Hygiene in the apiary

(A manual for hygienic beekeeping)

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Edited by Dalibor Titera, BRI Dol 2009

Editor

Dalibor Titěra

Team of authors

Michal Bednář
Jakub Dolínek
Marcela Haklová
Tomáš Jaša
František Kamler
Dalibor Titěra
Vladimír Veselý

Written on the basis of research results
of the Bee Research Institute, Dol
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INTRODUCTION

Every good bee-keeper's wish is to have healthy and strong bee colonies to bring him/her a lot of honey. And he/she wants to produce honey of the best quality to be offered to his/her customers. This will definitely be helped by bee-keepers having, apart from other things, good basic knowledge of the principles and procedures of disinfection. Indeed, in our opinion, such knowledge of disinfection techniques is absolutely necessary.

Micro-organisms are everywhere around us, and in huge quantities. One yoghourt pot contains more microbes than people on the Earth, and the weight of micro-organisms in one hectare of soil is equal to tons. Most micro-organisms are useful; only some of them are pathogenic. There are also pathogenic microbes which may cause diseases under certain conditions or which may aggravate the course of another disease. Disinfection and sterilization are aimed at eliminating these micro-organisms.

The significance of disinfection for beekeeping is ever increasing. The existence of antibiotics and other efficient medicines has meant that interest in cleanliness and hygiene has diminished. This applies also to beekeeping. Nowadays it is not justifiable to use antibiotics against diseases of bees for many good reasons. So we have to protect bees and bee products from extraneous substances and agents, which is where hygiene is of paramount importance.

Publication of this Manual was one of the goals of project PL 022568 ‘BeeShop’, supported financially by the EU’s 6th framework programme. In it, we follow up the useful methodological manual ‘Disinfection in bee-keeping’ which was produced by M. Peroutka in 1981 but which has been out of print for a long time. The procedures we describe here have been verified in the laboratory and have been tested in practice by our institute. Theoretical knowledge in the initial part of this publication draws on scientific and expert literature, mainly from the quoted book written by Vera Melichercikova, M.D.

The editor as well as the team of authors express their thanks to all other colleagues from the BeeShop participating institutes and the Czech Beekeepers Association who participated in experiments and contributed with numerous questions, advice and experience.

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Basic principles of disinfection

Disinfection is understood as such provisions which paralyze micro-organisms by means of physical, chemical or combined procedures with the goal of stopping the route of infection from its source to a susceptible individual. Disinfection can be defined as the destruction of or killing of micro-organisms or inanimate objects, in water, in the air and on intact skin. The goal of disinfection is to achieve a situation where no pathogenic microbes are present in the environment where they might cause disease or weakening of susceptible individuals, which in our case are bees.

If a disease is also spread in another way, via contact with infected individuals (e.g. in-flying bees to a hive) or via an intermediary carrier (e.g. *Varroa* mites), disinfection has, of course, also to be completed with therapeutic procedures. Disinfection has sometimes to be undertaken when carrying out rodent control and disinfestation (elimination of rodents and insects), mainly inside residential buildings and farm buildings.

Just to make the picture complete, it is necessary to define differences between disinfection and sterilization. Whereas disinfection removes pathogenic micro-organisms, sterilization is a process which liquidates all micro-organisms as well as other forms of life. This is, of course, more complex and more expensive. Disinfection is sufficient in many cases.

When considering a particular epidemiological situation, we distinguish the following kinds of disinfection:
a) preventive (prophylactic) and
b) focal (repressive), continuous and final.

Preventive disinfection

Preventive disinfection is performed when an infectious disease does not appear in a beekeeping operation or in the vicinity of an apiary. Preventive disinfection is usually a part of regular and comprehensive hygienic tasks. These tasks in beekeeping operations are mostly connected with the cleaning of sites (e.g. hive stands), tools and equipment. Care in the provision of clean water for bees is also included here. Preventive disinfection is also to be practised when handling and processing bee products. All trained persons may carry out preventive disinfection.

Focal disinfection

Focal disinfection is aimed at paralysing germs at the focus of an infection, with the aim to stop further spread of infection. At the same time, we have to:
- be aware of the ways and mechanisms in which infections are spread
- know the efficiency of disinfectants

- consider adverse side effects of disinfection on the material being treated as well as on the environment (this also applies to preventive disinfection).

Focal disinfection can be performed:

a) *continuously*, meaning repeated and systematically from the moment when the focus of disease appears. It may be combined with other actions which either have been imposed in form of extraordinary veterinary provisions (destruction and elimination of infected bee colonies and burning of equipment) or which logically complete the effort to get the disease under control (e.g. tracing and registration of the movement of diseased hives).

b) *finally*, meaning a one-off operation which precedes elimination of the focus and withdrawal of extraordinary veterinary provisions.

General requirements for disinfection

Disinfection requires expert knowledge. Only approved preparations and procedures may be used[1]

The following has to be taken into consideration in the course of disinfection:

- activity spectrum, i.e. whether the selected disinfectant and disinfection procedure work against those micro-organisms we need to eliminate
- how the disinfectant is applied (wiping, dipping, spraying, foam)
- recommended concentration of the disinfectant
- exposure time, i.e. the time necessary for the disinfection treatment to be effective
- disinfected environment, (i.e. how the expected effect is modify by other substances present, acidity, adsorption, inactivation, temperature, light, UV radiation and so on)
- effect of the disinfecting procedure on the material to be disinfected, mainly with regard to its damage (corrosion, colouring, change of properties)
- financial cost of the disinfection procedure.

Disinfection is a complex process which is also affected by microbes. Their survival is affected by many internal factors such as age, growth phase, pigment content, substrate they grew on and, of course, kinds of microbes, their resistance and other effects. Even a population of one kind of micro-organism may have differences in resistance that are dependent upon circumstances or that are genetically encoded.

Complexity is also given by the fact that disinfectants sometimes have a selective effect, so they do not act universally against all microbes. Two disinfectants may mutually inhibit when in mixtures or, on the contrary, they may intensify (synergy).

[1] List of preparations for disinfection, disinfestation and rodent control, referenced in the list of literature.

The success of disinfection also depends on the disinfectant used. It is thus not possible to rely on general recommendations given by manufacturers but it is necessary to duly verify the disinfective effect experimentally for a particular effect (validation) and also adhere to the verified procedures. Success is then dependent on conscientiousness of staff who perform disinfection.

