Using Epidemiological Methods to Improve Honey Bee Colony Health

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Abstract

Epidemiologists emphasize that health is a common good. By focusing research on health at the population level, epidemiology can make great positive impact on health. Using real-world examples, we hope to give you a quick overview of what epidemiology is, how it works and should be interpreted. First off, epidemiology is all about measuring (1) how much disease there is and (2) what factors contribute to the occurrence or absence of disease. So if you are a beekeeper, and you want to keep your bees alive (and why wouldn't you?), you should first understand the ways disease and risk are calculated and used to develop strategies to maximize bee health. This chapter is meant to do just that—gives a quick primer of epidemiology—so you and your fellow beekeepers have some way of self-evaluating new and old research about bee health and management and figure out how to apply new knowledge when managing your colonies to maximize their health.

Honey bees have been dying in the USA and in other countries at high rates for over a decade (Laurent et al. 2015; Seitz et al. 2015). Beekeepers have questions: *How many managed honey bee colonies died last winter in the USA? How did my operation compare? How many Nosema spores per bee are needed to justify treatment? What number of Varroa mites can live in my colony without hurting it? How should I treat an outbreak of European foulbrood? What can I do to reduce the chances of getting American foulbrood?* These are the kinds of questions epidemiologists try to answer.

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Using real-world examples, we hope to give you a quick overview of what epidemiology is and how it works. First off, epidemiology is all about measuring (1) how much disease there is and (2) what factors contribute to the occurrence or absence of disease. So if you are a beekeeper, and you want to keep your bees alive (and why wouldn't you?), you should first understand the ways disease and risk are calculated and used to develop strategies to maximize bee health. This chapter is meant to do just that—gives a quick primer of epidemiology—so you and your fellow beekeepers have some way of self-evaluating new and old research about bee health and management and figure out how to apply new knowledge when managing your colonies.

1 What Is Epidemiology?

Epidemiology is the study of disease levels in a population. Epidemiologists use a broad definition of "disease": any departure from perfect health. Honey bees pose a particular problem for epidemiologists as it is hard to define what a colony in perfect health would look like. Fortunately, diseased colonies are easier to identify.

When measuring disease or a departure from perfect health—we use both direct and indirect measures. **Direct** measures are easiest to understand. You go to a hive and see symptoms that look like American foulbrood (AFB), you take samples and send them to a laboratory, and the samples come back positive: Your colony has AFB. But as all beekeepers know, honey bee colonies are complicated, and since we can't ask the bees how they are feeling we have to use several **indirect** measurements to assess how healthy a colony is. Good beekeepers do this every time they inspect colonies—what does the brood pattern look like? How many frames of bees and brood and food are present? How is the queen doing? Is the colony alive? Obviously, a dead colony is the worst and most extreme "disease" outcome there is.

To be honest, epidemiologists are not really interested in how disease might affect a single individual (another epidemiological challenge—What is an individual in the beekeeping context? A bee? A colony? An apiary?—but more on that later), rather, epidemiologists focus on how disease spread and persist (or not!) in a population. The ultimate goal of epidemiologist is disease prevention, and so epidemiologists also evaluate prevention strategies and devise and evaluate ways to get the proven best practices widely adopted.

At the core, there are two different types of epidemiological studies, descriptive studies and analytic studies. As their name suggests, **descriptive studies** are designed to describe a disease, how widespread it is, where it occurs, when it occurs, etc. These studies do not necessarily try to link disease outcomes with cause (s). **Analytical studies,** on the other hand, are designed to determine which factors are related to disease outcomes. By measuring "exposure variables" (also called, "risk factors") and the occurrence (or not!) of a disease, analytical studies quantify the chances an individual will develop a particular disease after a certain exposure.

So if we think again about AFB, a descriptive study would endeavor to find out how many diseased colonies there are in a certain area over a certain period of time, while an analytical study would attempt to find factors that increased the chances that a colony would have AFB. Sometimes these risk factors are self-evident, e.g., buying used equipment from a neighbor who had a major AFB problem and did not know it.

The key point of analytical studies is to measure the **risk** or **chance** of developing disease after an exposure. It is important to remember that in many cases not all colonies exposed to a risk factor will get the disease, and not all colonies suffering from a disease will have been exposed to the same risk factor(s). Using our AFB example again, after being exposed to AFB spore-contaminated brood comb (Lindström et al. 2008a), two of five colonies developed AFB infections, meaning, three colonies on five did not show clinical symptoms of AFB even when sharing the same exposure. Conversely, not all cases of AFB are the result of introducing contaminated comb into uninfected colonies, for example bees can rob honey from infected colonies and bring the pathogen home starting an infection (Lindström et al. 2008b).

a. Surveillance and monitoring in honey bee health

Surveying a population for disease is the most basic form of a descriptive study. Systematic surveys conducted over time can help define "normal" disease rates in a population. Importantly, once we know what normal disease levels are, survey results help identify outbreaks and/or hotspots of disease occurrence. As one can imagine, finding a new disease early, before it spreads widely, is the reason many surveillance efforts are implemented. Knowledge of where and when a disease emerges is the starting point for many epidemiological investigations.

Beekeepers do this informally all the time. Every time you open and inspect a colony and look for evidence of brood disease you are "surveying" your operation. Of course other surveillance efforts, such as conducted by state apiary inspectors, are more structured and systematically look for disease within the operations of their purview on a regular and long-term basis. For survey data to have the most value, clear protocols are required so that data from many different inspections are comparable. Such data, aggregated over time and space, has huge value, as it can compare bee health over time and also provide insight on the relationship between colony health measures.

