Bee Medicine for Bee Vets

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1. Honey bee biology

Honey bees are social insects. According to E. O. Wilson, the following three criteria are required for insects to be called truly social (eusocial): 1) caste division of labor, 2) cooperative care of brood/offspring, 3) overlapping generations (offspring stay with colony and contribute to general welfare). Under normal circumstances, honey bee colony consists of a queen, drones and worker bees. Both queen and workers are diploid females and have 32 chromosomes and drones are haploid males with 16 chromosomes.

1.1. Castes

1.1.1. Queen is the only mated egg-laying female with developed reproductive tract in a colony. She is slightly larger than a worker bees and her abdomen is elongated and only partially covered by the wings because it contains fully developed reproductive tract in addition to other abdominal organs. Three days after oviposition, a diploid larva hatches from a fertilized egg and is fed abundant amount of royal jelly during the entire larval period (5 days) after which queen cell is sealed with wax (8-9 days post-oviposition) and pupation occurs until eclosion (emergence) at 16 days post-oviposition. The newly emerged virgin queen will destroy other queen cells, if they exist, and during the first two weeks of life will fly out from hive to mate with 10-15 drones (polyandry) in the air. The more drones the queen mates with, the greater the genetic diversity of her offspring, and the greater the robustness of the entire colony - *[important for the overall health of colony]*. The queen stores the collected sperm from drones in the sperm-storing organ, called spermatheca, for the entire life (3-4 years). Once mated, the queen does not leave the hive again except during natural swarming. There are two major roles that the queen performs. The first role is laying eggs that develop into workers, drones and queens (if needed). During the peak season, queen can lay ~1500 eggs a day (close to her own weight). The second role is production of multiple pheromones that maintain functionality of the entire colony. Pheromones are produced in mandibular (and other) glands and distributed throughout the entire colony via direct contact and trophallaxis (food exchange) - *[important behavior -> spread of diseases]*. Pheromones have many important effects on the colony, including suppression of ovary development in worker bees, suppression of queen rearing (swarm and supersedure cells) and enhancement of worker activity necessary for growth, productivity and overall health of the colony *[important behavior -> spread of diseases]* [1].

1.1.2. Drones are male bees whose main function is to mate with virgin queens after which the drones die. Drones are larger than worker bees but they are not as long as the queen. Three days after oviposition of a non-fertilized egg, a haploid larva hatches and is fed royal jelly during the first ~3 days and then pollen and nectar until the end of larval stage (day 9-10 post-oviposition). After pupation is complete, drones emerge at day 24 post-oviposition. During the first ~2 weeks of life, drones become sexually mature, and then fly out of the hive during the day to seek drone congregation areas where they join drones from other colonies which are waiting to mate with virgin queens. Drones that succeed to mate with a queen will die during copulation, while those that did not succeed to mate live for a few months during the summer and are expelled out of hive and die during the fall. During the summer, drones are accepted by all colonies, therefore they often drift from colony to colony - *[important behavior -> spread of diseases].*

1.1.3. Worker honey bees are ‘sterile’ females that have hypoplastic ovaries. Both queen and workers hatch from the identical fertilized eggs as diploid female larvae, and accordingly, they have the same genetic composition. The quality and quantity of feed available to these diploid female larvae determines their phenotypic development. Namely, if larvae are fed with abundant quantities of royal jelly during the entire larval period, they will develop into queens within 16 days post-oviposition; conversely, if the same larvae are fed restricted amount of royal jelly for first ~3 days and then with pollen and nectar, they will develop into worker bees within 21 days post-oviposition. The worker honey bees are responsible for all activities in colony except reproduction. The honey bee workers are divided into two major types based on seasonality and tasks: summer and winter workers.

