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Original article

Molecular epidemiology of *Paenibacillus larvae* infection in Finland*

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Abstract – The epidemiology of a *Paenibacillus larvae* infection, causing American Foulbrood (AFB), was studied regionally in Finland and temporally in the apiaries. Altogether 156 *P. larvae* isolates from honey or brood samples supplied by 114 beekeepers were analysed by *Not*I pulsed-field gel electrophoresis (PFGE) and biotyping, as well as for tetracycline resistance. Fifty-two *Not*I macrorestriction profiles (MRP) and three biotypes were detected. All isolates were susceptible to tetracycline. Most MRPs and biotypes IV and V were widely spread throughout the country, though a few MRPs and biotype I were clustered around certain areas. Analysis of subsequent isolates from 25 beekeepers usually showed persistence of the infection in the apiaries, but in six cases unrelated infections emerged. Typing of contemporary isolates from one beekeeper revealed the presence of the same infection in distant apiaries, thus indicating that the spread of infection was through apicultural practices instead of neighbourhood. *Not*I PFGE allowed efficient strain discrimination and can be recommended for epidemiological surveillance of AFB.

***Apis mellifera* / *Paenibacillus larvae* / American foulbrood / *Not*I pulsed-field gel electrophoresis / biotyping**

1. INTRODUCTION

American foulbrood (AFB) is the most serious brood disease of the honey bee (*Apis mellifera* L.). The disease is caused by the Gram-positive spore-forming bacterium *Paenibacillus larvae* (Ashiralieva and Genersch, 2006; Genersch et al., 2006). AFB has spread around the world and causes serious financial losses to beekeepers. It is the most important bee disease in the Finnish apiculture. It is a notifiable disease and there are regulations for its control. The infection is highly prevalent all over the country. For instance, 22% of examined 2428 honey and 43 brood samples were found to contain *P. larvae* spores in 2007 (unpubl. data).

AFB is spread vertically in colonies and horizontally by the bee trade, contaminated equipment or insertion of used or contaminated brood or honeycombs into the hives (Fries et al., 2006). Bees can be fed with contaminated honey or pollen, or they become infected when robbing honey from weakened colonies (Hansen and Brødsgaard, 1999). The infection can remain subclinical in a colony for several years (Fries et al., 2006). The development of a disease outbreak is thought to depend on the resistance of the bees, the infection load, as well as on the virulence of the infective strain (Otten, 2003; Genersch et al., 2005; Ashiralieva and Genersch, 2006; Behrens et al., 2007).

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Pulsed-field gel electrophoresis (PFGE) can be regarded as "golden standard" for molecular subtyping of bacterial pathogens (Barrett et al., 2004). We previously employed the method which utilizes *Not*I restriction to re-evaluate the taxonomy of *P. larvae* subsp. *pulvifaciens* and *P. larvae* subsp. *larvae* (Genersch et al., 2006). The two subspecies were reclassified as one species, but in PFGE the orange-pigmented field isolates of the former subspecies *pulvifaciens* clustered separately from the other strains. PFGE using *Xba*I was employed to identify the origin of oxytetracycline resistant *P. larvae* strains in Australia (Wu et al., 2005). At present, PFGE has not been used for epidemiological studies of AFB. Most reports on molecular subtyping of *P. larvae* isolates are based on PCR of conserved motifs in bacterial repetitive REP, ERIC and BOX elements (Alippi and Aguilar, 1998; Genersch and Otten, 2003; Neundorf et al., 2004; de Graaf et al., 2006; Peters et al., 2006; Antúnez et al., 2007).

The aim of this study was to develop a highly discriminatory PFGE method and use it for molecular epidemiological characterisation of *P. larvae* infection regionally in Finland and temporally in the apiaries of individual beekeepers. The isolates were also biotyped as described by Jeliński (1985) to analyse the relation between genotype and biotype. In addition, the susceptibility of *P. larvae* isolates to tetracycline was determined.