Various disinfection effects

Disinfectants represent a very diversified group of chemical substances which evoke changes that are adverse for the survival of micro-organisms. An action indicated as:

-cidal means 'killing of organisms';

-static means temporary loss of multiplication or drop in growth activity.

Table 1 Disinfection effects

Effect		Against
bactericidal	bacteriostatic	bacteria
fungicidal	fungistatic	fungi, mould
sporicidal	sporistatic	bacterial spores
virucidal		viruses

Other preparations used in agriculture (biocides) have similar specialized names but they are not used to perform disinfection. They are, for example: herbicides (against weeds), insecticides (against insects), acaricides (against mites) and rodenticides (against rodents).

Routes to disinfection

Active substances usually affect the metabolism of micro-organisms and their enzymes. This takes place via physical or chemical methods or via a combination of the two. Effects, of course, also depend on the technical means by which we apply the disinfectant to the microbes to be destroyed. An overview of the main routes to disinfection is in the table 2.

Table 2 Routes to disinfection

dipping	items are dipped (without air bubbles) for a certain period of time into the disinfecting solution

wiping	with a sufficiently soaked cloth, mop or swab under adherence to the prescribed period or until drying
spraying	pressurised and mechanic sprayer suitable for small areas; efficient concentration is difficult to achieve; they may irritate the operator
gassing	complex from the aspect of equipment and occupational safety; suitable for veterinary and manufacturing application
evaporation	more complicated to achieve the required concentration of vapour in the whole space being treated, especially if the space is large
foam	products are applied with foam generators; this way is suitable to clean walls and ceilings

Physical methods of disinfection

Physical methods of disinfection are generally friendly to the environment than chemical methods. They are based on the use of dry or wet heat and on application of radiation. Not all the methods mentioned below are sufficient to kill bee pathogens. Detailed recommendations are in the part of this manual dealing with specific disease organisms.

We can consider the use of the following options in beekeeping operations:

■ Burning. The oldest and yet still an excellent method of physical disinfection. Very resistant bacteria (such as American foulbrood (AFB)) are destroyed along with animal corpses, combustible items and general residues following mechanical cleaning. This includes burning tools which cannot be heated sufficiently as well as scorching surfaces of hives etc. with a flame.

■ Boiling in water under normal (atmospheric) pressure for a period of 30 minutes. The disinfecting effect is increased by adding 1 - 2 % of crystalline soda to the water.

■ Boiling in a pressure cooker for a period of 20 minutes.

■ Disinfection with hot water in washers and washing machines at a temperature of 90 °C, as per the manufacturer's instructions.

■ Hot air, flowing at best, at a temperature of 110 °C to 150 °C in ovens and dryers for a period of 30 minutes. Higher temperatures and humidity increase the disinfection effect. Hot air disinfection is suitable only for small items and materials which can be sufficiently heated through to a depth of at least 3 mm.

■ Pasteurisation consists in quick warming up to 85 - 90 °C for a period of several seconds and subsequent cooling down. It is rather used as a preservative method to increase durability of drinks.

■ Steam disinfection by a fixed or mobile steam generator. It is useful to realise that steam, unlike hot water, may even be warmer than 100 °C. Steam has to be under moderate pressure and at a minimum temperature equal to 110 °C with effective exposure of 40 - 45 minutes for it to be effective in disinfection. Usable for frames and small utensils. The effect of steam and pressure is the functional basis of so-called autoclaves.

■ Ultraviolet radiation. Light of wavelengths equal to 253 - 280 nm has a bactericidal effect. Maximum efficiency is achieved at 265 nm. Radiation of wavelength shorter than 185 nm evokes formation of ozone. Sources in addition to the sun include various so-called germicidal fluorescent lamps. The efficiency of ultraviolet lamps drops quickly as they age. Therefore, the duration of operation of a lamp has to be recorded. Good lamps are able to record automatically the duration of usage. Ultraviolet radiation does not penetrate into the objects being treated; the radiation acts only on the surface directly irradiated. A layer of dust reduces the efficiency. Microbes in water are killed only up to a depth of 0.1 to 1 mm. Sporulating bacteria are resistant to ultraviolet radiation. Ultraviolet radiation destroys some viruses very well. It is not still sure whether these viruses include also those afflicting bees.

■ Ionising radiation (radioactivity). Ionising radiation within a certain range of wavelengths (around 10 picometres) has considerable power to kill bacteria. Radiation of this wavelength is known as gamma radiation. The minimum dose to kill bacteria is equal to 5 kGy (kilogrey, i.e. KJ/kg).

Gamma radiation comprises waves like, for example, light, radio waves or X-rays. Irradiated objects have to stand or move for a certain period of time near the source of radiation. Chambers for exposing equipment to gamma radiation are complex; it is not possible to construct or operate them as an amateur. (Contacts to some radiation plants can be found on special web sites for each EU country). Irradiating equipment does not result in any radioactive contamination of that equipment. Also, the irradiated objects do not become further sources of radiation. As this method of disinfection is widely used, for example in the manufacture of all bandages and in packing common spices, there is no doubt that it could also be very useful in beekeeping. Its disadvantage is its relatively high cost for treatment and the need to transport materials to be disinfected to radiation plants. It is not possible to perform the treatment while one waits; two trips to a radiation plant are thus necessary.

Chemical methods of disinfection

Chemical methods of disinfection prevail over physical methods in many ways. The following chemical reactions are employed most often in the disinfection effect:

Table 3 Mechanism of chemical disinfection

Mechanism	**active substances**
oxidation	chlorine, hydrogen peroxide, other peroxide compounds, ozone, ethylene oxide
hydrolysis	acids, caustic soda, hot water
formation of salts of proteins	salts of alkali metals and heavy metals
coagulation of proteins in cells	quaternary ammonium salts, metals, phenols, alcohols
changes in permeability of cell membrane	quaternary ammonium salts
penetration into the enzymatic system	metals, formaldehyde, phenol
mechanical disruption of cells	quaternary ammonium salts

A two-stage procedure may be employed during disinfection - mechanical cleaning and disinfection as such. Both stages may be joined by using disinfectants with washing and cleaning properties.