Illustration 1. **Apiary Inspections: An Example of Surveillance Program**. In the early 1900s, in response to the high prevalence of the highly contagious "foul brood" (this was before the bacteria responsible was identified and the condition re-named "American Foulbrood") in the USA, many USA states enacted bee laws that mandated the inspection of honey bee colonies on a regular basis to help find and then destroy colonies that were contaminated (Burgess and Howard 1906). The Pennsylvania Department of Ag kept records of AFB prevalence that date back to the 1940s. When the survey was

first conducted, AFB was found in 12% of all the apiaries inspected. In the 2000s, it was well below 1% (unpublished). Note that this data says nothing about why the disease rate went down.

Systematic monitoring allows for early detection—and reaction—to the appearance of newly emerging diseases. Let's be honest, bees have faced and continue to face a lot of threats. Over 100 years ago, one of the biggest problems faced by North American beekeepers was Wax moth. Since then, we have had to face AFB, chalkbrood, sac brood, honey bee tracheal mite, *Varroa*, small hive beetle, more than a dozen virus; and we are now threatened by Asian hornets (racking havoc in France and other places in Europe), even more viruses, and the Tropilaelaps mite. The accidental introduction of Tropilaelaps mite into any country is the one threat that should keep beekeepers awake at night. Like *Varroa*, it evolved on a different species of honey bee and jumped host. In some places in Asia where they keep European honey bees, they have to treat for the mite once every 2 weeks (Pettis et al. 2013)! The value of a surveillance system (reviewed in Lee et al. 2015a) is to ensure that if Tropilaelaps mites are introduced into the USA, detecting it early would allow for interventions which would hopefully eradicate the problem before it became wide spread.

Box 1: Measures of disease frequency

Epidemiologists have their own jargon, which you will likely encounter in study summaries or reports. Here are some of the most common and useful terms defined.

Prevalence and *incidence* are two measures of disease in a population, and so usually the main result of descriptive studies (see Fig. 1). **Prevalence** is the proportion (usually expressed as percentage) of existing cases in a known population. It indicates how frequently a disease is present in a population during the survey time frame. If the survey randomly selected colonies to inspect, one way to interpret prevalence is the probability that any subject in a population has the disease. **Incidence** is the number of new cases that developed over a specified period of time in the population at risk. It specifically relates to the transition from a healthy state to a diseased state rather than just the number of diseased individuals. In the apiary depicted in Fig. 1, the prevalence of the "disease" was 37.5% (three of eight) on Date 1. The second inspection found that four of the seven were infected, so the prevalence was 57.1%. During the interval between Date 1 and Date 2, the incidence of the disease was 60% (three new infections in the five that were "at risk"—those that were not infected during the first inspection). Mortality rates are also a form of incidence. In the case we have just discussed, all eight colonies originally inspected were at risk of dying, one did die so the incidence (or mortality) rate was 12.5% (one of eight) for the period of time between Date 1 and Date 2.

Fig. 1 Incidence and prevalence. Fictitious apiary represented at two different dates. *Legend Green* colonies = disease absent; *Red* colonies = disease present; *Black* colonies = colony died. One Date 1, three of the eight colonies are diseased. The prevalence of the disease is 3/8 = 37.5% on Date 1. On Date 2, one of the diseased colonies has recovered, one is lost and three previously healthy colonies became diseased. The prevalence of the disease on Date 2 is 4/7 = 57.1%. The incidence of the disease between Date 1 and Date 2 is of 3/5 = 60% (three new cases among the five healthy, at risk, colonies on Date 1)

Disease Loads

Disease frequency (see Box 1) is useful in understanding bee health in some context but not others. When a disease is ubiquitous, then not much useful information is gained by just knowing if colonies have or do not have the disease. In other words, sometimes it is not the prevalence that matters, it is the disease's **load** that matters. For most diseases, and in particular infectious disease, the gravity of infection in diseased individuals provides a more complete picture than the simple presence or absence of the disease.

For example, nearly every colony in the continental USA has *Varroa*, specifically between 2011 and 2015 *Varroa* were detected in 91.7% of colonies sampled (i.e., 91.7% prevalence, Traynor et al. 2016). Please note we specifically and deliberately used the word *detected*—as, in all likelihood, the prevalence was much greater and the negative detections probably were the result of recent *Varroa* treatment applied by the beekeeper before inspection, so that, while mites were present they were at very low—undetectable—levels. We discuss the idea of test sensitivity and specificity in Box 2. If about every colony is infected with *Varroa*, then there is little value in knowing the prevalence (mere presence/absence) in order to help understand bee health. The real data of import is how many *Varroa* are found in a colony—the colonies mite **load** (Fig. 2). This information is predictive and actionable.

Values related to disease loads may be used as the trigger for management decisions (such as deciding which colonies to use as breeder stock, or when to apply a chemical or non-chemical control strategy). The *Varroa* load that currently warrants action—the **action threshold**—is a little tricky to pin down. It depends—on the time of the year (high levels early in the season are more concerning then a comparable level latter in the season), on the viruses the mites are vectoring

