



Oxytetracycline as a predisposing condition for chalkbrood in honeybee

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Received 15 December 2003; received in revised form 14 July 2004; accepted 14 July 2004

Abstract

Antibiotics, particularly oxytetracycline, have been discussed as a possible predisposing condition in the appearance of chalkbrood in the honeybee (*Apis mellifera* L.). Nevertheless, the scientific data to support this belief have been insufficient. We have developed a method to study the effects of this antibiotic as a predisposing factor under different circumstances. We conclude that oxytetracycline does not increase the risk of chalkbrood in susceptible worker brood in the short or mid-term. © 2004 Elsevier B.V. All rights reserved.

Keywords: *Apis mellifera*; *Ascosphaera apis*; Oxytetracycline; Predisposing conditions

1. Introduction

Ascosphaera infection in honey bee (*Apis mellifera* L.) is an invasive mycosis produced by *Ascosphaera apis* Maassen ex Claussen (Olive et Spiltoir), affecting stretched larvae, killing and drying them. Dead larvae become like black or white mummies, similar to little pieces of chalk, hence is known like chalkbrood disease (Heath, 1982).

Chalkbrood is a factorial disease requiring the presence of *A. apis* spores inside the larvae guts at the time of capping and the performance of a predisposing condition for the disease to manifest itself (Heath,

1982). For decades, several predisposing factors have been considered. Nevertheless, the lack of appropriate techniques to reproduce chalkbrood in a controlled way, while respecting the natural conditions of beehives as much as possible, has prevented the real importance of these possible predisposing conditions from being evaluated scientifically. In a previous research (Puerta et al., 1994; Flores et al., 1996), the development of a technique to study the chilling effect on infected susceptible worker brood, greatly enhancing chalkbrood, and thus confirming the findings by Bailey (1967). These investigators demonstrate that a high level of humidity does not in itself constitute a substantial factor to induce chalkbrood, although high relative humidity combined with slight cooling did enhance mummification (Flores et al., 1996).

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The excessive use of antibiotics, fundamentally oxytetracycline, to control and prevent bacterial diseases in beekeeping has also been considered a possible predisposing condition (Heath, 1982). This hypothesis however, was based on observations and subjective deductions than on scientific demonstrations, making it necessary to confirm the real importance of this factor as a possible predisposing condition.

The objective of this research was to study the possible role of the over-administration of oxytetracycline as a predisposing condition in the appearance of chalkbrood disease under three different temperatures, 25, 30 and 35 °C.

2. Materials and methods

The experiments were performed in the Andalusian Center for Ecological Beekeeping in Córdoba (Spain) from May to October 2002, coinciding with the beekeeping season. Trials were carried out in 10 Langstroth hives. This colonies have never been treated with antibiotics. Each colony was inoculated weekly for a total of four times (29 April, 6, 13, and 20 May) with the spores of 15 esporulated mummies in 150 g of pollen, which was sprinkled on the tops of the combs (Flores and Puerta, 2001).

The inoculated colonies formed two groups of five beehives. Each beehive of the first group was given an oxytetracycline preparation weekly (0.4 g oxytetracycline, 9.6 g sugar powder dusted on combs) for 3 weeks prior to evaluating the disease. This same treatment was repeated 3 days before each evaluation (7, 14, 21 and 29, July 19 June, 2 August, 6 September and 4 October). The second group was a control group was not treated.

The incidence of chalkbrood disease was evaluated in newly capped brood (worker brood capped in a period of 14 h). Unsealed fifth instar larvae (Rembold et al., 1980) were marked on plastic sheets. The broodcombs were subsequently returned to the beehives and again removed after 14 h. Portions of combs with newly sealed brood were removed and maintained in incubators at three different temperatures (25, 30 and 35 °C) and 60% relative humidity. Five days after sealing, the cells were opened and the percentage of mummified larvae was checked (Flores et al., 1996). Evaluation of the disease began 3 weeks after treatment

Table 1
Evolution of the percentage of chalkbrood disease (mean ± S.E.) in honeybee larvae from inoculated beehives treated with oxytetracycline and untreated beehives maintained at three temperatures: 25, 30 and 35 °C, and ANOVA (SPSS 8.0) between treatment and between dates within treatment

