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Agroscope
Swiss Bee Research Center

Resurgence of European foulbrood in Switzerland: epidemiology and diagnosis

J.D. Charrière; D. Grossar

The 2nd of October 2017, Istanbul



Brood diseases

- European foulbrood





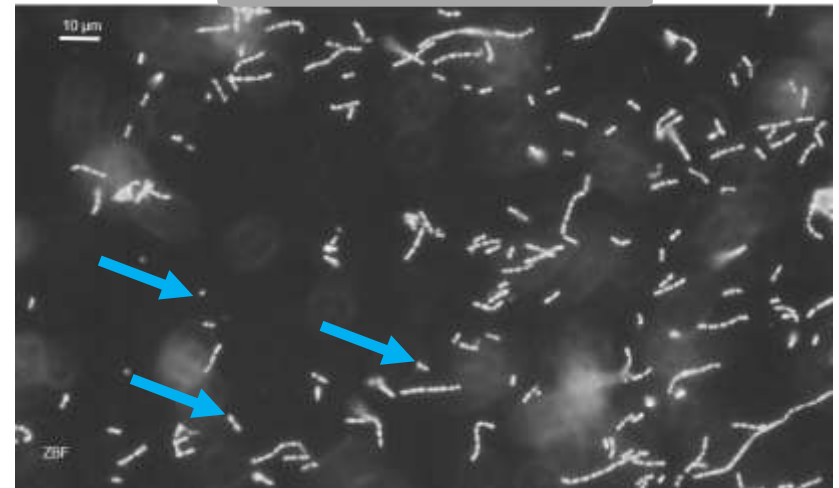
European Foulbrood (EFB): Pathogenesis

Bacterial brood disease of honey bees

Melissococcus plutonius

(Enterococcaceae; White, 1912)

- lanceolate cocci
- non-spore-forming





European Foulbrood (EFB): Pathogenesis

Melissococcus plutonius

- infects the intestinal tract of young honey bee larvae
- Competition for nutrients
- Secondary infections
 - *Paenibacillus alvei*
 - *Enterococcus faecalis*
 - *Achromobacter eurydice*

Forsgren, 2010





European foulbrood (EFB)

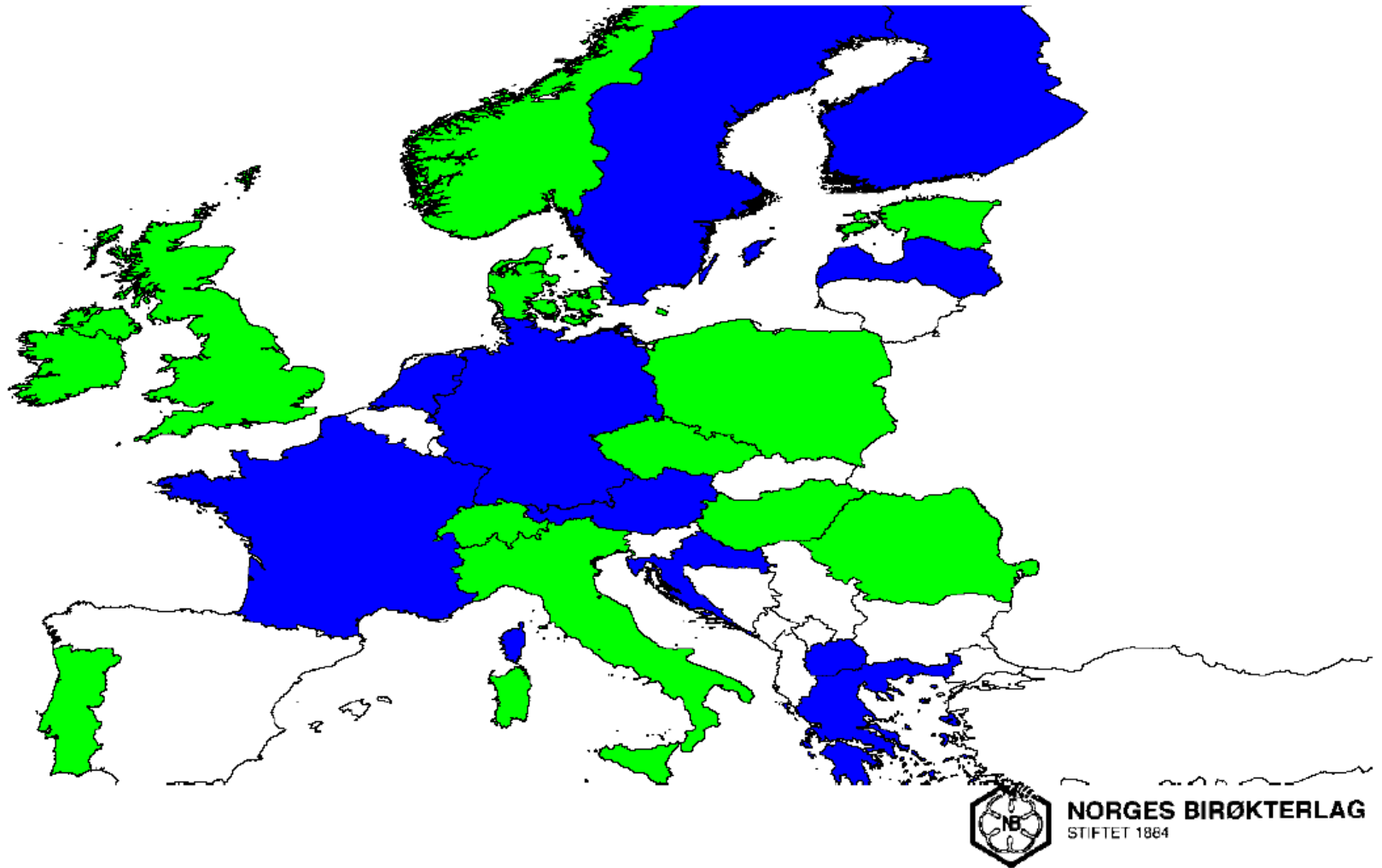
How to recognize colonies affected by EFB?

