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BEEVITAL®

Protecting bees

FIGHTING VARROOSIS

with

HIVE CLEAN

by Dr. Gheorge Dobre

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Experienced beekeeper and passionate
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the Federation of Beekeeping Associations
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FIGHTING VARROOSIS WITH BEEVITAL HIVECLEAN

Dr. Gheorge Dobre

INTRODUCTION

Varroosis is an external parasitosis, difficult to fight, which causes considerable economic losses to *Apis cerana* and *Apis mellifica* bee species.

In the current context (environment urbanization, intensive exploitation and chemical processing of agricultural lands, expanding of the forage area by reducing biodiversity, transhumance, etc.), the classical methods of fighting varroosis have become ineffective and because of this Apis mellifera can no longer maintain its balance (homeostasis) and cope with parasitic aggression.

The questionable efficiency of the currently used acaricides allows the persistence of residual infestation and the recovery of the Varroa population.

This is why it is important for the used substances to be as little aggressive to bees as possible, for the fighting methods to be oriented towards alternative "bio" methods, and, by technological means, the bees should be stimulated to defend themselves also by using their native protection mechanisms. (8, 21)

There are several drugs traders in the market providing an entire range of miticides; however, in many cases, these products are obsolete, contaminate beekeeping products with residues or create resistance. Sometimes they are toxic for the operator and the beekeeper needs protective gear or sophisticated application devices.

Overdosing, in most cases, may be hazardous. Many of these treatments can be applied only in the active season, in a combined or alternative manner, and most of the times success depends on external temperature, type of hive and the strength of the bee colony.

This is why we care what products we use. In a professional apiary, with very many bee colonies, one must use a small number of treatments, easy to apply, by simple methods, without leaving residues in beekeeping products, and, moreover, effective.

The continual use of synthesis products is limited in time, as strategic programs may control antibiotic, insecticide and pesticide residues in beekeeping products. (3, 13, 24)

The fight against varroosis and associated diseases is carried out on a global scale and is an action comprised both in European Union Programs and in the National Beekeeping Program (NBP) implemented in EU member states.

By analyzing the medicine it results that BeeVital HiveClean meets most of these requirements.

BeeVital HiveClean

BeeVital HiveClean is a product manufactured by the company Food & Beverage Handels GesmbH.-Austria, authorized for selling and distributed in 15 countries.

Presentation

BeeVital HiveClean is available for sale in 500ml containers, sticks (15 ml) and larger volume containers together with a special liquid dispenser provided by the manufacturer. Control (sticky) paper is available under the same brand.

Composition

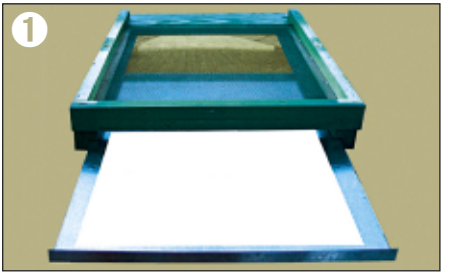
Besides natural extracts of propolis and etheric oils, the composition of **BeeVital HiveClean** also comprises the three main organic acids used in fighting varroosis (oxalic, formic and citric). All compounds

are stabilized in sweet water. By composition, **BeeVital HiveClean** is an ecological pest control product [28], recommended for bio treatments by CERES GmbH, Germany. (Annex 3).

Mechanism of action

The acarian has adapted so well to the parasitic life and to the biology of the bee that any change initiated from outside disrupts the parasite-host cohabitation system (beneficial for pest control). **BeeVital HiveClean** operates in a complex manner, both on the olfactory and gustatory chemoreceptors of the acarian, which modifies its behavior, and on the bee colony, which triggers the grooming behavior. The correct use of **BeeVital HiveClean** does not have any negative influence upon the bees, the brood or the queen. The association of components in view of enhancing the antiparasitic effect has proven advantageous.

All the substances in the composition of **BeeVital HiveClean**, taken separately, have been or are used in fighting varroosis [11, 14, 18], but the efficiency of each of these is rarely close to 90%. However, this association reduces the quantity of active substancesintroduced in the hive, thus enhancing their effect. By using very laborious detection techniques, it has been found that **BeeVital HiveClean**, applied according to instructions and by taking into consideration the biology of the parasite, is not addictive, does not leave residues and in time its efficiency is close to 100%. By changing the main microclimate characteristics in the bee colony, the product disorients the parasites, which, given the fact that they are blind, perceive this situation as a change of the habitat and leave or disturb their hosts. Recent observations show that after treatment, the bees become calmer, and the colony triggers the grooming behavior. Acarians are removed by grooming (crushed or mutilated); they



Mesh bottom board *Foto: BeeVital*

fall on the bottom of the hive and die [22, 23]. The result may be noticed only on the bottom of the hive, by using the screened bottom board (also known as open mesh floor-photo 1).

Main Characteristics of Product BeeVital HiveClean [16]

We consider that Table 1 synthesizes the main qualities of **BeeVital HiveClean**, each star representing an unquestionable value, and ultimately these summed values signify a 5-star value, a symbol that is also present on the label of the product. [15]

When to use BeeVital HiveClean

Main treatments are administered at the end of summer-beginning of autumn, when the first wintering bees appear and in winter during the broodless period, over the wintering cluster, when there is no more sealed brood in the colony.

Intervention (secondary) treatments are applied as an immediate solution, in February-April, when pollen is foraged and, as the case may be, when one finds a large number of acarians in the active growth period of the brood (May-June).

Control treatments (diagnostic) are necessary when one notices the involution of the bee colony. By taking into consideration the fact that the clinical signs of varroosis are noticeable only in the advanced stages of infestation, it is important for the diagnosis to be performed in the apiary

and, as the case may be, for the disease to be confirmed in a laboratory. The treatment is applied by periodical repetition, at adequate time intervals, by taking into consideration the extent and intensity of the Varroa infestation, the specific nature of the parasite's biology and the evolution of the bee colony (Table 2).