2. MATERIALS AND METHODS

2.1. *Paenibacillus larvae* isolates

P. larvae isolates from Finnish honey and brood samples were chosen from the culture collection of the national reference laboratory at Veterinary Bacteriology Unit, Research Department, Evira, to represent the whole country, though still taking into account the main apicultural areas. The isolates were from years 1997, 1999 and 2001, and had been stored at –70 °C. They had been identified from pure cultures on blood agar. The identification criteria were typical colony morphology and "potato-cellar" odour, typical Gram-staining, and negative catalase test and positive oxidase test. All isolates were non-pigmented. During the study period, only one beekeeper had orange-pigmented *P. larvae* isolates in his apiaries. These isolates which were previously classified as subspecies *pulvifaciens* were not available for this epidemiological study. From a previous study it is known that they form their own cluster in PFGE (Genersch et al., 2006).

Altogether 157 isolates were analyzed; 156 isolates from 114 Finnish beekeepers covering all beekeeping areas in the country, and type strain ATCC 9545. Twenty-five beekeepers (T1-T25) had isolates from different years (Tab. I). Six of them had *P. larvae* isolates from three years. One strain was selected from each beekeeper from each year. From one beekeeper (T26) we analyzed 13 isolates from samples taken from 11 regionally diverse apiaries in the same year, 1999.

2.2. PFGE

Preparation of bacterial chromosomal DNA in agarose plugs, the *Not*I digestion and PFGE analysis were performed as previously described (Genersch et al., 2006). In addition to *Not*I, ten other restriction enzymes were tested (*Apa*I, *Alw*44, *Bln*I, *Mlu*I, *Ngo*MIV, *Sfi*I, *Sma*I, *Spe*I, *Xba*I and *Xho*I), but their banding patterns were not so discriminatory or easy to interpret as those obtained using *Not*I. Macrorestriction profiles (MRPs) were analyzed visually and with the GelCompar II (Applied Maths, Belgium) program using UPGMA with the Dice coefficient to create a dendrogram. Each profile showing a one-band difference from another profile was regarded as a MRP and was given a number (MRP1 to MRP53).

2.3. Biotyping

Fermentation of salicin and mannitol was performed by inoculating 2 to 3 colonies of *P. larvae* to tubes containing 1% (w/v) of salicin or mannitol, 0.01% (w/v) of bromthymolblue, 1% (w/v) of neopeptone (Difco), 1% (w/v) of tryptone (Difco) and 0.5% (w/v) of NaCl in distilled water. The nitrate reduction test was conducted by inoculating 1 to 2 colonies of *P. larvae* to nitrate tubes containing 0.1% (w/v) KNO_3, 0.5% (w/v) tryptone, 0.5% (w/v) neopeptone, 0.05% (w/v) glucose and 0.25% (w/v) agar in distilled water. The tubes were incubated at 35 °C for at least 10 days. Biotypes were named according to Jeliński (1985) with Roman numerals.

Table I. *Not*I MRPs and biotypes of subsequent *P. larvae* isolates from 25 beekeepers in 1997–2001 and of 13 contemporary isolates of beekeeper T26.

Bee-keeper	Year 1997		Year 1999		Year 2001	
	MRP	Biotype	MRP	Biotype	MRP	Biotype
T1	2	V	2	V		
T2	31	I	31	I		
T3			31	I	37	I
T4	27	IV	27	IV		
T5	20	V	20	V	47	V
T6			15	V	15	V
T7	20	V	22	V	20	V
T8			1	V	1	V
T9	22	V	22	V		
T10	23	I	2	V		
T11	2	V	2	V		
T12			2	V	2	V
T13			47	V	47	V
T14			47	V	47	V
T15	1	VI	1	V	45	IV
T16	25	V	20	V		
T17	34	IV	34	IV		
T18			21	V	1	IV
T19			6	V	1	V
T20			7	V	7	V
T21	1	V	1	V		
T22	38	V	39	V	40	V
T23	27	IV	45	V	27	IV
T24	11	V	50	V	50	V
T25			34	IV	34	V
T26			2*, 3, 4, 44	V		

*10 isolates with MRP2.

2.4. Tetracycline susceptibility testing

The MIC values of *P. larvae* isolates for tetracycline were determined using E-test (AB Biodisk). Colonies from 2 to 3 day-old agar culture were suspended with a cotton swab in 0.9% NaCl (w/v) to obtain a turbidity of 0.5 McFarland. Blood agar plates were inoculated and E-test strips were adjusted according to the manufacturer's specifications. Plates were incubated at 35 °C for 2 days before reading the result.

