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# Nosemosis

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## Definition

Nosemosis is a parasitic disease affecting adult bees, due to the evolution and proliferation of the specific protozoan *Nosema apis* in the midgut epithelial cells. It causes digestive disorders (diarrhoea or constipation) and may impair the ability to fly.

## General epidemiology

*Nosema apis* was first described by Enoch Zander, a German scientist, in 1907, but earlier, the spores found in the intestine of diseased bees had been held responsible for the disease by Doenhoff (cited by Borchert 1970), although he concluded to the fungal origin.

The parasite has a cosmopolitan geographic distribution. In tropical and sub-tropical countries, it is considered of little pathological importance, however little information is available regarding its prevalence and pathological potential.

By contrast, in countries with a temperate climate, nosemosis is considered a severe disease. All European countries are affected, especially central Europe and the coldest regions of the continent. Many publications report serious adult bee losses in colonies of these countries, causing a significant decrease in productivity, and even the death of some colonies during winter. The economic consequences of the disease are often aggravated by difficulties in replacing infected queens in the colonies.

## Etiology

### The pathogenic agent

#### Systematic position

Realm Kingdom: Protist  
 Sub-realm: Protozoan  
 Phylum: Microspora  
 Order: Apansporoblastida  
 Family: Nosematidae  
 Species: *Nosema apis*

It is an intracellular parasitic protozoan, characterised by the production of complex single-celled spores.

#### Spore morphology (see Fig. 1)

Small-sized: 4.5 to 6 x 2 to 3 µm. Thick and refringent shell, constituted by a substance similar to chitin, and formed by a single piece. The inner structure is revealed by electron microscopy:

(i) A sporoplasm: the live part of the spore, filling the whole cavity, with 2 nuclei; it is a cell without mitochondria.

(ii) A spiralled, tubular, extremely long polar filament (100 to 400  $\mu\text{m}$ ), evaginable, and ending with a rectilinear part at the front end of the spore. It is the most characteristic element of these parasites and the part that transmits the infection.

(iii) A polar bag resembling the top of a mushroom, with a polar cap in its centre, reached by the front end of the polar filament.

(iv) A polaroplast formed by a series of superimposed layers, crossed by the rectilinear part of the polar filament.

(v) A vacuole is often present at the base or rear of the spore.

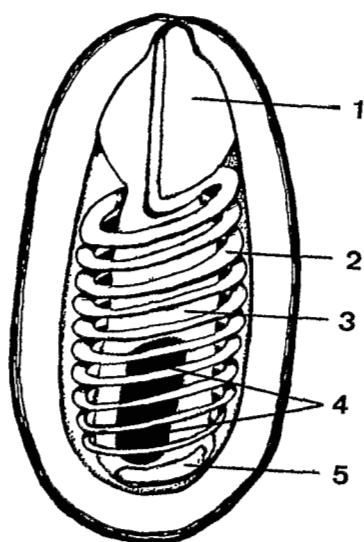


Fig. 1. Diagram of a spore: 1 polar bag; 2 polar filament; 3 sporoplasm; 4 two nuclei, vacuole.

## Biology and developmental cycle

### *Habitat*

*Nosema apis* is an obligate intracellular parasite, almost exclusively found in the midgut epithelial cells of the honeybee.

The host specificity of *Nosema apis* is quite narrow, although some authors seem to have been able to experimentally infect other insects. The site of multiplication in the midgut tissue also is highly specific; however, the parasite is sometimes found in the haemolymph.

### *Developmental cycle*

After the spores are swallowed by the bees, they reach the crop (proventriculus) and then the midgut (ventriculus) 10 minutes after ingestion. Spore germination, stimulated by the chemical environment of the intestine, causes an increase in the inner pressure, resulting in a sudden evagination of the polar filament as if it was the finger of a glove. In this way the rear end of the filament penetrates a midgut cell.



The sporoplasm and the 2 nuclei are enclosed by a plasma membrane as they pass through the polaroplast region and enter the filament duct, and after travelling along the whole length of the duct are inoculated in the host-cell.

Afterwards, the intracellular development of the parasite occurs in two different ways:

(i) Either a rapid and uninterrupted development during the active period of bees in fine weather:

- The parasite increases in size.
- The sporoplasm matures into a mother-cell, called a meront.
- Two successive schizogonies lead to the formation of 2 schizonts and a number of merozoites.
- Sporogony follows: the merozoites mature and become sporonts as a thick material is deposited at their periphery.
- Immediate division of the sporont into 2 sporoblasts.
- Maturation into thick-wall spores, formed 36 h after infection and expelled into the intestinal cavity. These spores germinate immediately and, by means of their evaginating polar filament, they infect the neighbouring epithelial cells (Fig. 2).

