CHAPTER 3.2.4.

NOSEMOSIS OF HONEY BEES

SUMMARY

To date, two microsporidian parasites have been described from honey bees: Nosema apis (*Zander*) *and* N. ceranae. Nosema apis *is a parasite of the European honey bee* (Apis mellifera), *and* N. ceranae *of the Asian* (Apis cerana) *and the European honey bees. Both parasites are cross-infective between host species.* Nosema ceranae *has recently been detected in several geographically separated populations of European honey bees in Europe, South and North America and Asia. The pathological consequences of* N. ceranae *in* Apis mellifera *are not well known. Both types are presumed to be very similar, but* N. ceranae *seems to be more sensitive to low temperatures and to be able to reproduce even in high temperatures.* Nosema apis *and* N. ceranae *invade the epithelial cells of the ventriculus of the adult honey bee. Infections are acquired by the uptake of spores during feeding or grooming. The disease occurs throughout the world, but treatment of bees can help to prevent the spread of infection to unaffected bee colonies.*

Nosema *levels generally increase when bees are confined, such as in the autumn and winter in colder climates. The disease is transmitted among bees via the ingestion of contaminated comb material and water, and by trophallaxis; honey stores and crushed infected bees may also play a role in disease transmission. Spores are expelled with the faeces. The relative importance of faeces, honey and cadavers as reservoirs of infective spores is not fully understood. The spores of* N. apis *are inactivated by acetic acid or by heating to 60°C for 15 minutes. To be effective, these treatments can be combined with feeding colonies with the antibiotic fumagillin to suppress infections in live bees. Many countries prohibit the use of antibiotic treatments of honey bees.*

Detection of the agent: *In some acute cases, brown faecal marks are seen on the comb and the front of the hive, with sick or dead bees in the vicinity of the hive. However, the majority of colonies show no obvious signs of infection, even when the disease is sufficient to cause significant losses in honey production and pollination efficiency. During winter, there may be an increase in bee mortality. In affected bees, the ventriculus, which is normally brown, can be white and very fragile. Microscopic examinations (×400 magnification) of homogenates of the abdominal contents of affected bees will reveal the oval spores of* Nosema *spp., which are approximately 5–7 × 3–4 µm with a dark edge* (N. ceranae *is slightly smaller, but species differentiation is difficult using light microscopy, especially as mixed infections occur). Their internal contents can be distinguished after staining with Giemsa's stain.* Nosema *spp. spores have a distinctive appearance, with a thick unstained wall and a blue-stained featureless interior. The nuclei within the spores are not visible. Staining can help to distinguish* Nosema *spp. from other microbes found in bees.*

The appearance of Nosema *spp.spores can be confused with yeast cells, fungal spores, fat and calciferous bodies or cysts of* Malpighamoeba mellificae*. The latter are similar in size to* Nosema *spp. spores, being 6–7 µm in diameter, but are completely spherical instead of oval.*

Positive identifications can be made only by observation of typical spores in the ventriculus or faeces. Very mild infections may not be demonstrable. The extent of infection is determined by counting the spores on a microscope grid and calculating the average number of spores per area and estimating from that the number of spores per bee. Identification to species level is difficult by light microscopy; polymerase chain reaction methods are preferred for this purpose.

Serological tests: *There are no applicable serological tests.*

Requirements for vaccines: *No vaccines are available.*

A. INTRODUCTION

The microsporidia *Nosema apis* (Zander, 1909) and *N. ceranae* (Fries *et al*., 1996) are parasites exclusive of the epithelial cells of the ventriculus of adult bees and both parasites occur throughout the world (Klee *et al.,* 2007). Based on molecular evidence, microsporidia are now included in the cluster Fungi (Adl *et al.,* 2005); thus, taxonomically, microsporidia are highly specialised parasitic fungi. Infection occurs by the ingestion of spores in the feed (Bailey, 1981; Webster, 1993), via trophallaxis (Webster, 1993) or perhaps after grooming of the body hairs (Bulla, 1977; Fries, 1993; Webster, 1993).