Table 1 **Main characteristics of product HiveClean** [16]

The treatment acts in time and its efficiency is over 95%. The medicinemaintains its effect between 6 and 10 days after administration.	★
BeeVital HiveCean has no adverse effects on brood, adult bees or queens and does not cause resistance to treatment. Accidental overdosing creates no problems in the bee colony.	★
BeeVital HiveCean is easy to apply, it does not require difficult technologies or protection measures for the operator, it is beneficial also as an adjuvant in fighting other diseases (mycoses, other parasitoses, etc.)	★
It may be used all year round (when the outside temperature is between -1° and 30°C) nomatter the physiological state of the bee colony.	★
Its formulation is based only on natural ingredients (ecological product) and leaves no residues in honey or in other beekeeping products.	★

How to use BeeVital HiveClean

When one uses **BeeVital HiveClean**, it is important to comply with the instructions of the manufacturer. Only in this manner can the beekeeper maintain the bee colonies in a good state of health, with high productivity, reducing the Varroa population to the minimum.

The product must be heated at the temperature of the human body and the container shaken well before application.

Dribble approximately 15 ml of **BeeVital HiveClean** into 7-8 gaps between frames in the middle of the hive, directly onto the bees (photo 2). One application is effective until day 8, but acarian falls may continue for several days, depending on the degree of infestation.

When there is sealed brood in the colony, it is necessary to execute three successive applications, at one-week intervals, by dribbling the liquid over the bees, in doses of 15 ml of **BeeVital HiveClean** per colony, in order to remove the acarians that appear at eclosion of the brood. In the active period, in case of hives heavily populated with bees, it is necessary to remove wax bridges. In the case of frames that are not covered by bees, the beekeeper has to reduce the nest by eliminating these frames. On hot days, the treatment should be applied late in the afternoon, when all the bees are in the hive. One must avoid treating largely depopulated bee colonies, until one finds out the cause for which the colony has devolved.

Treatment with BeeVital HiveClean stick.
Foto: BeeVital Archive

Table 2 **Treatment calendar (Northern Hemisphere)**

Month	Period	No. of treatments	Observations
February-April	When the pollen foraging begins	1-3 treatments with **BeeVital®HiveClean** every 7 days	Depending on the presence ofacarians in the detritus ("residue" on the bottom of the hive) during the spring apiary overhaul
May-June	During foraging	1-3 treatments with **BeeVital®HiveClean** every 7 days	Only when needed. Depending on acarian falls
Late July-August	After honey extraction	3 treatments with **BeeVital®HiveClean** every 7 days	**Main treatment**
November-December	When there is no more sealed brood	1 treatment with **BeeVital®HiveClean**	**Main treatment** Outside Temperature over -1°C

Legend: *Efficient control of Varroa infestation may be obtained only by good knowledge of the acarian biology and by observing the treatment calendar, conceived according to the several periods of the bee colony development. Determining the level of infestation prior to treatment is needed and it is advantageous from economical viewpoint. However, performing control treatments is possible at any time of the year, even in the presence of the wintering cluster. The result may be observed by any beekeeper that has screened bottom boards and control paper, in the conditions of an outside temperature exceeding -1° Celsius.*

Success in fighting varroosis depends on how fast beekeepers, by taking into consideration EU requirements on the quality of beekeeping products, shall implement modern pest control strategies.

THE BEE COLONY

Worldwide, bees live from close to the Arctic Circle to the Equator. One cannot describe the annual biological cycle of a bee colony without specifying the latitude of the location where the respective bee colony lives.

A bee colony can grow harmoniously only when it is in biological balance.

The bee colony annual cycle

At the beginning of the active period, the main activity of the bee colony consists of nursing the brood and its subsequent evolution is related to the existence of food and the possibilities of procuring and accumulating reserves. In the temperate climate area (parallels 44-46) the queen starts laying eggs in January, the colony reaches its growth peak at the end of June, the decline begins in July, when the day duration starts decreasing, and in the middle of October the queen stops laying eggs. The last eclosion takes place at the end of November. Correlated with altitude, in the mountains, at over 1,000m, the active stage is shorter, but also more intense, and in the field regions, the wintering stage is shorter, but in this case, summer corresponds to a regression caused by the drought. The rains at the beginning of autumn, if any, may generate a revitalization of vegetation and the activity of bees may be resumed. In this case, the growth curve has two peaks: one at the beginning of summer and another in autumn.

Labor relationships between bees

The social life and the organization of in-

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dividuals in the colony depends on the season and on their physiological necessities. Thus, the division of labor, the functions of nutrition, breathing and defense manifested both individually and socially entitle us to consider the bee colony as a "superorganism", and we may define its swarming as an "asexual reproduction".
Labor relationships between bees, within the colony, are related to: building combs, cleaning cells, feeding the brood, processing nectar, storing pollen, ventilating the nest and defense. Outside the colony, bees perform orientation flights, bring water and take part in foraging nectar, pollen and propolis. In addition, a situation of danger triggers the defense mechanism.

Instinctual activities and behavior patterns

From the multitude of the bee colony activities and behavior patterns, we may enumerate several instinct-driven ones: foraging, nectar processing, pollen storage, swarming, recognizing hive mates, caste differentiation, supersedure, driving out of drones, bee dance, hygienic and grooming behavior, propolisation, etc. Or activities and behaviors generated by factors which are external to the colony, such as: defense, states of alarm which lead to agitation and even aggressiveness, or those generated by human intervention, when normal or unexplainable behavior patterns occur. These occur by actions triggered or regulated by the action of ancestral stimuli and mechanisms, instinctually guided, usually by specific chemoreactions, and the beekeeper attempts to control them by various interventions (smoke, sprays with water or various other substances, etc.)

Bee colony evolution and involution

In order to keep and use the bees according to their natural growth, the main preoccupation of the beekeeper is to maintain the biological balance (homeostasis) of the bee colony. When a bee colony starts devolving, one must carefully examine and establish the cause that has generated this state. The existence of queenless, weak, depopulated families, incapable of defending themselves against aggression, with wide open hive entrances, with cracks or with inadequate nest organization, constitute a predisposition to or sign of disease. The causes which lead to devolution are either an inadequate queen bee (exhausted or mated with inbred drones), or old, deformed or moth-attacked combs, brood diseases (foul brood, chalk brood, stone brood, etc.), or a strong attack of Nosema or Varroa.