Summer workers live ~6 weeks. Approximately the first half of life of an adult summer worker (3 wks) will be dedicated to housekeeping tasks in hive (cleaning brood cells, nursing brood, attending to and feeding queen, building wax comb and sealing brood and honey, processing nectar into honey, processing pollen into bee bread, removing dead brood and adults from the hive, ventilating and defending the colony) - *[many of these behaviors are associated with spread of diseases]*. The second half of an adult summer worker's life (~ last 3wks) is dedicated to field work which consists of collection of nectar, pollen, water, and propolis. The most intense harvesting occurs within 2 miles but the forager bees can fly twice as far if nectar or pollen is not available closer - *[important behavior -> spread of diseases]*. Drifting of honey be workers from hive to hive is not as common as in drones; however, it exists. During the honey and pollen flow any worker bee carrying the load will be allowed into the hive - *[important behavior -> spread of diseases].* There are seasonal variations in the total number of worker bees per colony. To maximize honey production, the highest number of worker bees in a colony (50,000-70,000 bees) should coincide with the major blooming season of nectar producing plants (e.g. canola and alfalfa in Saskatchewan). In contrast, there are only 15,000 to 30,000 bees in a colony during the late winter.

Winter honey bee workers emerge at the end of summer and the beginning of fall (September to October in SK). During that time there is limited need for nursing (due to markedly reduced egg-lying activity) and foraging (reduced availability of flowering plants), so workers are not 'worn out' with these demanding tasks and their life span is on average 150 days [2]. The main role of winter worker bees is to ensure that queen survives during the winter until spring season starts and a new population of bees are generated. During the fall, surviving drones are expelled from the colony in preparation for winter. Honey bees do not hibernate. Instead, when the outside temperature falls, bees form a cluster surrounding the queen and generate heat by vibration of thoracic muscles and abdomens using energy from consumption of stored honey reserves. The density of the cluster is used as a mechanism for thermoregulation to achieve an optimal temperature of 29˚C in the center around the queen and ~6-8˚C at the periphery. Constant circulation of the bees within the cluster ensures exchange of the peripheral vs central positions of worker bees to freezing of bees at the periphery. The winter bees usually do not defecate within hive; instead, they accumulate waste within the rectum which can distend and occupy a substantial portion of the abdominal cavity. On sunny winter days when the temperature is above 0˚C, bees will fly out of the hive for a short period to empty the waste from their rectum (cleansing flights).

1.1.4. Development stages of brood are the same for all three castes even though the total development time is different. The queen lays non-fertilized eggs in drone comb cells (which have a larger diameter than worker cells) and fertilized eggs in worker comb cells and queen cups. From fertilized eggs, diploid larvae emerge 3 days after oviposition and will develop into workers or queens depending on the larval food they are provided by nursing bees. Non-fertilized eggs will develop into haploid drones. After hatching, larvae are attended and nursed by many young nursing bees visiting each larva many times per day - *[important behavior -> spread of diseases].* After completion of the larval stage (5-6 days), the comb cells containing larvae are sealed with wax (capped) and prepupal and pupal development occurs for 8-15 days depending on caste. Eclosion (emergence) of the imago (adult) stage occurs at day 16, 21 and 24 post-oviposition for queen, worker and drone, respectively. Larvae and pupae that die during the development process are removed and cells are cleaned by housekeeping worker bees - *[important behavior -> spread of diseases].* In addition, worker bees have the ability to detected infected or infested pupae and remove them; this is called 'hygienic behavior' which interferes with disease progression (e.g. reproduction of Varroa) and improves colony resistance to disease. Hygienic behavior is a part of social immunity and contributes significantly to the overall health of a colony. This is an inherited trait and there are various queen-breeding programs that selectively enhance this behavior - *[important management practice -> enhance disease resistance and colony health]*.

1.2. Reproduction

1.2.1. Natural propagating and reproduction of honey bee colony is accomplished by swarming during late spring and early summer. There are several predisposing factors that will result in swarming, including: 1) concentration of queen's pheromones become too low to maintain colony cohesion – this could be due to rapid expansion of colony during the spring or decreased production of pheromones; 2) decreased/inadequate space for colony expansion and food storage – colony becomes too crowded; 3) decreased space for egg laying and brood rearing. Once the colony enters the "swarming mood", queen cells with young queen larvae are produced. The old (mother) queen reduces egg-laying activity and her abdomen becomes smaller due to atrophy of ovaries in preparation for a swarming flight. Swarming will occur during favorable weather conditions (sunny and warm early afternoon), usually when the new queen cells are capped and the young queens are in the pupal stage. The old mother queen will leave hive with a substantial proportion of worker bees and form a transient swarm cluster, usually on a tree branch close to the original hive (less than 100 meters) until scout workers find a suitable location for their permanent new home, which could be a few kilometers away from the original hive - *[important behavior -> spread of diseases]*. Swarmed bees are docile and do not exhibit defensive behavior. The remaining portion of the original colony will wait for the new queen to emerge, mate and re-stablish functional order of the colony.

