National Institute of Apiculture

Trial of the application of the VITA AFB Test for diagnostic confirmation of American foul brood.

Final Report – November 2003

Introduction

The purpose of the experiment is to verify the sensitivity and conclusiveness of the **VITA AFB Test** in the identification of American foul brood in comb affected by American foul brood.
This experiment used a method involving comparison of the results obtained using the standard test and those of the VITA AFB Test, using the same biological samples.

Experimental procedure

Approximately 20 VITA AFB Tests sent to the INA in September 2003 were performed.
The tests were applied to samples from hives infected with American foul brood; in some cases, the test was carried out directly in the field.
The result (absence of T line, T line more or less dark) was evaluated using a four-value scale (negative, +, ++, +++). The reference tests consisted in the counting of the spores under an optical microscope and culture confirmation where necessary.

Diseased larvae of various ages (L5, prepupae, pupae) and at various stages of the disease (dark larvae, liquid larvae, scale, etc) were tested. Healthy larvae were also tested.

Results

The following table shows the results:

SAMPLE	LEVEL OF AFB IN THE COMB (*)	LARVA ANALYSED	RESULT OF THE AFB TEST (+ / ++ / +++)	NO. OF SPORES PER mL OF HOMOGENEATE (million)
4	high	Pupa. Scale with pasty, ropey consistency. Colour – dark brown.	++	150
		Prepupa Scale quasi-dry. Colour – dark brown.	++	110
5	mean	Prepupa. Liquid, ropey consistency. Colour – light brown.	++	230

		Pupa. Scale quasi-dry, ropey. Colour – dark brown.	+++	360
6	very high	Pupa. Ropey consistency. Colour – brown.	+++	400
7	high	Pupa. Ropey consistency. Colour – brown.	++	400
		Pupa. Scale with pasty consistency. Colour – dark brown.	++	240
		Prepupa. Liquid, ropey consistency. Colour- light brown.	+++	260
		Pupa. Liquid, non-ropey consistency. Colour – yellow.	++	190
8	high	Pupa. Ropey consistency. Colour – brown.	+++	280
		Pupa. Liquid, non-ropey consistency. Colour – yellow.	++	100
		Prepupa. Liquid, non-ropey consistency. Colour – whitish.	negative	50 (including the vegetative forms)
		Prepupa. Liquid, non-ropey consistency. Colour – whitish.	+	120
9	high	Pupa. Ropey consistency. Colour – brown.	+++	400
		L 5 prior to capping. Colour – whitish.	negative	absent
		Pupa without specific symptoms.	negative	absent

Verification of the detection limit

The test was performed using serial dilutions of the initial homogeneate.

Dilutions	No. spores/mL	Result
0	800×10^6	++
1/10	80×10^6	++
1/20	40×10^6	+
1/40	20×10^6	+ (weak)
1/100	8×10^6	-

Observations

Although limited, the data under examination admit of the following considerations:

- The application of the test to larvae with typical symptoms produces results in line with those of the reference method (absence of false positives). The age of the larva has no influence on the quality of the response.
- In only one case was a positive result in the reference test not confirmed by the AFB Test; however this was a case of a larva presenting a symptomatology that was not well defined and was probably at a non-advanced stage of the infection.
- The AFB Test was easy to use and therefore suitable for rapid verification in the field. It is important to underline the value of the test as diagnostic confirmation in clinical situations already indicative per se of American foul brood (ie larvae with symptoms that are already sufficiently defined); it does not however appear suitable in identifying sub-clinical situations in which there may be present only larvae in the initial stages of infection (a situation that is in any case transitory, and which in general leads to the confirmed pathology in which it is not difficult to identify the larvae suitable for the test).

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