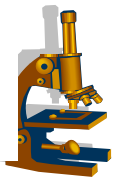
ICENI MICROSCOPY  STUDY GROUP

Quantifying *Nosema* infections 7th April 2018

Summary.

I quote from Ingemar Fries *et al* in the COLOSS *BEEBOOK* 2.1.1 [For brevity, references have been removed].
"The degree of *Nosema spp.* infection in a colony has most commonly been described through the average number of spores per bee in a pooled sample. However, some studies suggest that the best way to determine the degree of infection is to estimate the proportion of infected bees in the colony. Nevertheless, there is a good correlation between the proportion of infected bees and the average number of spores in a pooled sample of bees, but not in all cases. Evaluating the proportion of infected bees is much more laborious than to count the number of spores in a pooled sample, so pooled sampling will probably remain an important tool for quantifying infections in colonies. Because there is a wide variation in the numbers of spores found in individual bees in pooled samples, when the highest precision is needed, it may still be motivating to investigate the proportion of infected bees. The highest proportion of infected bees is found in foraging bees as is the greatest infection (spores per bee). Recent studies suggest the importance of determining the proportion of infected house bees to establish the viability and impact from infection on colonies."

Aims.

1. To establish Nosema infection levels and discover which colonies need help to combat either or both the two *A. mellifera* Nosemas by:
    a. establishing the degree of infection and/or
    b. determining the relative proportion of infected to healthy bees.
2. To compare the two methods.

Please bring with you:

Bees: 30 per colony to establish the degree of infection (Aim 1a).
10 per colony to determine the relative proportion of infected to healthy bees (Aim 1b).
For comparison, both samples should come from the same colony (Aim 2).
Record sheet - this includes suggestions how to collect the bees.
Compound microscope (*Nosema* needs to be viewed x400)
Slides, coverslips, Practamount or alternative, nigrosin negative stain, spirit lamp, etc.
Parallel sided bottle or similar for use as a rolling pin.
Small bowl or container for at least 30 ml water.
If you prefer not to use the plastic teaspoon provided, a way to measure small quantities of water.
Glass rod, spatula or item of plastic cutlery for transferring drop of slurry to slide.

We will provide small plastic teaspoons, for use as measures, and zip-lock plastic bags in which bees can be crushed. Some may prefer to bring the more usual pestle and mortar.

The session will begin with a presentation.

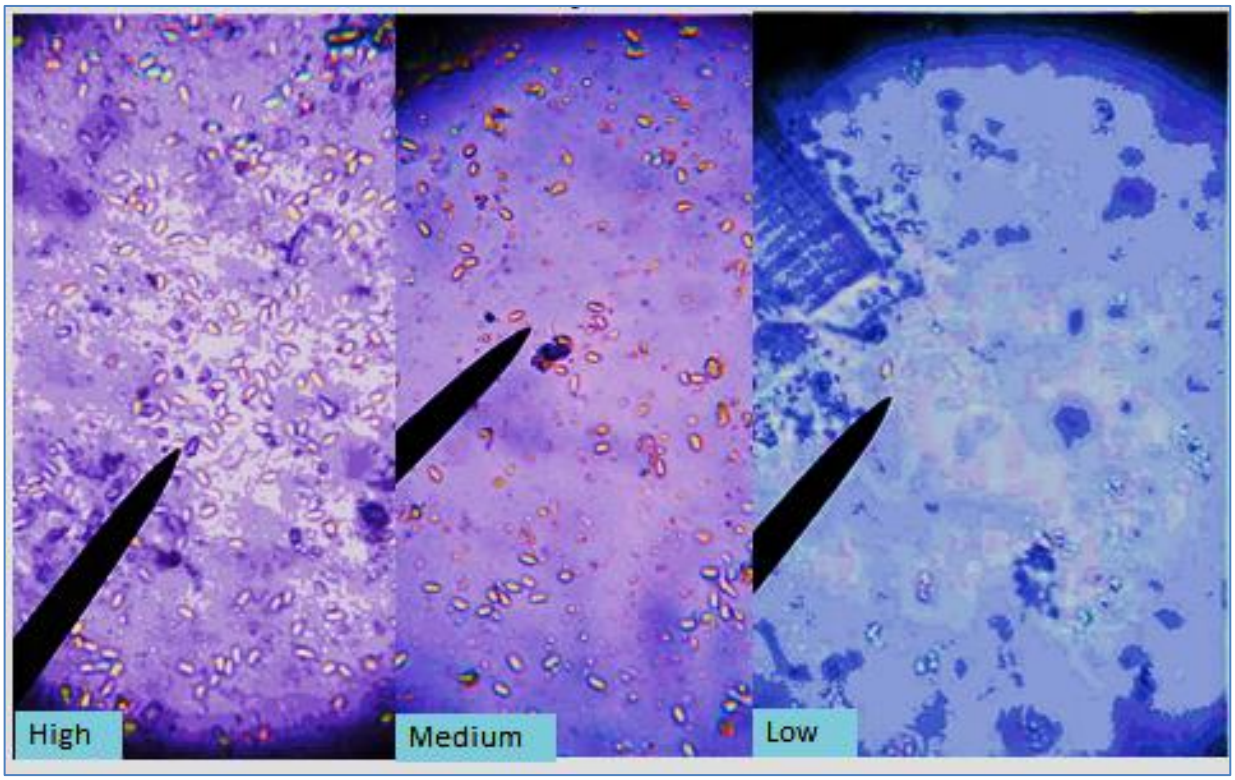
Suggested work.