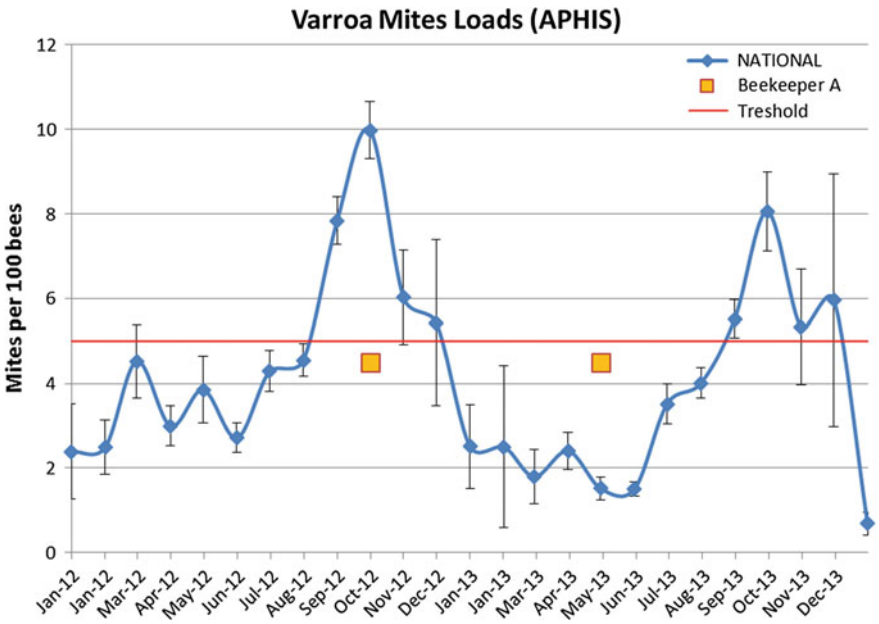

Fig. 2 Average *Varroa* loads in the USA (2012–2013). The *blue line* represents *Varroa* mite loads observed from the *USDA APHIS National Honey Bee Disease Survey* 2012–2013 (n = 1515 operations sampled; data from Traynor et al. 2016), averaged by month of observation (with error bars as standard errors). The two *orange squares* represent fictitious samples sent in by a beekeeper (used in the discussion above)

(*Varroa* can spread very fast which can wipe out much of an operation even when mite levels remain low), and on the region (with or without an interruption of brood due to winter). Disease loads often have a seasonality (Fig. 2). Knowledge of this seasonality is helpful when designing management tools to reduce losses. Mite levels peak in the USA population in late summer/early fall. This is also the time that colonies begin to crash from heavy mite infestations. When a colony crashes from *Varroa*, many of those mites are spread to neighboring colonies by the drift of the collapsing colonies last bees or by robbing bees that pick up *Varroa* while plundering the honey reserves of the collapsing colony (Frey and Rosenkranz 2014). For this reason, beekeepers are urged to check mite levels in hives at least once a month, particularly in the fall, when mite pressure increases from natural population growth, shrinking of the brood nest, and invasion from neighboring colonies. This is also the time when bees kept in northern locations switch from the production of short-lived summer bees to long-lived winter bees, and so heavy parasitism of the developing winter bees will increase the risk that colonies will die even if mites are controlled after these bees emerge.

So if for some conditions, such as *Varroa*, disease load is more informative than prevalence, it is the opposite for diseases that are highly contagious or swift acting. AFB is highly contagious and persistent, and so some state laws require total

destruction of diseased colonies even if the colony has just one AFB scale present. This makes sense when one considers that one AFB scale can contain several billion spores, that these spores remain infectious for at least 50 years, and it only takes less than 10 spores to kill a larval bee if it was fed the spores in the first day of its larval life (LD_{50} = 8.49, Brødsgaard et al. 1998). So in the case of AFB, a disease load of 1 scale is a sufficient threshold to implement control strategies.

Illustration 2. The National Honey Bee Disease Survey (NHBDS), funded by USDA APHIS (Animal and Plant Health Inspection Service), is an example of surveillance program designed to ensure early detection of invasive pests (i.e., *Tropilaelaps clareae* mites) which are not presently found in the USA. At the same time, this survey effort provides an opportunity to describe the prevalence and load of pathogens and parasites across the country and over time. One parasite monitored was the *Varroa* mite. In Fig. 2, the blue line represents the trend of *Varroa* mite loads in the USA throughout the 2012–2013 seasons. This graph shows the cyclic nature of the infestation loads, with a peak in end of summer to fall. In this context, let us imagine a beekeeper monitoring mites who observes 4.5 mites per 100 bees. Such a load is rather high, very close to the threshold of 5 mites per 100 bees which is sometimes referred to as the damaging level of mites in a colony. However, depending on the time of the year the interpretation will vary considerably. If the sample was taken in October, the NHBDS trend curve allows us to compare this single result to an estimated average load of 10 mites per 100 bees in the USA population at that time. This does not let us know if that level is acceptable, as the survey did not collect information about survivorship of those colonies, but it allows us to say that this beekeeper's sample would be below the norm. However, if the sample was taken in May, though it is still below the same threshold of 5 mites per 100 bees, we can see that it is far above the levels reported in the USA for that time of the year. Using only a threshold criterion would have failed to detect this anomaly, while comparing it to a descriptive study of *Varroa* loads in the USA gave us a more complete and useful story.

Box 2: Sensitivity and specificity

Whenever a test is performed to identify a disease (presence or absence), there is a certain risk of error in the diagnostic. Sometimes the test will fail to identify the presence of the disease (false negative), or sometimes the test will incorrectly detect the presence of the disease (false positive). A good test method should minimize those errors. How good a test is, is quantified as the sensitivity and specificity of a diagnostic test.

The **sensitivity** of a test is its ability to correctly identify samples with the disease. A highly sensitive test minimizes false positives. In other words, if a highly sensitive test identifies a sample as negative, we are nearly certain it is indeed negative (disease free).

The **specificity** of a test is its ability to correctly identify samples without the disease. A highly specific test minimizes false negatives. In other words, if a highly specific test identifies a sample as positive, we are nearly certain it is indeed positive (diseased).

Ideally, we would want all our diagnostic tests to be both highly sensitive and specific, but that is usually something of a tradeoff.

b. Identification of risk factors in honey bee health

While descriptive studies explain the prevalence and load of disease, analytical studies aim to identify and quantify the effects of exposure variables (or **risk factors)** on the prevalence of disease. Typically, epidemiologists look for association between exposure to a risk factor and a disease outcome by comparing populations with different exposures and see how they fare in respect to the disease of interest. Once identified, modifying risk factor exposure is the corner stone of preventive programs.