Defense complex in bees

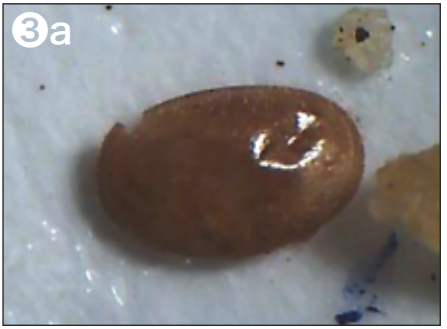
The basis for maintaining the health of living organisms in the animal regnum consists of systems and complexes of defense against pathogens (protozoa, fungi, bacteria, viruses, etc.), which have evolved and perfected on the phylogenetic scale. In insects, these systems, although existent, have been scarcely researched [7].
Disease evolution and pathogenic impact are influenced by the host-parasite relationship, both on an individual and on a population level, and the defense complex is manifested both on an individual level and on the level of the colony as a collective defense system. On an individual level, defense mechanisms are internal and protective (external). Internal mechanisms (immunity) are congenital and acquired, are based on humoral and free cellular elements and represent the capability of the bee organism to know the self and reject the not self. The hemolymph contains free cells, with phagocytizing role, but also humoral components with antibacterial action (lysozyme, apidecines). The food administered to the lar-

vae contains antimicrobial substances. In the intestinal cavity, but also on the exterior, there are cells that secrete anti-infectious substances.

External (protective) mechanisms are based on the integrity of anatomical and physiological barriers (chitin covering, intersegmental membrane, intestinal wall). These mechanisms can be annihilated on the exterior by ectoparasites such as acarians Varroa and Acarapis and on the inside by Nosema protozoa.

Besides these individual defense mechanisms, the bee colony ensures its anti-infectious protection and homeostasis on a superorganism level (collective defense), by maintaining thermal conditions in the nest, by interrupting the egg laying in drought and nectar scarcity periods, by larval food containing anti-infectious substances and by phytoncides foraged from nature, found in pollen and nectar.

The colony also reacts as a social community in order to face infectious aggression, by defense behavior, by hygienic behavior (removing infected bees and sick brood), by self-cleaning behavior, grooming behavior (photos 3a, 3b, 3c, 3d) or by leaving the strongly infected or parasitic nest. [5, 7, 20] Behavioral mechanisms occur by instinct and are part of the patrimony (the genetic heritage of the species). The way in which these mechanisms occur is little known and cannot be totally controlled by man, but nevertheless, by knowing them, one can influence them (hinder, trigger or favor). The grooming behavior is present in several animal species and is known and described in bees, especially in Apis cerana. Acarians (parasites) can be removed and harmed (mutilated), a process which is triggered by individual grooming action or by a collective activity (grooming dance), and in Apis mellifera by natural mechanisms or by using **BeeVital HiveClean**. [22, 23] The individual defense mechanisms of the bee colony, its hygienic behavior and its grooming behavior may be enhanced and consolidated by selection or stimulated by various techniques and methods. [5] Selection is a laborious, costly and long-lasting action, while measures of disease prevention and of stimulating these instincts are available to beekeepers, are less costly and may be applied to any bee colony.



Mutilated acarian, damage of shield, dorsal view.

Photo: NRL of Bee Diseases-IDAH Bucharest

Mutilated acarian, damage of shield, ventral view

Photo: NRL of Bee Diseases-IDAH Bucharest

One must treat the colony as a whole, not the individual

The bee colony, as a whole, has multiple reaction capabilities, which is understood or not by the beekeeper who supervises

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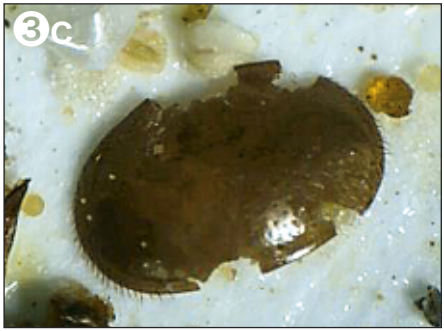


Mutilated acarian, multiple damage, dorsal view.

Photo: NRL of Bee Diseases-IDAH Bucharest

Mutilated acarian, immature stage.

Photo: NRL of Bee Diseases-IDAH Bucharest

their activity. Before starting a treatment, one must estimate the strength of the bee colony and its reaction capability (prognosis). Depending on the result of the appreciation (diagnosis), one decides whether to maintain it as such, to unify it under treatment or suppress it. Bees in a devolving colony represent a source of infection for the apiary, as they can no longer satisfy their individual or collective necessities and they die.

Integrated pest management

Integrated fighting is based on knowing the environment and the living population dynamics that exists in a certain area.

In applying the strategy of integrated fighting against parasitoses, one must take into consideration all the ectoparasites that may coexist (Varroa, Braula, Acarapis, etc.) and establish, by laboratory examinations, adequate fighting procedures. For fighting, one must use all accessible means (mechanical, physical, chemical and biological) depending on the season, the physiological state of the bee colony, and all the growth stages of the parasitic biological cycles. The concept of integrated pest management implies maintaining the pest population on levels that do not cause economic damages. The uncontrolled use of chemical products has not eradicated varroosis but induced the acarian resistance, and the presence of residues has generated unfavorable (adverse) effects both on man and on the environment. Integrated Pest Management (IPM) is a scientifically justified principle with the main objective of reducing residues in agricultural and alimentary products, by limiting the use of pesticide treatment.

BIOLOGY AND BEHAVOIUR OF *VARROA SP.* ACARIAN

Currently, varroosis is a panzootic which has engulfed the entire world and endemically threatens the existence of the honeybee as a species.