Both parasites first invade the epithelial cells in the posterior region of the ventriculus. They produce a fully developed infection throughout the epithelium within 2 weeks. The disease is transmitted among bees via the ingestion of contaminated comb material and water, and by trophallaxis; honey stores and crushed infected bees may also play a role in disease transmission. The infection mechanism of microsporidian parasites is based on mechanical injection of a polar filament protruding from the germinating spore. With physical force, the filament penetrates a host cell membrane into the host cell (ventricular epithelial cells for *N. apis* and *N. ceranae*). Through the filament, the infective sporoplasm is entered into the host cell cytoplasm where parasite replication, and later spore production is initiated (Larsson, 1986).Auto-infections can occur at the same time as new infections. Three days post-infection, mature spores start to develop in large quantities for *N. apis*, approximately a day later for *N. ceranae* (Forsgren & Fries, 2010). Both parasites have a temperature-dependent multiplication rate. *Nosema* spp. levels generally increase when bees are exposed to prolonged confinement, which increases the risk of in hive defecation. In the spring the infection levels may increase rapidly as the bees clean the combs for the expanding egg laying of the queen (Bailey, 1955) and a larger proportion of the bees are exposed to brood temperatures, where parasite replication, at least for *N. apis* is optimal (Lotmar, 1943). *Nosema ceranae* may grow better at slightly higher temperatures compared to *N. apis* (Fenoy *et al.,* 2009).

Any inherent natural defence by a bee colony against a heavy infection with the parasite depends on the colony size as well as on the prevailing weather conditions during the early part of the autumn of the previous year (Steche, 1985). If these conditions are unfavourable, the overall life expectancy of the colony is reduced. This may lead to the premature death of bees during winter or early spring. In a typical case of a colony being depleted because of a *Nosema* infection, the queen can be observed surrounded by a few bees, confusedly attending to brood that is already sealed.

In faecal droppings, spores of *N. apis* may retain their viability for more than 1 year (Bailey, 1962). Spores may also remain viable for up to 4 months after immersion in honey (White, 1919) and for up to 4.5 years in the cadavers of infected bees (Steche, 1985). The spores may lose viability after only 3 days when submerged in honey at hive temperature (Morgenthaler, 1939). Faecal contamination of wax, especially in combs used for brood rearing or other hive interior surfaces, provides sufficient inoculum for *N. apis* to be successfully transmitted to the next generation of bees and is probably the primary source of infection (Bailey, 1955). For *N. ceranae,* the durability of spores in different situations remains to be investigated, but they appear to withstand desiccation and heat better than *N.* apis spores (Fenoy *et al.,* 2009) whereas they are more sensitive to freezing temperatures (Forsgren & Fries, 2010). The relative importance of faeces, honey and cadavers as reservoirs of infective spores is not fully understood and it seems that temperature may have a marked effect on the rates at which spores lose viability, regardless of their medium (Morgenthaler, 1939).

Spores of *N. apis* may be killed by heating hive equipment or tools to a temperature of at least 60°C for 15 minutes. Combs may be sterilised by heating to 49°C for 24 hours (Cantwell & Shimanuki, 1970). This cannot be used for *N. ceranae,* which can survive up to 60°C (Fenoy *et al*., 2009). Fumes from a solution of at least 60% acetic acid will inactivate spores of *N. apis* within a few hours, depending on the concentration; higher concentrations are even more effective and will kill spores within a few minutes (Bailey, 1957). The corresponding data are lacking for *N. ceranae*. Such procedures come under the jurisdiction of national control authorities with protocols that vary from country to country. Disinfection can be carried out, for example, by putting acetic acid solution into bowls or on to sponges that can soak up the liquid on top of a sealed stack of boxes with combs. Following disinfection after an outbreak, all combs should be well ventilated prior to use. Suppression of *Nosema* disease can also be achieved by feeding an antibiotic, fumagillin, in sugar syrup to the colony (Cantwell & Shimanuki, 1970). Use of antibiotics for honey bees is forbidden in many countries and in the European Union.

B. DIAGNOSTIC TECHNIQUES

Table 1. Test methods available for the diagnosis of nosemosis and their purpose

Method	Purpose					
	Population freedom from infection	Individual hive freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Identification of the agent[(a)]						
Bacterial isolation						
Microscopy						
Antigen detection						
Conventional PCR						
Real-time PCR						
Mass spectrometry						

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose. PCR = polymerase chain reaction.
[(a)]A combination of agent identification methods applied on the same clinical sample is recommended.