Illustration 3. An example of cross-sectional study was recently completed in Argentina. Researchers quantified the *Varroa* loads in colonies and also asked beekeepers about the management practices they used. The results showed that colonies with a mite load of 3 or more mites per 100 bees were 4.9 times more likely to die over the winter (Giacobino et al. 2015) compared to colonies with mite loads below 3 mites per 100 bees. Beekeepers who did not monitor mite loads after they applied treatment, or did not requeen colonies the previous year were also more likely to experience higher rates of colony mortality.

It is important to remember that *association* (correlation) is not the same as *causation*. Most epidemiological studies are **observational** rather than experimental. This means that they take advantage of "natural experiments" in which the exposure (and sometimes the outcome) has already occurred, or occurs without the intervention of the researcher. Such "natural experiments" provide no guarantee that the two groups being compared are identical in all aspects other than the exposure/lack of exposure of interest. Experimental studies, on the other hand, try to ensure that all aspects are similar before applying the exposure themselves to a random subset of the experimental subjects. Epidemiologists strive to identify and control for all extraneous variables ("confounders") that may correlate in

unexpected ways with the observed results. Whenever possible, confounders are accounted for when analyzing results from observational studies, because, if unchecked, they can bias the interpretation of the results. Even when cofounding variables are identified and controlled, scientists rarely identify risk factors as "causal" of an outcome without experiment-based evidence of these associations. Observational studies can also serve as a basis for identifying the origin (etiology) of new problems and for helping to formulate hypothesis for later experimental testing.

Illustration 4. A recent study set out to document risk factors associated with increased risk of colony mortality in three migratory beekeeping operations (vanEngelsdorp et al. 2013). The researchers found that "queen events" (evidence of a queen replacement or queen failure) was associated with an increased risk of colony death in the short term (~50 days following the event). In this study, "queen events" were the exposure variable of interest, and "colony death" the disease or outcome of interest. This is an example of observational (non-experimental) study as the researcher did not induce any of the queen events to follow their impact on the mortality rate. Instead, they took advantage of natural events, carefully recorded, to get insights into honey bee health mechanisms.

Measures of association

When designing studies, epidemiologists plan how they will select the subjects, follow them over time, and analyze their results. This is referred to as the "study design." There are many different study designs meant to identify possible associations between exposure and disease outcome. Three of the most widely used of these study designs include *cohort* studies, *case-control* studies, and *cross-sectional* studies (and are illustrated and explained in Figs. 3, 4 and 5).

Each study design has its strengths and weaknesses, and a detailed explanation of these differences is beyond the scope of this chapter. At the core, these differences revolve around how the subjects of the study are selected (either based on their disease or exposure status), which affects how results should be interpreted. Either way, they compare the "risk" (or probability) of disease occurring in two different groups—one exposed to the risk factor and the other not—from the same population. The results are usually presented as relative risk (also called **risk ratio**, RR) or relative odds (also called **odds ratio**, OR) (see Figs. 3, 4 and 5 for details).

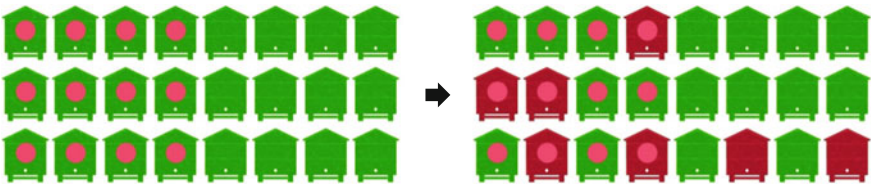

Fig. 3 Fictitious cohort study. *Legend Green* colonies = disease absent; *Red* colonies = disease present; *Dot* = exposure present (before the start of the study) to a certain factor X. The *arrow* represents the passage of time. In a cohort study, a group of disease-free subjects (the cohort) is selected based on their exposure status (both exposed and non-exposed) to a risk factor of interest (*left panel*). All of the hives would then be followed for a set period of time, and the incidence of disease in both the exposed and unexposed subgroups are monitored (*right panel*). This kind of study allows for the calculation of relative risk (RR) which is the ratio of incidence of disease (or risk) in the exposed population (R_{Exp}) divided by the incidence of disease in the unexposed population (R_{NExp}). In other words, it is a measure of the increased (or decreased) risk subjects have of developing a disease after being exposed to a risk factor. For this example, the probability, or risk, of a colony in the exposed group (*with dots in the figure*) to develop the disease during the study period would be 0.42 (5 on 12). Not all colonies exposed will develop the disease. This rate should be compared to the risk for colonies without a known exposure (*without dots in the figure*) to develop the problem, which is about 0.17 (2 on 12). In this example, the disease does also occur in colonies that were not exposed to the risk, but at much lower rate than for exposed colonies. The relative risk is calculated at 2.47 ($RR = R_{Exp}/ R_{NExp} = 0.42/0.17$), which represents an increased risk of 147% ($(2.47-1) \times 100$). This means that exposed colonies were 147% more likely to become diseased than non-exposed colonies. The conclusion is that colonies exposed to the product X present a higher risk of developing the disease than the non-exposed colonies, and the recommendation would be that beekeepers avoid the use of product X

A RR of OR of 1 indicates that both groups show similar risks of disease, irrespective of the level of the exposure. So the exposure seems unassociated with the disease. A RR or OR greater than 1 indicates that the group exposed shows higher levels of disease, so the exposure is associated with the disease. A RR or OR less than 1 indicates that the group exposed shows lower levels of disease, which suggest the exposure reduced disease prevalence. The greater the magnitude of the difference, the greater the "strength of the association": The more one group shows an increased risk for the disease compared to the other group.