- Brood appearance:
 - colour change
 - position
 - flaccid/decomposed
 - scales
- Foul/sour odor
- Hygienic behavior
 - Brood area patchy





Notifiable disease (green)

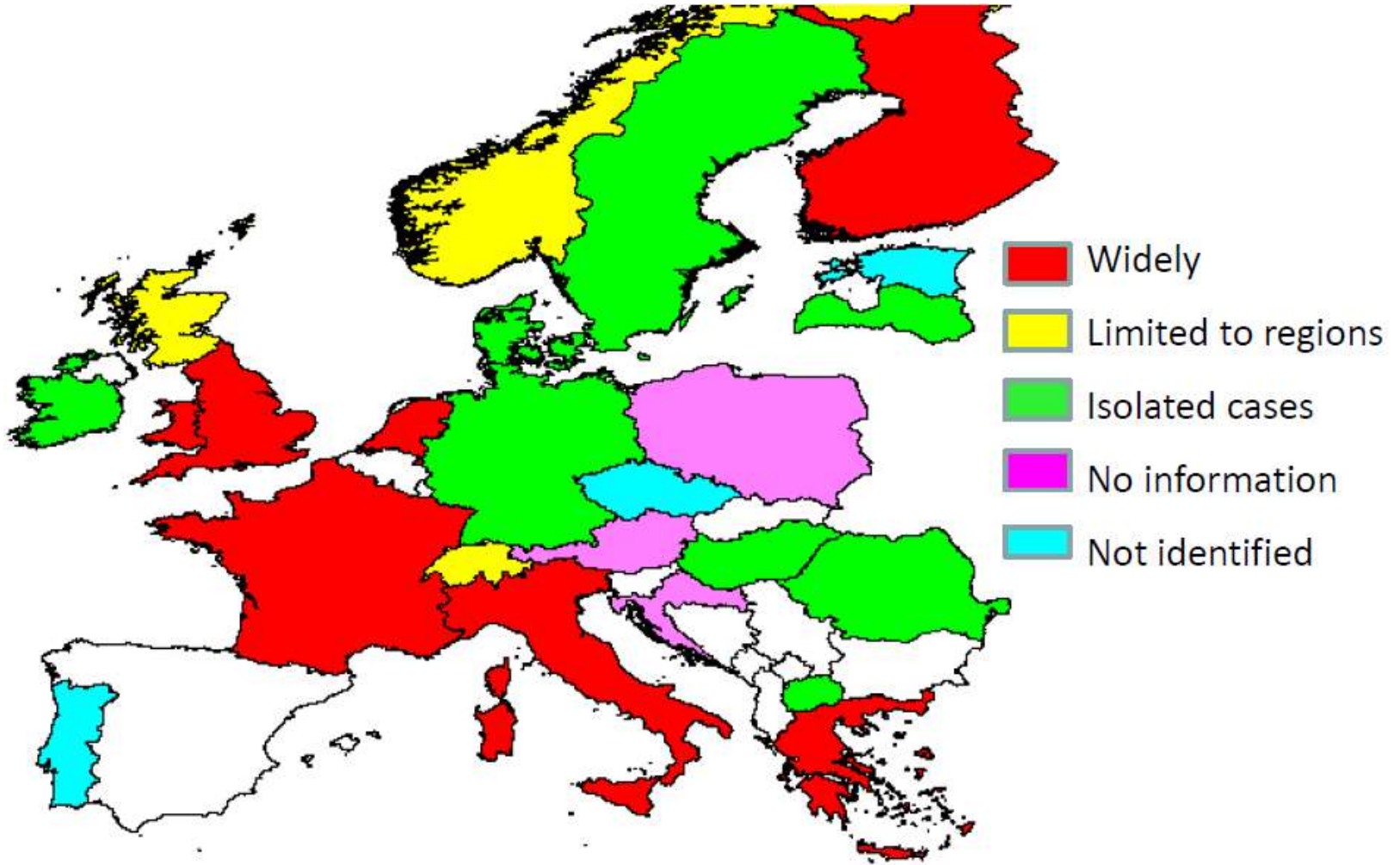