Taxonomically, the acarian is part of Varroidae family, Varroa variety. There are various species in bees: *V. jacobsoni* (Oudemans 1904); *V. underwoodi* (Delfinado—Baker & Aggarwall 1987); *V. rindereri* (Guzman & Delfinado-Baker 1986); *V. destructor* (Anderson & Trueman 2000). [7]

Acarian *Varroa destructor*, present also in Europe, is the most aggressive external parasite of the honeybee, as it affects the entire adult population (worker bees, drones, queen), as well as the juvenile

stages of growth, starting with that of larva, 9 days of age. The development of the acarian starts with the birth of the brood and ends with the end of the egglaying process of the queen bee. [1]

The parasite inserts itself between the chitin plates of adult bees, where it penetrates the intersegmental tegument and ingests the hemolymph. By perforating the intersegmental membranes, it is responsible for disseminating and maintaining viruses in the bee colony. The parasitic activity of the mite is very damaging, it weakens the organism of the bee, reduces its lifecycle and favors the occurrence of bacterial and mycotic diseases. [1, 7, 11]

For reproduction, it penetrates drone or worker bee brood cells shortly before sealing. The acarian is attracted towards the bee chemically, thermally and by vibration [11, 12]. After sealing, it lays up to 7 eggs at 1-2-day intervals. Some of them, by metamorphosis, reach the stage of adulthood. The global fertility of the acarian is influenced by the development cycle of the bee colony and by the temperature of the environment. The female has an oval-shaped body, with the anterior-posterior diameter smaller than the lateral diameter. The anatomical peculiarities of the female certify its strong adaptation to parasitic life. The shape of the body and its suckers ensure a solid attachment of the acarian on the body of the bee. Moreover, during feeding, its attachment is completed by the action of chelicerae. Both the body and the appendix (pedipalps and legs) are covered by numerous hairs, which form an elastic covering, preventing the immobilization of the female (between the cocoon and the nymph), and between the chitin plates (tergites / sternites) of the adult bee. The attachment of the acarian on the bee is facilitated by the featherlike conformation of the hairs on the ventral side of the Varroa female, which operates as a zipper with the also featherlike hairs on the body of the bee. The absence of the anal valvula facilitates excretion in limited space, and the mobility of the proximal extremities of the perithermal tube ensures proper breathing in various conditions. The acarian hides between abdominal sternites, where they overlay over the wax mirror, where it is usually protected against the cleaning actions of the bee and the beekeeper's eyes. [1, 11]

The male is smaller than the female and remains in the sealed cells, with the juveniles. The color of the tegument is greywhitish or yellowish and lacks chitin. The mating takes place in the cell, before the eclosion of the bees. After mating the males die. The females lay eggs only on bee larvae. Acarian eggs are milky-white in color. The first-laid egg is a female, the second is a male, and the rest are females. [30] The determination of the gender of the descendants depends on the fecundation or non-fecundation of the oocyte. During the active period, the fecundated females are found on bees or in sealed cells, with brood (in a ratio of 1/3 – 1/6). They may be founding females, which may initiate a new cycle, young females, fecundated by their brothers and which will initiate their first cycle or unmated females who, in the next stage will initiate their own first partenogenetic cycle, followed by normal ones.

Dissemination in the hive is performed only by females, the acarian moves along the frames and to the bees, of which they attach by their suckers. On the body of the bees the Varroa females move very easily and find their favorite attachment position, usually between the first abdominal chitin segments (photo 4).

Sensory organs on their first pair of legs perform orientation; their main stimuli are vibrations, smell, taste and temperature. The first pair of legs has a small sensory cavity (photo 5), with chemoreceptor sensiles which act as olfactory organs. At the

Bee with Varroa mite. *Foto: Archive*

base of the first pair of legs there are olfactory and gustative sensiles and mobile pedipalps with a tactile role (photo 6).
These represent sensory organs that control the reactions of the female acarian in the environment (attachment to the bees, penetration in the cell before sealing, perforating the tegument and feeding, etc.) [12]
The juveniles (larva, protonymph, deutonymph) and the males do not leave the comb cells, while female may leave them.
The feeding is performed by repeated

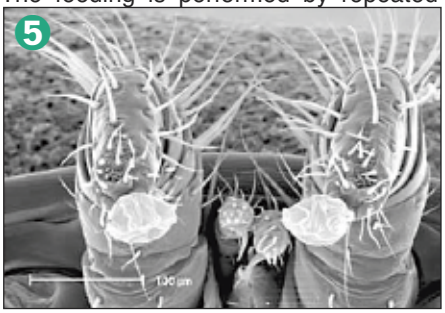
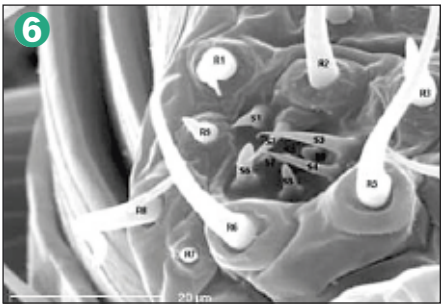


Photo: Schweizerisches Zentrum für Bienenforschung.



contact with the hemolymph, by perforating the intersegmental tegument of the worker bee or the drone, or by perforating the larval epidermis. For reproduction, the female prefers drone brood to worker bee brood. When a female penetrates a drone cell, the evolutionary cycle becomes longer, and there may be as many as 7 eggs, and during the wax removal there may be as many as 2 young fecundated females and one unfecundated one. Thus, in a drone cell, especially in July, there are at least 1-3 female acarians, or even more, while in a worker bee cell usually there is one female. By the rapacious action on the bee brood, the Varroa female prolongs the sealing period, favoring the growth of its descendants and thus enhancing its prolific nature. The number of reproductive cycles of a Varroa female in one year is 3-4, but it may be as many as 7. The lifecycle of the female is 2-3 months in summer and 6-8 months in winter.
The maximum number of parasites is at the end of the active season, when the first signs of disease also appear.
In autumn the females focus on the last worker bee brood, and in a cell there may be several females. The critical level of infestation of the worker bee brood is 10% of the cells, and over this level the family faces extinction. In the colonies which are not treated in autumn the degree of infestation of the worker bee brood can be almost 50%, and in every worker bee cell there can be several adult females [1].
An average infestation of 10% of the worker bee brood cells corresponds to an average infestation of 40% of the drone brood cells. Our observations according to the mesh bottom board show that the natural mortality (acarian falls) intensify starting with May and reach a peak depending on the intensity of the invasion in July-August. It appears that the Varroa female can survive at a temperature of 25^0 Cel-

sius and a relative humidity of 50%, without food, for 3-5 days, even more. At the end of the beekeeping season, the fecundated Varroa females attach between the abdominal chitin plates (tergites/sternites) of young bees, where they will winter. They will immediately penetrate the cells of the spring brood.
In a bee colony that is not treated in autumn, the number of acarians may vary from hundreds to thousands - the stronger the bee colony and the more brood, the more acarians there are.