Illustration 4 (continued). The study of migratory operations is an example of cohort study where groups with different exposure histories (queen events) were followed and compared in terms of disease incidence (in this case, colony mortality). A total of 284 inspections were performed, from which 35 showed signs of a queen event. The colonies were inspected again after ~50 days to determine the outcome status for the whole colony. The table of incidences is shown below. From it, we can determine that colonies that underwent a queen event showed a risk of 0.31 or 31% ($R_{Exp} = 11/35$) of

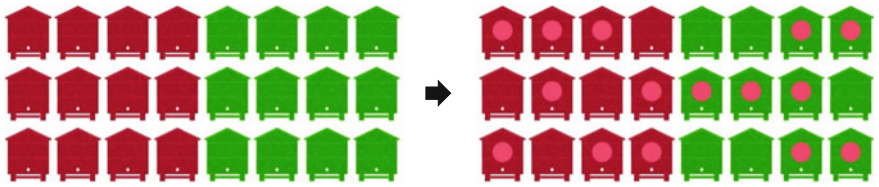

Fig. 4 Fictitious case-control study. *Legend Green* colonies = disease absent; *Red* colonies = disease present; *Dot* = exposure present (before the start of the study) to a certain factor X under study. The *arrow* represents the passage of time. In a case-control study, a group of diseased subjects (the cases) are compared to a group of disease-free subjects (the controls) (*left panel*). Ideally, control subjects resemble the disease subjects as closely as possible. Their history is then compared (usually through surveys, or tests are performed) to establish which of them were exposed to the risk factor under study (*right panel*). Because the proportion of cases to control is unlikely to be representative of their proportion in the source populations (we actively looked for the diseased colonies), it would not be fair to calculate totals, probabilities, and risk ratios. However, we can compare odds: The probability that some event will occur compared to the probability that it will not occur. Odds ratios (OR) are tricky and easily misinterpreted, even by professionals. For this example, of the 24 colonies selected based on their disease status (12 diseased and 12 controls non-diseased), 15 of the colonies were found to have been exposed to the factor X under study (*with dots in the figure*). The odds of an exposed colony being a case are 8 to 7. The odds of a non-exposed colony being a case are 4 to 5. The odds ratio (OR) would therefore be 1.4 (8/7 divided by 4/5). We would be interpreted as a 40% increase of odds of developing the disease in the exposed population compared to the non-exposed population. When the disease is uncommon, OR will be reasonably good estimates of risk ratio and can be interpreted similarly

dying over the next ~ 50 days. Colonies that did not experience a queen event showed a risk of 0.10 or 10% (R_{NExp} = 25/249) of dying over the next 50 days. In this study, the relative risk is calculated at 3.1 (RR = R_{Exp}/R_{NExp} = 0.31/0.10), which represents an increased risk of 210% ((3.1−1) × 100). This means that the risk of dying for colonies who experienced a queen event was more than two times more likely to die than those colonies that did not experience a queen event (vanEngelsdorp et al. 2013).

Table of incidences from Illustration 4 cohort

		Outcome		
		Dead	Alive	Total
Queen event	Yes	11	24	35
	No	25	224	249
	Total	36	248	284

It is critical to remember that association is not causation, so it would be incorrect to say that the queen event caused the increase in colony loss. Based on this study, it is not possible to determine if that queen events caused the increased mortality, or if some other factor caused colonies to die also caused an increase in queen events.

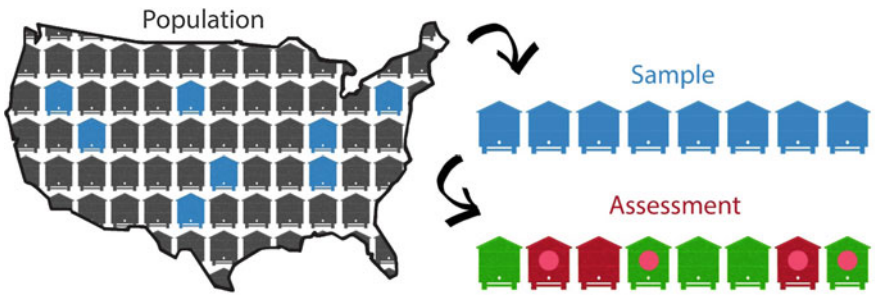

Fig. 5 Basic design of a cross-sectional study. In cross-sectional studies, subjects are first selected according to a particular sampling scheme (random, convenience or other), without regard for their disease or exposure status. They are referred to as the **sample**. Then both exposure status and outcome status are assessed at the same time. Sometimes, the investigators try to determine past levels of exposure through retrospective surveys. For instance, they might be able to glean significant information from beekeeping records. Those studies have the advantage that both outcome and exposure levels are representative of their true prevalence in the target population (subjects exposed and/or diseased are not more likely to be selected). Another variant is to follow the same population over time in a series of snapshots of cross-sectional studies. While ***cohorts*** start with disease-free subjects and investigate *incidence* of problems (i.e., development of new cases), ***cross-sectional*** studies focus on the *prevalence* within (i.e., the existing cases) a population at the time of the study. Therefore, in cross-sectional studies, the measure of risk is based on the prevalence of disease outcome in groups that have had or have not had an exposure to the risk factor of interest. This is expressed as a relative risk (RR) and is calculated exactly like the relative risk in Cohort studies. The difference, however, is that in this case relative risk relates to the risk of having the disease rather than the risk of developing it. This is an important distinction when interpreting the results: If a factor improves the survivorship of diseased colonies compared to non-exposed diseased colonies (but without curing them), it could be misinterpreted as being associated with the disease, because most disease colonies still alive would be most probably exposed (the others being already lost)

2 Significance of Epidemiology for Your Beekeeping Management

Before collecting any data, epidemiologists plan their experiments and decide which exposures and outcomes they will investigate. This is because the real world is complex. Multiple causes can exist for almost every outcome and every exposure variable can affect many different diseases (Dohoo et al. 2003). Epidemiologists have to focus on a specific problem. Many times even minor unrecognized factors can dramatically impact outcomes. Colonies managed by different beekeepers will be subjected to very different regimens (equipment, feeding, treatment, migration…). Even within the same beekeeping operations, apiaries will differ between each other in terms of availability of resources. Further within the same apiary, colonies can experience very different microclimates (for instance, some colonies are predominantly in the shade while others in the sun). A careful study would try to control these extraneous variables; for instance making sure all apiaries were all in full sun, so that any potentially cofounding effects are minimized.