3. RESULTS

3.1. *Not*I macrorestriction profiles

The analysed 156 *P. larvae* isolates and the ATCC strain could be classified into 53 *Not*I MRPs (Fig. 1). The profiles consisted of more than 20 bands. The exact counting of the bands was difficult, and only bands between 50 to 400 kbp were included in the GelCompar II analysis. The profiles were also compared visually. The profiles had many conserved fragments and their genetic similarity was over 0.85. The most extensive variation was seen in the bands corresponding to fragment sizes of 50 to 130 kbp. The dendrogram generated from the MRPs had two major clusters: MRP1-MRP44 containing 128 isolates and the ATCC strain, and MRP45-MRP50 with 25 isolates. The three remaining profiles MRP51-MRP53, each with one isolate, could not be assigned to either of the major clusters.

Two closely related profiles, MRP1 and MRP2, were the most common with 24 and 25 isolates, respectively (Fig. 1). However, MRP1 was the most widely spread in the

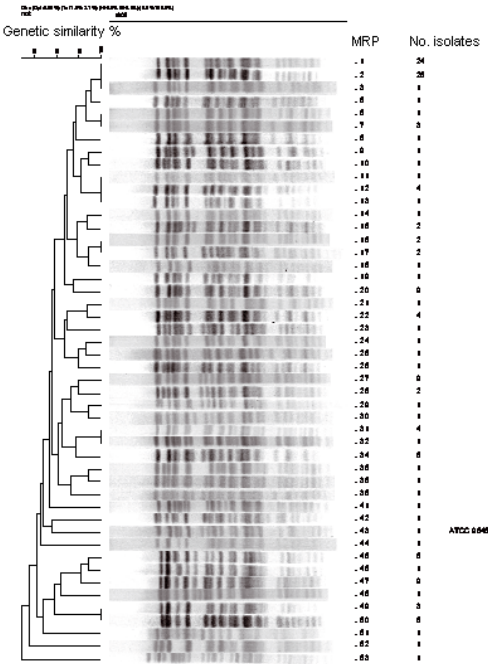

Figure 1. Macrorestriction profiles (MRP) obtained from *Paenibacillus larvae* by *Not*I PFGE and the number of isolates within profiles.

country since these isolates were obtained from 21 different beekeepers. MRP2 isolates originated from the apiaries of 13 different beekeepers. Three profiles, MRP20, MRP27 and MRP47, occupied the third place, each with nine isolates. Other MRPs had only a few or one isolate, and they were isolated only from a single beekeeper. None of the Finnish isolates was genetically identical with the ATCC strain.

Most genotypes that were found from several beekeepers were spread over geographically distant parts of Finland. However, some genotypes showed clustering in certain areas. The most common genotype MRP1 was found from the apiaries of six beekeepers in the city of Lapua in the western Finland (Fig. 2). All the six MRP50 isolates were from the hives of five beekeepers in North Carelia. Three beekeepers in the city of Orimattila had closely related or identical strains with MRP types 31 and 37 in their apiaries.

3.2. Biotypes

Biotype V (positive for salicin, negative for mannitol, positive for nitrate reduction) was the most common, accounting for 117 isolates (75%) and included 38 MRPs. Biotype IV (positive for salicin, negative for mannitol, negative for nitrate reduction) was represented by 28 isolates (18%) and included 13 MRPs. Biotype I (negative for salicin, negative for mannitol, negative for nitrate reduction) had 14 isolates (7%) and included 10 MRPs. Sixteen MRPs were shared by two or more *P. larvae* isolates; half of these MRPs contained more than one biotype. Five biotype I-isolates were from three apiaries in Orimattila (Fig. 2), four isolates with profile MRP31 and one with a closely related profile, MRP37. Identical or closely related MRP types (31, 35, 36 and 37), all of biotype I, were also found in southern, central and northern Finland.

