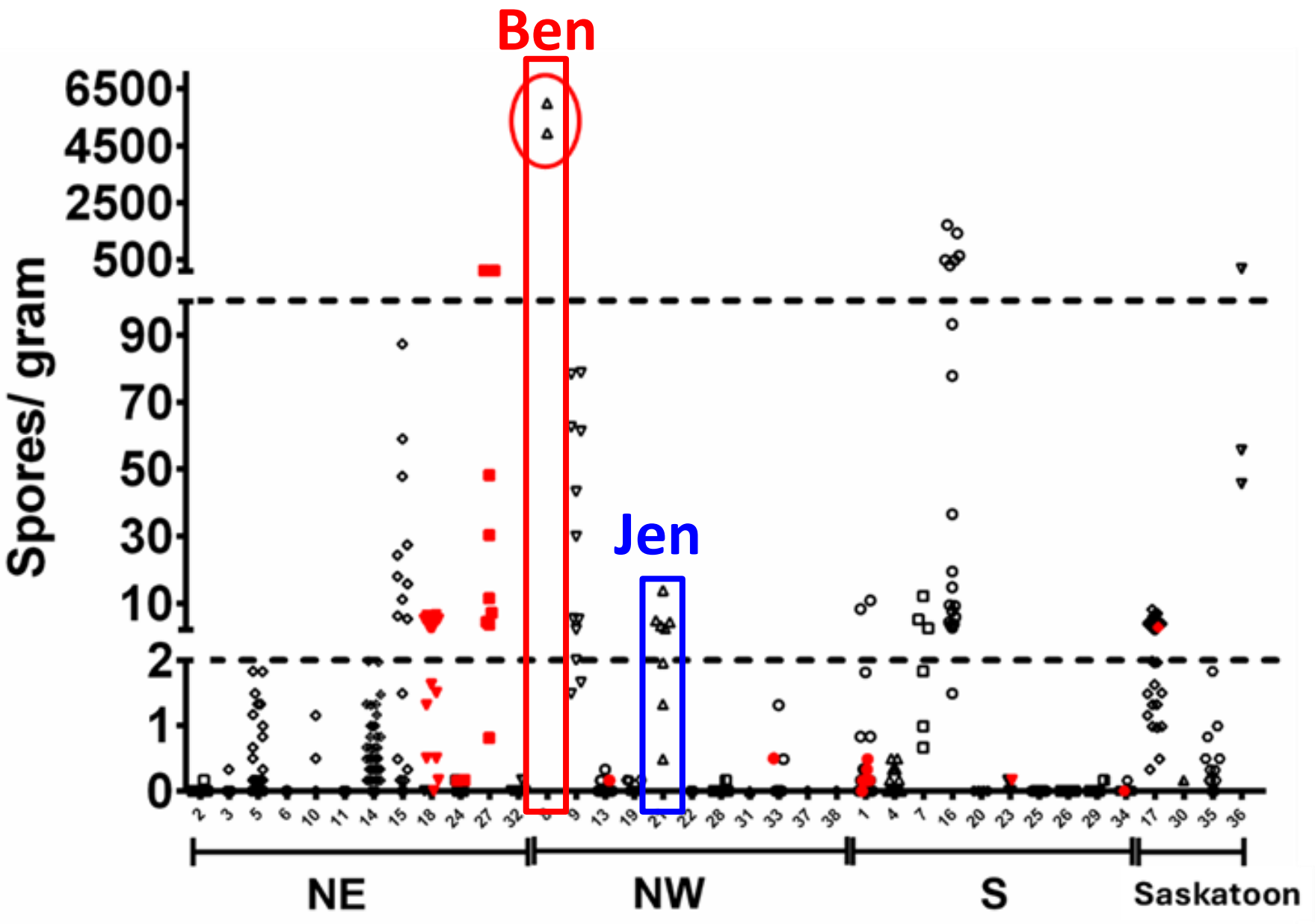
AFB risk assessment: **Extracted Honey**

- Does not correlate well with the presence of clinical disease (unless very high count)
- Easy to obtain sample
- Multiple samples can **predict AFB risk at operation**
- **Low risk thresholds well defined** (2 spores/gr)
- The most feasible for “herd risk assessment”