1. Identification of the agent

In acute forms of infection, especially in early spring, brown faecal marks may be noted on the comb and the front of the hive (Bailey, 1967). Lack of seasonal prevalence and symptoms such as faecal deposits have been reported for *N. ceranae* (Higes *et al.,* 2008). At the entrance to the hive, sick and dead bees may be seen, although other causes, such as pesticide poisoning and diseases of adult honey bees (such as acarapidosis should be eliminated first if this is the case. The detection of these infectious diseases requires microscopic examination. During winter, *N. apis*-infected colonies may become severely depleted of bees or die out altogether. The majority of *N. apis*-infected colonies will appear normal, with no obvious signs of disease even when the disease is sufficient to cause significant losses in honey production and pollination efficiency (Anderson & Giacon, 1992; Fries *et al.,* 1996). A proper diagnosis can be made by microscopic examination of adult bee abdomen or ventriculus, by molecular tools (polymerase chain reaction [PCR]) or by transmission electron microscopy (TEM). To diagnose a *Nosema* spp. infection using microscopy the posterior pair of abdominal segments is removed with a forceps to reveal the ventriculus, complete with the malpighian tubules, the small intestine and rectum. The ventriculus is normally brown but, following a *Nosema* spp. infection, it can become white and fragile. However, this appearance is given by other causes of intestinal disturbance, for example feeding on indigestible food stores, such as syrup containing actively growing yeast. For a reliable diagnosis, a number of bees in a sample should be examined. For example, 60 bees examined in a composite sample will detect a 5% infection level with 95% probability.

1.1. Microscopy

It is necessary to attempt to distinguish between a *Nosema* spp. infection and an infection caused by *Malpighamoeba mellificae* (Webster, 1993). There is quite often an indication of dysentery in a *N. apis* infection. In an *M. mellificae* infection, there may be diarrhoea, often of a sulphur-yellow colour and with a distinct odour. Characteristics of *M. mellificae* cysts are described later. Secondary mixed infections may occur (Morgenthaler, 1939). A simple, non-quantitative method for detecting *Nosema* spp. infection is as follows: sampled bees should be obtained from the hive entrance in order to avoid sampling young bees that are less likely to be infected. At least 60 bees should be collected in order to detect 5% of diseased bees with 95% confidence (Fries, 1993). Before sending to the laboratory, the bees should be

fixed in 4% formol, 70% ethyl alcohol or frozen in a standard freezer in order to prevent them from decomposing and to improve their reception and organisation in the laboratory. The abdomens of the bees to be examined are separated and ground up in 5 ml of water. Then water is added representing a total volume of 1 ml per bee in the sample. A drop of the suspension is placed on a slide under a cover-slip and examined microscopically at ×400 magnification, under bright-field or phase-contrast optics.

The spores are about 5–7 μm long and 3–4 μm wide (*Nosema ceranae* is slightly smaller than *Nosema apis*). They are completely oval with a dark edge. Their contents, consisting of nucleus, sporoplasm and polar tube, cannot be seen. Dyes are usually not necessary. *Nosema* spp. spores must be differentiated from yeast cells, fungal spores, fat and calciferous bodies, and from *M. mellificae* cysts, which are spherical and approximately 6–7 μm in diameter.

When air-dried, ethanol-fixed smears of infected tissue are stained with Giemsa's stain (10% in 0.02 M phosphate buffer) for 45 minutes. *Nosema* spp. spores will have a distinctive appearance, with thick unstained walls and an indistinct blue interior, without visible nuclei. Insect cells, fungal spores and other protozoa stained in this way will generally have thinner walls, blue/purple cytoplasm and magenta-coloured nuclei.

To quantify the average infection level, spore counts in a haemacytometer can be used (Cantwell, 1970) or bees can be diagnosed individually to yield the proportion of infected bees. A standardised procedure such as the following must be used.