The success of chemical disinfection is reliant upon the following principles:

■ Disinfection solutions have to be prepared via careful measurement or weighing of active substances and water in order to achieve the correct, prescribed concentration.
■ During the preparation of solutions, one should not confuse whether the formula specifies concentration of the product (which mostly has a trade name) or that of the active substance.
■ The solution should be prepared just before use - its efficiency drops following preparation.
■ Increased efficiency of some preparations can be achieved under a higher temperature of the solution. For chlorinated, phenolic and quaternary ammonium salts, up to 50 - 60 °C, for hydroxides up to 80 °C.
■ It is very good to alternate disinfectants at one workplace with active substances of a different chemical nature in order to prevent the build-up of resistance of microbes to any active substance.
■ Protective equipment, i.e. gloves, goggles, suitable clothing and footwear should be used in the course of work with disinfectants.

■ Disinfectants should be selected with regard to their efficiency, smell (honey can acquire foul odours within the hive), residues on disinfected items, biodegradability (option for easy disposal), shelf life and price.

Preparation of disinfection solutions

Disinfection solutions should be prepared using a precise measurement of concentrated active ingredient, water or other solvent. Solutions should always be prepared fresh, if possible. Therefore only prepare sufficient quantity which one is able to use immediately. Dilution should be performed in the well-known order: water first, disinfectant after.

Required volume of solution	Required concentration of solution			
	0.5 %	1 %	2 %	5 %
1 litre	5	10	20	50
2 litres	10	20	40	100
5 litres	25	50	100	250
10 litres	50	100	200	500

Numbers in bold-framed part of the table represent the quantity of disinfectant in grams or millilitres which is added into the required quantity of water.

Various kitchen measuring jugs can be used in practice. A glass usually holds ca. 200 ml, a tablespoon about 12 grams. A common bucket has a volume of 8 - 10 litres. Some plastic vessels have scales or gauge marks which indicate volume.

TYPES OF CHEMICAL SUBSTANCES WITH DISINFECTIVE PROPERTIES

Hydroxides and alkali salts

Solutions of hydroxides and alkali salts act through their high concentration of hydroxylic anions (OH^-). Solutions with a pH higher than 12 are very efficient. The pH of a solution can be measured by pH test papers. They are often used as additions to other means of disinfection because they increase total disinfection efficiency. They can be used while hot, but then the more caution has to be used. Chemical burn with caustic soda causes deep lesions. We also appreciate that in beekeeping they dissolve wax and lipids. They are readily available and relatively cheap.

This group includes

- potassium hydroxide, KOH
- sodium hydroxide (caustic soda), NaOH
- sodium carbonate (washing soda), Na_2CO_3

they are used in 2 up to 6 % concentration, at best while hot (they also destroy spores of AFB at 80 °C)

- calcium hydroxide, $Ca(OH)_2$

is used in 10 up to 20 % concentration to disinfect soil, waste traps and so on.

Inorganic (mineral) acids

These are relatively efficient but are also caustic and can corrode materials. They act in aqueous solutions via a high concentration of reactive hydrogen ions. Acidity of solutions is expressed in pH units. The lower the pH, the stronger is the solution. They are not used very much in beekeeping. Hydrochloric acid at a concentration of 10 % (HCl) is used for general cleaning. Phosphoric acid (H_3PO_4) and sulphuric acid (H_2SO_4) in concentrations of 0.5 up to 5 % are used in the processing of beeswax. Boric acid is usually available in home first-aid kit boxes. It is a relatively mild disinfectant used to treat eyes.

Attention! Acid should always be poured into water in course of dilution (and not *vice versa*). Solutions become very warm when diluted.

Organic acids

Formic acid and oxalic acid are used in beekeeping for their acaricidal effect against the parasitic mite *Varroa destructor*. Acetic acid and sulphuric acid (formed in a humid environment when thick candlewicks are burnt) are used against the larvae (caterpillars) of waxmoths in stores of combs. Besides their uses described above, all these acids also have disinfecting effects against some bacteria and fungi (the causative agents of stonebrood, chalkbrood and nosemosis of adult bees). Peracetic acid (also known as peroxyacetic acid) (trade name Persteril) is a good disinfecting agent to clean glass vessels, for example carboys/demijohns during production of mead. It is used in concentrations equal to 0.2 - 0.5 % and equipment needs to be treated for at least 10 minutes.

Oxidizing substances

Oxygen is very reactive and thus in fact toxic, principally when in its monatomic form. All substances that dissociate from monatomic nascent oxygen have very good disinfecting effects. However, they only have a short-term action in the presence of organic substances. The disinfecting effect of oxygen is increased in the presence of some metals, for example silver and magnesium.

The following may be used for disinfection in beekeeping practices:

- Hydrogen peroxide (H_20_2). It is stored as a solution in water. It is sold as 3%, 10% as well as 30% solutions. Solutions of concentration equal to 0.5 to 3 % can be used. A hydrogen peroxide solution releases oxygen, though unfortunately only the molecular form and not the monatomic form. Solutions of hydrogen peroxide turn old very quickly.

- Potassium permanganate, also called permanganate ($KMnO_4$). Dark-violet crystals which are easily soluble in water. Oxygen is released in contact with organic substances and the solution turns brown due to the manganese monoxide which is formed (brown solutions already do not have any disinfecting effect). An aqueous solution (0.3%) of permanganate acts against bacteria and viruses. It was formerly used to disinfect hands. Permanganate is mostly replaced today with colourless agents with different bases.

- Organic peroxides are modern but rather expensive disinfectants. They act against spores of bacteria (AFB). An advantage is the fact that they are environmentally friendly; they disintegrate completely into innocuous substances. Dismozon (active substance magnesium monoperoxyphthalate) is a time-tested product, which is efficient against spores of AFB but expensive for daily use in beekeeping practice.

- Ethylene oxide is a liquid with a low boiling point (11 °C). Gaseous ethylene oxide disinfects the surface of objects very efficiently. It is used in beekeeping mainly in large commercial plants which have the necessary equipment and which can ensure the safety of operators.

Halogens

The mechanism of action of halogens is based on a combination of the effect of highly reactive halogene compounds and nascent oxygen (see the previous group of compounds) which is released in an alkaline environment.