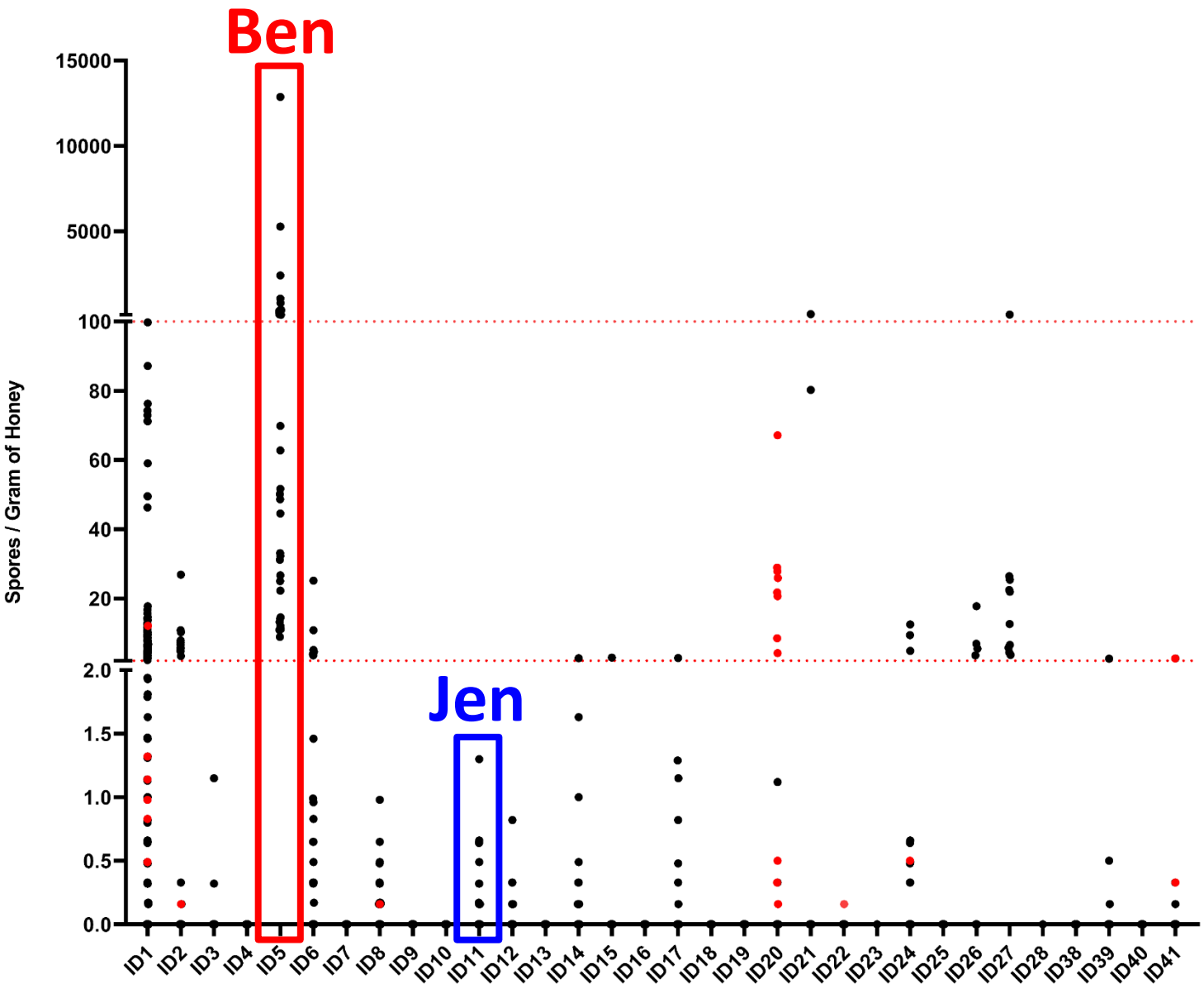
AFB risk assessment: **Feasibility**

Test	BK 2000	BK 500	BK 10
Multiple visual inspection	X	?	√
Bees	X	?/√	√
Brood honey	X	?	√
Bottom board debris	X/?	√	√
Pooled extracted honey	√	√	X

2023 AFB surveillance in extracted honey in SK



2024 AFB surveillance in extracted honey in SK



AFB risk assessment: **Feasibility**

Test	BK 2000	BK 500	BK 10
Multiple visual inspection	X	?	√
Bees	X	?/√	√
Brood honey	X	?	√
Bottom board debris	X/?	√	√
Pooled extracted honey	√	√	X

Paradigm shift from "pet health" to "herd health"

Pooled extracted honey enables AFB risk assessment at operation level

https://cdn.shopify.com/s/files/1/0361/0973/t/1/assets/blog_Listening-to-the-bees.jpg?78