Epidemiologists work at the population level, trying to estimate the difference the implementation of preventive or curative practices would have for the whole population. In some respects, epidemiologists ask "what if." What if the risk factor associated with a disease was removed? How many fewer cases of the disease could we expect?

As epidemiologists deal with calculating the chance of something occurring or not, they cannot make predictions for individuals, rather they can make predictions at the population level. Thus, a large part of epidemiology involves the application of risk easement strategies in order to reduce disease prevalence for the whole population.

There is a common saying: "a poll is only as good as its sample size." The same holds true for epidemiological studies. Whether the interest is in knowing the prevalence of a disease in a population or the strength of its association to an exposure, it is important that the sample is **representative** of the overall population. Populations have variability, and a good sample has the same variability. Epidemiologists usually convey this idea with a measure of uncertainty around their results, such as the **confidence intervals** (CI). Usually, the greater the sample size (the more subjects in the study), the smaller the CI around the estimate (the smaller the incertitude).

Illustration 2 (continued). The US National Honey Bee Disease Survey report (Traynor et al. 2016), summarizing the results from 2009 to 2014, indicated that migratory beekeepers had significantly lower *Varroa* prevalence than stationary operations (84.9% [81.4–87.8%] versus 97.0% [95.6–97.9%]). The estimates are followed by a bracket indicating the breadth of the confidence interval. Because those two intervals (the ones for stationary and for migratory) do not overlap, we are confident in saying their prevalence are significantly different.

Traditionally, statisticians employ a "95% CI," which indicates that, if we were to repeat the study 100 times, with 100 samples drawn randomly from the same population, and that a CI was calculated for each trial, 95 of those CI would contain the population's true *Varroa* prevalence.

3 Current State of Honey Bee Colony Population and Health

There are many ways to monitor honey bee health. One measure is the total numbers of managed honey bee colonies over time. Honey bee populations have increased globally by 64.7% since 1961, reaching a total of 81 million managed honey bee colonies in 2013 (Food and Agriculture Organization of the United Nations (FAO) 2015). This global increase is largely driven by increases in colony numbers in some regions of the world (Asia and South America) which masks significant decreases experienced in other regions, such as that documented in

Europe (−20.3%, Potts et al. 2010a) and the USA (−52.1%, vanEngelsdorp and Meixner 2010) (see Fig. 6). While total colony counts are good indicators of managed pollinator availability, they inadequately represent honey bee health. Managed honey bee colony population trends are mostly driven by socioeconomic factors (such as number of beekeepers, price of honey, political disruption) (Aizen and Harder 2009; Potts et al. 2010a, b; vanEngelsdorp and Meixner 2010) rather than biological. Total colony counts, estimated once a year, ignore the beekeeper practice of replacing dead-outs to keep operational numbers up. Beekeepers divide healthy colonies and/or buy and install packages in order to replace dead-out colonies or to increase operational size, so that the absolute number of colonies can be stable or even increasing year after year, even if colonies are subjected to high mortality rates (vanEngelsdorp et al. 2007).

Because of the ability to replace dead-out colonies quickly, which is particular to managed systems (as opposed to wild pollinators), honey bee health is better represented by measuring the rate of colony mortality over a defined time frame. In 2008, the COLOSS (prevention of honey bee Colony LOSSes) network—formed of honey bee experts from Europe, North America, and some other regions around the world—developed a standardized questionnaire to gather information about colony losses in an effort to enable comparison between participating countries (van der Zee et al. 2012). While at first these survey efforts focused on winter mortalities,

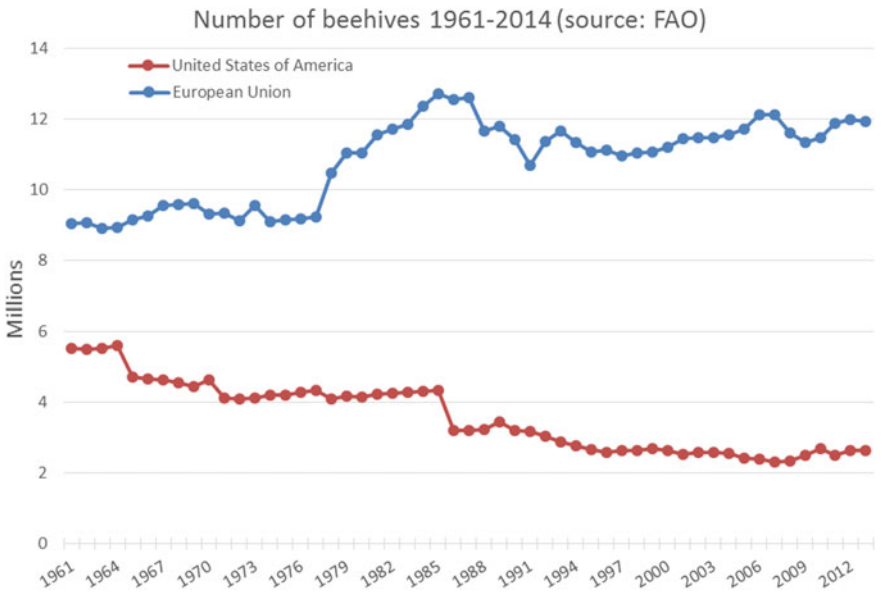

Fig. 6 Population trend. Estimates of the total number of managed honey bee colonies in the USA and European Union between 1961 and 2014 (Food and Agriculture Organization of the United Nations (FAO) 2015). © FAO 2015 Production/Live Animals/Beehives. This is an adaptation of an original work by FAO. Views and opinions expressed in the adaptation are the sole responsibility of the author of the adaptation and are not endorsed by FAO

more recent USA efforts have included calculating summer loss rates as well. It has long been assumed that summer loss rates are minimal; however, survey efforts have shown that in the USA summer losses are not negligible (Steinhauer et al. 2014) and so should also be considered when attempting to describe the status of honey bee health.