NORGES BIRØKTERLAG
STIFTET 1864

Dahle et al. Murcia 2014



Distribution within countries

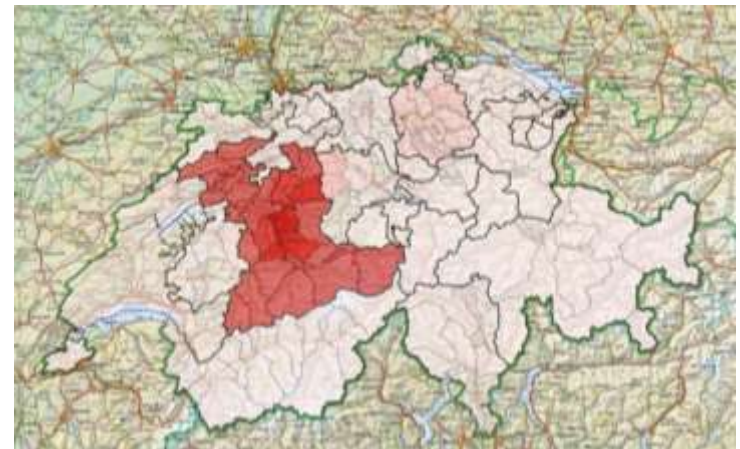
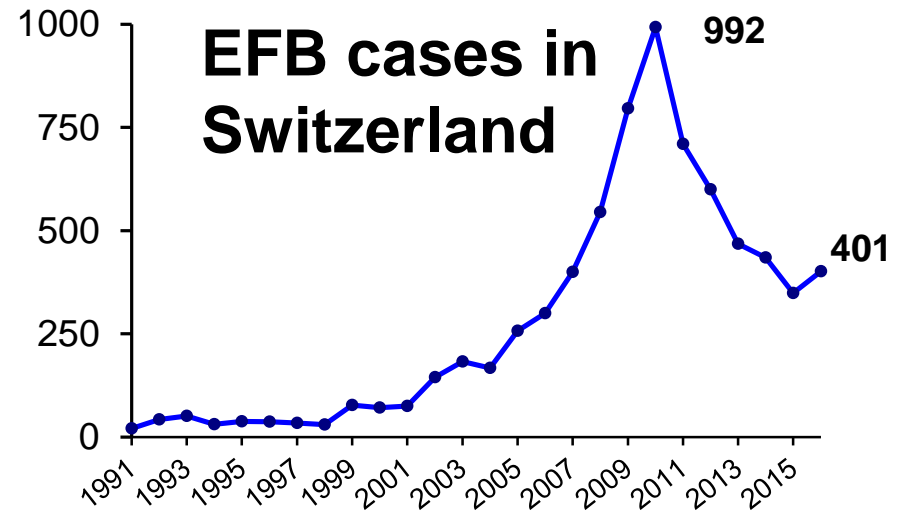


