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Original article

Controlling European foulbrood with the shook swarm method and oxytetracycline in the UK

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Abstract – Colonies infected with European foulbrood (EFB) were treated with the shook swarm method in combination with oxytetracycline (OTC) and compared with those treated with OTC alone, the usual treatment for EFB in England and Wales. Success rates and instances of recurrence in the following season were recorded in the seasons 2000 and 2001 respectively. Both treatments had similar success rates with respect to elimination of EFB in 2000. Shook swarm plus OTC treatment resulted in a lower level of EFB recurrence in the 2001 season than OTC treatment alone. Colonies treated with the shook swarm plus OTC method showed a recurrence rate of 4.8%, whereas those treated with OTC alone had a recurrence in 21.1% of cases. The differences were shown to be significant at the 10% level. These results suggest that the shook swarm plus OTC method could be a valid method for EFB treatment and control in the UK.

European foulbrood / shook swarm / oxytetracycline / disease control / alternative method

1. INTRODUCTION

European foulbrood (EFB) is a serious disease of honeybee (*Apis mellifera* L.) colonies, caused by the bacterium *Melissococcus plutonius*, previously known as *M. pluton* (Bailey and Collins, 1982). There are several other bacteria that are associated with the disease, including *Paenibacillus alvei*, *Brevibacillus laterosporus* and *Enterococcus faecalis*, although their role in the infection process is unclear (Bailey, 1963; Alippi, 1991). The disease affects honeybee brood and in serious cases may lead to colony loss. The bacterium is ingested by the larva during feeding by adult bees, then resides in the larval gut, and competes with it for food (Bailey and Locher, 1968; Bailey, 1983). If food is in short supply, the brood food will be scavenged by the bacteria rather than consumed by the larva, causing larval death by starvation and visible signs of EFB in the colony (Bailey, 1960). However, if there is plenty of food, the larva will develop normally and pupate, excreting the bacteria during development. This will leave potentially infective bacteria in the cell after the bee has hatched.

Relatively light infections, with few larvae within a colony showing clinical symptoms, can be treated with the bacteriostatic antibiotic oxytetracycline (OTC), which has been used since 1967 to treat EFB in the UK. OTC was found to be effective against EFB by Katznelson et al. (1952). In some countries OTC is also used against American foulbrood (AFB), another honeybee brood infection caused by spores of *Paenibacillus larvae* subsp. *larvae* (the two diseases are unrelated despite the similar names). In the US, beekeepers have used OTC as a cure and also a prophylactic measure against both AFB and EFB since the 1950s (Moeller, 1978; Lehnert

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and Shimanuki, 1980; Hoopingarner and Nelson, 1987; Kochansky, 2000), and resistance of *P. larvae* subsp. *larvae* to OTC has recently been reported in the US and South America (Alippi, 2000; Miyagi et al., 2000). Colonies with AFB are destroyed in the UK as the disease is highly virulent and treatment with OTC can also mask signs of EFB (Oldroyd et al., 1989). Spores of *P. larvae* subsp. *larvae* will not be affected by the antibiotic, which may lead to a further infection in the colony (Thompson and Brown, 1999). In both diseases, colonies are defined as being infected if they have overt clinical symptoms of disease; the presence of bacteria is not sufficient for them to be regarded as having an infection.

EFB is well established in England and Wales, with an average of 773 cases per year between 1991 and 2001. The major problem is the very high rate of recurrence of infections within apiaries after treatments, which averages at 27% for a subsequent season (Thompson and Brown, 2001). It is predictable as to which apiaries will have cases of EFB season after season, leading to the assertion that EFB is an apiary disease rather than one affecting individual colonies.

Under UK Bee Health legislation (The Bees Diseases Control Order, SI 1982 No. 107, 1982), infections of EFB can be treated with OTC (formulated as Terramycin®) if the colony is likely to recover from the infection. A recommendation for treatment will depend on the severity of infection and the size of the colony; if small (fewer than six frames of adult bees) or heavily infected (if more than half the brood in the colony show clinical signs of infection), colonies are usually destroyed. Routine treatment with OTC involves feeding the colony with a 1 g active ingredient dose, as described by Bailey and Ball (1991). However, this may not eliminate all of the bacteria present in larvae and on the brood comb, which may leave a 'reservoir of infection' leading to further infection in subsequent seasons. In recent years, an alternative form of treatment known as the 'shook swarm' plus OTC method has been used for EFB-affected colonies which aims to eliminate this potentially infective reservoir of bacteria. This old technique, used before the advent of antibiotics, involves the transfer of adult bees from the diseased colony into a new hive box with new foundation (Morse and Shimanuki, 1990). None of the brood comb is removed to the new colony; this is all destroyed. This 'new' colony is fed with sugar syrup containing a dose of OTC, which is thought to limit carry-over of bacteria on adult bees. The feeding of sugar stimulates the colony to draw out the foundation in order for the queen to start laying and the colony to re-establish itself. Removal of the potentially infective material should reduce the possibility of further EFB recurrence. It does not, however, affect the probability of infections being brought in from external sources.

