

Activity of Oxytetracycline against *Bacillus larvae*

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American Foul Brood (AFB) is an important disease of larval European honeybees (*Apis mellifera*) caused by the bacterium, *Bacillus larvae*. Only the spore stage of *B. larvae* can initiate the disease. One-day-old larvae are highly susceptible, whereas two-day-old larvae are highly resistant (Chen *et al.*, 1997). In Taiwan, AFB was reported in 1967 (Yen and Chyn, 1971) and is still an important disease. Oxytetracycline (OTC) has been widely used for AFB control, and methods of use have been postulated by Delaplane and Lozano (1994). Considerable work has also been carried out on the stability of OTC in honey stored by bees (Lehnert and Shimanuki, 1981; Matsuka and Nakamura, 1990; Wilson, 1974) and the stability of OTC in diets fed to bees for disease control (Gilliam, 1975). However, less work has been aimed at the control efficiency of OTC, and how long OTC can prevent effects of AFB in field colonies. This paper surveyed the activity of OTC against *B. larvae* *in vitro* and in apiary colonies to formulate an AFB control strategy.

Materials and Methods

In vitro test

Three antibiotics, OTC, chlorotetracycline (CTC) and lincomycin, were tested for bacteriostatic and bactericidal activities against *B. larvae* spores

(obtained from AFB scales) and vegetative cells (ATCC 9545) by broth dilution method. Each tube contained 4×10^6 cells (spores) per ml and were tested in BHIT broth at doses ranging from 0–400 μg per ml to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

Control efficiency in field colonies

Eight healthy hives with no history of AFB each of nine frames and approximately the same strength were selected. Four were fed 1 kg of sugar syrup containing 50 mg of OTC, and four were fed 125 mg of OTC. To determine the control efficiency, 90 one-day-old larvae were marked and inoculated with 21 spores, 442 spores or water only. Each colony was inoculated on Day -14, -2, 0, 3, 7, and 10. When treated larvae reached capping stage, all adult bees were removed and capped frames were placed in an incubator at 37°C and 70% R.H. Nine days later, treated bees were examined for signs of AFB.

Therapeutic effects on diseased colonies

Twenty-one diseased hives with either light (AFB signs <50) or heavy infection (AFB signs >500) were selected. Adult bees only were kept, all frames were discarded and replaced by disease-free combs. OTC medication was carried out on Day 5 after frame replacement. Doses were 50 mg per colony or 125 mg per colony. Colonies

Table 1. Minimal inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC) of three antibiotics to vegetative cells and spores of *Bacillus larvae*

Antibiotics	Vegetative cells		Spores	
	MIC	MBC	MIC	MBC
Oxytetracycline	0.016 µg/ml	0.125 µg/ml	0.125 µg/ml	>8.0 µg/ml
Chlorotetracycline	0.008 µg/ml	0.250 µg/ml	0.004 µg/ml	>8.0 µg/ml
Lincomycin	0.063 unit/ml	0.063 unit/ml	0.016 unit/ml	1.0 unit/ml

were surveyed weekly for AFB recurrence over 15 weeks.

Results and Discussion

In vitro test

The antimicrobial activity of OTC, CTC, and lincomycin is shown in Table 1. All these antibiotics may have good potential for controlling AFB in hives; however, OTC is the only approved medication in the USA and some other countries.

Control efficiency of OTC in field colonies

Although Delaplane and Lozaho (1994) postulated a control method—200 mg of OTC per colony repeated three times 4–5 days apart—this dose is probably too high for one-storey hives used in Taiwanese apiaries. In a preliminary study (Chen *et al.*, 1997), the pathogenicity of *B. larvae* to one-day-old larvae is 21 spores (LD₅₀) and 442 spores (LD₉₅). Treatment groups fed 125 mg of OTC showed good prevention of AFB with 0 % infection from Day (-2) to Day 7 of spore inoculation. Even at Day 10, treatment groups showed only 0.8 % infection. There were lower preventive effects in treatment groups fed 50 mg of OTC. They showed 0.8 % infection at Day 7 of inoculation.

Recovery of diseased colony

OTC has no sporicide effect, so all frames of diseased colonies should be discarded, if colonies are to be kept. By this method, treatment groups with light infection recovered without any drugs (Table 2). In groups with heavy infection, a dose of 125 mg OTC per colony was required to prevent recurrence of AFB.

Table 2. Date of AFB recurrence of infected colonies after comb replacement and OTC syrup treatment

Hive no.	Treatment ¹	AFB class before treatment ²	Date of AFB recurrence (weeks)
P ₁₋₄	125 mg OTC + R	**	n ³
O _{5-6,9}	50 mg OTC + R	**	n
O ₇₋₈	50 mg OTC + R	**	9
C ₁₋₃	R	**	3
C ₄	R	**	N
O ₁₋₄	50 mg OTC + R	*	n
C ₅₋₈	R	*	n
C ₉₋₁₂	N	-	N

Notes: (1) R = all combs replaced by disease-free combs; 125 mg OTC = fed 125 mg of OTC five days after R; 50 mg OTC = fed 50 mg of OTC five days after R; N = no treatment (control). (2) * = AFB signs < 50; ** = AFB signs > 500; - = AFB free. (3) n = no AFB recurrence.

References

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