Dahle et al. Murcia 2014

European foulbrood (EFB)

Prevalence in Switzerland

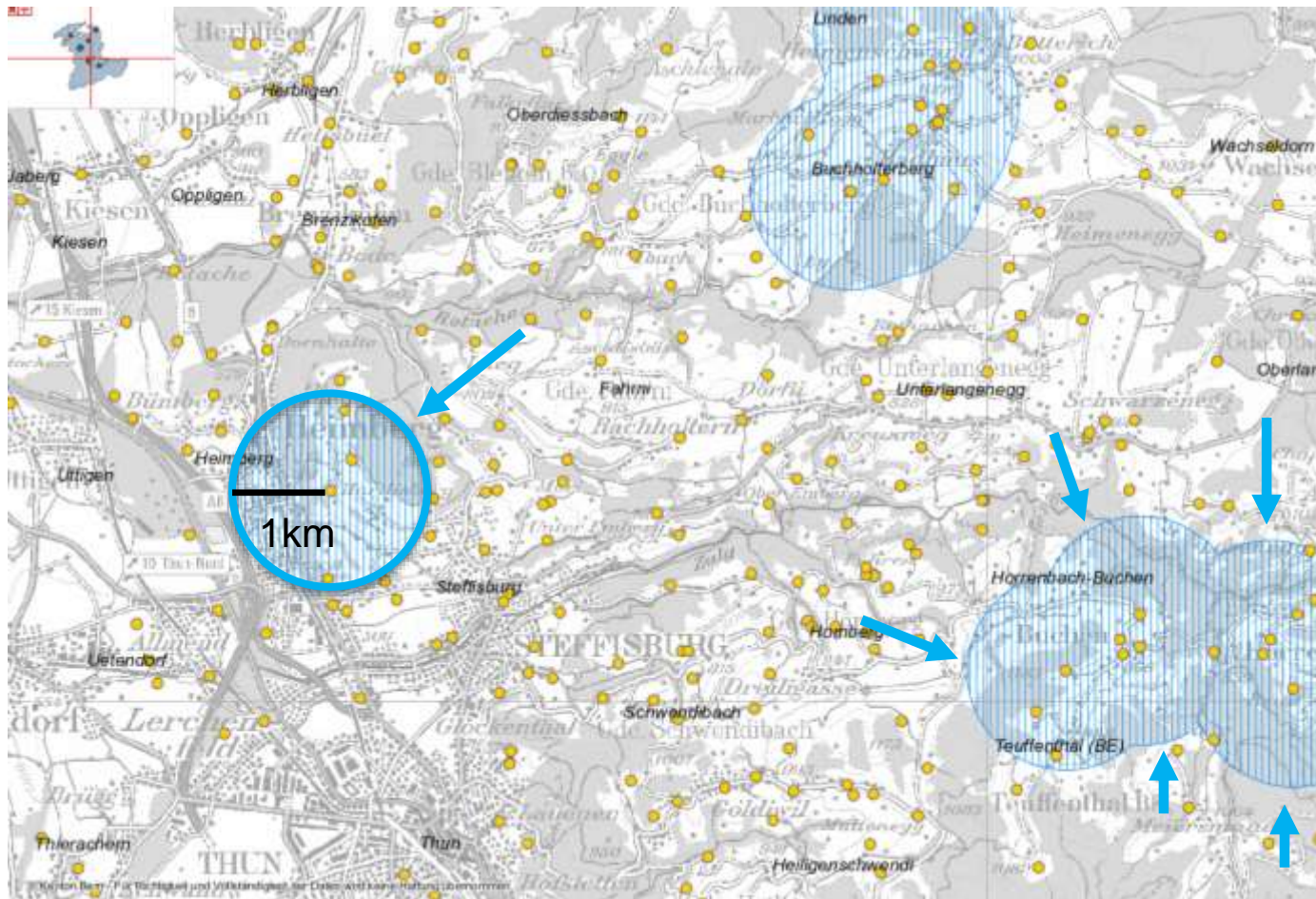
- Until 2002 the number of EFB-cases registered in Switzerland was below 80/year
- 992 cases in 2010 - Since then decreasing tendency
- Most cases are detected in the canton of Bern and Thurgau





Sanitation measures

- Destruction of all colonies with clinical symptoms
- Restriction zones of 1 km radius