3.3. Persistence and diversity of infective strains

The persistence of certain genotypes and biotypes in the apiaries over a four-year period was investigated by analysing isolates from subsequent samples supplied by the beekeepers. Nineteen beekeepers had isolates from two different years and six beekeepers had isolates from every investigated year (Tab. I). In most cases, the genotype remained the same or the isolates were genetically closely related (Fig. 3, T22). In the case of six beekeepers (T5, T10, T15, T19, T23 and T24), there were 6 to 7-band differences in the banding patterns between subsequent isolates, indicating that these isolates were not closely related (Tenover et al., 1995). The isolates from apiaries T5, T15, T23 and T24 even belonged to different major MRP clusters (Fig. 3; T23 shown). The same biotype was consecutively isolated from 20 beekeepers (Tab. I).

Thirteen isolates from 11 distantly located apiaries of beekeeper T26 in 1999 were divided into four closely related genotypes. Ten isolates were identical (MRP2) and the profiles MRP3, MRP4 and MRP44 of the remaining three apiaries differed from MRP2 by one

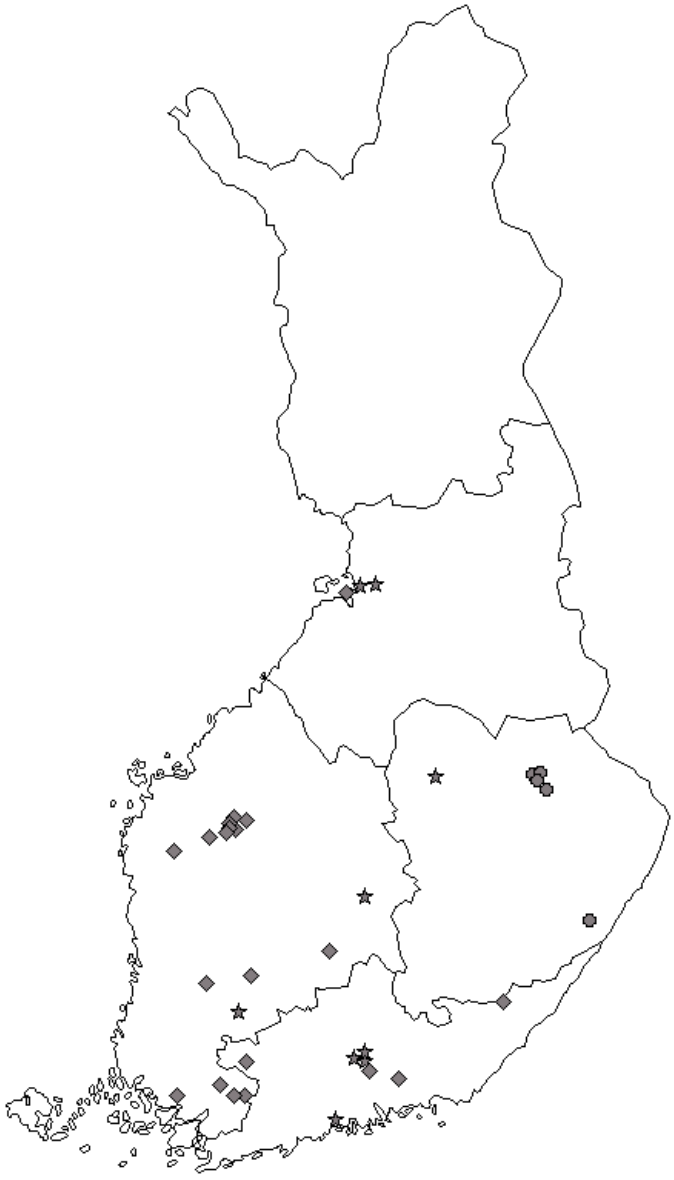

Figure 2. Occurrence of *Paenibacillus larvae* types MRP1 (squares), MRP50 (crosses), and MRP31 or MRP37 (stars) in Finland.

or two bands (Fig. 3). All isolates belonged to biotype V.

3.4. Tetracycline susceptibility

All *P. larvae* isolates were susceptible to tetracycline with MIC values ranging from 0.023 to 0.19 μg/mL.