1.2.2. Swarming is detrimental for a beekeeping operation due to loss of bees and subsequent decrease in honey production. Accordingly, good beekeeping management practices aim to decrease/eliminate swarming by removing predisposing factors for this natural reproductive and propagating behavior. There are numerous management beekeeping techniques used for multiplying colonies and some of them use queens produced by commercial queen breeders who ship queens worldwide. Large-scale commercial queen breeding and production by comparatively limited number of companies is considered to be a threat to genetic diversity - *[important management practice -> impact on colony health].*

2. Seasonal cycle in honey bee biology and beekeeping

2.1. Winter

European honey bees are adapted to a temperate climate and will survive winter providing that they are healthy, have abundant food stores and proper ventilation (to prevent condensation within the hive). At the end of winter and beginning of spring (March in SK), the bee population is at its lowest and the colony will start to rear brood to replace the old population of winter bees. *[Disease management note: At this stage, there is no or very little brood hence the great majority of Varroa mites are in phoretic stage – this is the most effective treatment time with miticides]*.

2.2. Spring

During the winter, the colony consumes just enough food to generate sufficient heat in the cluster to protect the queen. However, when egg-laying and brood rearing resumes, requirements for energy and protein rapidly increase, and consequently, consumption of both stored honey and pollen is also substantially increased. Initiation of brood rearing (during early spring in SK) is the most critical time for overwinter survival of a colony, because if the colony does not have sufficient food stores it will most likely run out of supplies and die of starvation considering the lack of external sources of nectar and pollen in the environment at this time of year. Once spring blooming commences (crocuses and willows in SK -> Apr-May), colonies start to expand rapidly. During this time, additional therapy against *Nosema* sp. and/or *Paenibacillus larvae* (American foulbrood) may be considered if necessary, to make sure that there is sufficient withdrawal time before major spring blooming (e.g. dandelions and caragana in SK -> May-Jun) if honey is to be harvested for human consumption. This is also a time period (May-Jun in SK) of intense beekeeping activity in the apiary, including, *inter alia,* spring inspections and clean-up, preparation for queen rearing, nucleus (replacement colony) establishments, and queen replacement. All of these activities are crucial for prevention of swarming, breeding of new queens and multiplication of colonies to be used for replacement or expansion of the operation. Under normal conditions, replacement of queens is done every second year, but this depends on management practices of each beekeeper. Nevertheless, the importance of high quality queens cannot be overemphasized, not only for optimal colony production, but also for the overall health of colony – *[important management practice -> enhancement health of colony by high quality of queens]*. An old or poor queen that does not produce sufficient quality and quantity of pheromones will compromise colony homeostasis and cohesion through alteration of several physiological and behavioral modifications in the worker bee activities such as reduced cleaning, guarding, foraging and brood care [1] that ultimately results in a weak colony and increased susceptibility to disease. Following natural instinct, the colony will try to replace the queen by supercedure but the progeny queen will still have the same poor genetics as its mother.

2.3. Summer

During the summer (end of June to August in SK), the colony is at the peak of its strength and the majority of colony activities are centered on intense harvesting of food reserves (i.e. nectar and pollen) to be stored as honey and beebread for use during times of dearth (winter). However, "clever" beekeepers exploit this prolific behavior to generate profit from "stolen" honey☺ using well established beekeeping practices.