Acarian pathogenicity

Varroa acts on adult bees, larvae and pupae by: weight loss; morphological deformity; reduction of the secretion of hypopharyngeal glands; reducing the protein level of the hemolymph; contamination with viral particles or other infectious germs; reducing wax secretion and life-cycle, etc., and over the bee colony by: depopulation, abnormal brood, attack of opportunistic germs. The pathogenicity of the acarian is not only a complex individual colony phenomenon, but it also varies depending on the factors that may influence the homeostasis of the bee colony [6, 7, 17].

Population unbalance of the bee colony

The weakening of the bee colony occurs because of the reduced viability of descendants, which are not capable of ensuring the normal activity of the colony. The seriousness of the effects on the newly eclosioned bees is also influenced by the number of founding Varroa females, and by their descendants that have fed with the hemolymph of the nymph. Severe infestation may cause its death before eclosure. The overloading of the body of the bee with acarians and the consumption of hemolymph reduces the flight capabilities of the bee and influences biological performance within its colony. The capability of the acarian to be a vector for pathogens is another hazard that must not be neglected [1, 6, 8, 11].

Epizootology

Parasitic families represent infestation sources. Abandoned and sanitarily unchecked apiaries, as well as feral swarms, are focal points for spreading the disease. The bees carrying Varroa females facilitate the infestation of vulnerable colonies. The spread of the acarian is ensured by lost bees, robbing bees, drones, by the introduction in the apiary of swarms with unknown origin, by the introduction of queens with uncontrolled accompanying bees. The diffusion of the disease on large territories is performed especially by migratory bee activities, in which untreated or insufficiently treated bees take part. It is possible that wasps also play a vector role in transmitting varroosis.
The disease is unnoticeable in the first year, becomes apparent in the second year and threatens the extinction of the colony in the third year, when the acarian-bee ration may be 20:100 [1].

APIARY HEALTH SUPERVISION (Varroosis Diagnosis)

Only the permanent sanitary-veterinary supervision and continuous prevention and fighting of disease will help one diagnose and control the degree of infection, avoiding in this way the surprise of explosive manifestations of these conditions. Laboratory testing is a must, as many diseases cannot be diagnosed clinically, evolve in a hidden, asymptomatic manner, and once the bee family starts collapsing, there is no more recovery.
The experience of previous years, even in less deleterious conditions, shows us that the lack of action drastically diminishes the livestock and may compromise bee-

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keeping growth over the following 3-5 years.

What does one determine and how does one interpret the results?

The health state in case of varroosis is monitored in apiary or in laboratory, on live bees, by direct observation, by uncapping drone and/or worker bee brood and in a permanent manner by using the screened bottom board. In fighting Varroa we have to take into consideration the physiological state of the bee colony and the biological cycle of the acarian.

Prevalence: Total number of cases or focal points (frequency of the disease);

Invasion extension: Presence of parasites in the environment (on the level of the apiary) = Examined samples / positive samples (%);

Incidence: Number of cases or focal points in a population;

Intensity: (host/parasite ratio on the level of the bee colony), amplitude, degree of parasitic infestation. Counted acarians are related to the number of bees calculated to exist in the hive at the moment of the determination. The quantity of bees in a colony is to be estimated taking into consideration the number of intervals between frames. In average there can be between 10,000 and 40,000 bees in a production colony (150-270 grams/interval)

Host/parasite ratio: must be known in its dynamics, and the intensity may be considered weak, average or strong (devastating) [25]

Not supervising the evolution of a disease leads to a situation of not really knowing its extension and how important the losses might be. The analysis and evolution of these indicators allow us to appreciate in an efficient manner the efficacy of the applied fighting means, and the success in keeping varroosis under control.

DIAGNOSIS PROCEDURES AND METHODS OF FIGHTING

Apiary examinations

At the beginning ofwintering, onemust perform a control treatment. Overwinteringmust be supervised by brief revisions; at the end ofwintering period, the main revision must be performed and the detritus collected on the bottom of the hive must be examined. During the active season acarian falls must be checked by means of screened bottom board and the sealed brood (photo 9) has to be examined microscopically. Depending on the level of infestation and taking into consideration the calendar (see table 2), one or several treatments need to be applied.

Observations on the screened bottom board

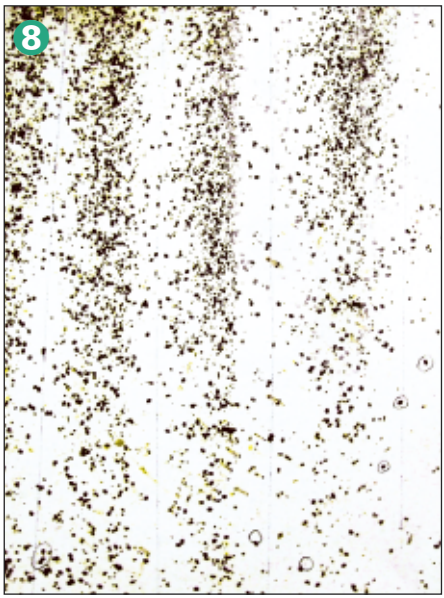
The screened bottomboard collects the acarians fallen off the bees (photo 7), retains insect larvae (moths) and all the residues removed or fallen from the hive. It also offers the benefit of elimination of carbon dioxide during winter and ensures proper ventilation during transportation (the bees never cover the bottom mesh with propolis). In order to evaluate the degree of Varroa parasitic infestation, one

Control sheet from mesh bottom board with debris.

