APIMONDIA SCANDINAVIA 2025
SWEDEN | DENMARK | NORWAY

PP-077 [Bee Health]

WAGENINGEN UNIVERSITY & RESEARCH

Prevalence of *Paenibacillus larvae* in *Apis mellifera* in the Netherlands

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Background

American Foulbrood (AFB) is a globally prevalent brood disease in honey bees caused by the spore-forming bacterium *Paenibacillus larvae*. The disease leads to the death of infected larvae following oral spore uptake and is characterized by ropy decay and foul odor in the brood cells. Transmission occurs both within and between colonies through spores spread by adult bees and contaminated equipment. Notably, asymptomatic colonies carrying high spore loads can serve as infection sources. As in many countries, AFB is a notifiable disease in the Netherlands. In 2005, control of AFB in the Netherlands shifted from the government to beekeeper associations, with bee health coordinators managing suspected cases. Mandatory destruction of all colonies in affected apiaries was replaced by isolating and treating only symptomatic colonies using the shook swarm technique. A 2008 study assessed the impact of policy changes on AFB prevalence, finding *P. larvae* in only 0.6% of sampled apiaries and 3% of honey samples.

Introduction

Given the low number of reported AFB outbreaks in the Netherlands over the past two decades and the absence of recent prevalence data, this study aimed to assess the current presence of *Paenibacillus larvae* in Dutch apiaries. Understanding the present prevalence is essential to evaluate the long-term effects of the 2005 policy changes on disease control and to inform ongoing management strategies.

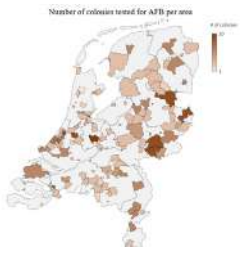

Figure 1. Map of the Netherlands providing an overview of the locations of the colonies participating in the survey based on the 4 digit postal code of the area where the colony was kept. Darker colors indicate a higher number of colonies in a postal code area.

Figure 2. Picture of a brood nest and the site of sampling for the brood comb honey as part of the instruction leaflet for participating beekeepers in our study.

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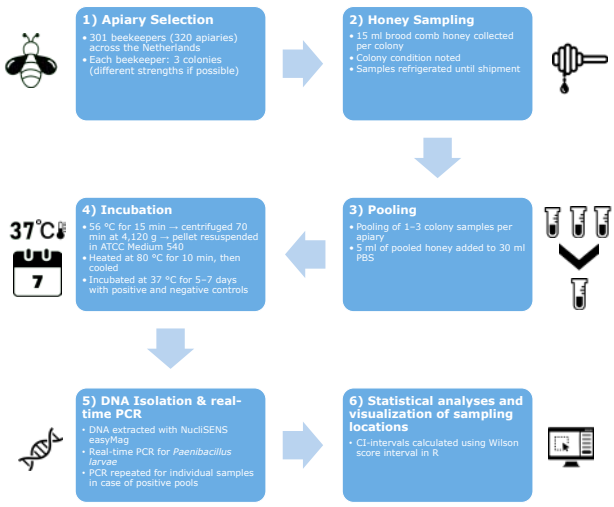
Methods



Results

One pool tested positive, indicating an apiary-level prevalence of 0.4% (95% CI: 0.02–2.9%). When the individual samples from this pool were tested, two of the three were positive, corresponding to a colony-level prevalence of 0.3% (95% CI: 0.06–1.3%).

Number of samples in pool	Number of PCR-positive pools/tested pools	Number of PCR-positive individual samples/number of individual samples tested
1	0/14	
2	0/21	
3	1/188	2/3
Total number of positive tested pools/total number of tested pools	1/223* (0.4%, 95% CI: 0.02-2.9%)	
Total number of positive tested colonies/total number of colonies tested in pools	2/620* (0.3%, 95% CI: 0.06- 1.3%)	

Figure 4. Overview of the number of samples per pool and the number of pools and individuals samples tested positive for *P. larvae* by real-time PCR. *One pool of honey samples was lost due to a technical failure and there was no material left to repeat the test.

Conclusions

These results confirm that the prevalence of *P. larvae* in Dutch *A. mellifera* colonies remains very low, consistent with the study in 2008 and the very few to no reported outbreaks of clinical AFB reported annually.

Acknowledgements

We thank Annebel Claassen, Rita de Man, Jonathan Kooi and Saskia Bouwmeester for their help and the EURL Bee Health for providing their SOP for the real-time PCR assay for *P. larvae*

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Background
Paenibacillus larvae is the causative agent of American Foulbrood (AFB), a disease of the brood in Western honey bees (Apis mellifera). Following changes in policy regarding the control of AFB in the Netherlands in 2005, a prevalence study was conducted in 2008, which served as a baseline measurement. The prevalence of P. larvae was found to be very low.

Problem description
In order to determine whether the changes in policy have had an effect on the prevalence of P. larvae in the Netherlands, we conducted a study, which allowed us to compare the current prevalence with the prevalence in 2008. In addition, we were interested if the very few outbreaks of AFB reported annually over the past decades reflected the current prevalence of P. larvae.

Methodology
A total of 623 brood comb honey samples originating from 224 apiaries across the country were analyzed for the presence of P. larvae by real-time PCR. Samples were pooled per apiary, resulting in 223 pooled samples.
Results and conclusion
One pooled sampled tested positive for P. larvae, resulting in a prevalence of 0.4% (95% CI: 0.02-2.9%) on the level of apiaries. When tested individually, two out of the three samples from this pool yielded a positive result, resulting in a prevalence on the level of colonies of 0.3% (95% CI: 0.06- 1.3%). Based on these results, we can conclude that the low prevalence of P. larvae in the Netherlands has not increased since the changes in policy and is in accordance with the very few clinical outbreaks reported annually.

Practical implications for beekeepers
The low prevalence of AFB in the Netherlands in both 2008 and 2023 is likely due to Dutch beekeeping practices. Most beekeepers use artificial swarming in spring, splitting the colony into nukes and leaving periods without brood, which limits P. larvae growth. While this method reduces honey production, it may explain the lower prevalence of P. larvae in the Netherlands compared to other countries.