

DIAGNOSIS OF *Nosema apis* INFECTION BY INVESTIGATIONS OF TWO KINDS OF SAMPLES: DEAD BEES AND LIVE BEES

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S u m m a r y

All the techniques of diagnosis of *Nosema apis* infection, recommended in the OIE (World Organisation for Animal Health) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2004, are based on the investigation of samples of live bees collected at the entrance of the hive. However, sometimes, it is impossible to get such samples - for instance when the colony has died. On the other hand, it would often be useful for beekeepers to know the health status of the colonies in early spring. At this time obtaining samples of live bees at the hive entrance is very difficult in many areas. In this work the method of investigation of the dead bees collected from the bottom board of the hive just before the time of the first spring flight, or from the dead colony, is described. In many countries with a climate similar to or cooler than in Poland such samples are easy to get. The method consists of the grinding of 100 whole bees in 50 ml of water, passing the suspension through a filter made of about 25 GSM (g/m^2) Nonwoven PP (polypropylene) cloth, and microscopic examination of the filtrate, under bright-field. The comparison of the method in relation to the simple nonquantitative method of investigation of live bees, from the OIE Terrestrial Manual, was performed.

Keywords: *Nosema apis* infection, diagnosis, method.

INTRODUCTION

The impact of *Nosema apis* infection on beekeeping economics is enormous but it is often underestimated by beekeepers. The OIE (World Organisation for Animal Health) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2004 contains the nonquantitative and quantitative methods of diagnosing the infection based on the investigation of samples of live bees collected at the entrance of the hive (Terrestrial Manual 2004). Described in the OIE Terrestrial Manual the nonquantitative method is quite reliable (95% confidence when 5% of bees are infested) (Fries 1988) and gives information about the current status of the colony's health. However, collecting

a live bee sample at the entrance of the hive is sometimes very difficult or even impossible, for instance, in the early spring or if a colony has died. On the other hand, from the point of view of disease control in the apiary, it would be good to have a possibility of examining bee samples in such cases. In the literature descriptions of the methods based on the investigation of dead bees can be found (Fingler et al. 1982, Kauko et al. 2002, Shimanuki and Knox 2000). However, the samples used in these methods are too small to give reliable results since they consist of 10-30 bees (Hartwig and Topolska 1995).

Since 1995 in our laboratory we have been investigating the samples consisting of 100 dead bees collected from the hive bottom board at the end of wintering, for

instance, before the first spring flight (it is best done in March). In Poland and in countries with a similar climate such samples are easy to get. We advise beekeepers to send to the laboratory all the bees collected at the bottom board. In the laboratory we choose 100 insects that have died most recently, grind whole bees in distilled water, filter the suspension and examine the filtrate microscopically.

In 2005 we investigated three apiaries for *Nosema apis* by both methods: investigation of dead bee samples and investigation of live bee samples.

MATERIAL AND METHODS

1. Investigation of dead bee samples (own method)

The samples were collected in the middle of March. 100 whole bees (chosen from the insects that have died recently) were ground in 50 ml of distilled water. The suspension was passed through a filter made of about 25 GSM (g/m^2) Nonwoven PP (polypropylene) cloth. Next, the filtrate was examined microscopically at x 400 magnification, under-bright-field.

We designated the results of the examination as:

- - if no spores were found
- + - single or few spores not found in each field of vision
- ++ - single or few spores found in each field of vision
- +++ - numerous spores found in each field of vision

The method of such an investigation is not quantitative but the constant proportion water/bee allowed us to make a very rough estimation of the level of infection. This can be very important for the beekeeper.

2. Investigation of live bee samples (method from OIE Manual)

The samples were obtained in the first days of May (there was a very cold spring

this year and building up the size of colonies was delayed). Bees were collected at the hive entrance with the help of a car vacuum cleaner, anaesthetized with carbon dioxide and put into an envelope. Next steps followed the procedure for the nonquantitative method of diagnosis described in the OIE Terrestrial Manual. These were then: killing the bees in a freezer and fixation in 4% formol, separating the abdomens of 60-80 bees, grinding up the abdomens in water (we used the constant proportion: 3 ml of water for 60 abdomens) and microscopic examination of suspension at x 400

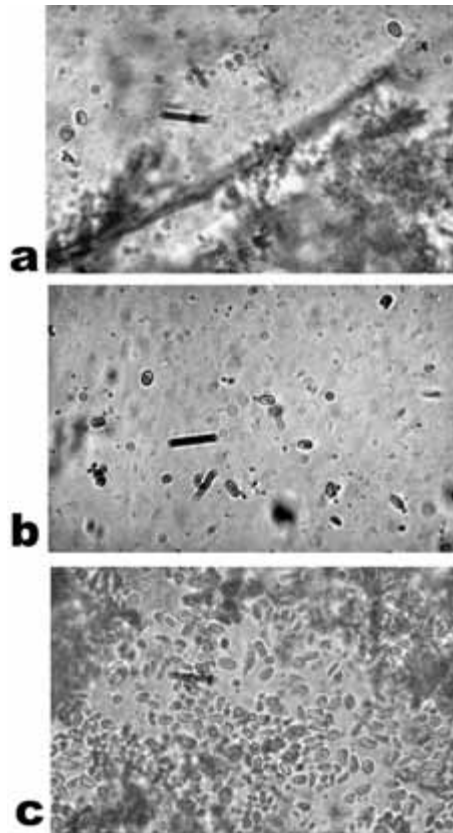


Fig. 1. - Microscopic examination (x 400 magnification) of live bees samples for *N. apis* spores – different groups of infection. a) slightly infected [S]; b) moderately infected [M]; c) heavily infected [H]

Table 1.