1. Average number of spores per bee in a pooled sample.

"Spore counts. To determine the degree of infection of *Nosema spp.* in a sample, it has been suggested that subjective judgement on an arbitrary infection scale can be used (Fries)" and examples appear on the next page. For research, a haemocytometer is needed. Enquiry has shown that the specific recommendations of Yates & Yates command little support among the beekeeping scientific community so the guidance is: if it looks bad, do something about it!

Method:

a. Count the bees. For a statistically satisfactory answer at least 29 are needed.
b. Assuming the plastic teaspoons provided hold 2.5 ml, measure 1 ml of water per bee and set aside.
c. In 1/3 of the measured amount of water, grind the bees (or, if you prefer, their abdomen or ventriculus) thoroughly. If using a plastic bag, roll from the corner.
d. Add the remaining water and mix thoroughly.
e. Add a small drop of the slurry (smear guidance suggests 4 mm in dia) to a slide and a cover-slip. The spores are easily seen under the microscope, but if desired, an equal amount of nigrosin may be added and mixed into the drop before the cover-slip is lowered; this extra volume will reduce the number of spores to be seen per view but is thought unlikely to affect your conclusion.
f. View x400. Phase contrast may help.
g. Take several views.
h. If spores are seen, use the guide below to make an estimate and record it on the record sheet.



To make a reference slide

Stating at point 'e' above:

e. Add a small drop of the slurry (smear guidance suggests 4 mm in dia) to a slide and a cover-slip. The spores are easily seen under the microscope, but if desired, an equal amount of nigrosin may be added and mixed into the drop before the cover-slip is lowered; this extra volume will reduce the number of spores to be seen per view but should not affect your conclusion.

f. Smear the drop - how to do this is shown in the accompanying paper.

g. Dry, kill and fix by passing the back of the slide over a spirit lamp flame once or twice.

h. Ensure really dry, perhaps by putting onto a warm hot-plate.

i. Apply a mountant such as Practamount or Histoclear. Allow to dry (some weeks). Ring.

2. To discover the proportion of infected bees

a. Take ten bees.

b. Add one to a plastic bag; add a drop of water (as near 1 ml as possible).

c. Grind, working from the corner.

d. Add a drop of the resultant slurry to a slide and cover with a cover-slip. View x400. Phase contrast may help.

e. Take several views.

f. Record whether diseased or not.

g. When all have been checked, record the percentage diseased.

Compare the results of taking first pooled and then individual samples. What is your conclusion? Which method do you prefer?

For information and comparison, Bedfordshire BKA results on 10 Mar 18 were:

For pooled samples:

53% None (9)
29% Slight (5)
6% Moderate (1)
12% High (2)

Time did not allow them an attempt to discover the proportion of infected bees.