The shook swarm technique (or a variant of it) has been used for the control of AFB in several countries, including France, Denmark and Australia (Jean-Prost, 1987; Brødsgaard and Hansen, 1999; Hornitzky and White, 2001). In Denmark, the approach without the use of antibiotics is routinely used for AFB control, apparently successfully. In Australia, several methods were investigated, including shaking bees onto irradiated combs from colonies that had AFB previously or onto foundation, either with or without a dose of OTC. Many of the colonies involved in the study died, and there was a relatively high level of AFB recurrence, although the most promising method appeared to be shaking bees onto foundation then feeding with OTC. This approach was taken in the current study.

This study aimed to determine whether shook swarm plus OTC treatment was at least as effective as OTC treatment alone within a season, and to determine rates of EFB recurrence in the following season in colonies treated with either of the two methods.

2. MATERIALS AND METHODS

2.1. Selection of colonies

Inspections for foulbrood diseases are carried out routinely as part of the Defra (Department for Environment, Food, and Rural Affairs) Bee Health Programme in England and the NAWAD (National Assembly for Wales Agriculture Department) scheme in Wales by Appointed Bee Inspectors (ABIs). Samples from suspect colonies are sent to the National Bee Unit diagnostic laboratory for

disease confirmation. If positive for EFB, recommendations for treatment or destruction are given depending on factors such as size of colony, extent of infection and possible recurrence of a previous infection. At the time of inspection by the ABI, beekeepers were asked if they would like to participate in the shook swarm plus OTC treatment trial if any of their colonies were positive for EFB. Selection was based on these indications from the ABI, therefore decisions were based both on the expertise of the field personnel and the agreement of the beekeeper.

2.2. Field trial set-up

The trial compared the two different treatments within apiaries to limit external factors such as availability of forage and weather conditions; these should be uniform for colonies in the same apiary. To take part in this trial, at least two colonies per apiary had to be visibly infected with EFB with both showing less than 50% affected brood. Ideally, colonies were also of similar size and had a similar extent of infection. One colony in the apiary was treated with the shook swarm plus OTC method, and the other corresponding colony with routine OTC treatment; allocations of treatment were chosen at random and both were carried out at the same time within the apiary. In total, 25 colonies were treated using shook swarm plus OTC and a corresponding 21 colonies were treated with OTC alone in 16 apiaries across England and Wales in 2000 in this trial. Treatments were carried out between the months of April and July. All colonies were also inspected in the 2001 season for recurrence of disease.

2.3. Treatment regimens

2.3.1. Routine OTC treatment

In England and Wales, all OTC treatments were carried out by an ABI, as the antibiotic is a controlled substance and can only be applied by an authorised person. The following protocol was used for routine treatment. A honey jar (approximately 250 mL) was filled with thick sucrose syrup (1 kg sucrose to 568 mL water) and a single dose of OTC (supplied by a designated veterinarian at the Veterinary Laboratories Agency, Weybridge, England) was added to the jar and mixed thoroughly. The resulting suspension, containing 1 g of active ingredient, was trickled into cells on an empty frame from the edge of the brood nest. Any remaining dosed sucrose was trickled onto the top bars away from unsealed brood. Direct application to the brood is not desirable, as the antibiotic is toxic at the concentration used. However, as the adult bees move the sugar syrup around the colony thus diluting the antibiotic, such effects are not evident. The colony was left for 8 weeks and then assessed for any further signs of disease, during which time the beekeeper was advised to feed the colony with sugar syrup if there was no nectar flow. A successful treatment was regarded as no clinical signs of EFB infection in a follow-up inspection after this period of time.