Over the last 10 years, the rate of honey bee losses over the winter in the USA has ranged from 22.3% to 35.8%, averaging around 28%. Over the 6 years for which summer (as defined by the period between April and October) numbers are available, summer losses ranged from 16.2% to 25.3% and averaged 21% (vanEngelsdorp et al. 2007, 2008, 2010, 2011, 2012; Spleen et al. 2013; Steinhauer et al. 2014; Lee et al. 2015b; Seitz et al. 2015; Kulhanek et al. 2017). Those loss estimates are far above the levels beekeepers themselves judge acceptable (16%, average of 10 years).

The causes of high levels of managed honey bee colony losses are multiple and probably interacting (Potts et al. 2010b). Honey bees face a very diverse array of threats (reviewed in Potts et al. 2010b; vanEngelsdorp and Meixner 2010) from diseases and parasites to reduced quality and quantity of bee forage due to land-use change, climate change, contaminations by pesticides (applied both outside and inside the hive) and, at least for USA populations, potential loss of genetic variability (but see Wallberg et al. 2014).

High levels of colony loss throughout the year seriously threaten the sustainability of beekeeping operations. Replacing dead colonies is costly, both directly (e.g., purchase of queens and bees) and indirectly, resulting from reduced productivity of split colonies. Weak and unhealthy colonies are also more costly to maintain as they need more feed, more frequent inspection and disease treatments. Weaker colonies also do not generate the same return as healthy strong colonies. Almond producers commonly have provisions in their pollination contracts that pay premiums for strong colonies while enforcing penalties for weak colonies. In fact some pricing schedules are now based on frame counts instead of the number of hives (Champetier 2011).

4 Summary

Epidemiology emphasizes that health is a common good. By focusing research on health at the population level, epidemiology can make a great impact in improving health. Large-scale epidemiological studies are important both to produce reliable accounts of the status of honey bee health and also to react efficiently to abnormal health events, develop and test hypotheses on disease etiology and to inform prevention and control strategies.

The same key principles that make epidemiological studies successful at population levels apply at apiary or operational levels and should be applied by every beekeeper. These recommendations include:

1. Carefully apply the preventive recommendations developed locally.
2. Monitor disease levels as often as practical throughout the year.
3. Compare the levels present in your own apiary to a quality baseline to detect abnormalities.
4. Apply the proper recommended control strategies when problems are detected.
5. Assess the efficacy of such control methods whenever they are used. This means always doing a recheck for the problem to be sure the control method(s) used were effective.
6. Always keep quality records and when possible participate in national surveys.

References

Aizen MA, Harder LD (2009) The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Curr Biol 19(11):915–918. doi:10.1016/j.cub.2009.03.071

Brødsgaard CJ, Ritter W, Hansen H (1998) Response of in vitro reared honey bee larvae to various doses of Paenibacillus larvae larvae spores. Apidologie 29(6):569–578. doi:10.1051/apido:19980609

Burgess AF, Howard LO (1906) The Laws in force against injurious insects and foul brood in the United States. Washington, D.C. : U.S. Department of Agriculture, Bureau of Entomology, 236 pp

Champetier A (2011) The foraging economics of honey bees in almonds. Presented at the Agricultural & Applied Economics Association's 2011 AAEA & NAREA Joint Annual Meeting, University of California, Davis, Pittsburgh, Pennsylvania

Dohoo IR, Martin SW, Stryhn H (2003) Veterinary epidemiologic research. 706 pp, AVC Inc., Charlottetown

Food and Agriculture Organization of the United Nations (FAO) (2015) FAOSTAT [WWW Document]. http://faostat3.fao.org/. Accessed 13 Aug 2015

Frey E, Rosenkranz P (2014) Autumn invasion rates of Varroa destructor (Mesostigmata: Varroidae) into honey bee (Hymenoptera: Apidae) colonies and the resulting increase in mite populations. J Econ Entomol 107(2):508–515. doi:10.1603/EC13381

Giacobino A, Molineri A, Cagnolo NB et al (2015) Risk factors associated with failures of Varroa treatments in honey bee colonies without broodless period. Apidologie 1–10. Doi:10.1007/s13592-015-0347-0

Kulhanek K, Steinhauer N, Rennich K, Caron DM, Sagili RR, Pettis JS, Ellis JD, Wilson ME, Wilkes JT, Tarpy DR, Rose R, Lee K, Rangel J, vanEngelsdorp D (2017) A national survey of managed honey bee 2015–2016 annual colony losses in the USA. J Apic Res 56(4):328–340. DOI: 10.1080/00218839.2017.1344496

Laurent M, Hendrikx P, Ribiere-Chabert M et al (2015) A pan-European epidemiological study on honeybee colony losses 2012–2014. European Union Reference Laboratory for honeybee health (EURL)

Lee K, Steinhauer N, Travis DA et al (2015a) Honey bee surveillance: a tool for understanding and improving honey bee health. Curr Opin Insect Sci 10:37–44. Doi:10.1016/j.cois.2015.04.009

Lee KV, Steinhauer N, Rennich K et al (2015b) A national survey of managed honey bee 2013–2014 annual colony losses in the USA. Apidologie 1–14. Doi:10.1007/s13592-015-0356-z