• Apiary

1 km



European Foulbrood (EFB): Control measures applied in CH

- Notifiable disease in Switzerland
- Routine checks by bee inspectors
- Restriction zone of 1 km radius
- Destruction of diseased colonies
- Total sanitation of the apiary if more than 50% of the colonies are affected (killing or artificial swarms)
- Visual check of all colonies within the restriction area by bee inspectors
- Follow-up checks by the bee inspector after sanitation (also next Spring)
 - Control measures are costly



European Foulbrood (EFB): Diversity of *M. plutonius*

Journal of Apicultural Research 32(2): 80-88 (1993)

The cultural
characteristics
and serological
relationships of
isolates of
*Melissococcus
pluton*

M F ALLEN; B V BALL



FEMS Microbiology Letters 173 (1999) 311-318



Geographically diverse Australian isolates of
Melissococcus pluton exhibit minimal genotypic diversity
by restriction endonuclease analysis

Steven Philip Djordjevic *, Lisa Annette Smith, Wendy Ann Forbes,
Michael Alan Hornitzky

*NSW Agriculture, Microbiology and Immunology Section, Elizabeth Macarthur Agricultural Institute, Private Mail Bag 8, Camden,
N.S.W. 2570, Australia*

Received 29 October 1998; received in revised form 27 January 1999; accepted 4 February 1999

***M. plutonius* isolates were
thought to be homogenous...**

J. gen. Microbiol. (1962), 28, 385-391

Printed in Great Britain

Cultural Characters of *Streptococcus pluton* and its
Differentiation from Associated Enterococci

By L. BAILEY AND A. J. GIBBS

Rothamsted Experimental Station, Harpenden, Hertfordshire

(Received 24 March 1961)

SUMMARY

Strains of *Streptococcus pluton* (White) from widely separate parts of the
world were very similar culturally and serologically and differed greatly in



European Foulbrood (EFB): Diversity of *M. plutonius*

Contents lists available at ScienceDirect



Veterinary Microbiology

Journal homepage: www.elsevier.com/locate/vetmic

Short Communication

Typing of *Melissococcus plutonius* isolated from European and Japanese honeybees suggests **spread of sequence types across borders and between different *Apis* species**

Daisuke Takamatsu^{a,b,c}, Keiko Morinishi^c, Rie Arai^{b,d}, Aya Sakamoto^c, Masatoshi Okura^a, Makoto Osaki^a

OPEN ACCESS Freely available online

PLoS one

Diversity of *Melissococcus plutonius* from Honeybee Larvae in Japan and Experimental Reproduction of European Foulbrood with Cultured Atypical Isolates

Rie Arai^{1,2}, Kiyoshi Tominaga³, Meihua Wu⁴, Masatoshi Okura³, Kazutomo Ito⁵, Naomi Okamura⁷, Hidetaka Onishi⁶, Makoto Osaki^{2*}, Yuya Sugimura⁹, Mikio Yoshiyama⁸, Daisuke Takamatsu^{2,5*}



GENOME ANNOUNCEMENT

Complete Genome Sequence of *Melissococcus plutonius* DAT561, a Strain That Shows an Unusual Growth Profile and Is Representative of an Endemic Cluster in Japan

Kayo Okumura^{a,b}, Rie Arai^{c,d}, Masatoshi Okura^e, Teruo Kirikae^f, Daisuke Takamatsu^{c,g}, Makoto Osaki^{h,*} and Tohru Miyoshi-Akiyama^a

...but new studies contradict this opinion.

OPEN

The ISME Journal (2014), 1-10
© 2014 International Society for Microbial Ecology. All rights reserved. 1751-7362/14
www.nature.com/ismej

ORIGINAL ARTICLE

Molecular epidemiology and **population structure** of the honey bee brood pathogen *Melissococcus plutonius*

Giles E Budge¹, Mark DF Shirley², Benjamin Jones¹, Emiline Quill¹, Victoria Tomkies¹, Edward J Feil³, Mike A Brown¹ and Edward G Haynes^{1,4}

environmental microbiology reports



Environmental Microbiology Reports (2013)

doi:10.1111/1758-2229.12057

A typing scheme for the honeybee pathogen *Melissococcus plutonius* allows detection of disease transmission events and a study of the **distribution of variants**

Edward Haynes^{1,2}, Thorunn Helgason^{1*}, J. Peter W. Young¹, Richard Thwaites² and Giles E. Budge²

genetic diversity (Allen and Ball, 1993; Djordjevic *et al.*, 1999). Indeed, isolates from the UK and Australia have proven indistinguishable by RFLP (Restriction Fragment

Multilocus Sequence Typing (MLST)

Multilocus sequence typing scheme (MLST) for *M. plutonius* developed recently (Haynes *et al.*, 2013).