Sodium hypochlorite ($NaClO$) has excellent bactericidal and virucidal properties. Its is widely used. It is an efficient constituent of many commercial preparations. Commercially available preparations mostly contain about 5% sodium hypochlorite and they are further thinned for use as per instructions for use. The solution has to act for approximately 30 minutes in order to achieve a disinfecting effect. The effect is considerably weakened in a mixture with 5% sodium hydroxide. Caution! Mixing of sodium hypochlorite with acids and acidic disinfectants is highly dangerous and should not be undertaken because gaseous hydrogen chloride may be released which is irritating and toxic to humans when inspired.

Metals and their compounds

Some metals have considerably toxic effect on live cells. Ions of metals pass into solution in insignificant quantities, but they may nevertheless be very efficient as disinfectants. Modern technologies are able to prepare and use suspensions of fine particles of metals (so-called nanoparticles) which have further noticeable and unexpected effects. Compounds of silver (there are various commercial preparations) are used to disinfect drinking water. Bees tolerate relatively well colloidal silver and nanosilver. Water in swimming pools is often disinfected with preparations containing copper. Bees do not mind copper, either. Compounds of tin are added against moulds into paints and painter's colours.

An excellent antiseptic agent, mainly for festering burns, is the silver salt of sulphadiazine (the preparation is called DERMAZIN).

Alcohols, ethers

Many commercial disinfectants are based on alcohols (ethanol, isopropyl alcohol and other ones). They have their best effect in aqueous solutions of about 70%, and in combination with other active substances. Concentrated alcohol (pure alcohol, 99%) as well as too diluted alcohol (e.g. rum, whisky, plum brandy) do not have any good disinfecting effects. Alcoholic preparations are not sufficient to destroy spores, e.g. those of AFB. They are reported to be reasonably efficient against some viruses. However, it has not been verified yet whether alcoholic solutions also destroy propagating bee viruses and under what conditions.

Aldehydes

The disinfecting effect of aldehydes is based on chemical reduction and alkylating reactions which denature proteins and damage cells.

Formaldehyde (HCHO) is a gas. When in solution, at 35 up to 40 % solution in water, it is known as formalin. It was largely used in the past in the health service as well as in agriculture because it is efficient and cheap. Very strict rules governing its use are in force now with regard to its harmful effect on human health (potential carcinogen). In theory, it might be used in beekeeping only to disinfect equipment such as honey separators, barrels and so forth which can be rinsed very thoroughly with water several times. Porous objects such as wood and also combs and wax must not be disinfected with formaldehyde as the treatment leaves considerable residues in their surface and it is not possible to remove these residues easily. These harmful substances must not come into contact with food, i.e. with bee products.

Glutaraldehyde is the basis of very efficient disinfectants. Glutaraldehyde reliably destroys bacteria, viruses and spores following prolonged exposure. It is used as a part of disinfection mixtures. It is thinned before use to a 2% concentration and alkalinized with the addition of 0.3 % $NaHCO_3$ (sodium bicarbonate). The solution can be used for several days. Glutaraldehyde 0.25% - 0.5% is used in the form of sprays, for example in the preparation of STERILIUM.

Cyclic compounds

This is a group of substances damaging enzymes and protoplasm. They often irritate the skin and they have a characteristic smell. Therefore, they are not used very much for beekeeping purposes despite their efficiency. Some, like pheonol, are suspect carcinogens.

Phenol is one of the oldest and well-known disinfectants. It is badly soluble in water though easily soluble in alcohol (ethanol). Phenol and similar compounds have been used as constituents of disinfectants to this day. It is interesting to note that phenol is used as a standard to assess the efficiency of other disinfectants. A so-called phenol coefficient is a number which gives how many times is a product is more efficient that phenol. Medicinal manuca honeys from New Zealand are offered on the market and their effect against some bacteria is comparable to that of a 16 % phenol solution.

Phenol is a good disinfectant. It used to be an efficient and cheap substance for rough disinfection, sold as LYSOL. Preparations based on phenol - Orthosan and Kresosan - have been used in the past.

Surface-active substances - tensides, detergents, wetting agents

These are natural as well as synthetic substances (soaps, detergents which are used in washing. They reduce the surface tension of water, which increases wettability of surfaces of the objects being cleaned. As they dissolve grease, they help a lot during disinfection.

Tensides from the group of quaternary ammonium compounds, and which are sold under the names of Ajatin and Septonex, have especially disinfecting effects. Quaternary ammonium compounds particularly act on gram-positive bacteria; they are only ineffective on gram-negative bacteria. Their disinfecting effect is eliminated by soap. Their importance in disinfectants is considered to be questionable today.

Combined preparations

Disinfectants based on several active substances can be suitably combined and are manufactured nowadays for commercial sale. Higher efficiency is thus achieved at lower concentrations of preparations. More and more attention is paid to the environmental burden of disinfectants, so lower concentrations are to be welcomed. The composition, dilution as well as spectrum of agents against which they can be usefully employed are usually given on instruction labels. However, details of the effects of active ingredients can slide into promotional wording. Experiments, however, show that combined preparations are not miracle disinfectants. Some traditional, time-tested and considerably cheaper products bring at least comparable results.

Confirmation of the efficiency of products

Commercially marketed products, including their instructions for use, have to undergo official registration. Lists of disinfectant products are published by local health authorities in each EU country. Laboratory assessment of the products is carried out by means of fixed comparative methods on selected strains of bacteria, fungi or viruses. However, the micro-organisms we fight against in beekeeping include several highly resistant ones, particularly AFB and its resistant spores. This bacterium even survives the action of many preparations which are described as efficient against its spores. The description of some products states that they are virucidal, but this is true only against the virus species/strains being tested. We still know little about the form and conditions under which bee viruses survive; they belong mostly to the group of so-called picornaviruses. Therefore, the laboratory of the Bee Research Institute continually develops and checks disinfection procedures designed specifically for small and large bee operations.