Foto: BeeVital

places white sticky paper on the metallic tray of the mesh bottom board and counts the number of parasites fallen on it. The natural mortality of the acarians observed in summer time as well as analyses of the collected detritus in winter time supply us valuable data regarding the intensity of the infestation [7, 8, 11]. If, at the end of summer, the number of fallen parasites exceeds 30 in 24 hours, it means that the situation requires immediate action.

Table 3 shows the correlation between natural fall of acarians, the degree of parasitic infestation of the bee colony in the active season between June 15 and August 15, as well as the necessary actions to be performed. In order to diminish the losses of bee colonies due to Varroa infestation the evolution of the disease must be monitored all year round using the mesh bottom board.



Control sheet *Foto: BeeVital*

Table 3 **Appreciating the degree of infestation by natural acarian falls**

Daily acarian falls	Parasitic infestation degree	Measures
0 - 8	Weak = under 100 acarians	Control repetition
8 - 15	Moderate = 100 – 1,000 acarians	Treatment
15 - 30	High = 1,000 – 2,000 acarians	Immediate treatment
Over 30	Over 30Critical = over 2,000 acarians	Complex fighting measures

WARNING! *During the winter, when one notices on the bottom board more than one acarian in every two days, the bee colony may perish until the end of the season and in this case a treatment in the wintering cluster with a substance which is adequate to that moment is required.*

In February – March, the level of infestation of the bee colony is examined by method of sieving or flotation of the samples taken (collected detritus). The collection is dried for 24-48 hours. One can examine it by separating the impurities by sieving (system of sieves with 3; 2; 1.6; 1 mm mesh) and/or the detritus is mixed with ethanol; the residues from wax, propolis, wood, bee remains, sediment on the bottom of the container, and the parasites remain on the surface of the washing (immersion) liquid, in this way they may be examined and counted. The de-

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gree of infestation is determined by relating the number of found acarians to the estimated bee population in the hive [7].

Control treatments with acaricides

At any time during the active period we may diagnose the intensity (amplitude) of the Varroa infestation of the bee colonies by the method of control treatments with acaricides, at over 90% accuracy.

The control treatments with acaricides may be applied in any period of the year by using **BeeVital HiveClean**, even in the wintering cluster. The result is noticeable by any beekeeper who has screened bottom boards, adhesive paper to retain parasites and adequate temperature conditions (over -1^0 Celsius).

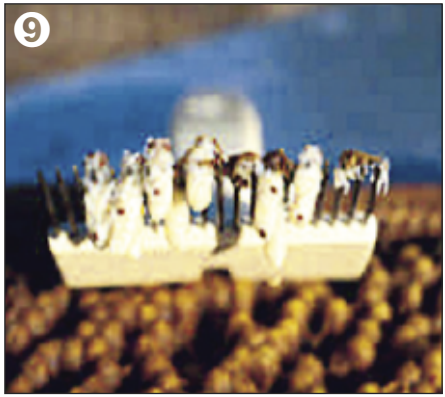
The control treatment must be applied to at least 5% of the hives. If, at the end of summer, the number of acarians fallen in 24 hours exceeds 30 individuals, it is necessary to start the treatment immediately.

Observations on the sealed brood

By uncapping drone brood, one determines the infestation percentage examining 100 cells and counting the number of infested ones (photo 9).

Correlating this number with the surface of existent sealed drone brood we find out the number of infested drone cells in the colony. In order to determine the number of acarians in the colony, we use 10 as a multiplication factor (mentioned in the specialized literature). A parasitic infestation level higher than 4% requires the immediate application of the treatment.

By uncapping worker bee brood (n=200) one identifies not only the infestation on the nymphs, but also immature bees and/or bees with deformed wings which might unbalance the bee colony after eclosion [3, 8, 16].



Uncapped drone brood.
Photo: National Refernce Lab of the National Institute for Diagnosis and Animal Health, Bucharest

Control on live bees

This is the most frequently used method both by specialists and by beekeepers. By clinical observation one determines the presence or absence of the acarian on bees. In order to reach the detection threshold, the observation should be performed early in the morning, when foraging bees have not left yet. In order to apply the control method on live bees, the live bees may be harvested by a portable aspirator or by shaking them into a paper

bag. In order to be significant, the sample must contain at least 200 bees. Both in the apiary and in the laboratory one may use several examination techniques: the ether method, stirring in alcohol, in water with various solvents, with detergent 0.1%, by using acaricide strips or dusting the bees with powder sugar in a recipient with a perforated cap.
More simply, one can freeze them or dry them and determine the parasitic percentage by sieving and counting the mites, thus establishing how many parasites have fallen from 100 bees.
In the active period, when the egg laying of the queen is at its maximum capacity, the ratio between the number of acarians on the bees (approximately 15%) and the number of acarians in the brood cells (about 85%) is 1 to 6. When the presence of brood diminishes, this ratio reaches 1 to 3, and when it is absent, we no longer need a correction factor. Between July and September, when the egg laying is significantly reduced, the number of bees decreases, the colony depopulates, and the percentage of acarians on 100 live bees may increase from 6% to over 35%. By knowing the biology of the parasite, when one appreciates the number of acarians in a bee colony, one must take into consideration that this number may be influenced by the current season, by the strength of the colony and by the manner and conditions in which the treatment has been or is applied.

Laboratory examinations

If one does not know the epidemiological situation of an apiary and the administered treatments, in order to determine as exactly as possible the degree of Varroa infestation it is necessary to perform clinical examinations in the apiary, which need to be performed by means of laboratory examinations: sugar dusting, washing in alcohol, washing with 0.1% detergent, examining the detritus. Laboratory examinations performed within the strategic program of supervision, prevention and fighting of bee diseases will confirm and complete the observations made in the apiary [15].
Of all the diseases affecting the bees, varoosis (and associated diseases) represent one the most important causes of losses in apiaries, worldwide. For this reason, the Varroa control methods must take into consideration both the incidence and the prevalence of varroosis, the biology of the parasite and the dynamics of the disease.