4. DISCUSSION

PFGE using *Not*I restriction enzyme was found to be an efficient method to achieve strain differentiation of *P. larvae* isolates. DNA degradation was prevented by keeping the samples chilled and working swiftly before proteinase K treatment. Alternatively, DNA

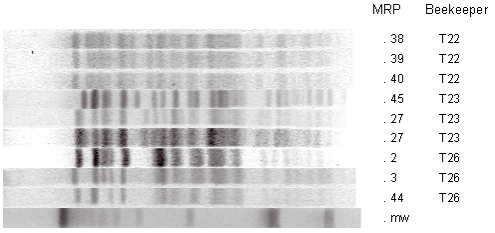

Figure 3. MRPs of subsequent isolates from beekeepers T22 and T23, and of contemporary isolates from beekeeper T26.

degradation could be prevented with formaldehyde treatment (Hielm et al., 1998). Djordjevic et al. (1994) and Alippi and Aguilar (1998) also reported difficulties in preserving bacillus DNA. Eleven restriction enzymes were tested to obtain a DNA banding pattern which was easy to interpret. *Not*I cut the genome into more than 20 fragments, but it was still possible to interpret the banding patterns.

Altogether 53 MRPs were detected using *Not*I restriction, when a one-band difference separated one profile from another. Among 156 Finnish *P. larvae* isolates there were 52 MRPs. Most of the *P. larvae* isolates would have been closely related according to the interpretation criteria proposed by Tenover et al. (1995) devised mainly for hospital outbreak investigations. Experience from PFGE subtyping in epidemiological studies has shown that specific criteria are needed for each organism or even their subspecies, as genetic diversity can vary extensively between organisms (Barrett et al., 2004).

The genetic similarity between all banding patterns was over 85%, indicating high genetic homogeneity among the *P. larvae* strains. Eight strains of this study were previously compared with *P. larvae* strains from Germany and Sweden using *Not*I PFGE (Genersch et al., 2006). The Finnish strains, all with different MRPs, clustered together with fourteen strains from Sweden, three strains from Germany and type strain ATCC 9545. All these strains were non-pigmented and typed to the ERIC I pattern by repetitive element PCR. The genetic similarity within the cluster was 85%, the same value as observed among all Finnish isolates in the present study. Inclusion of pigmented variants of *P. larvae* of ERIC II, III and IV groups in the *Not*I PFGE analysis declined the genetic similarity value to 77% (Genersch et al., 2006). To fully characterise the genetic diversity of *P. larvae* species, more isolates from world-wide collections need to be analysed with a harmonised PFGE method.

*Not*I PFGE was highly discriminatory compared to the number of genotypes reported using BOX-PCR and ERIC-PCR, ribotyping or analysis of sequence heterogeneities of 16S rRNA gene (Alippi and Aguilar, 1998; Genersch and Otten, 2003; Peters et al., 2006; Antúnez et al., 2007). As PFGE is widely used and available in most reference laboratories analysing bacterial diseases, it can be recommended for epidemiological studies of AFB.

Most genotypes that were found from several beekeepers were spread over geographically distant parts of Finland, probably by trade. However, some genotypes showed clustering in certain areas. Most probably AFB infection had been introduced to those areas and then spread to other apiaries in the region. Other molecular epidemiological studies using different methods have shown similar clustering of genotypes in certain geographical areas (Djordjevic et al., 1994; Alippi and Aguilar, 1998; Genersch and Otten, 2003; Peters et al., 2006).

When subsequent isolates from 25 beekeepers were analysed from the years 1997, 1999 and 2001, the genotypes in most cases were identical or only exhibited minor changes. The biotype remained the same in 80% of the apiaries, and in these cases the MRP of the isolates remained also closely related. These results reflect the long persistence of *P. larvae* spores in beehives (Fries et al., 2006). Six beekeepers, however, had hives which had clearly been infected with different strains during the study period. The strains found in samples from four beekeepers even belonged to different major MRP clusters. These six beekeepers are located in different parts of the country, which illustrates that new infections attack the apiaries both in intensive (south-west) and extensive (central-eastern) apicultural areas.

