SANITIZATION OF EUROPEAN FOULBROOD THROUGH DIFFERENT BEEKEEPING PRACTICES

G. Formato[1], A. Giacomelli[1], F. Nisi[1], S. Bassi[2], S. Pongolini[2], E. Carra[2], and S. Saccares[1]

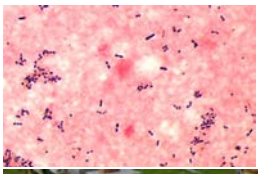
[1]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana (Italy)

[2] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Sezione di Modena (Italy)

Introduction

European foulbrood (EFB) is a disease that affects honey bee larvae (*Apis mellifera*) and produces heavy economic losses in Apiculture sector bringing to death the affected colonies or producing a reduction of activity of the bees at the starting of the productive season. The causative agent of this pathology is the Gram positive bacterium *Melissococcus plutonius.*

In Italy, in order to control the spread of EFB, the disease is put under official veterinary control. During the years 2008 and 2009, the *Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana,* in collaboration with the *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna,* carried out field trials to verify the adoption of different beekeeping practices for sanitization of hives affected with EFB.

Objective

The present work aimed at finding new proposals for the Veterinarian Authorities on the method for the diagnosis of EFB and on the management of the infected hives.

Materials and Methods

On April 2008 the health status of 52 hives placed in Latium region with suspect symptoms were analyzed.

Diagnosis of EFB in the hives was achieved both for the presence of the clinical symptomps (yellow and twisted larvae) in the uncapped brood, and for the positive reaction at the immune enzymatic tests (EFB kit of Vita Europe); EFB was diagnosed in 23 hives that were treated on the same April 2008 with different methods for the sanitization.

In April 2008, the 23 sick colonies were split in 4 different groups containing families homogeneous in force and disease's severity:

- ✓ one group of 6 families were treated with tetracycline hydrochloride;
- ✓ one group of 6 families were treated with the halt in egg-deposition obtained with the segregation of the queens for 30 days into specific queen-cages named *Scalvini®*;
- ✓ one group of 6 families were treated with a single shook -swarming;
- ✓ one group of 5 families remained untreated.

In June 2008 (i.e. 60 days after the treatments) and in March 2009 (i.e. about one year later), the hives were evaluated for: the presence of clinical signs of EFB, the results to the Vita Europe kit EFB and, finally, the results to the PCR analysis.

MOLECULAR METHOD

DNA EXTRACTION FROM 5 LARVAE	
Homogenization	with 1 ml of TE buffer (10mM tris-HCl, pH 8.0, 1mM EDTA) + 0.9% NaCl
Centrifugation	13.000 X *g*, 2 min
Pellet	washed with 1 ml of the same solution
Centrifugation	13.000 X *g*, 2 min
Pellet	suspended with 400µl of Lysozyme solution + over-night proteinase K treatment (*T. Bakonyi et al. - App. Env. Micr., 2003*)
DNA	purified by Nucleon Spin tissue minikit (Macherey - Nagel), eluted in 100µl of elution Buffer, stored at -20°C

PCR PROTOCOL

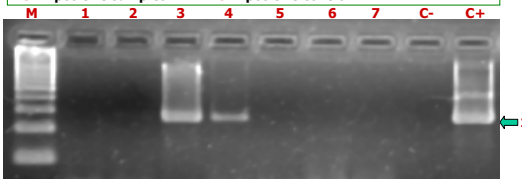
Primers: MP1: 5'-CTT TGA ACG CCT TGA AGA -3' MP3: 5'-TTA ACT TCG CGG TCT TGC GTC TCT C -3' Amplification product: 276bp		specific for 16S rDNA sequence of M. *plutonius* (*Djordjevic S P et al.* J. Apic. Res., *1998*)
Reaction volume: 25 µl (20 µl of reaction mix + 5 µl of template DNA)		according to Fast Start Taq kit (Roche)
Amplification protocol	activation of HotStart Taq polymerase	95°C for 5 min
	40 cycles of denaturation	94°C for 30 s
	40 cycles of annealing	56°C for 15 s
	40 cycles of elongation	72°C for 30 s
	final extension	72°C for 7 min
10µl of amplification product were electrophoresed on 1,8% agarose gel in Tris-Borate-EDTA buffer. The gel was stained in a solution of EtBr (1µg/ml) and visualized by UV transillumination (Fluor-S, Bio Rad)		

IMMUNE ENZYMATIC METHOD

The VITA® EFB Diagnostic kit was used in according to the manufacturer's instructions

DETECTION PCR ON LARVAE

M: molecular size marker (100bp ladder)
1-2, 5-7: negative samples C-: negative control
3-4: positive samples C+: positive control



Results

Treatment	April 2008	June 2008			March 2009			
	Hives with EFB	Clinically healthy	Immune enzymatic test	PCR	Deaths	Clinically healthy	Immune enzymatic test	PCR
Shook swarming	6	6	6 negatives	1 positive; 5 negatives	5 (83%)	1 (100% of the survived hives)	1 negative (100% of the survived hives)	1 negative (100% of the survived hives)
Halt in deposition	6	6	6 negatives	1 positive; 5 negatives	4 (67%)	2 (100% of the survived hives)	2 negatives (100% of the survived hives)	1 positive and 1 negative (50% of the survived hives)
Tetracicline HCl	6	6	6 negatives	6 negatives	3 (50%)	3 (100% of the survived hives)	3 negatives (100% of the survived hives)	3 negatives (100% of the survived hives)
Not treated	5	5	1 positive; 4 negatives	5 negatives	1 (17%)	4 (100% of the survived hives)	4 negatives (100% of the survived hives)	4 negatives (100% of the survived hives)

Conclusion

The "shook swarming" method seems to be the worst in terms of survival for the colonies: 83% dead 1 year after the treatment. The "halt in deposition" method presents similarly a high percentage of mortality (67% dead) 1 year after the treatment, associated with a persistence of the pathogen that could be revealed i with the P CR analysis n the 50% of the survived hives one year after the treatment. Therefore, none of the mentioned treatments could be able to sanitize completely the hives 2 months after their application, although both of them could be able to lead to a disappearance of the clinical symptoms and to responde negative to the immune enzymatic test.

The treatment with tetracicline couldn't save the hives that presented a too high level of infection to survive the Winter season, but coul be able to lead to a negative responde for the clinical sympoms and to responde negative to the immune enzymatic test and PCR analysis.

Finally, the "untreated" group presented the best results, in terms of survivals of families and in terms of negativization of the families sustaining the seasonal trend of EFB and the spontaneous recovery of the hives at the same time with the increase of the nectar flow and with the colonies strenght.

In conclusion, the "shook swarming" method and the "halt in deposition" method, don't seem to be strategies practicables for the management of the hives infected with EFB.

ACKNOWLEDGEMENTS: The Authors wish to thank Dr. Pietro Arculeo for photographs and Dr.Gian Luca Paganelli for technical assistance

CORRESPONDENCE: gioformato@yahoo.es