2.4. Fall

At the end of August (in SK) all honey stored in honey supers (above brood chambers) is removed for extraction. Fall treatment and feeding is initiated to ensure that overwintering colonies are as healthy as possible and have sufficient food stores. The most important, and very often necessary treatment is against Varroa mite. For many beekeepers in North America, metaphylactic treatment against American foulbrood is equally as important and it is also done at this time (September in SK). The third potential fall treatment is against *Nosema apis* and *Nosema ceranae*; this last treatment is recommended/applied based on infection rates determined in forager bees, or based on history of Nosema disease in this particular operation. Since beekeepers harvest the majority of colony honey stores accumulated during summer, in September, honey bee colonies are provided with abundant feed in the form of sugar syrup to ensure that colonies have enough food stores during the winter. During mid-October in SK, miticide strips are removed from colonies and colonies are prepared for overwintering (according to the local winter climate).

<u>3. Transmission of disease</u>

3.1. Mode of transmission of disease within a colony

A honey bee hive contains thousands of bees with biological behavior that requires close interaction (e.g. trophallaxis), direct contact (e.g. pheromone spread) and housekeeping duties (e.g. removal of dead brood and adult bees). These behaviors facilitate horizontal transmission of pathogens between individual bees. In addition, there are certain pathogens (e.g. viruses) that can be transmitted vertically from queen to progeny.

3.2. Mode of transmission of disease between colonies

Once the disease is established within a colony it can spread from colony to colony by natural or anthropogenic means.

3.2.1. Natural transmission of diseases between different colonies may be facilitated by 1) drones and workers drifting to adjacent colonies [3], 2) foragers from different colonies foraging on the same crops [4], 3) queens mating with infected drones [5], 4) colony swarming, and most importantly 5) foragers robbing infected, weak or dead colonies.

Robbing behavior of honey bees is the most important natural mode of transmission of honey bee diseases between colonies. Robbing is a special behavior of forager bees that find an unprotected source of honey that is collected and brought back into their hive. This behavior intensifies at the end of summer when there is reduced availability of nectar from flowering plants and large number of foraging bees. Unprotected stores of honey could be available in dead colonies that died due to various diseases, hence the "robber" bees become contaminated and bring infectious pathogens back to their hives. Weak colonies are also often targeted by robber bees because their guard bee population is depleted and, consequently, easily overpowered. One of the major causes of colony weakness is disease; accordingly, pathogens from weak colonies are transmitted to healthy and strong colonies by their strong foraging population (robber bees). Thus, it is extremely important to remove dead-out colonies from the apiary and, if infectious disease is identified, destroy or disinfect equipment (frames, comb, etc.) to minimize disease spread. In addition, if infectious disease is not identified as the cause of weak colonies, weak colonies should be re-queened and/or merged to create strong colonies. It should be emphasized that robbing is not restricted to colonies in the same yard, but it can occur anywhere within the ~5 km (flight radius of forager bees) <u>*[important behavior -> spread of diseases]*</u>.

3.2.2. Anthropogenic transmission of diseases (e.g. fomites, equipment, trade, etc.) is also extremely important. Using contaminated fomites and equipment, beekeepers can spread diseases from hive to hive or from yard to yard within the same operation if optimal biosafety practices are not implemented. Potentially devastating disease outbreaks can occur due to the sale and purchase of contaminated equipment or infected bees among beekeepers. National and international trade of potentially infected bees and products (e.g. packaged honey bees, queens, semen, honey, etc.) has been a major contributor to the global spread of honey bee pathogens during the last several decades [6] in spite of best intensions, strict regulations and high quality inspections. Migratory beekeeping practices also contribute significantly to transmission of diseases among colonies and dispersal of pathogens over wide geographical areas.

4. Treatment and prevention of diseases

In beekeeping industry, the integrated pest management (IPM) strategy is a commonly used term for prevention and control of diseases that includes: 1) genetic selection for resistance to disease (e.g. hygienic behavior); 2) management practices to reduce incidence and spread of diseases (e.g. frequent inspection, maintenance of strong/healthy colonies, prevention of robbing); 3) physical control (e.g. destruction of infected equipment/colonies, segregation of infected colonies, "shaking" method for control of brood disease, regular replacement of equipment/frames, interruption of parasite cycles, screened bottom boards for Varroa management etc.); 4) chemical control (e.g. chemical therapy of infected colonies and disinfection of contaminated equipment).