Beekeeper T26 had nearly 100 apiaries in western Finland. Of the 110 honey and 15 brood samples he sent to us during the

summer of 1999, 70% were infected with *P. larvae* spores. The apiaries had on average three hives. We analysed 13 isolates from 11 distantly located apiaries sampled in 1999. Ten isolates were genetically identical and closely related with the remaining three isolates. Thus there seemed to be only one infection causing AFB in all apiaries of the beekeeper. The apiaries were located in an intensive apicultural area (south-west) where AFB is very common and genotypically diverse *P. larvae* strains were detected. Genetic analysis of the *P. larvae* isolates demonstrated that the infection had spread within apiaries through contaminated beehives or equipment and not from other bees in the neighbourhood.

The Finnish *P. larvae* strains were divided into three different biotypes (I, IV and V) described by Jeliñski (1985). According to Heyndrickx et al. (1996) *P. larvae* produces acid from salicin, but not from mannitol. Nitrate reduction varies between strains. In our study, 94% of the isolates produced acid from salicin, but none produced acid from mannitol. Nitrate was reduced only by 75% of the isolates. Only 7% of the Finnish *P. larvae* isolates were salicin and nitrate-negative. Both nitrate positive (biotype IV) and nitrate negative (biotype V) phenotypes were associated with genotype MRP1 among the strains originating from the same area. This suggests that closely related or identical strains may be classified to biotype IV or V because of the variation in nitrate reduction.

The use of antimicrobial drugs for honey bees is not recommended in Finland, and no antibiotics are registered for use in bees. Drugs can be used according to the cascade rule. The most common drug prescribed by veterinarians is oxytetracycline. In this study all the Finnish isolates were susceptible to tetracycline. The highest MIC value was 0.19 μg/mL which is well below the reported upper limit <4 μg/mL for susceptible strains (Alippi et al., 2007).

This study characterises only infections caused by non-pigmented strains since pigmented *P. larvae* strains are extremely rare in Finland; only one beekeeper had orange-pigmented strains in his apiaries in 1997–2001. We have recently shown that orange-pigmented isolates cluster together in *Not*I PFGE analysis and differ from the non-pigmented strains (Genersch et al., 2006). In biotyping, the Finnish pigmented *P. larvae* strains have the characteristics of biotype III: mannitol positive, salicin negative and nitrate negative (unpubl. data). The biotype III was not detected among the isolates examined in this study. Thus the orange-pigmented *P. larvae* strains differ by both their biotype and genotype from the non-pigmented strains found in Finland.

In conclusion, the epidemiology of *P. larvae* infection could be efficiently studied by using *Not*I PFGE analysis. We showed that several strains of *P. larvae* had spread throughout the country, possibly by several routes such as trading of contaminated bees and beehives. Once the disease had become established in a new area, both beekeepers and honey bees easily spread it further. Molecular typing also demonstrated the spread of a particular infective strain within distantly located apiaries of one beekeeper, which had occurred most likely through apicultural practice. *Not*I PFGE analysis can be recommended as a complementary epidemiological tool for the surveillance of AFB, to trace infection sources, to plan control measures and to assess their efficacy.

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Épidémiologie moléculaire de l'infection à *Paenibacillus larvae*.

***Apis mellifera* / *Paenibacillus larvae* / loque américaine / biotypage / électrophorèse en champ pulsé *Not*I**

Molekulare Epidemiologie der Infektion mit *Paenibacillus larvae* in Finnland. Die Amerikanische Faulbrut (AFB) ist die schwerwiegendste Brutkrankheit der Honigbienen (*Apis mellifera*). Sie wird durch das Gram-positive, Sporen bildende Bakterium *Paenibacillus larvae* verursacht. In Finnland ist AFB eine häufige Krankheit, deren Behandlung durch das finnische Gesetz festgelegt ist.