Dates	25 °C			30 °C			35 °C			ANOVA (p)					
	Oxytetracycline		Controls	Oxytetracycline		Controls	Oxytetracycline		Controls						
	No of checked cells	Percentage of chalkbrood cells	No of checked cells	No of checked cells	Percentage of chalkbrood cells	No of checked cells	No of checked cells	Percentage of chalkbrood cells	No of checked cells						
10th June	556	78.44 ± 3.75	436	51.06 ± 4.33	0.005	140	46.13 ± 6.83	139	34.60 ± 12.96	0.475	129	7.56 ± 4.87	149	12.76 ± 0.16	0.346
17th June	516	51.39 ± 10.26	564	47.39 ± 10.18	0.791	199	34.79 ± 2.41	200	26.61 ± 4.61	0.167	134	13.69 ± 8.06	208	11.29 ± 3.87	0.781
24th June	295	39.15 ± 13.43	344	53.39 ± 10.57	0.425	307	37.61 ± 12.15	403	30.71 ± 10.28	0.675	293	6.84 ± 2.72	293	3.56 ± 0.85	0.243
1st July	338	26.68 ± 6.16	358	35.77 ± 12.63	0.571	320	13.55 ± 2.14	411	27.51 ± 10.68	0.292	307	2.59 ± 0.48	403	9.14 ± 3.80	0.174
8th July	292	31.74 ± 5.66	400	32.43 ± 12.43	0.962	251	20.79 ± 1.97	414	24.66 ± 14.89	0.852	250	5.29 ± 1.78	393	10.77 ± 5.77	0.509
22th July	311	31.08 ± 12.26	359	46.33 ± 15.42	0.461	302	31.08 ± 19.26	402	30.37 ± 12.30	0.975	250	9.29 ± 5.98	382	18.89 ± 10.08	0.47
5th August	292	26.47 ± 9.04	296	19.87 ± 10.07	0.643	177	14.13 ± 7.82	301	18.62 ± 6.69	0.707	150	1.54 ± 1.54	294	6.81 ± 3.66	0.397
9th Septem	136	23.18 ± 9.38	217	28.53 ± 18.98	0.793	134	9.55 ± 7.16	209	20.08 ± 16.32	0.541	28	7.14 ±	215	10.19 ± 8.79	0.878
7th October	173	22.32 ± 11.86	187	6.76 ± 1.93	0.243	161	13.78 ± 10.08	192	5.45 ± 2.27	0.452	125	2.27 ± 2.27	149	0.53 ± 0.53	0.497
ANOVA (p)		0.009		0.192			0.247		0.694			0.509		0.514	

was initiated with the antibiotic and continued for a period of 5 consecutive weeks according to the method described above. The sixth and seventh evaluation of the disease were done biweekly and the eighth and ninth, monthly (Table 1 for dates). The readiness brood descended after the ninth repetition, making it necessary to conclude the experiment.

The percentage of chalkbrood disease in the beehives that received periodical treatment with oxytetracycline and the control beehives was recorded in each evaluation and compared. Data obtained were evaluated statistically by descriptive parameters, analysis of variance (ANOVA) and the “Tukey Honest Significant Difference (HSD) Test” ($p < 0.05$) (SPSS 8.0 for windows).

3. Results

The results are shown in Table 1 and in Fig. 1.

Significant differences were only found in the first date of the brood maintained at 25 °C. Other significant differences were not detected between the treated group and the control group maintained at 25, 30 or 35 °C.

When considering the successive evaluations in time for each group and each temperature, significant differences were only detected in the percentage of mummification for the brood from the treated beehives maintained at 25 °C. A higher percentage of disease was observed in the first date than in the last dates (evaluations 4, 6, 7, 8 and 9), which had a lower percentage of mummification. Significant differences were not detected between dates for the brood from treated beehives maintained at 30 or 35 °C, or when the brood came from the control beehives. In all cases, there was a tendency for the disease to diminish with successive dates. This tendency was observed for both treated and control beehives.

4. Discussion

Antibiotics, and particularly oxytetracycline, are frequently used for the control and prevention of bacterial diseases in honeybee colonies. Giauffret and Taliercio (1967) and Samsinaková et al. (1977) point out the possibility that this antibiotic

could be a predisposing condition in chalkbrood disease. On the contrary, Menapace and Wilson (1979) found that this antibiotic did not aggravate chalkbrood. Many other authors have discussed the role of antibiotics in general and oxytetracycline in particular, but have always based their observations on previous investigations or by extrapolating the results found in other animal species or in humans. The use of antibiotics in the honeybee can upset the balance of intestinal microflora, favoring the growth of fungi such as *A. apis* (Menapace and Wilson, 1979).

We have evaluated the effect in the short and mid-term of the risk of enhancing chalkbrood disease in beehives due to the over-administration of oxytetracycline under three possible circumstances:

- (1) The brood from both treated and untreated infected beehives was maintained at 35 °C. In accordance with previous results (Flores et al., 1996), temperature was not considered a predisposing condition and any increase in the appearance of chalkbrood in the brood coming from beehives supplied with the antibiotic should be attributed to this cause. Nevertheless, the results show that significant differences were not found between groups. We, therefore, concluded that the antibiotic did not act as a predisposing condition to produce the disease.

On the other hand, Fig. 1 shows the irregularity of the disease over time, with frequent peaks and falls throughout the experiment. However, the same observations were made for both treated and untreated groups. Unfortunately, in the absence of scientific observation and control groups, these peaks and falls can lead to erroneous conclusions. This fact has been clearly demonstrated in our study and the findings could be extrapolated to the study of this and other diseases of the bees.
- (2) When the susceptible brood was maintained at 25 °C, chilling acted as a predisposing condition and was itself able to trigger the disease and mask the possible effects of exposure to the antibiotic. Under these conditions, we thought about the possibility that oxytetracycline could upset the microflora balance of the beehives by inducing chalkbrood in a natural manner, thus leading to an increase in *A. apis* spores in the beehives that could be detected when the brood is exposed to chilling.

