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Scientific note

A scientific note on the presence of *Paenibacillus larvae larvae* spores in sub-Saharan African honey

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***Apis mellifera* / honey / *Paenibacillus larvae larvae* / American foulbrood**

The bacterium *Paenibacillus larvae larvae* causes American foulbrood (AFB) in honey bee larvae. According to Matheson (1996) the disease is reported worldwide, except in sub-Saharan Africa. The only report of the disease in South Africa is by Davison et al. (1999) who mentioned that W. Ritter identified one sample from South African bee colonies as AFB. Following this, honeys collected from 57 apiaries were examined for *P. l. larvae* using PCR techniques. No *P. l. larvae* was found in this survey and it was concluded that AFB was not present in South Africa (Davison et al., 1999).

In October 2001, we sampled ten South African honeys, all retailed by wholesale-dealers. In January 2002, four honeys were sampled in Gambia. Three of the samples were from Gambian apiaries and one sample was imported from Guinea Bissau. The honey was examined for *P. l. larvae* spores using direct inoculation on J-agar plates as described by Hansen (1984) with the modification that nine plates were used and the honey solution used contained 0.24 g honey per three plates. Furthermore, 5% CO_2 was added to the incubation atmosphere to improve the sensitivity of the method (Hornitzky and Nicholls, 1993). 3 µg/mL nalidixic acid was added to the medium to prevent overgrowing by other bacteria (Hornitzky and Clark, 1991). Four repetitions were made. If colonies resembled *P. l. larvae* the strains were characterized by morphology of colonies (Hansen and Rasmussen, 1986), microscopy of Gram stained cells, phase contrast microscopy of spores, catalase activity (Gordon et al., 1973), and by their ability to use 50 carbon sources tested by the API50CH-system (bioMérieux) as described by Carpana et al. (1995). The identity of *P. l. larvae* was further confirmed by polymerase chain reaction (PCR) using specific primers as described by Govan et al. (1999). Determination of the bacteria was made on the basis of all these diagnostic features.

In two of the analyzed South African honeys (SA06 and SA10) and in one sample from Guinea Bissau (GNB10) contamination with *P. l. larvae* spores were found. In all three honeys only a light contamination was found. Table I shows the result of the api50CHβ analysis, including the reference strain ATCC9545 and the Danish reference strain JT-79. The GNB10 sample fermented the same sugars as SA06 and JT-79. The South African strain SA10 differed from SA06 in its ability to ferment mannose and tagatose but resembled ATCC in this way. The identity of the three identified *P. l. larvae* was confirmed by PCR according to Govan et al. (1999) as an amplicon of the expected size was generated for all three isolates.

All honey imported to South Africa is irradiated before it is marketed. This is done to ensure that honey bee pathogens are killed and infection of South African honey bee colonies is prevented. Thus, it should not be possible to find viable *P. l. larvae* spores in honey imported into and retailed in South Africa. The limited survey of honeys presented here is the first confirmed registration of the presence of *P. l. larvae* in honey from African bee colonies south of Sahara, in South Africa and Guinea Bissau, respectively. In all three contaminated honeys, only a light contamination was found. It is has previously been demonstrated that even a few batches of contaminated honey can contaminate a whole batch of blended, bottled honey retailed by wholesale-dealers (Hansen, 1984). Thus, the light contamination found suggests that

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Table I. Results of carbohydrate acidification by 5 *P. l. larvae* strains using the API 50CHß system (Carpana et al., 1995). The increasing strength of positive reactions in 3 steps is marked by 3, 4, 5 – indicates no reaction. The rest of the carbohydrates were not acidified.

Origin	ATCC	Denmark	South Africa	South Africa	Guinea Bissau
Strain	9945	JT-79	SA06	SA10	GNB10
Glycerol	4	4	4	5	3
Ribose	5	5	5	5	4
D-Glucose	4	4	4	4	4
D-Mannose	4	-	-	3	-
N Acetyl glucosamine	5	3	4	4	4
Salicine	4	-	-	-	-
Trehalose	5	3	4	4	4
D-Tagatose	5	-	-	3	-
5 ceto-gluconate	3	3	3	3	3

only a few batches may have been the contamination source of the South African honeys in this study. The strains were very similar in their ability to acidify certain sugars.

Several studies report that colonies without clinical symptoms of AFB may contain honey contaminated with *P. l. larvae* spores (e.g. Hansen and Rasmussen, 1986). Field experiments with inoculation of *P. l. larvae* spores have also shown that infected colonies may eliminate the infections and that no simple correlation exists between the number of spores in the honey and the first visible signs of AFB in capped brood cells (Hansen and Brødsgaard, 1997). Therefore, our study only indicates the presence of *P. l. larvae* in sub-Saharan African bee colonies and not that colonies with clinical symptoms of AFB are present.

Note scientifique sur la présence de spores de *Paenibacillus larvae larvae* dans les miels d'Afrique sub-saharienne.

Eine wissenschaftliche Notiz zum Vorhandensein von *Paenibacillus larvae larvae* Sporen in den Honigen der afrikanischen Sub-Sahara.

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