Biggest mistakes in disinfection

- selection of wrong preparation or combination of preparations
- use of wrong concentration
- use of old or depleted solution
- insufficient exposure time for disinfection
- poorly cleaned surface of the object being disinfected

PATHOGENS AND PESTS: SENSITIVITY OF INFECTIOUS AGENTS

Viruses

Viruses are classified as simple micro-organisms or complex organic molecules. They are not able to reproduce on their own but they can multiply in host cells. About ten virus diseases of adult bees and bee brood have been described in terms of their symptoms, and several tens of viruses have been isolated which are connected with these problems. This number is not definitely final. The majority of bee viruses known so far belong to the small, uncoated, single strand RNA viruses. They are called picorna viruses or picorna-like viruses (i.e. similar to picorna viruses). These viruses are very resistant to disinfectants; they are resistant, for example, to alcohol, presumably because they do not have a protein coating. As there is still little knowledge about these issues, it is possible to deduce from wider experience that the principal means of disinfection will be mechanical cleaning (removal and burning of hive debris) and in the subsequent use of oxidizing preparations to disinfect equipment. Fire/scorching helps to disinfect equipment.

Bacteria

Bacteria are microscopic single-cell organisms; they are extremely variable in form and function. They are mostly useful and necessary for life on earth. Only some are pathogenic.

As regards disinfection, it is important to divide bacteria in sporulating and non-sporulating types. All bacteria can be relatively easily destroyed in the so-called vegetative stage, i.e. in their common form when they are growing and dividing. As regards sporulating bacteria, besides their vegetative forms we also have to fight against their spores, which are produced by bacterial colonies as their tough and resistant form. Spores of bacteria can survive adverse conditions and it is very difficult to destroy them by means of disinfection procedures.

An example is represented by the non-sporulating bacteria *Leuconostoc mesenteroides*, which causes sugar solutions become mucilaginous. This bacteria does not survive half an hour at a temperature above 60 °C. The exact opposite in resistance is represented by sporulating bacteria. Ten per cent of AFB (*Panibacillus larvae*) spores survived in our experiments at a temperature equal to 108 °C for a period exceeding fifty hours.

AFB and EFB are considered the main bacterial diseases of honey bees. They are both highly contagious. Therefore emphasis is put on disinfection. We need to maintain cleanness and fight against bacteria, as doe the food industry, even in those bee operations where these diseases have not been found. In general, bacteria can be destroyed by high temperature, UV radiation and by chemical agents that vary considerably in their efficiency. A 5 % solution of caustic soda, which was recommended in the past, acts against AFB spores only while hot (80 °C). However, caustic soda is efficient in a mixture with sodium hypochlorite even at room temperatures. Frost does not destroy the majority of bacteria, neither does extreme cold (e.g. the boiling temperature of liquid nitrogen, which is -196 °C) kill AFB spores.

Fungi and microsporidia

Fungi are larger organisms compared to bacteria, unicellular and multicellular, and they are often fibrous. They also have their irreplacable role in nature and only some of them cause economic problems. We face nosemosis in beekeeping (caused by the microsporidia *Nosema apis* and *Nosema ceranae*, and formerly classified among the protozoa). The fungus *Ascosphaera apis* causes chalkbrood. Yeast is also classified among fungi. Yeasts, along with moulds and bacteria, enter honey in the course of processing, particularly in an unclean environment. Though most fungi have vegetative stages as well as dormant stages (spores), they are not so resistant as bacteria and disinfection is more feasible. High temperatures and the action of the majority of the chemical disinfectants described above will destroy fungi.

Further infectious agents

Further originators of bee diseases are not among the micro-organisms but rather they belong to more complex organisms (mites *Varroa, Acarapis, Tropilaelaps*, the beetle *Aethina tumida*). Reducing their parasitic effects is often focused on destruction of the adults, which is thus not disinfection in the narrow sense of the word.

Preventive (prophylactic) disinfection

Cleaning, cleanliness and disinfection

Micro-organisms are found in astronomical numbers in disintegrating organic matter. Colloquially said: dirt, dust, mess. Beekeeping operations, small and large, aim to produce food. Therefore they should be at the level of hygiene expected in food production. Dirty environments may contain spores of nosema, bacteria and bee viruses. Their occurrence is reduced by cleaning.

Cleaning should mainly be mechanic, completed with wet wiping of firm floors and with wiping of surfaces. A detergent which dissolves dirt and grease well should be added to lukewarm water. Washed surfaces should then be wiped thoroughly with a disinfectant. Disinfection has to act for the prescribed period of time in order to be efficient. If the disinfection gets dry too soon, the process has to be repeated.

Rodent control

Cleanliness also includes fighting against harmful rodents - rodent control. An animal whose behaviour may cause devaluation or destruction of beekeeping equipment and bee products is clearly a pest in beekeeping. However, it is very important to realize that a pest represents only a human definition for a certain group of animals which were, are and will be a part of nature, independent of humans.
Reasons for rodent control are the following:

- Prevent the hazard of contamination of beekeeping operations and food with micro-organisms.
- Avoid contamination of food with excrement, urine, hairs as well as with dead bodies of pests.
- Avoid material damage to equipment.

Preventive provisions

Beekeeping storerooms, honey extracting rooms and warehouses should not provide pests with shelter, food, or conditions for reproduction. Control of rodents is already helped by the correct arrangement of rooms as well as by regular cleaning of them. Equipment which is not in use, and rooms where nobody enters, represent ideal shelters for rodents. Preventive provisions include inspections and checks, which should be carried out by the beekeeper but which may also be carried out by representatives of a veterinary service or hygiene service. Direct as well as indirect evidence of the presence of pests need to be looked for in course of inspections (dead bodies, excrement, prints of paws in dust, bitten objects, black smudges near floor alongside walls). Monitoring and rodent control should be scheduled according to the results of these inspections.

Rodent control

So-called rodent control stations with traps are used most often in food processing premises for monitoring purposes and rodent control (mainly brown rats and mice).

These stations protect traps against mechanical damage (squashing, breaking, drenching) as well as against consumption of the bait by a family pet - cat or dog. Plastic stations are the most suitable to use. They can be purchased or hand-made. Professional stations (with commercial traps) have to be used for official monitoring of controlled sites. These stations are sealed so that only authorized staff members or inspectors may handle them. They are, in fact, boxes with openings. A toxic bait is fastened inside in such a way that a rodent may nibble it but nor carry it away. Stations must be checked on a regular basis and records of these checks kept. If the baits are not nibbled, everything is in order. If they are nibbled, cleaning of the site and placing of more baits is necessary. In order to catch mice or brown rats, we can also use classic spring traps with baits. These traps should kill rodents immediately. In accord with EU Acts against cruelty to animals, the manufacture, import and sale of jawed traps and adhesive traps are nowadays prohibited.

