

DETECTION METHOD OF PAENIBACILLUS LARVAE LARVAE FROM BEEHIVE WINTER DEBRIS

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Summary

In Czech republic all bee colonies in protection areas around American foulbrood focuses are obligatorily inspected. Clinical inspections performed by specialists are very high-powered and expensive.

We developed a method of *Paenibacillus larvae larvae* detection in winter debris from beehives for purposes of exact localization of diagnostic clinical inspections.

This method has been validated in comparison with results of clinical findings and microbiological evaluations of sugar stores in colonies.

Described method is suitable for rapid elimination of healthy colonies in suspicious areas.

Introduction

In Czech republic all AFB-positive bee colonies are killed and all colonies in protection areas around American foulbrood focuses are obligatorily inspected.

Clinical inspections performed by specialists are very high-powered and expensive. The probability of false-negative results is high.

As all Czech beekeepers collect obligatory debris for *Varroa* inspection, we developed a method of *Paenibacillus larvae larvae* (P.I.I.) detection in winter debris from beehives

Method

The Method step by step

- debris collecting from the bottoms of hives and weighing (1,5 g)
- dissolution of probe in organic solvent (10 ml toluene)
- put 2 ml of liquid part into 6 ml of 0,7% NaCl
- shake very good
- Petri-dishes inoculation and cultivation 7 days on MYPGP agar with growth suppression of non-target microbes by nalidixic acid (30 mg/litre)
- P.I.I. verification
- reading of results and interpretation

Results

4 areas with 3 various infestation levels have been inspected

Area No. 1

The district Česká Lípa in north Bohemia, no focus of AFB since 1990.

No. of inspected colonies:	4500
No. of positive P.I.I. finds from debris:	1
Honey P.I.I. positive:	0
Clinical occurrence:	0

Area No. 2

Protective zones in 2 km circles around AFB focuses found 1999 - 2001

No. of inspected colonies:	360
No. of positive P.I.I. finds from debris:	48
Clinical occurrence:	100% stands, 80% colonies during 2 years

Area No. 3

Protective zones in 2 km circles around AFB focuses found 2002

No. of inspected colonies:	862
No. of positive P.I.I. finds from debris:	215
Clinical occurrence:	still under inspection

Area No. 4

AFB positive apiaries (outside Czech Republic)

No. of inspected colonies:	160
No. of positive P.I.I. finds from debris:	38
Honey P.I.I. positive:	29
Clinical occurrence:	15

Discussion

The presented method of *Paenibacillus* larvae detection in winter debris from beehives detects early stages of AFB. Some "debris positive" hives are without clinical symptoms for following reasons

- A) found P.I.I. is possibly not virulent - the typisation of these microbes is to be required
- B) the infection pressure is lower than infection dose
- C) the colony is resistant
- D) human error when colony inspected

Conclusion

Described method is suitable for rapid elimination of healthy colonies in suspicious areas.

This elimination provides more power for next clinical and laboratory examinations. This method be an aid in process of radical eradication of AFB focuses.