INFORMATION ON VARIOUS INTERVENTIONS WITH BEEVITAL HIVECLEAN

Currently **BeeVital HiveClean** is manufactured in Austria and sold in over 20 countries, most of them located in the European Union. The qualities of this product have been tested and compared with other antivarroa products (Apiguard®, Thimovar®, Perizin®, Apioxal®, Apilaifvar®, Bipin, Fumisan, etc.) by numerous experiments at Hochenheim University of Germany (H. Horn - 2003), at Ages Institute of Vienna (R. Moosbeckhofer - 2005), at the National Zootechny and Veterinary Medicine Institute of Chișinău (Valentina Cebotari - 2006), at Nigde University of Turkey (E. Akyol et al - 2008), at Apiculture Section of ISK in Puławy, Poland (K. Pohorecka - 2009, the Institute for Small Animal Research Gödölo, Hungary) etc.
Extended research on **BeeVital HiveClean** is currently carried out (September 2011) in the Institute for Diagnosis and Animal Health in Bucharest, Romania due to a collaboration protocol with the Romanian National Reference Laboratory for Honey Bee Diseases in order to investigate the existence of a relation between hygienic behavior of bees, crippled or squashed Varroa mites and **BeeVital HiveClean**

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treatments. Fighting varroasis with **BeeVital HiveClean** was monitored for a long period in apiaries in Austria, Hungary, Poland and Romania [2, 9, 10, 17, 22, 23, 25].
Using **BeeVital HiveClean** was recommended for ecological beekeeping by the organic agriculture inspection and certification organism (CERES GmbH, Germany, Annex 3.).

CONCLUSIONS

Supervision of bee colonies, evaluation of the extent and intensity of the Varroa infestation and applied treatments.

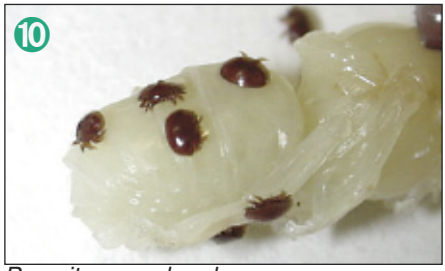
We benefit from the experience of more than four decades of fighting this acarian but we see how production capabilities diminish, the mortality of affected families remains at alarming levels, varroosis and associated diseases continue to spread and cause damages.
Fighting has become difficult and complex because of the multitude of epizootological factors. It is difficult to control the evolution of the disease, because of the biological peculiarities of the bee colony and often with poor results, because of the lack of reliability of acaricides or difficult to foresee epizootological situations, as the disease passes from one apiary to another or it is transmitted by wasps. Without controlling the efficacy of treatments and adequately using the available techniques, "blind" treatments will induce a false view of security regarding the health of bees. Precious information may be obtained by use of screened bottom boards, but also by uncapping drone brood and by examinations of adult bees.

Importance of the screened bottom board

In order to minimize the damages caused in apiaries, the evolution of varroosis in the bee colonies must be supervised at all times, and fighting measures must be applied by taking into consideration the physiological state of the bee colony and the biological cycle of the acarian. The permanent monitoring of the health state of the bee colony (the degree of infestation) is performed by using the screened bottomboard (photo 7). The natural mortality of acarians, observed during the summer, but also the analysis of the detritus collected in winter provide concrete data regarding the intensity of the infestation [7, 8, 11].

How to choose medication?

One cannot randomly use any registered medicine, imposed or analyzed only by the price view point.
The medicine must be highly efficient and maintain its effect for a long period after administration, as during the active season only 15% of acarians are to be found on bees, and the remaining 85% or even more are in the brood cells (photo 10).



Parasites on a bee larva.
Photo: BeeVital Archive

The medicine must not influence the health of bees

An anti-parasitic treatment is effective if it ensures maximum results with minimum risks for the bee colony.
The application (administration) techniques must be easy and must not create either adverse reactions in the bee population or dysfunctions for the operator. Fumigation, evaporation, sublimation or spraying implies constant temperatures

and specialized devices.

The application period must not be influenced by the period of activity or rest of the bee colony, and the treatment must be available at all times.

The medicine must not contaminate the products of the hive

The compliance with the strategic programs of preventing and fighting diseases and the requirements of the European Union on ensuring the quality of beekeeping products is a vital, non-negotiable necessity. Conversion from conventional to ecological apiaries is a major objective for any European country [4, 24].

The medicine must not induce resistance to acarians

Both pyrethroids (fluvalinate, flumethrin) and other synthesis chemical substances (amitraz) induce resistance. Moreover, amitraz induces cross resistance [18].

Why we recommend BeeVital HiveClean

Currently there is no other product on the market, tested and registered with the Institutes of Biological and Veterinary Product Control that may be applied risk-free, both in winter and in any other period of the year (Table 1).

By using **BeeVital HiveClean** maximum results are obtained with minimum risks to the bee colony as a whole, and on an individual level (worker bee, queen).

With **BeeVital HiveClean** we act against Varroa with efficient and nonpolluting means, even in the wintering cluster. In EU the National Beekeeping Program subsidizes the antivarroa treatments; in some EU member states organic apiaries benefit from additional financial support and an important component consists in financial aid for bio means of controlling Varroa, **BeeVital HiveClean** being one of the few options in this case.

Ensuring the quality of beekeeping products

European Union consumers' general orientation towards the consumption of natural products and the extreme sensitivity to the issue of food residues demand us, as suppliers of agricultural products, extreme care in fighting bee diseases. Unlike many other food products, the main risks for beekeeping products are chemical ones, especially because of medicine treatments applied on bee colonies.

By definition honey is considered as natural and pure must be free of any residues. By using **BeeVital HiveClean**, which is an ecological product, we ensure this desiderate. The quality of beekeeping products is also ensured by complying with strategic programs of preventing and fighting diseases and the requirements on beekeeping best practice guides and product traceability [4, 24].