2.3.2. Shook swarm plus OTC treatment

The shook swarm plus OTC treatment was rather more complicated and involved preparation by the beekeeper. The diseased bee colony was moved a short distance from its original position, and a new hive box, full of new foundation and with a queen excluder under it, was put in its place. The infected colony was opened, and if possible, the queen was found and caged (to prevent loss). All the adult bees were shaken into the new hive box and the queen released once this was complete. All brood from the colony was destroyed by incineration. The 'new' colony was fed with sugar syrup containing antibiotic, at the same concentration as for routine OTC treatment. This should have ensured that any bacteria carried over by the adult bees were exposed to the antibiotic, and the feeding stimulated colony growth. In times of no honey flow, beekeepers were advised to keep feeding the colony until it was at full strength. As for routine treatment, colonies were assessed after 8 weeks and if free from disease, the treatment was considered successful.

2.4. Statistical analysis

The recurrence data was analysed using Fisher's Exact Test to determine whether the recurrence rates were significantly different between the treatment groups.

3. RESULTS

3.1. Success of treatments within the season

The results from the year 2000 season are shown in Figure 1. This shows the percentage of colonies that were successfully treated with either OTC alone or shook swarm plus OTC. Only OTC-treated colonies included in the shook swarm plus OTC treatment trial are included. The vast majority of treatments were

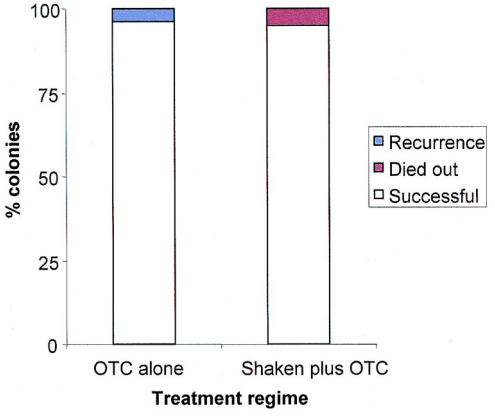

Figure 1. Shook swarm trial in 2000; outcomes of treatments. All colonies were recommended for treatment with OTC.

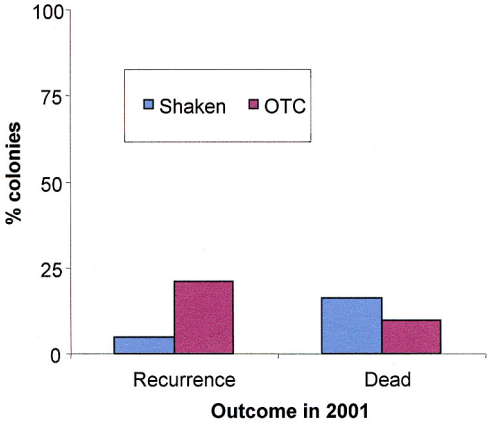

Figure 2. Recurrence of EFB in 2001 in surviving colonies either shaken or treated with OTC alone (as a comparison) in 2000. Colonies that died out before the assessment in 2001 but after the second inspection in 2000 are also shown.

successful within the season although some colonies died out or had a recurrence of EFB within the season, with most of the unsuccessful treatments being carried out towards the latter part of the season (data not shown).

3.2. Recurrence of infections in the subsequent season after treatments

Colonies involved in the shook swarm plus OTC treatment trial were assessed for recurrence of EFB after treatment. Figure 2 shows the percentage recurrence on a colony basis in the following season. Recurrence is defined as a further case of EFB in 2001 in a surviving colony treated in 2000. EFB recurred in colonies treated with OTC alone in 21.1% of cases. For colonies that were treated with shook swarm plus OTC, the recurrence rate was just 4.8% and the difference between the groups was significant at the 10% level, with a mid *P*-value of 0.08. However, it is notable that some of the colonies died out; four of the colonies treated with shook swarm plus OTC died out, compared to just two of those treated with OTC alone.

When EFB recurrence was examined across whole apiaries involved in this trial, a slightly different picture emerged (Fig. 3). This figure shows whether a subsequent EFB infection in the apiary was found in a colony that was treated with shook swarm plus OTC, one treated with OTC alone or a new infection

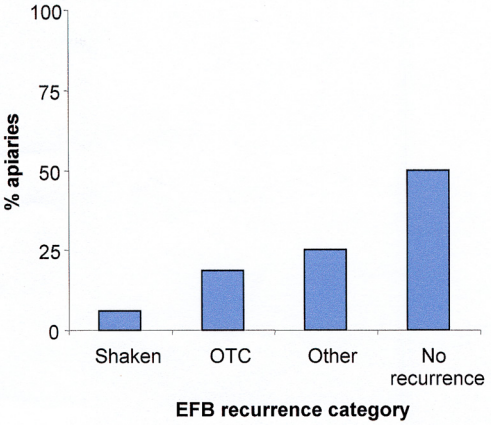

Figure 3. Recurrence of EFB in 2001 in apiaries with both shook swarm and OTC treated colonies. Figures indicate where in an apiary recurrence occurred, either in the treated colonies (shaken or OTC) or in a different colony (other). Apiaries with no recurrence are also shown.