AFB wird durch verunreinigte Ausrüstung und Bienenfutter, durch infizierte Honigbienen und die Honigräuberei zwischen Bienenvölker übertragen.
Die Epidemiologie von AFB kann durch molekulare Subtypisierung von *P. larvae* Isolaten untersucht werden. Pulsfeld-Gelelektrophorese (PFGE) gilt als der „Goldene Standard" für die molekulare Subtypisierung bakterieller Pathogene, diese wurde allerdings bisher nicht für epidemiologische Untersuchungen von AFB eingesetzt. Ziel dieser Untersuchung war die Entwicklung einer hochgradig unterscheidungsfähigen PFGE-Typisierungsmethode und darüber hinaus die Beschreibung der Epidemiologie von *P. larvae* sowohl regional in Finnland als auch zeitlich in den Völkern einzelner Imker.
Eine *Not*I PFGE-Analyse unterteilte die Isolate in 52 Subtypen und unterschied sie von der ATCC-Typ Linie. Auf Grund ihrer genetischen Ähnlichkeit gruppierten sich die meisten Subtypen in zwei Hauptzweigen, von denen einer 44 Subtypen mit 128 Isolaten und die ATCC Linie enthielt, der andere enthielt 6 Subtypen mit 25 Isolaten. Drei der Subtypen konnten keinem der Hauptzweige zugerechnet werden. Die Biotypen der Jeliñski-Klassifikation wurden anhand der Fermentation mit Salizin und Mannitol und der Reduktion von Nitrat bestimmt. Es wurden nur die Biotypen I, IV und V vorgefunden, 75 % der Isolate reduzierten Nitrat. In allen Subtypen kamen verschiedene Biotypen vor. Die MIC-Werte gegenüber Tetracyklin wurden mit dem E-Test bestimmt und bewegten sich zwischen 0,023 und 0,190 μg/mL, dies zeigte eine hohe Empfindlichkeit an.
Die meisten der bei einigen Imkern gefundenen PFGE-Subtypen hatten sich über geografisch entfernte Gebiete von Finnland verbreitet, es wurden aber auch regionale Häufungen beobachtet. Bei der Untersuchung von über vier Jahre aufeinander folgenden Isolaten von 25 Imkern zeigte sich, dass die Genotypen und Biotypen in den meisten Fällen gleich blieben oder nur geringfügige Änderungen aufwiesen. In sechs Fällen traten Infektionen ohne Bezug auf. Die Typisierung von 13 gleichzeitigen Isolaten aus 11 Bienenständen eines Imkers ergab die gleiche infektiöse Linie in allen Bienenständen, dies belegte die Verbreitung der Infektion innerhalb der Bienenstände, aber nicht aus der Nachbarschaft. Die *Not*I PFGE Analysis erlaubte eine effiziente Linienunterscheidung und kann daher als epidemiologisches Werkzeug der Überwachung von AFB empfohlen werden, um damit die Ausbreitung der Infektionen zu untersuchen oder um Behandlungsmaßnahmen zu planen und ihre Wirksamkeit zu erfassen.

***Apis mellifera* / *Paenibacillus larva*e / Amerikanische Faulbrut / *Not*I Pulsfeld-Gelelektrophorese / Biotypisierung**

REFERENCES

Alippi A.M., Aguilar O.M. (1998) Characterization of isolates of *Paenibacillus larvae* subsp. *larvae* from diverse geographical origin by the polymerase chain reaction and BOX primers, J. Invertebr. Pathol. 72, 21–27.

Alippi A.M., López A.C., Reynaldi D.H.G., Grasso D.H., Aguilar O.M. (2007) Evidence for plasmid-mediated tetracycline resistance in *Paenibacillus larvae*, the causal agent of American foulbrood (AFB) disease in honeybees, Vet. Microbiol. 125, 290–303.

Antúnez K., Piccini C., Castro-Sowinski S., Rosado A.S., Seldin L., Zunino P. (2007) Phenotypic and genotypic characterization of *Paenibacillus larvae* isolates, Vet. Microbiol. 124, 178–183.

Ashiralieva A., Genersch E. (2006) Reclassification, genotypes and virulence of *Paenibacillus larvae*, the etiological agent of American foulbrood in honeybees - a review, Apidologie 37, 411–420.

Barrett T.J., Ribot E., Swaminathan B. (2004) Molecular subtyping for epidemiology: issues in comparability of patterns and interpretation of data, in: Persing D.H., Tenover F.C., Versalovic J., Tang Y.-W., Unger E.R., Relman D.A., White T.J. (Eds.), Molecular Microbiology: Diagnostic Principles and Practice, ASM Press, Washington DC, pp. 259–266.