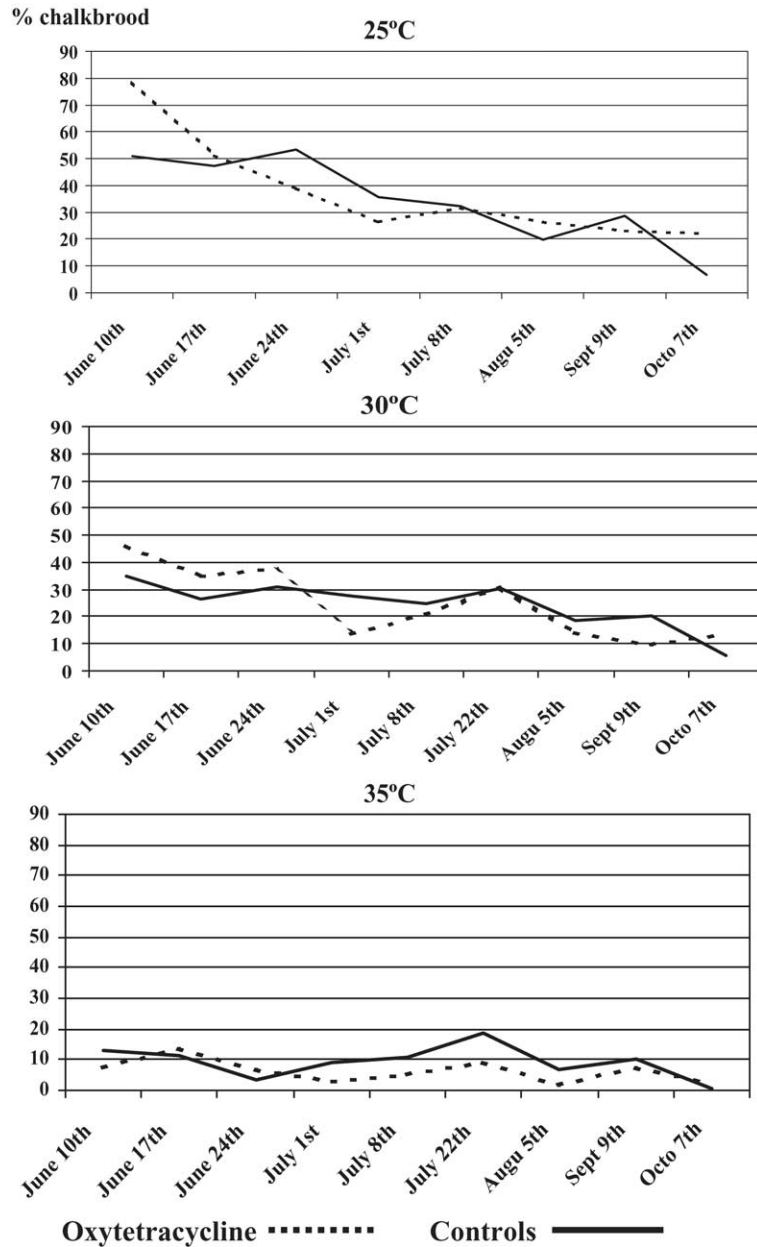


Fig. 1. Evolution of the percentage of chalkbrood disease in honeybee larvae from inoculated beehives treated with oxytetracycline and untreated beehives maintained at three temperatures: 25, 30 and 35 °C.

In this case, significant differences between treated and untreated broods were not found, suggesting a putative that an imbalance in the normal microbiota does not play a role in the appearance of chalkbrood disease in beehives.

On the other hand, since bacterial diseases are not common in the experimental area and the colonies have never been treated with antibiotics, we do not hope that bacterial microbiota resistance to the Oxytetracycline have an effect on the results.

- (3) When the brood is maintained at 30 °C, it is considered a situation of moderate cooling, in which the performance of a second predisposing condition such as excess humidity, together with temperature, could induce the disease (Flores et al., 1996). This investigation showed that exposure to oxytetracycline, together with moderate cooling did not increase chalkbrood disease. Once again, the same tendencies were observed for both the control beehives and those exposed to the antibiotic.

5. Conclusion

Based on these data, we conclude that the colonies of bees exposed to the over-administration of oxytetracycline were not at a higher risk of producing chalkbrood disease, at least under the conditions of our experiment in the short and mid-term. Nevertheless, it would be of great interest to verify this same effect in the long term in apiaries where this antibiotic is used systematically every year.

Acknowledgements

Funds were provided by the INIA (project API99-007) supported by the EC and the Spanish government (CE 1221/97).

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