

NOTE

American Foulbrood in Uruguay: Isolation of *Paenibacillus larvae larvae* from Larvae with Clinical Symptoms and Adult Honeybees and Susceptibility to Oxytetracycline

American foulbrood (AFB) is a severe bacterial disease of honeybee brood caused by the spore-forming bacterium *Paenibacillus larvae larvae* (Heyndrickx *et al.*, 1996). The disease had been suspected by beekeepers in Uruguay for some years and although *P. l. larvae* spores were detected in 44 honey samples for export (OIE, 1999), a microbiological diagnosis from dead larvae and worker bees was lacking. We report here the isolation and characterization of *P. l. larvae* strains from larvae with clinical symptoms of AFB and adult honeybees obtained from Uruguayan apiaries. Since oxytetracycline hydrochloride (OTC) is often used to prevent AFB in Uruguay, we also assessed the susceptibility to OTC of several isolates.

Dead larval remains were obtained from apiaries with clinical symptoms of AFB located in three Uruguayan provinces on the Uruguay River littoral (Table 1) and isolation of *P. l. larvae* was performed according to Alippi (1996). Samples of 20 worker honeybees, taken from brood combs of hives belonging to affected or healthy apiaries, were crushed using a Stomacher (Seward). The resultant homogenate was heated at 80°C for 15 min and aliquots of the liquid phase were spread onto J medium (Hornitzky and Nicholls, 1993) agar plates with nalidix acid (Alippi, 1995). We randomly selected 17 isolates from larval and adult honeybee samples that were characterized by colony shape and margins, microscopic examination of gram-stained smears, and biochemical features. Presence of spores was determined by phase-contrast and scanning electron microscopy (SEM) of 10-day-old cultures (Suzuki *et al.*, 1995). Under a low-power objective, colonies showed irregular edges with typical branches. All examined isolates obtained from larvae and adult bees were gram-positive rods and spores were observed after 10 days. SEM observation showed ellipsoidal spores of approximately 1.2 µm in length and 0.6 µm in diameter, with a smooth surface and without traces of sporangia (Fig. 1). All isolates exhibited negative catalase and Voges–Proskauer reactions and were unable to withstand serial transfer in nutrient broth. They liquefied gelatine but neither hydrolyzed starch nor produced indol and could not use mannitol. *P. l. larvae*

was even isolated from adult honeybees belonging to an apiary without clinical symptoms. As Hornitzky and Karlovskis (1989) suggested, the culture of adult honeybees for the detection of *P. l. larvae* spores could be used to track subclinical infections. Our finding could indicate that the pathogen is spreading through the region and that infection may be in a subclinical stage in apiaries that do not show AFB symptoms. Oxytetracycline susceptibility patterns of *P. l. larvae* isolates were assessed by the disk diffusion method following the general guidelines of NCCLS (1993a) using disks containing 5 µg of OTC. Minimal inhibitory concentrations (MIC) of OTC were determined by the broth macrodilution susceptibility test (NCCLS, 1993b).

All isolates were susceptible to 5 µg of OTC, and MIC were <2.5 µg/ml in all assessed cases (Table 1). These

TABLE 1
Oxytetracycline HCl Inhibition Zones of *P. l. larvae* Isolates from Different Sources

Isolate	Diameter*	MIC ^a	Source	Location (Province)
OL1	45.5	<2.5	Larvae	Ombúes de Lavelle (Colonia)
OL4	50	—	Larvae	Ombúes de Lavelle (Colonia)
OL5	52.5	—	Larvae	Ombúes de Lavelle (Colonia)
OL6	53.7	—	Larvae	Ombúes de Lavelle (Colonia)
OL8	46.8	—	Larvae	Ombúes de Lavelle (Colonia)
NP1	49	<2.5	Adults	Nueva Palmira (Colonia) ^b
NP2	45	<2.5	Adults	Nueva Palmira (Colonia) ^b
NP3	52	<2.5	Adults	Nueva Palmira (Colonia) ^b
NP4	50	—	Adults	Nueva Palmira (Colonia) ^b
NP5	50.3	—	Adults	Nueva Palmira (Colonia) ^b
NP6	55	—	Adults	Nueva Palmira (Colonia) ^b
P4	50	<2.5	Larvae	Paysandú (Paysandú)
P7	49	<2.5	Larvae	Paysandú (Paysandú)
P10	47.3	—	Larvae	Paysandú (Paysandú)
P11	55	—	Larvae	Paysandú (Paysandú)
P1	55	<2.5	Adults	Paysandú (Paysandú) ^c
P5	52.3	<2.5	Adults	Paysandú (Paysandú) ^c

Note. The data represent one experiment showing the mean of diameters of inhibition zones of triplicate plates. Similar results were obtained from an additional experiment.

* Inhibition zone (mm) including disk.

^a Minimal inhibitory concentration (µg/ml).

^b Adult bee samples from a healthy apiary.

^c Adult bee samples from an affected apiary that showed AFB symptoms.

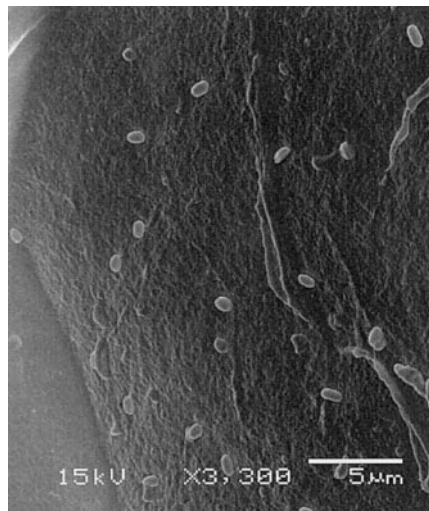


FIG. 1. Scanning electron microscopy of spores of *Paenibacillus larvae larvæ* P1 strain.

results could indicate that OTC resistance is still not a problem in the studied region. Recently, development of resistant strains of this pathogen has been reported in the United States by Miyagi *et al.* (2000), and the authors suggest that other antibiotics should be investigated for future control of AFB. Although studied Uruguayan *P. l. larvæ* isolates were OTC susceptible, care must be taken to avoid the application of antibiotics of poor quality, in inadequate doses, and without technical supervision.

We thank Eduardo Corbella and Ariel Arrambide for their assistance in sampling and fieldwork. This work was partially supported by Grant LIA 029 INIA-BID.

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Received March 14, 2001; accepted October 18, 2001

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