Behrens D., Forsgren E., Fries I., Moritz R.F.A. (2007) Infection of drone larvae (*Apis mellifera*) with American foulbrood, Apidologie 38, 281–288.

de Graaf D.C., Alippi A.M., Brown M., Evans J.D., Feldlaufer M., Gregorc A., Hornitzky M., Pernal S.F., Schuch D.M.T., Titera D., Tomkies V., Ritter W. (2006) Diagnosis of American foulbrood in honey bees: a synthesis and proposed analytical protocols, Lett. Appl. Microbiol. 43, 583–590.

Djordjevic S., Ho-Shon M., Hornitzky M. (1994) DNA restriction endonuclease profiles and typing of geographically diverse isolates of *Bacillus larvae*, J. Apic. Res. 33, 95–103.

Fries I., Lindström A., Korpela S. (2006) Vertical transmission of American foulbrood (*Paenibacillus larvae*) in honey bees (*Apis mellifera*), Vet. Microbiol. 114, 269–274.

Genersch E., Otten C. (2003) The use of repetitive element PCR fingerprinting (rep-PCR) for genetic subtyping of German field isolates of *Paenibacillus larvae* subsp. *larvae*, Apidologie 34, 195–206.

Genersch E., Ashiralieva A., Fries I. (2005) Strain- and genotype-specific differences in virulence of *Paenibacillus larvae* subsp. *larvae*, a bacterial pathogen causing American foulbrood disease in honeybees, Appl. Environ. Microbiol. 71, 7551–7555.

Genersch E., Forsgren E., Pentikäinen J., Ashiralieva A., Rauch S., Kilwinski J., Fries I. (2006) Reclassification of *Paenibacillus larvae* subsp. *pulvifaciens* and *Paenibacillus larvae* subsp. *larvae* as *Paenibacillus larvae* without subspecies differentiation, Int. J. Syst. Evol. Microbiol. 56, 501–511.

Hansen H., Brødsgaard C. (1999) American foulbrood: a review of its biology, diagnosis and control, Bee World 80, 5–23.

Heyndrickx M., Vandemeulebroecke K., Hoste B., Janssen P., Kersters K., de Vos P., Logan N.A., Ali N., Berkeley R.C.W. (1996) Reclassification of *Paenibacillus* (formerly *Bacillus*) *pulvifaciens* (Nakamura 1984) Ash et al. 1994, a later subjective synonym of *Paenibacillus* (formerly *Bacillus*) *larvae* (White 1906) Ash et al. 1994, as a subspecies of *P. larvae*, with emended descriptions of *P. larvae* as *P. larvae* subsp. *larvae* and *P. larvae* subsp. *pulvifaciens*, Int. J. Syst. Bacteriol. 46, 270–279.

Hielm S., Björkroth J., Hyytiä E., Korkeala H. (1998) Genomic analysis of *Clostridium botulinum* group II by pulsed-field gel electrophoresis, Appl. Environ. Microbiol. 64, 703–708.

Jeliński M. (1985) Some biochemical properties of *Bacillus larvae* White, Apidologie 16, 69–76.

Neuendorf S., Hedtke K., Tangen G., Genersch E. (2004) Biochemical characterisation of different genotypes of *Paenibacillus larvae* subsp. *larvae*, a honey bee bacterial pathogen, Microbiology 150, 2381–2390.

Otten C.A. (2003) A general overview on AFB and EFB pathogen, way of infection, multiplication and outbreak, Apiacta 38, 106–113.

Peters M., Kilwinski J., Beringhoff A., Reckling D., Genersch E. (2006) American foulbrood of the honey bee: Occurrence and distribution of different genotypes of *Paenibacillus larvae* in the administrative district of Arnsberg (North Rhine-Westphalia), J. Vet. Med. B Infect. Dis. Vet. Public Health 53, 100–104.

Tenover F.C., Arbeit R.D., Goering V., Mickelsen P.A., Murray B.E., Pershing H., Swaminathan B. (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field electrophoresis: criteria for bacterial strain typing, J. Clin. Microbiol. 33, 2233–2239.

Wu X.-Y., Chin J., Ghalayni A., Hornitzky M. 2005. Pulsed-field gel elecctrophoresis typing and tetracycline sensitivity of *Paenibacillus larvae* subsp. *larvae* isolates of Australian origin and those recovered from honey imported from Argentina, J. Apic. Res. 44, 87–92.