A sample of older worker honey bees is taken from the hive entrance or from peripheral frames if weather does not permit flight conditions. The abdomens of 60 individuals are macerated in 5 ml of water using a mortar and pestle and 50 ml of water is added for a total volume of 1 ml per bee (5 ml is added later). When tissue pieces have become quite fine, the suspension is filtered through two layers of muslin (thin loosely woven cotton fabric) in a funnel leading to a graduated centrifuge tube. A second 5 ml of water is used to rinse the pestle, swirl around the inside of the mortar and pour through the subsample in the funnel. When the suspension appears to be homogenous after shaking, a sample is taken to fill the calibrated volume under the cover-slip of a haemocytometer (blood cell counting chamber). After a few minutes the spores will have settled to the bottom of the chamber. *Nosema* spp. spores appear transparent but with a very distinct dark edge and are 5–7 μm long and 3–4 μm wide. They are best seen using a magnification of ×400 and bright-field or phase-contrast optics. The number of spores in each square is counted. Where a spore lies over the edge of a square, count only those spores that straddle the left and upper edges of the square, not the right and bottom edges. The size of these chambers can vary with manufacturer but they mostly consist of two separate chambers, each with a defined volume ($0.1 mm^3$) containing a marked counting grid with an area of $1 mm^2$. The whole grid consist of 3 × 3 large squares, separated by triple lines. Each large square is further subdivided into 16 smaller squares subdivided by double lines, in total 144 squares. The spores are counted in the smaller squares with the area of $1/25 mm^2$. When the counting is completed, the number of spores per bee in the sample can be calculated according to the formula:

$$Z = \alpha/\beta \times \delta \times 250{,}000$$

Where

Z = spore numbers per bee
α = total number of spores counted
β = number of squares counted
δ = dilution factor

The number 250,000 is used because the volume in each counted square is 1/250 000 ml and the equation uses the average number of spores per counted square. If no spores are seen, the result should be designated 'not detected', but that does not mean that the bees are not infected. Regulatory agencies will decide on the level of infection useful for their purposes.

A laboratory method for the simultaneous detection of *Nosema* spp. spores and *M. mellificae* cysts consists of the individual examination of the colonies using 60 bees per colony. A suspension of the abdomens of dead bees is prepared by grinding with 5–10 ml water; the volume of water depending on the number and condition of the bees. The suspension must be filtered to remove debris that would

interfere with the examination, first through a 100 μm and then a 40 μm filter. Parts of the malpighian tubules pass through the 100 μm filter, but are collected on the 40 μm filter. They are placed on a slide or bacterial counting chamber and examined at ×400 magnification. Only a few tubules are filled with cysts after an *M. mellificae* infection. The normal structure of malpighian tubules is not visible in this case. Only cysts inside the malpighian tubules can be taken as a positive result, because *M. mellificae* cysts are often confused with fungal spores and yeast cells.

1.2. Culture

Several lepidopteran cell lines have been shown to be susceptible to both *N. apis* and *N. ceranae* infection. Susceptibility was recently demonstrated for the following cell lines (Gisder *et al.,* 2010): MB-L2 (*Mamestra brassicae*), Sf-158 and Sf-21 (*Spodoptera frugiperda*), SPC-BM-36 (*Bombyx mori*), IPL-LD-65Y (*Lymantria dispar*), and BTI-Tn-5B1-4 (*Trichoplusia ni*). All these cell lines can be obtained through national cell culture collections together with protocols on how to maintain and passage the cell lines. However, the available protocols do not yet allow the continuous propagation of *Nosema* spp. in cell culture. Thus, it is not yet possible to replace infection of bees for the production of spore suspensions.

1.3. Polymerase chain reaction (PCR)

Different methods have been developed to distinguish *N. apis* from *N. ceranae*. A multiplex PCR is described below with which both pathogen types along with *Nosema bombi* can be clearly identified at the same time (Fries *et al.,* 2013).

1.3.1. Sample preparation for PCR

Place a maximum of 30 bees in a filter grinding bag. Add 0.5 ml (DNAase/RNAase free) ddH_2O per bee and homogenise the mixture using a homogeniser. Flash-freezing in liquid nitrogen is possible prior to homogenisation to aid in mechanically breaking open cells. Without access to a robot, a pestle can be used to crush the bee tissue (frozen tissue if flash-frozen) to generate a homogeneous homogenate. Transfer 100 μl of the liquid homogenate into a microcentrifuge tube and centrifuge for 3 minutes at 16,100 ***g*** to precipitate the microsporidia and other cellular material. Discard the supernatant. Freeze the pellet by using liquid nitrogen and crush using a pestle until pulverized (in order to break open *Nosema* spore walls), and repeat 2–3 times so that *Nosema* DNA goes into solution. The DNA extraction can be easily carried out using routine procedures or commercial kits. Complete the final elution step in 100 μl AE buffer.