in the apiary. The overall recurrence level of EFB was 50.0% across the apiaries. However, if this figure broken down to where in the apiary EFB actually recurred, this presents some very interesting data. In just one case (6.3%) was a colony that had been shaken then fed with OTC responsible for recurrence in an apiary; OTC treated colonies were responsible for 18.8% and new infections for 25.0% of cases.

4. DISCUSSION

Our results suggest that the shook swarm plus OTC treatment has potential to control both outbreaks of EFB and to prevent recurrence in subsequent seasons. Recurrence rates were 4.8% compared with 21.1% with OTC treatment alone. Another investigation (data not shown) indicated that shook swarm plus OTC treatment could also be used for colonies that had high levels of clinical symptoms (greater than 50% of larvae infected in a colony). Hives with this amount of infection are normally destroyed under UK legislation.

Shaking of colonies plus OTC treatment was received favourably by beekeepers, many of whom opted to have the shook swarm plus OTC treatment on their colonies, despite the extra time and effort required. This was particularly true for beekeepers who had continual problems with EFB in their apiaries.

There is a specific time-frame within which shook swarm plus OTC treatments should be carried out in UK conditions, as indicated by earlier studies by the NBU (data not shown). If too late, usually after July, the colony is unlikely to recover and build up sufficiently to overwinter due to lack of honey flows and other environmental factors. This may explain the few failures that occurred when colonies died out. By treating comparable colonies within apiaries, it was anticipated that any effects due to different bee races and their tolerances to brood diseases would be limited.

The shook swarm technique has been investigated for use in controlling AFB, but results have been inconsistent. Hornitzky and White (2001) carried out a similar trial to the current study in order to assess whether shook swarm treatment could be used to treat AFB. Their studies found a high colony mortality when colonies were shaken, and also a high level of AFB recurrence. Del Hoyo et al. (2001) found that shaking bees from AFB-affected colonies either into or in front of a new hive reduced the levels of spores detected on adult bees and in honey, although it did not eliminate them completely. An earlier study (Knox et al., 1976) found that shaking bees onto new equipment with or without a dose of OTC was effective for controlling AFB for 1 year (colonies were not maintained beyond this time). However, these colonies were relatively small, and some of the other colonies that did develop AFB in a similar trial (shaken onto ethylene oxide-fumigated equipment and fed a dose of OTC) did not show any AFB symptoms until 15 months after the treatment. If the "AFB-free" colonies had been maintained for a longer time period, they may have shown symptoms. Brødsgaard and Hansen (1999) found that using a modified shaking technique without antibiotics reduced the levels of *P. larvae* subsp. *larvae* spores in colonies, and also appeared to reduce symptoms of AFB. However, EFB and AFB are very different diseases, and require different control mechanisms. The shook swarm plus OTC method obviously has potential for controlling EFB in the UK.

Replacement of combs by beekeepers is a routine measure that should be adopted as a routine husbandry method. Nelson and Gochnauer (1982) and Koenig et al. (1986) showed that the level of chalkbrood infections (cause by the fungus *Ascosphaera apis*) in colonies with new comb was significantly lower than similar colonies with old comb. *A. apis* may reside on combs and may cause infections from year to year (Gilliam and Vandenberg, 1990). This indicates that shaking will lead to lower levels of chalkbrood and maybe reduced numbers of other potential pathogens, such as the varroa mite. This may lead to lowered disease levels and is a simple method that beekeepers can use.

The option to treat colonies with the shook swarm plus OTC method has continued for the seasons 2001 and 2002; in 2001 a total of 139 colonies were treated with shook swarm plus OTC, and these are currently being observed for rates of recurrence in the 2002 season. Preliminary data indicates a very low incidence of recurrence, even lower than in 2001. The next step will be to try the shaking technique without the addition of OTC to colonies.