Beehives, frames

Only such materials which are easy to clean and disinfect should be used to house bees, if possible. We also have to have an option to dispose of beehives and their parts in an environmentally friendly way, if necessary, or after their service life is over. Wood is the most common material used for beehives, which is absolutely ideal for bees. Disinfection is rather complicated by wood's porosity; living micro-organisms may hide in its pores. Plastics may be presented by manufacturer as washable but attention, please! They also have microscopic pores which host bacteria. Disposal of plastics, resilent as well as foam ones, is a difficult and expensive issue.

In order to maintain health and sanitation, and necessary cleanliness, all empty beehives and replacement parts have to be disinfected before bees are placed inside again. An exception may be allowed in case of supering healthy bee colonies. It is sufficient to disinfect them once every 3 years. Utmost attention has to be paid to the bottoms of beehives.

Disinfection of wooden supers/brood boxes

Mechanical cleaning is the first step. Inside and outside walls of the boxes should be thoroughly scraped with a steel scraper. We can also use a hot-air gun or blowtorch to help clean boxes. Scrapings and sweepings should be burnt. Cleaning is followed by disinfection as such.

Thermal disinfection

Fire

Blowtorches powered with propane (touristic cartridges) or blowtorches which can be connected to common pressure cylinders by means of a hose are available and safe. Petrol burners with pumps are less practical and more hazardous. Electric hot-air guns do not have the necessary power output. Scorching with flames is not sufficient for disinfection to be efficient. Material has to be heated up in every place, i.e. singed to brown. Despite that, singed wood is not enough to kill all AFB spores within the wood. Its spores are up to 3 mm deep in the wood structure. We have seen beekeeper professionals abroad who stack up to fifteen supers, one on another, in an open space and make a fire underneath the stack. The fire scorches the inside surface of the boxes within a short time due to the stack effect. This method requires a lot of experience and caution.

Long-term heating

If we are able to heat brood and super chambers in an oven to 50 - 52 °C and to maintain this temperature for at least 24 hours, we will free beehives as well as frames, honeycombs and all other equipment from spores of nosema and chalkbrood. Parts of beehives and other items have to be on pallets so that hot air can flow into them. Besides a source of heat, the oven also need fans which ensure good air flow and thermal probes which ensure the necessary temperature in all corners of the oven. Warmer air gathers near the ceiling of an oven and heating is insufficient near the oven floor without constant circulation. If no honeycombs are in the oven, there is no hazard of overheating. The temperature should not exceed 55 °C to prevent honeycombs from collapsing. A high temperature takes about one day to be reached in an oven, which needs to be borne in mind, and it is necessary to keep the oven hot for the next 24 hours after a temperature of 50 °C is reached. Temperatures can be checked by classical minimum-maximum thermometers, thermocouple probes, by means of wireless data loggers (www.cometsystem.cz) or by means of cheaper home-made meteorological stations with an option to connect several wireless sensors with a temperature range up to 70 °C (for example: the wireless thermometer WS-7050 is supplied by www.conrad.com).

Heating in hot paraffin wax

Paraffin wax is a mixture of solid hydrocarbons which is generated in the course of oil processing. It is manufactured in several kinds, which differ mainly in melting point, hardness and oil content. This material is shipped as liquid, plates, granulated, as little stones or in scales according to the technology of individual manufacturers. The following paraffin wax is suitable for penetration into and disinfection of wooden beekeeping equipment: Paraffin 60/62 up to 1.8 % of oil, melting temperature: 60-62 °C (boiling temperature: 330 °C, ignition temperature with a match is approximately 260 °C). The higher the melting temperature of paraffin wax, the more it will withstand the sun. The price of paraffin wax is approximately 1.20 Euro per kg. (see also http://www.parafin-wax.cz).

A heated metal vessel with a furnace fitted with fireclay brickwork has to be built in order to treat beekeeping boxes with paraffin wax. Another way of heating the wax is over a fire, but solid fuel is very expensive. The dimensions of a time-tested vessel for heating paraffin wax is 55 x 55 x 60 cm (length, width, height). The vessel should be made of stainless metal sheet of thickness 1.5 to 2 mm in order to be used also in the manufacture of combs (heated beeswax becomes contaminated in contact with iron or zinc).

Exhaust fumes from the furnace have to exit in such a way so as to prevent flames or hot gases from overheating the metal of the vessel above the level of the paraffin wax. If overheating occurs, liquid paraffin may splash onto the overheated metal, causing immediate ignition of the contents of the tub.

It takes about 75 minutes to heat 60 kg of paraffin up to 200 °C in a stainless steel tank. Application of paraffin disinfection requires temperature of 160 - 230 ^{o}C. We adhere to the higher limit, 220 – 230 °C, for disinfection purposes. If no water is in the vessel, the paraffin does not bubble, forming only a slight foam when wood is immersed in it. The temperature of the paraffin wax is checked with an electric thermocouple probe of adequate range, which needs to be fastened on a wooden bar and immersed in the paraffin wax. The temperature is read on the display of the digital multimeter. A suitable thermocouple is DT860E, which may be purchased for less than 10 Euro from the company GM-Electronic (www.gme.com), for example.

The dipping time of a brood box or other object is from 2 to 5 minutes, depending on whether it needs impregnation or disinfection. Heating for 5 minutes at 220 °C really has to be performed in case of suspicion of AFB. About 70 seconds is enough to heat brood chambers in such a way so that the paraffin wax absorbs into the wood and drips off well, though this depends on the humidity of the wood. Water is evaporated in the first stage after dipping. Bubbles of water vapour leave the wood first, fizzing stops after a while and the paraffin wax fills up pores of the wood. Consumption of paraffin wax for one brood box is 50 - 80 g. However, the difference in weight of the brood box before and after dipping is lower, about 20 - 30 g, as water has also been lost from the chamber. The output achieved in the course of paraffin wax treatment of new or recycled brood boxes is approximately 15 - 20 boxes per hour; if two boxes can be placed one above another, the output increases to 40 boxes per hour. Treatment with paraffin wax for disinfecting purposes results in lower throughput as the dipping time has to be adhered. Treatment of wooden beekeeping equipment has to be repeated after four to five years of operation.