1.3.2. Multiplex PCR

For multiplex PCR amplification of partial 16S rRNA (=SSU rRNA) gene fragments, the following primer combination can be used. Primers were designed based on alignment of all available sequence data in GeneBank of the 16S rRNA gene from *N. apis, N. bombi* and *N. ceranae*.

Mnceranae-F	forward primer: 5'-CGT-TAA-AGT-GTA-GAT-AAG-ATG-TT-3'
Mnapis-F	forward primer: 5'-GCA-TGT-CTT-TGA-CGT-ACT-ATG-3'
Mnbombi-F	forward primer: 5'-TTT-ATT-TTA-TGT-RYA-CMG-CAG-3'
Muniv-R:	reverse primer: 5'-GAC-TTA-GTA-GCC-GTC-TCT-C-3'

Note that the Mnbombi-F primer contains variable sites to account for the sequence diversity observed for this species.

PCR product size:

for *N. ceranae:*	143 bp
for *N. bombi:*	171 bp
for *N. apis:*	224 bp

PCR conditions:

1 μl of DNA (ca. 1 ng)
0.5 U of Taq polymerase
2× Taq reaction buffer (3 mM $MgCl_2$)

0.3 mM of each dNTP (dNTP mix)
0.4 µM of Mnceranae F
0.4 µM of MnapisF
0.5 µM of Mnbombi-F
0.5 µM of Muniv-R
in 10 µl total volume

Amplification is carried out in a thermocycler under the following conditions: Initial denaturation step of 95°C for 2 minutes, 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 60 seconds, with a final extension step of 72°C for 5 minutes. Visualisation of the amplification products is made using standard procedures.

2. Serological tests

There are no serological tests available.

C. REQUIREMENTS FOR VACCINES

No biological products are available.

REFERENCES

ADL S.M., SIMPSON A.G.B., LANE C.E., LUKEŠ J., BASS D., BOWSER S.S., BROWN M.W., BURKI F., DUNTHORN M., HAMPL V., HEISS A., HOPPENRATH M., LARA M., LE GALL L., LYNN D.H., MCMANUS H., MITCHELL E.A.D., MOZLEY-STANRIDGE S.E., PARFREY L.W., PAWLOWSKI J., RUECKERT S., SHADWICK L., SCHOCH C.L., SMIRNOV A. & SPIEGEL F.W. (2005). The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J. Eukaryot. Microbiol.,* **52**, 399–451.

ANDERSON D.L. & GIACON H. (1992). Reduced pollen collection by honey bee (Hymenoptera: Apidae) colonies infected with *Nosema apis* and sacbrood virus*. J. Econ. Entomol.,* **85**, 47–51.

BAILEY, L. (1955). The epidemiology and control of *Nosema* disease of the honey-bee. *Ann. Appl. Biol*. **43**, 379–389.

BAILEY L. (1957). Comb fumigation for *Nosema* disease. *Am. Bee J*., **97**, 24–26.

BAILEY L. (1962). Bee diseases. *In:* Report of the Rothamsted Experimental Station for 1961, Harpenden, UK, 160–161

BAILEY L. (1967). *Nosema apis* and dysentery of the honey bee. *J. Apic. Res*., **6**, 121–125.

BAILEY L. (1981). Honey Bee Pathology. Academic Press, London, UK.

BULLA (1977). *In:* Comparative Pathobiology. Vol. 1: Biology of *Microsporidia* (1976); Vol. 2: Systematics of the *Microsporidia,* Lee A. & Cheng T.C., eds. Plenum Press, New York, USA, and London, UK.

CANTWELL G.E. (1970). Standard methods for counting *Nosema* spores. *Am. Bee J*., **110**, 222–223.

CANTWELL G.E. & SHIMANUKI H. (1970). The use of heat to control *Nosema* and increase production for the commercial beekeeper. *Am. Bee J.,* **110**, 263.

FENOY S., RUEDA C., HIGES M., MARTÍN-HERNÁNDEZ R. & DEL AGUILA C. (2009). High-level resistance of *Nosema ceranae*, a parasite of the honeybee, to temperature and desiccation. *Appl. Environ. Microbiol.*, **75**, 6886–6889. Epub 2009 Sep 4.