4.1. Chemical treatments

Chemical therapy with synthetic or natural chemicals is used in the Canadian beekeeping industry against mites (Varroa and tracheal mites), fungi (*Nosema apis* and *N. ceranae*) and bacteria (*Paenibacillus larvae* -> American foulbrood, and *Melissococcus plutonius* -> European foulbrood). Miticides and antimicrobials are used as both therapy and metaphylaxis depending on disease conditions, season of production and management practices. Unfortunately, resistance to antimicrobial and antiparasitic synthetic drugs has become a big concern for beekeeping industry.

4.2. Administration of therapy

Two major routes are used to administer treatment to honey bees: 1) administration in feed (antibacterial and antifungal medication) and 2) external contact administration (direct contact between external surfaces of bees and therapeutic chemicals impregnated in plastic strips, dissolved in solution or vaporized in hive).

<u>5. Major Bacterial Diseases of Honey Bees in Canada</u>

Short summary of those diseases for which chemical therapy is approved in Canada.

5.1. American foulbrood [7, 8]

American foulbrood is a devastating, contagious brood disease that develops rapidly, kills the colony and spreads to other colonies by robbing, drifting bees and anthropogenic modes.

<u>Etiology:</u> *Paenibacillus larvae* is a Gram-positive, spore forming, rod-shaped bacterium. Spores survive in contaminated equipment for decades. Approximately 2.5 billion spores are produced in each infected larva [8].

<u>Pathogenesis:</u> Larvae (up to 2-day old) ingest spores which germinate and proliferate in the intestine and subsequently spread throughout the body causing fatal septicemia.

<u>Gross pathology:</u> Brood frames have spotty brood pattern (shotgun brood); punctuated and sunken capping of brood cells; color of dead larvae changes from dull white to brown at which stage 'ropiness' test* is positive; and desiccated, dead larvae which form dark brown, brittle scales firmly adhered to the 'ventral lateral' wall of the brood cell (scale cannot be removed without destroying the cell wall). In advanced stages of disease there may be a strong decaying odor when the colony is opened, hence the name of the disease, 'foulbrood'.

*‘Ropiness’ test – The large number of vegetative *P. larvae* bacteria within macerated dead larvae will generate a typical glue-like consistency that can be detected by the ‘ropiness’ test. A dead larva is macerated with a matchstick within a cell and then slowly withdrawn. If the macerated tissue can be drawn out and stretched more than 2 cm, it is indicative of AFB infection.

<u>Diagnosis:</u> Gross pathology, especially a postive ‘ropiness’ test and the presence of scales, are highly characteristic, or could be considered even pathognomonic, for AFB. Nevertheless, submission of samples of affected brood (including scales, if present) is recommended for confirmation of diagnosis by bacterial culture and/or PCR.

<u>Therapeutic treatment:</u> treatment with antibiotics of clinically affected colonies is not recommended, and in some jurisdictions, prohibited (contact provincial apiculture specialist for more information). The safest approach is to burn the entire colony and contaminated equipment. Alternatively, if infection rate is low, contaminated equipment could be irradiated, and in some jurisdictions, adult bees may be salvaged by the “shook-swarm method”*.

* The “shook-swarm method” is used to salvage adult bees from colonies affected by brood disease (e.g. EFB and AFB). Adult bees with the queen are transferred/shaken into a screened box and kept in a cool place for a several ours to allow time for consumption and digestion of contaminated honey present in the gastrointestinal tract. These adult bees are subsequently transferred to a hive with new frames/foundation. This artificial method of separating of adult bees from infected brood reduces substantially the number of spores within a newly established colony, terminating, but not eradicating the disease [9]. Concurrent antibiotic therapy of the newly established colony will enhance efficacy of disease termination.

<u>Metaphylaxis:</u> In certain countries, antibiotics are prohibited in the beekeeping industry. In Canada and the USA, metaphylactic use of oxytetracycline (Oxytet-25, Oxysol 62.5, Foul Brood Mix) and tylosin tartrate (Tylan Soluble) against AFB is permitted and used regularly by many commercial and hobby beekeepers. The label instructions for Oxytet-25 are as follows:“Thoroughly mix 454 g of OXYTET-25 with 3.5 kg of powdered sugar. Apply 32 g of medicated mix per colony on the outer parts or ends of the frames 3 times at 4 to 5 day intervals in the fall and in the spring at least 4 weeks before the main honey flow.” Administration of oxytetracycline in syrup is also possible but it is not practiced as commonly. Tylosin is recommended only in beekeeping operations in which *Paenibacillus larvae* developed resistance to oxytetracycline. Potential residues in honey for tylosin are much higher when administered during the spring than for oxytetracycline.