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Résumé – Traitement de la loque européenne à l'aide de la méthode de l'essaim artificiel et de l'oxytétracycline au Royaume-Uni. Le but de cette étude était de rechercher une méthode alternative de traitement de la loque européenne (EFB) et de la comparer au traitement de routine à l'oxytétracycline (OTC) utilisé au Royaume-Uni. Il est nécessaire de trouver une méthode alternative car cette maladie revient généralement d'une saison sur l'autre, même après application du traitement standard. La méthode étudiée consiste à créer un essaim artificiel (EA) en secouant dans une nouvelle boîte les abeilles adultes des colonies infectées. Un nourrissement fait de sirop et d'1 g d'OTC est donné à la colonie résultante pour essayer d'éliminer toute bactérie transférée ; tout le couvain est brûlé. On a comparé dans le même rucher les colonies traitées à l'OTC et celles traitées par l'EA et l'OTC. Dans la saison 2000 la majorité des colonies secouées ont récupéré : le taux de succès a été de 95,1 % pour le traitement EA + OTC et 96,1 % pour le traitement à l'OTC seul (Fig. 1). Les taux de récurrence ont été mesurés en 2001. Ils ont été respectivement de 4,8 % et 21,1 % pour les colonies traitées par l'EA + l'OTC et pour celles traitées à l'OTC uniquement (Fig. 2). Le taux habituel de récurrence d'une année sur l'autre est de 20–25 % ; la récurrence de l'EFB a donc été beaucoup plus faible chez les colonies traitées par l'EA + l'OTC. Lors de la détection de la récurrence de l'EFB dans un rucher où des colonies avaient été traitées à l'EA + l'OTC, c'était généralement une colonie non traitée par l'EA + l'OTC qui était atteinte ou bien il s'agissait de l'infection d'une nouvelle colonie (Fig. 3). On en conclut que la méthode essaim artificiel + oxytétracycline aboutit à un taux significativement plus faible de récurrence de la loque américaine que le traitement à l'OTC seul, à la fois sur le plan de la colonie et du rucher.

loque européenne / essaim artificiel / oxytétracycline / méthode alternative / maladie

Zusammenfassung – Behandlung der Europäischen Faulbrut mit der Kunstschwarmmethode und Oxytetracyclin in England. Ziel dieser Untersuchung war die Suche nach alternativen Behandlungsmethoden gegen die europäische Faulbrut (EFB) und ihr Vergleich mit der in England üblichen Oxytetracyclin (OTC) Behandlung. Eine Alternative ist notwendig, weil diese Krankheit immer wieder in den nachfolgenden Bienenjahren auftaucht, auch nach Anwendung der Standardmethode. Hier wurde die Wirkung untersucht, die das Abschütteln von adulten Bienen der infizierten Völker in eine neue Beute mit frischen Mittelwänden hat – also eine Kunstschwarmmethode. Das so entstandene Volk wurde mit 1 g OTC in Zuckerlösung gefüttert, um die letzten möglicherweise übertragenen Bakterien abzutöten. Die gesamte Brut wurde verbrannt. Ein Vergleich der OTC Behandlung und der Bildung des Kunstschwarms plus OTC Behandlung wurde am selben Bienenstand durchgeführt. Die Mehrzahl der Völker erholte sich von der Kunstschwarmbildung im nächsten Bienenjahr (2000), mit einem Behandlungserfolg von 95,1 % bei Kunstschwarmvölkern plus OTC, und 96,1 % bei alleiniger OTC Behandlung (Abb. 1). Die Häufigkeit des Wiederauftretens wurde 2001 bestimmt. Beim Vergleich von Kunstschwärmen mit anschließender Fütterung von OTC mit Völkern, die nur mit OTC gefüttert wurden, betrug die Anzahl der erneuten Infektion 4,8 % bzw. 21,1 % (Abb. 2). Die normale Rate des Auftretens von EFB betrug in jedem Jahr 20–25 %, entsprechend hatten die Völker nach der Kunstschwarmbildung in Kombination mit OTC Fütterung eine wesentlich geringere Befallsrate als im Durchschnitt. Wenn Bienenstände auf EFB untersucht wurden, in denen Völker mit Kunstschwarm und OTC-Fütterung behandelt worden waren, waren meist die Völker befallen, die nicht mit dieser Kombinationsmethode behandelt waren, sondern es war entweder ein Wiederauftreten in einem mit OTC behandelten Volk oder mit größerer Wahrscheinlichkeit eine Neuinfektion (Abb. 3). Demnach führt die Kombination von Kunstschwarm mit anschließender OTC Fütterung zu wesentlich geringerem Wiederauftreten von EFB als die alleinige Behandlung mit OTC, und zwar sowohl im Volk als auch im Bienenstand.

Europäische Faulbrut / Kunstschwarm / Oxytetracyclin / Krankheitsbekämpfung

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