Success in fighting varroosis will depend on how fast beekeepers, taking into consideration the EU requirements on the quality of beekeeping products, will adopt modern fighting strategies. Let us not forget that, in time, the disease was favored in its evolution by two subjective factors: the lack of knowledge on pathogenesis and the underestimation of the damages caused by the acarian due the lack of sanitary supervision.

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Annex 1

Control of varrosis in the apiary

Control by clinical examinations in the apiary
(n=200; *acarians in 100 individuals; **immature bees or bees with deformed wings %)

Observation method	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Observations
Uncapping of drone brood*					>10%	>10%	>10%						Immediate treatment
Examination of worker bee brood**						>5%	>5%						Immediate treatment
Control on 200 adult bees			>5%	>5%	>5%	>5%	>5%	>5%	>5%	>5%	>5%	>5%	Immediate treatment
Examination of detritus			>5%										Immediate treatment

Control by sceened bottom board

Observation method	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Observations
Control with acaricides													
Acarian fall (24 h)			>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	Immediate treatment
Natural falls													
Daily acarian falls			1	1	1	1	1	1					Control in autumn
Daily acarian falls			2	2	2	2	2	2					Control after 2 month
Daily acarian falls			>3	>3	>3	8	8	8					Immediate treatment
Daily acarian falls	>0.5	>0.5	>6	>6	>10	>10	>16	>30	>20	>20	>30	>0.5	Critical situation / collapse

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Annex 2

List of pathogens of bees, diseases, inter-colony transmission and virulence

Type of pathogen	Name of pathogen	Name of didease	Transmission horizontal	Transmission vertical	Virulence
Protozoa	*Nosema sp.*	Nosemosis	+	+++	Benign-lethal
	Malphigamoeba mellificae	Amoebiasis	+	+++	Benign
Fungi	*Ascosphaera apis*	Ascospherosis (chalk brood)	+	+++	Benign
	Aspergillus	Aspergillosis (stone brood)	+	+++	Benign
Bacteria	*Paenibacillus larvae*	American Foul Brood	+++	+	Lethal
	Melissococcus plutonius	European Foul Brood	+	+++	Benign
	P. alvei				
	B. laterosporus				
	E. faecalis				
Viruses	APV	Acute Paralysis Virus	+	+++	Benign*
	DWV	Deformed wing virus	+	+++	Benign*
	CPV	Chronic Paralysis Virus	+	+++	Benign*
	BQV	Black Queen Cell Virus	+	+++	Benign*
	SBV	Sac Brood Virus	+	+++	Benign*
Acarians	*Acarapis woodi*	Acarapisosis	+	+++	Benign-nearly lethal**
	*Varroa destructor*****	Varroosis	+	+++	Benign-lethal***
Pests	*Tropilaelaps sp.*		+	+++	Benign-lethal***
	Aethina tumida		+	+++	Benign-lethal***

Legend

1(+) or +++

* serious effects in the colonies infected only with Varroa parasites

** serious effects in the colonies where the parasite has recently appeared

*** serious effects in the colonies where the parasite has recently appeared or where the parasite is effectively fought with

**** Newly described species of *V. Jacobsoni* (Anderson and Trueman 2000)

(*Gabriela Chioveanu, 2008*)

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Glossary

Ancestral – character derived from ancestors.
Chemoreceptor – terminal part of an organ that, in contact with the molecules of a substance in the external environment, receives it and differentiates it depending on its chemical structure.

Chelicerae – either of the first pair of fanglike appendages ear the mouth of an arachnid, such as a spider, often modified for grasping and piercing.

Cross resistance to insecticides – capability of living organisms (acarians) to acquire, by natural selection, resistance to toxic doses of chemically or otherwise related substances, even if they have never been used as treatments upon these organisms.

Detritus – material collected in the tray of the screened bottomboard during the wintering of the bee colony, remains and broken pieces, larvae, insects, etc.

Endemic – infectious or parasitic disease that is permanently found in one region.

Enzootic – spread form of an infectious-contagious or parasitic disease in a certain territory, without extending tendency, but reoccurring periodically and sporadically.

Habitat (biotope) – living environment which provides favorable conditions, the complex of these conditions.

Hemolymph – circulating liquid in the organism of bees containing nutritious substances and in which cellular elements, with a phagocytizing role, and humoral elements, with an anti-infectious role (lysozyme, apidecines) freely float.

Homeostasis – property of the organism to maintain constant values of the internal environment within very close limits.

Panzootic – wide-spread disease (countries, continents) which affects a large number of individuals.

Parthenogenesis – growth of organisms from unfecundated eggs.

Phagocytosis – engulfing and digesting foreign bodies (microbes) by certain specialized cells (phagocytes).

Phylogenesis – process by which a group of organisms differentiates in time (classes, species, varieties, families, etc.) within the process of evolution of the living world.

Phytoncides – volatile substances generated and eliminated by certain plants, with bacteriostatic and sometimes bactericide action.

Sensiles – sensorial appendices.

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Annex 3
Confirmation for Organic Beekeeping

CERES
Certification of Environmental Standards GmbH

Confirmation

for Materials Used in Organic Agriculture

Document No: 18166

Issued for:

BeeVital GmbH
Wiesenbergstraße 19
5164 Seeham
Austria

CERES GmbH herewith confirms, that the materials listed below, which are produced or sold by the above mentioned company, can be used in organic beekeeping according to following standards:

Product	Use as	Standard	Restrictions
BeeVital HiveClean	Bee Care Product	EU Council Reg. (EEC) 834/2007	n/a
BeeVital MycoStop / Chalkbrood			

This confirmation is valid until 1st of September, 2013

Happurg, 18th of March 2013

Dr. Bernhard Schulz, CERES GmbH

Last inspection date: 0th of March 2012

CERES authorises the above mentioned company to use the CERES seal on the products specified above. The CERES Seal is property of CERES GmbH, Happurg, Germany. This confirmation is based on an onsite verification and laboratory analysis. However, the certificate holder's responsibility to ensure, that all batches sold under the above mentioned commercial names, or with reference to organic, or with the CERES seal, comply with the standard(s). CERES did not verify other aspects of quality, as e.g. the suitability of the materials for the proposed purpose. 4.8.6e(V210411)

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