...is a technique for the typing of bacterial isolates using DNA sequences of multiple housekeeping genes.

In total 148 Swiss samples analyzed:

97 *M. plutonius* isolates (2005-2007)

51 *M. plutonius* isolates (2013)

Summary

The 148 analyzed Swiss *M. plutonius* isolates belong to 13 different Sequence Types:

- 108 isolates could be assigned to Sequence Types, identical to isolates already described
(Haynes, 2013; Takamatsu, 2014; Budge, 2014)
- 40 isolates belong to six novel *M. plutonius* Sequence Types identified for the first time in this study
- No clear pattern in the distribution of the different sequencing types

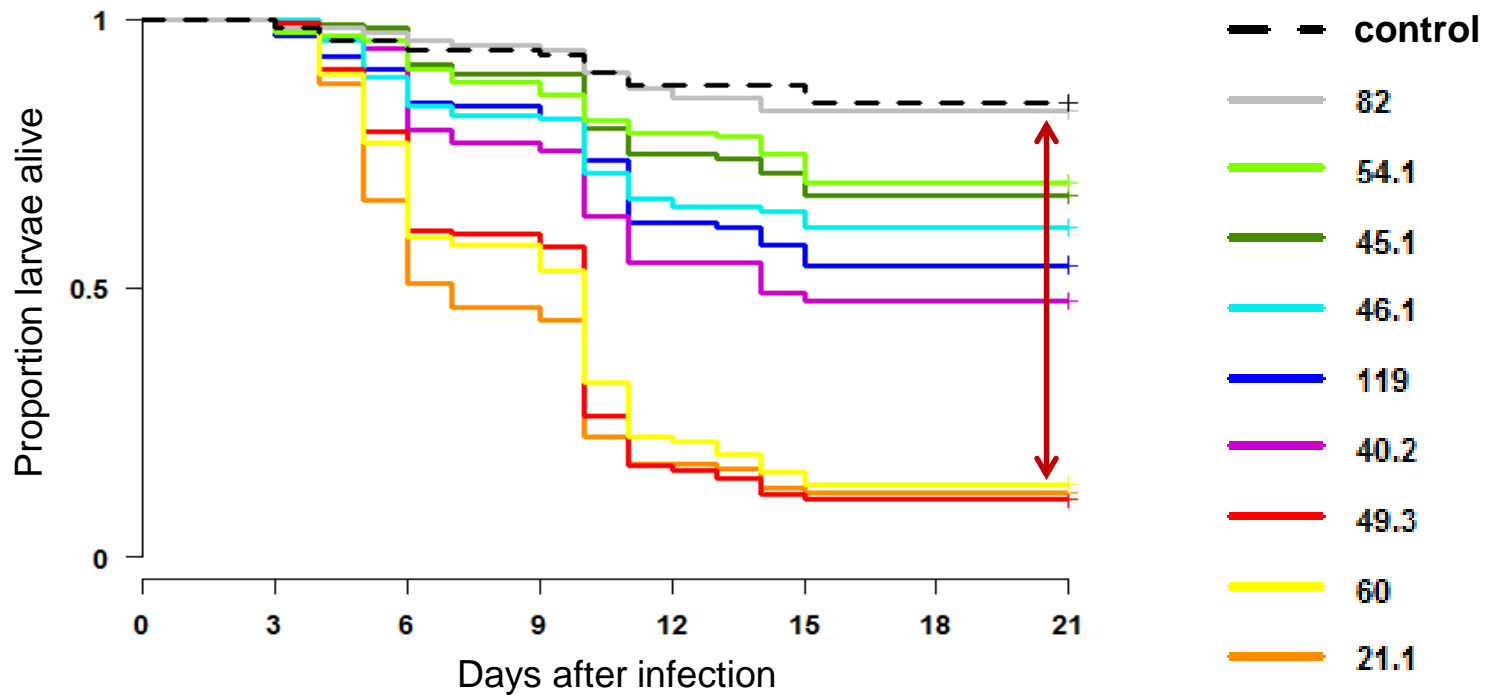
Comparison of the virulence between strains: *in vitro* larval rearing (Aupinel et al., 2005)