<u>Integrated Pest Management (IPM):</u> Strategies for AFB management include re-queening with hygienic genetics; routine renewal of comb in the brood chamber (20-30% per year) to minimize contamination; frequent inspection to identify early stages of disease; prevention of spread of disease by robbing, contaminated equipment or feed; destruction of infected colonies and equipment; irradiation of equipment to destroy both vegetative stages and spores.

5.2. European foulbrood [8]

European foulbrood is an often self-limiting brood disease that is a consequence of reduced/suboptimal larval feeding due to an insufficient number of nursing bees to care for rapidly increasing numbers of larvae. A deficiency of brood care and feeding is most likely to develop during vigorous spring build-up of colonies in temperate climates (usually during the first major nectar/pollen harvest) [8, 10].

<u>Etiology:</u> *Melissococcus plutonius*, a Gram-positive coccus, is the main causative agent of EFB. However, it is often isolated with other bacteria (*e.g. Paenibacillus alvei*, *Brevibacillus laterosporus*, *Enterococcus faecalis* etc.) that may be secondary pathogens or saprophytes that may contribute to the typical sour odor of the infected colony as well as to “pseudoropiness” of affected brood (see above ‘ropiness’ test) [8, 10].

<u>Pathogenesis:</u> Larvae (less than 3-day-old) ingest food contaminated with *M. plutonius* bacteria which proliferate in the intestinal tract, competing with the larva for nutrients. During certain stages of colony expansion, the nursing bee population is insufficient to feed the expanding larval population, which, if infected with *M. plutonius*, will die due to starvation. Once the deficiency in nursing bees and larval nutrition is corrected, the symptoms of EFB will disappear [8, 10].

Gross pathology: Brood frames contain spotty brood pattern (shotgun brood); color of dead larvae changes from dull white to brown at which stage tracheal network becomes visible; macerated dead larvae exhibiting 'pseudoropiness',but consistency of macerated larvae is granular and not as stretchable (less than 2 cm) as in AFB; and desiccated dead larvae which form dark brown, C-shaped, rubbery scales that are loosely attached to the bottom of brood cells. The presence of a 'sour' odor depends on the presence and composition of additional saprophytic bacteria [8, 10].

Diagnosis: Gross pathology could be used to distinguish EFB from AFB. Nevertheless, submission of samples of affected larvae is recommended for confirmation of diagnosis by bacterial culture and/or PCR. Submission of larvae affected at early stages (live larvae) will facilitate diagnosis because at early stages of infection, saprophytic bacteria are not as prevalent, and overgrowth by secondary bacteria in culture will be reduced [10].

Therapeutic treatment: Mild cases of EFB disappear once nectar follow becomes steady and/or nursing bee population is increased. Severe cases of disease can have a considerable impact on honey production due slow spring build-up of colonies and subsequent suboptimal population of foragers during the main honey flow. Heavily infected colonies (more than 50% brood affected) should be destroyed together with equipment. For low or moderate infections, therapy with oxytetracycline can be implemented (as described above for AFB) as long as an appropriate withdrawal period is observed. Nevertheless, the disease will usually recur the following year, therefore it is advised to use additional IPM strategies for prevention (e.g. shook-swarm method, re-queen, etc) [8, 10].

Beekeeping operations that use metaphylaxis against AFB are also protected against EFB in most instances.

Integrated Pest Management (IPM): Strategies for EFB control include re-queening with hygienic genetics, routine renewal of comb in brood chamber (20-30% per year) to minimize contamination, and the 'shook-swarm method' for colonies with low to moderate infections [8, 10].

Nota bene: These are the only two diseases of honey bees in Canada for which beekeepers will require a veterinary prescription to obtain antibiotics, because both tetracycline and tylosin are categorized by the Health Canada as medically important antimicrobials (MIA).

6. References:

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