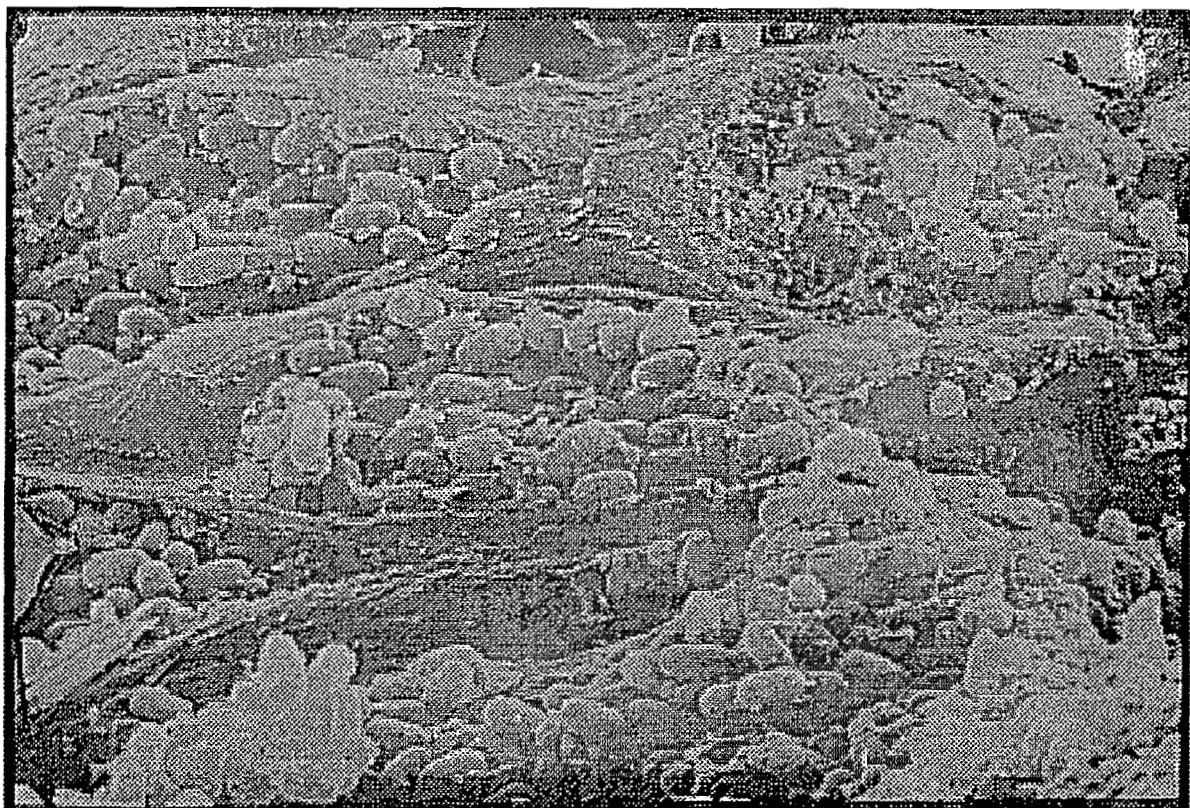


Fig. 2. Epithelial cells of the midgut, containing numerous spores.

(ii) Or a delayed development that occurs when winter is near:

- A second type of spore is formed from the sporoblasts: these are the late appearing thin-wall spores.
- These spores are not expelled but remain in an intracellular location where they directly become a mother-cell: the meront, which penetrates into the nucleus of the infected epithelial cell but ceases its development for the whole winter.
- In the spring, the parasite development resumes: 2 schizogonies, sporogony and production of thick-wall spores, found in the intestine.

In summary, the multiplication of the parasite leads to the formation of dimorphic spores:

- (i) Thick-wall spores occurring early, responsible for the transmission of the disease but also for the intercellular infection of the bee.
- (ii) Thin-wall spores occurring late, preserved in the digestive tract of the bees during the winter and responsible for the recurrence of the disease in the following spring.

## Spread and transmission

### Origin of the parasites

Spores are transmitted from infected bees through their excrement, especially when bees are dysenteric as the watery faecal matter is very sweet and attracts other bees. The ingestion of a single spore of *Nosema apis* could cause infection of the midgut of the bee; however, the average infectious dose is estimated to be from 20 to 90 spores per bee.

During the disease, 30 to 50 million spores can be found in the midgut. When the diseased individual is unable to leave the colony on a cleaning flight, spores accumulate in the rectum and more than 200 million spores can be found in a single bee.