Paraffin wax in the vessel, which was clear at the beginning, becomes gradually coloured with wood resins and with propolis. Old brood boxes treated with different paints may also be disinfected with hot paraffin wax. Wood exposed to the sun without painting for a longer period of time becomes darkened more quickly under increased temperatures of the paraffin wax. It is, of course, possible to treat only single-walled brood chambers, lids, bottoms and other objects without heat insulation.

Occupational safety:

Paraffin wax is combustible!

Never work indoor. It is most safe is to work in an open space. Do not leave the paraffin vessel without any supervision after you put the fuel in the furnace, and from temperature of 150 °C upwards. If the contents of the vessel burst into flames, the burning wax is best extinguished with a metal lid which is fastened to the vessel with hinges, so it is at hand at all times. If paraffin wax leaks from the vessel, use powder extinguishers to extinguish any fire. Do not use water to extinguish a wax fire. If water is poured in, it goes to the bottom because it is heavier and starts boiling immediately. Escaping steam is able to lift quickly the whole contents of a vessel which will burn intensively in the open tank. Even 100 ml of water is able to make a considerable mess. Gloves are to be worn - something which goes without saying - two-layered leather ones are best (approximately 2 Euro).

Chemical disinfection

Solutions

Disinfection with chemical agents is mostly connected with their use in solution. However, wood is a porous material and absorbs and releases water with changing humidity. Micro-organisms, including the causative agents of disease, thus gain access to capillary openings in wood. Well protected AFB spores can then survive in microscopic chambers of wooden hives for an unlimited period of time. We found spores up to 3 mm deep in some wooden structures. Despite the use of disinfection solutions, which are efficient disinfectants of absorbent objects (see below), it is not possible to disinfect wood efficiently with these solutions (especially AFB).

Vapours of acids

The literature gives as optional the prophylactic disinfection of beehives with vapours of formic acid, acetic acid, peroxyacetic acid and sulphuric acid. The first three above mentioned acids are unbearably expensive to disinfect beehives. Sulphuric acid, which is generated on humid surface from sulphur dioxide while burning candlewicks, is not sufficiently efficient.

Ethylene oxide

There is certain experience abroad with the use of gaseous disinfectants. Ethylene oxide (Etoxen 6/10/15 % of ethylene oxide in carbon dioxide) can be considered. However, this disinfectant requires special and very expensive equipment. Ethylene oxide also forms an explosive mixture with air, which also makes its use complicated. This procedure was tried in our experimental laboratory but has not been implemented in practice.

<u>Disinfection of honeycombs</u>

The latter two sections, on vapours and gases, could theoretically apply also to disinfection of honeycombs, mainly empty combs. Long-term heating of honeycombs is also described above. However, we recommend small beekeeping operations to ensure the safety of honeycombs by their regular replacement, performed as often as possible. Old honeycombs should be boiled into wax. If any beekeeper suffers from colony death or loss of a larger number of bee colonies, she/he will still retain many honeycombs, empty combs, honeycombs with reserves and also honeycombs with the residues of brood. Treat these honeycombs according to the reason for death, if known. If unsure, do not take any risk.

Reason for death loss	***What to do with the honeycombs?***
Starvation, loss of queen bee, laying workers, mice, birds	Remove brood; empty combs and the other honeycombs may be reused. Honeycombs may be processed into wax.
Intoxication with chemical spray	Only bees are mostly affected, honeycombs and reserves may be reused. Some active substances are also dangerous, for example: fipronyl and neonicotinoids. Consult agricultural advisor in unclear cases.
Nosemosis	Remove brood; empty combs and the other honeycombs may be used after long-term heating - at least 50 °C for 24 hours (see above). Honeycombs may be processed into wax.
Chalkbrood	As for nosemosis. Broodcombs with a high frequency of chalkbrood mummies should be burnt.
Viruses	Survival period of viruses in honeycombs is not known. Honeycombs may be processed into wax.
Varroa disease	The *Varroa* mite survives maximum two days without bees. Remove brood; empty combs and the other honeycombs may be used. Honeycombs may be processed into wax.
Bacterial diseases (AFB, EFB)	Burn honeycombs! Honeycombs must not be mixed with combs designed to be processed into wax.

Reason for death loss	What to do with the honeycombs?
Unknown	Consider different hazards, do not take any risk.

Beeswax

It is not necessary to disinfect beeswax and wax foundations from official manufacturers which are subject to official veterinary supervision. Beeswax is melted for a certain duration, if common processing technology is used. Solar radiation may also work well to melt beeswax. The most common causes of disease (nosema and chalkbrood as well as probably viruses) do not survive heating, and the occurrence of so-called ordinary microflora is also reduced by heating. Beeswax for food purposes may not contain any spores of bacteria (*Salmonela* as well as those of *Escherichia coli*). Neither of these bacteria survive if wax is properly processed. However secondary contamination from vessels has to be prevented as well as contamination of surfaces of wax cakes after they are cooled down.

Honeycombs which are known to come from AFB-affected bee colonies shall not be processed into beeswax but disposed of by burning. There is a risk in honey collection stations and honey processing plants that honeycombs with spores of AFB from undiscovered sources may get into the combs being processed. This should be taken into account when beeswax is processed. Plants which manufacture foundation and process combs from various sources should have a system of critical points in place (HACCP). Either mixing of melted wax with sulphuric acid or heating of wax without water for several hours to a temperature of at least 130 °C needs to be performed as an efficient provision to reduce the number of live spores of *Paenibacillus larvae*, the causative agent of AFB. Too long and too strong heating of wax results in a change of its physical and chemical properties and reduces the quality of foundation made from such wax.
Microbiological inspections of foundation should be performed on a regular basis at all official manufacturers. No case of AFB in foundation has been detected yet. The source of infection from foundation is not completely excluded but there has been no danger in practice so far.

Honey and sugar stores

We rarely face the problem of how to sterilize separated honey or sugar reserves. It is recommended to dilute, boil and feed reserves from separated honeycombs after the death or loss of bee colonies due to nosemosis. Reserves from bee colonies which die due to varoasis need not be disinfected (if the death was not caused by a combination of diseases). Reserves from apiaries with AFB are disposed of via burning together with the other flammable items. Separated honey which is found to contain spores of AFB must in any case never come into contact with bees. Veterinarians may order a suitable way of destruction of contaminated honey, starting from controlled processing into bakery or mead up to burning in a carcass disposal plant. Disinfection of pollen reserves is not performed. If we treat honeycombs containing pollen by heat, the pollen will not be disinfected.