Lindström A, Korpela S, Fries I (2008a) The distribution of Paenibacillus larvae spores in adult bees and honey and larval mortality, following the addition of American foulbrood diseased brood or spore-contaminated honey in honey bee (Apis mellifera) colonies. J Invertebr Pathol 99(1):82–86. doi:10.1016/j.jip.2008.06.010

Lindström A, Korpela S, Fries I (2008b) Horizontal transmission of Paenibacillus larvae spores between honey bee (Apis mellifera) colonies through robbing. Apidologie 39(5):515–522. doi:10.1051/apido:2008032

Pettis JS, Rose R, Lichtenberg EM, Chantawannakul P, Buawangpong N, Somana W, Sukumalanand P, Vanengelsdorp D (2013) A rapid survey technique for Tropilaelaps mite (Mesostigmata: Laelapidae) detection. J Econ Entomol 106(4):1535–1544. doi:10.1603/EC12339

Potts S, Roberts S, Dean R, Marris G, Brown M, Jones R, Neumann P, Settele J (2010a) Declines of managed honey bees and beekeepers in Europe. J Apic Res 49(1):15. doi:10.3896/IBRA.1.49.1.02

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010b) Global pollinator declines: trends, impacts and drivers. Trends Ecol Evol 25(6):345–353. doi:10.1016/j.tree.2010.01.007

Seitz N, Traynor KS, Steinhauer N, Rennich K, Wilson ME, Ellis JD, Rose R, Tarpy DR, Sagili RR, Caron DM, Delaplane KS, Rangel J, Lee K, Baylis K, Wilkes JT, Skinner JA, Pettis JS, vanEngelsdorp D (2015) A national survey of managed honey bee 2014–2015 annual colony losses in the USA. J Apic Res 54(4):292–304. doi:10.1080/00218839.2016.1153294

Spleen AM, Lengerich EJ, Rennich K, Caron D, Rose R, Pettis JS, Henson M, Wilkes JT, Wilson M, Stitzinger J, Lee K, Andree M, Snyder R, vanEngelsdorp D (2013) A national survey of managed honey bee 2011–12 winter colony losses in the United States: results from the bee informed partnership. J Apic Res 52(2):44–53. doi:10.3896/IBRA.1.52.2.07

Steinhauer NA, Rennich K, Wilson ME, Caron DM, Lengerich EJ, Pettis JS, Rose R, Skinner JA, Tarpy DR, Wilkes JT, vanEngelsdorp D (2014) A national survey of managed honey bee 2012–2013 annual colony losses in the USA: results from the bee informed partnership. J Apic Res 53(1):1–18. doi:10.3896/IBRA.1.53.1.01

Traynor KS, Rennich K, Forsgren E, Rose R, Pettis J, Kunkel G, Madella S, Evans J, Lopez D, vanEngelsdorp D (2016) Multiyear survey targeting disease incidence in US honey bees. Apidologie 47(3):325–347. doi:10.1007/s13592-016-0431-0

van der Zee R, Pisa L, Andonov S, Brodschneider R, Charriere J-D, Chlebo R, Coffey MF, Crailsheim K, Dahle B, Gajda A, Gray A, Drazic MM, Higes M, Kauko L, Kence A, Kence M, Kezic N, Kiprijanovska H, Kralj J, Kristiansen P, Martin Hernandez R, Mutinelli F, Nguyen BK, Otten C, Ozkirim A, Pernal SF, Peterson M, Ramsay G, Santrac V, Soroker V, Topolska G, Uzunov A, Vejsnaes F, Wei S, Wilkins S (2012) Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008–9 and 2009–10. J Apic Res 51(1):91–114. doi:10.3896/IBRA.1.51.1.12

vanEngelsdorp D, Meixner MD (2010) A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J Invertebr Pathol 103:S80–S95. doi:10.1016/j.jip.2009.06.011

vanEngelsdorp D, Underwood R, Caron D, Hayes J (2007) An estimate of managed colony losses in the winter of 2006-2007: A report commissioned by the apiary inspectors of America. Am Bee J 147(7):599–603

vanEngelsdorp D, Hayes J, Underwood RM et al (2008) A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. PLoS ONE 3(12). Doi:10.1371/journal.pone.0004071

vanEngelsdorp D, Hayes J, Underwood RM, Pettis JS (2010) A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. J Apic Res 49(1):7–14. doi:10.3896/IBRA.1.49.1.03

vanEngelsdorp D, Hayes J, Underwood RM, Caron D, Pettis J (2011) A survey of managed honey bee colony losses in the USA, fall 2009 to winter 2010. J Apic Res 50(1):1–10. doi:10.3896/IBRA.1.50.1.01

vanEngelsdorp D, Caron D, Hayes J, Underwood R, Henson M, Rennich K, Spleen A, Andree M, Snyder R, Lee K, Roccasecca K, Wilson M, Wilkes J, Lengerich E, Pettis J (2012) A national survey of managed honey bee 2010-11 winter colony losses in the USA: results from the Bee Informed Partnership. J Apic Res 51(1):115–124. doi:10.3896/IBRA.1.51.1.14

vanEngelsdorp D, Tarpy DR, Lengerich EJ, Pettis JS (2013) Idiopathic brood disease syndrome and queen events as precursors of colony mortality in migratory beekeeping operations in the eastern United States. Prev Vet Med 108(2–3):225–233. doi:10.1016/j.prevetmed.2012.08.004

Wallberg A, Han F, Wellhagen G, Dahle B, Kawata M, Haddad N, Simões ZLP, Allsopp MH, Kandemir I, De la Rúa P, Pirk CW, Webster MT (2014) A worldwide survey of genome sequence variation provides insight into the evolutionary history of the honeybee Apis mellifera. Nat Genet 46(10):1081–1088. doi:10.1038/ng.3077

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Find more on our wonderful team at BIP's website: www.beeinformed.org

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