### Resistance of the parasites

- (i) The thick walled spores allow the parasite to persist and remain infective in bee faeces from one year to the next. In the hive, the spores can survive in dead bees for 5-6 weeks, and in honey for 2-4 months.
- (ii) Action of physical agents: resistance for 2 months to desiccation, for 15-30 h to sunlight, for 20 minutes to a 60°C temperature (in water or honey).
- (iii) Action of chemical agents: phenol at 4% kills the spores in 10 minutes; formol is less effective.

### Means of transmission

Infection by ingestion of spores: (i) within the hive, by faecal matter directly ingested during cleaning activities or in contaminated food; and (ii) from hive to hive, by robbing, drifting and the contamination of flowers and water by diseased bees.

### Factors that favour outbreaks

*Nosema apis* infections are commonly present in adult bees in most apiaries without causing significant damage. The outbreak of an epizootic disease only takes place after the occurrence of certain environmental factors which cause stress. These factors are sometimes intrinsic but most of the time extrinsic, associated with the climate or beekeeping management.

#### *Intrinsic factors*

If egg laying by the queen slows or is inhibited, the emergence of young bees declines and the population of bees becomes older than average and more likely to be infected. Queen rearing is a beekeeping practice likely to aggravate infection.



### Extrinsic factors

(i) Season and climate are very important factors:

- During a good summer the rapid replacement of bees inhibits the development of the disease, even more so as the diseased bees die away from the colony (subsequently there is a lower risk of transmission).
- During a short and harsh winter, no problems arise.
- During a long and rainy winter, with variable temperatures, or even during a cool and rainy summer, the bees live longer, but are confined to the hive, and are more likely to defaecate on the combs and transmission increases.
- Spring is the critical period, when the colony is principally made up of old and diseased bees and when young bees clean the combs as brood rearing increases.

(ii) Secondary infections: essentially in association with *Malpighamoeba mellificae* and some viruses.

(iii) Beekeeping practices:

- Shortage of protein: measures to avoid protein deficit before the wintering period are beneficial. The excessive consumption of fresh pollen pellets or of pollen substitutes in the spring, favours nosemosis development.
- Using the same frames, on which the infective spores are preserved, over many years: in nature, feral colonies rarely remain for more than 2 years in the same location, consequently there is natural control.
- Movement of colonies early in the spring.
- Keeping too many colonies for the available forage in an area.

In summary, the influence of all the above factors leads us to distinguish: (i) slight infections, which are relatively frequent; and (ii) the outbreak of the disease nosemosis, which seems generally to result from poor weather conditions or bad beekeeping practices.

### Susceptibility

(i) Age: nosemosis almost exclusively affects older bees; newly emerged bees are always free of infection.

(ii) Bee race: certain races (Italian, Caucasian) seem to be more susceptible.

### Pathogenesis

The multiplication of *Nosema apis* causes a destructive and degenerative pathogenic action on the midgut epithelial cells, causing:

- (i) An inflammation of the digestive tract, which causes diarrhoea.
- (ii) A negative effect on nutrient uptake, as the midgut constitutes the only really active part of the digestive tract.
- (iii) The epithelial cells degenerate, showing large vacuoles, glycogen deposits and aggregated ribosomes, leading to a reduction of RNA synthesis and the suppression of enzyme secretion.
- (iv) A negative effect on the protein reserves of the fat body; a reduction in the levels of proteins and fatty acids in the haemolymph; the fatty acid composition of infected bees is modified and the corpora allata cells are affected.
- (v) Foraging activity occurs at an earlier age, which reflects a faster physiological ageing of infected bees.

(vi) Metabolic disorders affect the development of the hypopharyngeal glands, reducing the ability of the bee to secrete larval food.

On the other hand, constipation, which is sometimes observed, results from the obstruction of the digestive tract by the accumulation of spores.

The queen can become infected and the associated metabolic disorders cause degeneration of the ovaries and atrophy of the oocytes.

## **Clinical symptoms - Differential diagnosis**

### **Clinical symptoms**

Nosemosis exclusively affects adult bees. The disease can be inconspicuous for a long time, as the bees apparently continue to forage normally. In winter there is often a slow rise in infection and bee longevity decreases from 3-4 months to 4-5 weeks.

Symptoms are not very characteristic or specific. Nosemosis may occur as early as the end of the winter, but is most prevalent in spring:

(i) Lack of activity of foragers and decline in the adult bee population in spite of the existence of normal brood and good local conditions.

(ii) Many bees are unable to fly, crawling at the hive entrance, apparently paralytic.

(iii) Presence of pale, watery excrements, attractive to other bees (sweet excrements), covering the front and the inner parts of the hive.

