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Study of the depletion of lincomycin residues in honey extracted from treated honeybee (*Apis mellifera* L.) colonies and the effect of the shook swarm procedure

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ABSTRACT

Bee colonies were treated with 1.2 g lincomycin hydrochloride per hive (single treatment in sucrose solution) and samples of honey were then collected at intervals over a 41-week period. The samples were analysed for lincomycin using Liquid Chromatography–Mass Spectrometry/Mass Spectrometry (LC–MS/MS). The highest mean concentration of lincomycin (pooled analytical results for brood and super honey) was 24 $\mu g\,g^{-1}$ 3 days after treatment, a mean of 3.5 $\mu g\,g^{-1}$ after 129 days. The shook swarm procedure was investigated and resulted in a lincomycin concentration of 34 $\mu g\,g^{-1}$ in honey (pooled results for brood and super honey) 3 days after treatment, declining to 0.38 $\mu g\,g^{-1}$ 129 days after treatment. Lincomycin was persistent in the hive and detected in all over winter (290 days after dosing) samples of honey collected from both non-shook swarmed and shook swarmed colonies. The results overall indicate that lincomycin parent is a suitable marker compound to detect lincomycin misuse in apiculture.

1. Introduction

Lincomycin along with other lincosamides comprises a group of antibacterial compounds that have a wide range of applications in the field of veterinary medicine. In therapeutic applications lincomycin is used to control certain Gram-positive bacteria [1]. EU Maximum Residue Limits for lincomycin vary between 50 $\mu g\,kg^{-1}$ in eggs to 1500 $\mu g\,kg^{-1}$ in kidney [2]. Lincomycin represents all of the microbiological activity of incurred residues in tissues, milk and eggs; the parent compound is therefore the recommended marker residue in all of these species.

Lincomycin, clindamycin, tylosin, erythromycin and monensin have been reported to be effective in the treatment of oxytetracycline resistant strains of *Paenibacillus larvae* ssp. *larvae* which can cause American Foulbrood Disease (AFB) [3–5] and tylosin is approved for use in the USA to treat American Foulbrood [6]. Within the European Union no antibacterial compounds are authorised for use in apiculture and therefore the detection of lincomycin residues (or any other antibacterial veterinary medicine), in honey is indicative of misuse. During the period from 2003 to 2007 there were *ca.* 170 EU rapid alerts (RASFFs) relating to veterinary medicines that had been detected in honey, of which 6, 17, 26 and 27 alerts related to the residues of oxytetracycline, tylosin, streptomycin and chloramphenicol, respectively [7]. More recently residues of lincomycin were detected in honey imported into the EU from China [7]. Consequently, there is an ongoing

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requirement for the development of methods and subsequent surveillance of retail honey to establish the extent of misuse of antibiotics in apiculture. Some EU countries enforce a tolerable limit of 10 $\mu g\,kg^{-1}$ on unspecified materials, which would include lincomycin residues in honey. There are few published methods for the determination of lincomycin in honey, although Thompson et al. [8] described a Liquid Chromatography–Mass Spectrometry/Mass Spectrometry (LC–MS/MS) method capable of detecting lincomycin at 5 $\mu g\,kg^{-1}$. Chemically, lincomycin is a lincoside that has tertiary amine moiety. It is normally purchased and used as its hydrochloride salt, which dissociates freely to lincomycin.

The main purpose of this study was to investigate the depletion of lincomycin in a hive system treated with lincomycin hydrochloride to ascertain if parent lincomycin is a suitable marker compound to detect the misuse of lincomycin in apiculture. Additionally the shook swarm procedure was investigated as a possible method to reduce residue concentrations in honey after treatment. Thompson et al. [9] reported the use of the shook swarm technique to successfully reduce concentrations of oxytetracycline in honey collected from treated bee colonies. Thus, the second aim of the project was to determine the persistence of lincomycin in honey with and without the application of the shook swarm procedure.

2. Methods and materials

2.1. *Bee colonies and treatments*

The study was carried out from May 2006 to March 2007. Eight (standardised) approximately uniform colonies with similar numbers of brood, honey surplus and free flying UK honeybees (*Apis mellifera* L.) were used in this study. The colonies were housed in Smith double brood boxes with 11 British standard frames (33.6 cm by 20.4 cm giving 685.4 cm^2 per side of brood frame) per brood box and at least one super box, with 18–20 frames of bees. The colonies were owned and maintained by the Central Science Laboratory (CSL), National Bee Unit. At the start of the trial these colonies showed no clinical signs of European or American foulbrood, sacbrood or baldbrood and had only a low incidence of chalkbrood. Six colonies were treated with lincomycin and were located at an experimental apiary approximately 10 km from two undosed control colonies that were established in parallel at the CSL site. This was to reduce the risk of cross-contamination by bees either drifting between colonies or 'robbing' behaviour.

The six colonies were treated with a solution of 1.2 g of lincomycin hydrochloride in 200–250 mL aqueous sucrose solution (50–60%, w/v) by pouring into the marked top empty brood frame. The treatment comb was placed in the top brood box, two frames in (usually on the edge of the brood nest with the treated side of the frame out). The two control colonies were fed with untreated sucrose using the same method of application. Seven days after treatment and honey sampling, two of the treated colonies were randomly selected and shook swarmed (as described in Thompson et al. [9]). The shook swarm treatment involved the transfer of the adult bees onto clean foundation with the brood and original frames being removed and destroyed. During winter, i.e. after October sampling, the colonies were fed with 50% (w/v) sucrose using a rapid tray feeder.

2.2. *Sampling*

The sampling time points are summarised in Table 1.

In May 2006, 2–4 days before treatment (D2 to D4) samples of up to 100 g of nectar/honey were taken from each colony to establish a baseline response for the colonies, i.e. to confirm antibiotic residues were not present. Samples of brood honey were collected at eight different time points during the bee-keeping season. Post-wintering samples were collected in March 2007, at D290.

Each sampling day, four comb samples (approximately 8 cm by 10 cm) were taken from each hive, two from the brood chamber and two from the super. The four samples were taken

Table 1 – Honey sampling plan from the 8 experimental hives (n = total number of honey samples collected from the hive treatment set)

Time point from dosing (days)	Treated hives without shook swarm procedure ($n=8$)	Treated hives with shook swarm procedure ($n=4$)	Control hives ($n=4$)
−4 to −2 (baseline sample)	√	√	√
3	√	√	×
7	√	√	√
14	√	×	√
21	√	×	√
28	√	×	√
56	√[a]	√[b]	√[c]
84	√	√	√
129	√	√	√[d]
290 (over winter)	√	√	√[e]

√: sampling; ×: no sampling.
[a] $n=16$.
[b] $n=8$.
[c] $n=6$.
[d] $n=3$.
[e] $n=1$.