Sources of water

Bacteria as well as spores of nosema may survive on or in water. Wooden boards placed over sources of water should be replaced once a year, dried, singed and impregnated with hot paraffin wax. We can dip them into a solution of NaClO with caustic soda from time to time during operation (5% NaClO and ½ kg of little pellets of caustic soda per 10 litres of water).

Tools

Beekeeping tools represent rather small source of infection compared to very old brood chambers, honeycombs and abandoned beehives. Despite this, we try to keep tools clean. It is advisable to have one set of tools for every apiary - a hive tool, a brush and a smoker. We know from experience that this sound advice still remains largely theoretical amongst most beekeepers. Occasional singing of hive tools with the flames of burning newspaper in a smoker represents more magic than disinfection. A metal hive tool is heated during this procedure little more than if it were in our pocket. This is not sufficient. The case is similar with other small utensils such as cages for queen bees, queen excluders, pollen traps and others. Options for disinfection depend on the material: high temperature, paraffin wax, 5% NaClO with caustic soda.

Equipment for honey and beeswax processing

Honey extraction rooms and wax manufactures should be considered as if they were food-processing plants, and all hygienic rules of food-processing plants have to be adhered to. Hygiene has to be taken into account as soon as a plant is established. The best is to consult a veterinary inspector who performs regular inspections of such plants. The main focus is to have a cleaning and disinfection system to prevent hazardous micro-organisms from propagating. As recent years have shown, new resistant microbial strains appear as well as cases of lax disinfection, resulting in infectious diseases of people who ate products from faulty production plants.

Honey is not as sensitive as a fish salad with mayonnaise but caution should nevertheless be taken in its processing, particularly as microbes do well in all wet and warm places. Everything has to be washed, disinfected and left to dry after every work period or work-shift. Besides mechanical cleaning, various disinfectants should be used and changed. Regular changing of the disinfectant is important to prevent the propagation of resistant strains of microbes. For example: when wiping eqipment, we can change chlorinated 5% NaClO and acidic PERSTERIL at weekly intervals. A really efficient disinfectant which also acts against spores of AFB is an alkaline solution of sodium hypochlorite, the already mentioned mixture of 5% NaClO and caustic soda. One bottle of the preparation can be made from 5% NaClO and ½ kg of pellets of caustic soda per 10 litres of water. The solution only should be used on the day of mixing.

Records should be kept of cleaning and disinfection. The best form or record is a card placed on the wall of the plant. Too short a time for the disinfectant to act is a very frequent error during disinfection of equipment, surfaces of tables and floors. Adhere to the instructions given on labels or package leaflets of disinfectants.

Vessels and glass jars

There is no general rule which governs how to wash and disinfect glass jars for honey and other food. The requirement is clear - the package has to be absolutely clean and safe. Therefore, the majority of beekeepers use new glass packages directly from a manufacturer. While washing glass jars, we have to take into account the fact that anything could have been in the vessel before use. We rather choose a thorough washing procedure, hot rinse and continuous visual as well as olfactory check.

Water for syrup feed

Discussions are sometimes held as to how to disinfect water we use to prepare feeding solutions for bees. A problem may arise if we use a cold procedure to prepare feed, for example the preparation of solution with a slurry pump. Water should not be contaminated with the excrement of bees which may contain viable spores of nosema. Furthermore, there is a hazard of getting the solution infected with bacteria which causes the solution to become mucilaginous. The causative bacterium of micilage, *Leuconostoc mesenteroides*, is quite sensitive to heat; half an hour at a temperature of 60 °C will be sufficient for its destruction. We may add any alcohol propolis tincture at a quantity equal to 1 ml per one litre of solution into the water in watering places as well as in top feeders. Propolis also stabilizes weak stimulative sugar solution against fermentation. Bees tolerate without any problems water disinfected with silver as the active substance or with NaClO (active substances are chlorine and oxygen). Of course, the prescribed dose has to be adhered to.

Hands

Disinfection of hands is an important provision against the carryover of all germs or diseases. Carryover of diseases via beekeeper's hands is not very probable. Contrary to this, honey processing requires cleanliness of hands. Honey contains very little water. Therefore bacteria cannot propagate in honey. Hands should be thoroughly washed before disinfection. Dirt is a carrier of microbes. Substances of minimum irritation should be used for the disinfection of hands. However, they have to kill microbes within a very short period of time. We sometimes require active substances to have a very long disinfecting effect (for example, during the work of physicians). Residues of active substances in disinfectants can represent a hindrance - as in case of food processing, for example. Ajatin is an example of a product with a long-term disinfecting effect. Preparations based on alcohol, hydrogen peroxide and peroxoacids act quickly. Alcohol-based preparations may be applied only to dry hands, otherwise the alcohol will become diluted and its disinfecting effect will be lessened. The majority of alcohol-based preparations contains substances which protect skin, which is not the case of water-soluble preparations. The market offers sufficient selection of hand disinfectants. Even efficient preparations need at least one minute to achieve the necessary disinfecting effect.

It is far from being easy to achieve really clean hands. Just as a matter of interest, see below the procedure for so-called surgical washing of hands: Hands including forearm shall be washed for a period of 2 x 5 minutes with soap with the use of a sterile brush with intermediate rinsing with drinking water, and they shall be dried by means of a sterile gauze. Then they shall be washed with 70 % alcohol for 3 minutes, they shall be dipped into a disinfecting aqueous solution for 1 minute and they shall be dried by means of a sterile gauze.

Working with clean hands is an indication of thoroughness and quality. Demanding customers will notice and appreciate it.

Laundry

Working clothes and laundry which have been washed and dried or ironed in the usual way will not be sterile, but do not represent a source of infection for bees.

Disinfection of vehicles

Bee diseases will not be carried over on tyres of vehicles. The interior of a vehicle may sometimes be stained with honey. Excrement of bees may often be found on the bonnet and roof. Everyday washing is sufficient for cleaning, and a 5% NaClO solution is sufficient for disinfection. Mind the varnish.

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