The results of the investigation of dead bee samples and live bee samples from three apiaries for *Nosema apis* infection.

Apiary No.	Dead bee samples				
	No. of samples	Level of infection			
		-	+	++	+++
I	22	12	6	3	1
II	19	1	2	6	10
III	10	10	0	0	0

Apiary No.	Live bee samples				
	No. of samples	Level of infection			
		N	S	M	H
I	22	6	13	2	1
II	9	0	0	0	9
III	10	0	4	6	0

Designation:

[-] - no spores found, [+] -single or few spores not in each field of vision,

[++] -single or few spores in each field of vision, [+++] -numerous spores in each field of vision;

[N] - “not infected”, [S]-slightly infected, [M] -moderately infected, [H] -heavily infected

magnification under bright field. For our purposes, on the basis of the results, we divided the samples into four groups: “not infected” – it means no spores found – designation (N), slightly infected - (S) moderately infected - (M) and heavily infected - (H) (Fig. 1).

RESULTS

The summary results of the investigation are shown in the Table 1.

In the case of apiary I the level of infection was not high. Generally, colonies which were proved to be less infected in the first investigation remained less infected also in the second investigation. Six colonies which seemed to be healthy in the first investigation were slightly infected in the second investigation and one, whose infection was estimated as [+] in the first investigation, was found “not infected” in the second investigation. We think that the first investigation revealed the health status of the colonies quite well.

In the case of apiary II the infection of many samples was estimated as [+++] in the first investigation. The beekeeper agreed not to be informed about the results. In May most colonies from which these samples came were dead, and many others proved to be heavily infected (earlier, they had the possibility of robbing the food from heavily infected or dead colonies).

In the case of apiary III the first investigation did not reveal spores in any sample. Samples from the second investigation proved to be slightly and moderately infected. However, this apiary was located about 500 metres from the previous one and obviously the bees had become infected through robbing food from the severely ill or dead colonies.

If the beekeeper from apiary II had known the results of the investigation of his samples he would have been able to prevent the spread of the disease to other colonies and the neighboring apiary.

CONCLUSIONS

We suggest the method of investigation of dead bee samples described above is used to diagnose *Nosema apis* infection in the situation when the application of the nonquantitative method described in OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals is impossible, and the collection of dead bee samples from the bottom board is easy.

Intended purposes of the method are: confirmatory diagnosis of clinical cases of nosemosis and identifying infected bee colonies toward implementing disease control measures.

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ROZPOZNAWANIE INFEKCJI *Nosema apis* METODAMI OPARTYMI NA BADANIU PSZCZÓŁ ŻYWYCH I MARTWYCH

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S t r e s z c z e n i e

Wpływ infekcji *Nosema apis* na ekonomikę pszczelarstwa jest ogromny, choć często niedoceniany przez pszczelarzy. W opracowaniu Światowej Organizacji Zdrowia Zwierząt, dotyczącego zalecanych metod rozpoznawania chorób zwierząt lądowych („Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 2004”) zamieszczone są metody wykrywania *N. apis*. Techniki te oparte są na badaniu pszczoł żywych, zbieranych na wylotku ula. Czasem jednak uzyskanie takich próbek jest niemożliwe, np. w sytuacji, gdy rodzina pszczoła osypała się. Natomiast wczesną wiosną, na wielu obszarach, uzyskanie odpowiednio licznych próbek (60 owadów żywych) jest bardzo trudne, chociaż informacja o stanie zdrowia rodzin pszczoł w tym okresie jest dla pszczelarzy bardzo istotna. W literaturze można znaleźć metody wykrywania infekcji *N. apis* oparte na badaniu martwych pszczoł, jednak zalecane tam próbki owadów są zbyt małe (10-30 pszczoł), aby otrzymane wyniki były wystarczająco miarodajne.

W pracy zastosowano prostą nieilościową metodę badania pszczoł żywych – zalecaną przez OIE, oraz metodę opartą na badaniu pszczoł martwych. Martwe pszczoły zbierano z dennicy ula tuż przed pierwszym wiosennym oblotem, lub z rodzin zamarych. Do badania wybierano 100 osobników zamarych najpóźniej. Rozcierano je z 50 ml wody i zawiesinę przepuszczano przez filtr wykonany z fizelinowej tkaniny o gęstości 25 GSM (g/m²). Z filtratu wykonywano preparat i oglądano w mikroskopie świetlnym.

Na podstawie analizy przeprowadzonych wyników proponujemy stosowaną przez nas metodę do diagnostyki infekcji *N. apis* w przypadku, gdy zastosowanie metody zalecanej przez OIE jest niemożliwe.

Słowa kluczowe: infekcja *Nosema apis*, diagnostyka, metoda.