- Transferring young larvae into plastic cups with food
- Infection with *M. plutonius* added to artificial diet (sugar + royal jelly)
- Rearing in an incubator under constant conditions in the laboratory
- Daily feeding and monitoring of the mortality till emergence





Virulence of *Melissococcus plutonius* isolates



Isolates differ in their virulence on the larval level

N=116-130 larvae per strain



European foulbrood (EFB)

Environment



Colony and apiary density

Social immunity



Beekeeping practices, sanitation measures



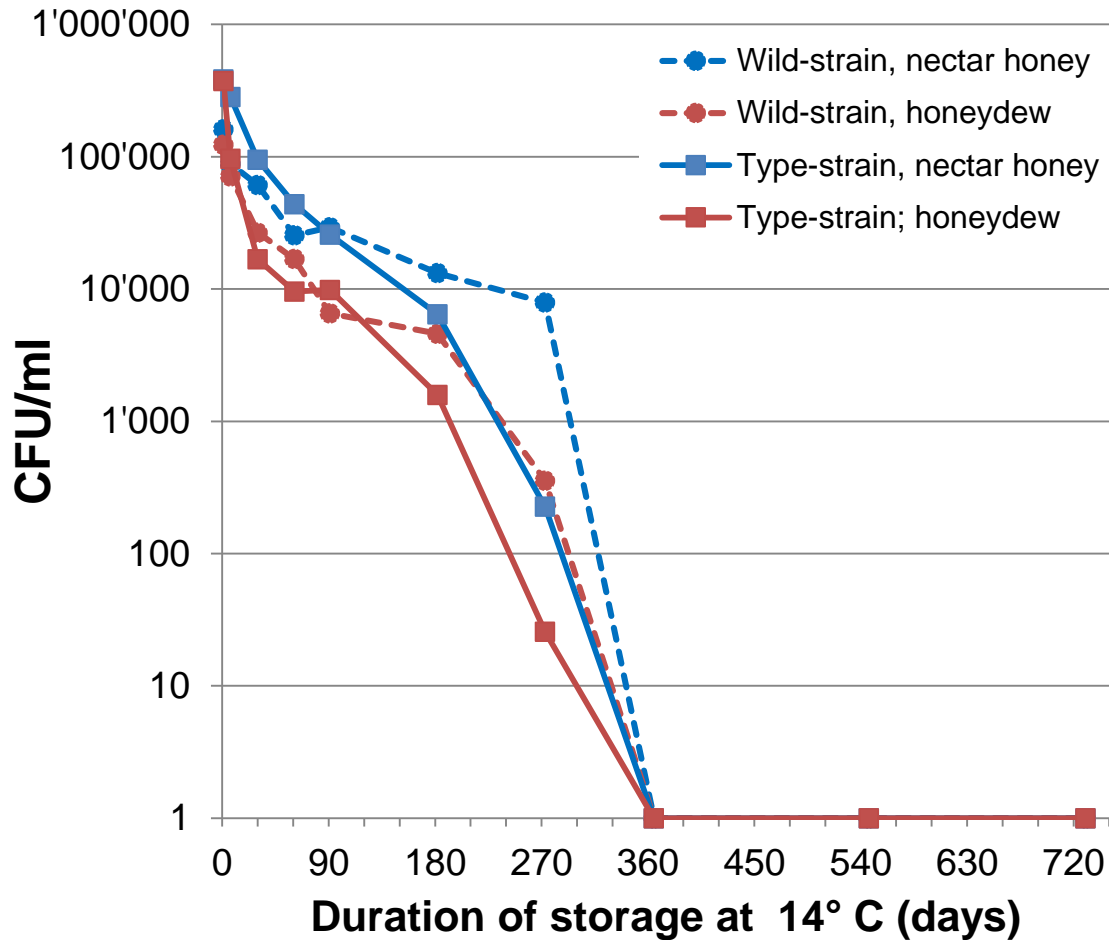
European Foulbrood outbreak

Individual immunity



M. plutonius

Survivorship of *M. plutonius* in honey





Thank you for your attention.



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www.apis.admin.ch