FORSGREN E. & FRIES I. (2010) Comparative virulence of *Nosema ceranae* and *Nosema apis* in individual European honey bees. *Vet. Parasitol.,* **170**, 212–217.

FRIES I. (1993) *Nosema apis* - a parasite in the honey bee colony. *Bee World,* **74**, 5–19.

FRIES I., CHAUZAT M.-P., CHEN Y.-P., DOUBLET V., GENERSCH E., GISDER S., HIGES M., MCMAHON D.P., MARTÍN-HERNÁNDEZ R., NATSOPOULOU M., PAXTON R.J., TANNER G., WEBSTER T.C. & WILLIAMS G.R. (2013). Standard methods for *Nosema* research. *In:* The COLOSS BEEBOOK: Volume II: Standard methods for *Apis mellifera* pest and pathogen research, Dietemann V., Ellis J.D. & Neumann P., eds. *J. Apic. Res*., **52** (1): http://dx.doi.org/10.3896/IBRA.1.52.1.14

FRIES I., FENG F., DA SILVA A., SLEMENDA S.B. & PIENIAZEK N.J. (1996). *Nosema ceranae* n.sp. (Microspora, Nosematidae), morphologycal and molecular characterization of a Microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). *Eur. J. Protistol*., **32**, 356–365.

GISDER S., MÖCKEL N., LINDE A. & GENERSCH E. (2010). A cell culture model for *Nosema ceranae* and *Nosema apis* allows new insights into the life cycle of these important honey bee-pathogenic microsporidia. *Environment. Microbiol*.. **13**, 404–413.

HIGES M., MARTÍN-HERNANDEZ R., BOTIAS C., BAILON E.G., GONZALES-PORTO A., BARRIOS L., DEL NOZAL M.J., PALENCIA P.G. & MEANA, A. (2008) How natural infection by *Nosema ceranae* causes honeybee colony collapse. *Environment. Microbiol.,* **10**, 2659–2669.

KLEE J., BESANA A.M., GENERSCH E., GISDER S., NANETTI A., TAM D.Q., CHINH T.X., PUERTA F., RUZ J.M., KRYGER P., MESSAGE D., HATJINA F., KORPELA S., FRIES I., PAXTON R.J. (2007). Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera. J. Invertebr. Pathol.,* **96**, 1–10.

LARSSON R. (1986). Ultrastructure, function, and classification of Microsporidia. *Progr. Protistol*., **1**, 325–390.

LOTMAR R. (1943). Über den Einfluss der Temperatur auf den Parasiten *Nosema apis*. *Beih. Schweiz. Bienenztg*., **1**, 261–284.

MARTIN-HERNANDEZ R., MEANA A., PRIETO L., SALVADOR A.M., GARRIDO-BAILON E. & HIGES M. (2007). Outcome of colonization of *Apis mellifera* by *Nosema ceranae. Appl. Environ. Microbiol*., **73**, 6331–6338.

MORGENTHALER D. (1939). Die ansteckende Frühjahrsschwindsucht (*Nosema-Amoeben*-Infection) der Bienen. Erweiterter Sonderdruck aus der Scheizerischen Bienenzeitung Heft 2, 3 und 4.

STECHE W. (1985). Revision of ZANDER & BOTTCHER. Nosematose. *In:* Krankheiten der Biene, Handbuch der Bienenkunde.

WEBSTER T.C. (1993). *Nosema apis* spore transmission among honey bees. *Am. Bee J.,* **133**, 869–870.

WHITE G.F. (1919). *Nosema* Disease. United States Department of Agriculture Bull., No. 780, 54 pp.

ZANDER E. (1909). Tierische Parasiten als Krankenheitserreger bei der Biene. *Münch. Bienenztg*. **31**, 196–204.

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NB: There is a WOAH Reference Laboratory for nosemosis of honey bees (please consult the WOAH Web site: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the WOAH Reference Laboratories for any further information on diagnostic tests and reagents for nosemosis of honey bees

NB: FIRST ADOPTED IN 1989 AS BEE NOSEMATOSIS. MOST RECENT UPDATES ADOPTED IN 2013.