(iv) Sometimes constipation: the bees have a distended abdomen and tremble or shake.

(v) Finally, the bees cluster together in small groups on the ground and on the upper part of the frames in the warmest part of the hive.

When the queen is severely infected, she becomes sterile and egg laying ceases. The loss of the queen in winter, when they are often not replaced, is quite frequently caused by nosemosis; in spring, the diseased and sterile queen is often superseded.

### **Differential diagnosis**

Nosemosis is difficult to distinguish by symptoms from the diseases that provoke digestive disorders and leave the bees unable to fly:

(i) Acarapisosis: increased mortality also occurs in the early spring. The crawling symptom is more pronounced.

(ii) Dysentery: results at the end of the summer time in a red or blackish diarrhoea, due to an accumulation of excrement in the rectum caused by intestinal disorders following an excessive intake of pollen.

### **Sampling**

It is possible to take samples of either live bees (which is preferable) or dead individuals for diagnosis, but it is best to collect foraging bees coming back to the hive (young bees often are disease-free) or to take bees from inside the hive on top of the cluster.

## Laboratory diagnosis

Since there are no clear symptoms, the diagnosis of infection can only be confirmed by microscopic examination of the intestinal content of bees for the presence of spores.

In the case of live bees, grasp the end of the abdomen with a pair of forceps and pull to withdraw the digestive tract. Alternatively, remove the abdomen and macerate in a known volume of water.

In the case of dead bees, either dissect out the gut from the abdomen or grind the whole abdomen in a mortar in a known volume of water.

A brief examination of a droplet of liquid from the macerated abdomen sometimes shows a characteristic milky-white colour. In healthy bees, the colour is rather yellow-green or brownish due to the presence of pollen which fills the intestine.

Under the microscope, the spores are readily recognised by their refringent character which is more evident by examination under phase contrast microscopy or after the addition of some droplets of Indian ink to the spore suspension. The spores can be differentiated from yeasts, which are commonly present in the bee intestine, by their larger size (6x3 µm) and the absence of budding forms.

The level of infection in colonies is highly variable during the year: low in summer, it increases in autumn and winter and becomes very high in spring, when the number of spores per individual in a large proportion of the adult bee population, often exceeds 50 million.

Microscopical diagnosis is generally very easy, but for practical purposes an estimate of the level of infection gives a useful indication of the condition of the colony.

The results of analysis may be expressed:

- (i) Either as a percentage of infected bees, after individual examination of a sample of some twenty live bees per hive. It is useful evaluation but is laborious and time-consuming.
- (ii) Or as the number of spores in pooled samples of a known number of bees extracted in a known volume (counting them by haemocytometer). It is possible to classify these results on scale of, for example 5, infection levels, previously determined by the laboratory.

## Treatment and prophylaxis

### Treatment

When a colony is very severely infected and weakened, it is preferable to destroy it. Only one effective pharmaceutical, which is well tolerated by bees, is recommended for use: fumagillin. This antibiotic substance, whose chemical structure is a bi-cyclo-hexyl-ammonium, is produced by the fungus *Aspergillus fumigatus*. Its mode of action seems to be to inhibit the replication of the DNA of the intracellular parasite without affecting the host-cell.

Fumagillin is used at a concentration of 0.0025%, that is 25 mg per kilo of syrup. One kilo of syrup (1/2 l water + 500 g sugar) is used per colony: half of it is put in the feeder, the other half can be sprayed on the adult bees covering the frames. The treatment is applied once a week, for 4 weeks from the beginning of spring, but not during honeyflows.

### Prophylaxis

As *Nosema apis* forms resistant spores which can persist in the hive and on equipment from one season to the next, it is necessary to diagnose it, to eventually notify it and to take measures to reduce its incidence.



## In a contaminated environment: Treatment and disinfection

All colonies, except the weak ones, may be treated by feeding fumagillin and the treatment must be repeated the following year. The dead and crawling bees must be destroyed by burning. The strong surviving colonies must be transferred to clean or disinfected equipment and must be treated. Hive bodies and contaminated combs can be disinfected with acetic acid vapour: after stacking up the boxes of empty comb as "chimneys" a cup filled with 80% acetic acid is placed at the base, the quantity being one ml per litre of volume to be disinfected; proceed at a temperature higher than 15°C -18°C for 48 h. Air well before use. Disinfection of the honey by heating it to 60°C, and the wax to 100°C for 30 minutes.

## In a healthy environment

Avoid the introduction of bees of unknown health status and contaminated beekeeping equipment. Comply with hygienic measures and avoid beekeeping management practices that aggravate infection.

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