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Honey bee surveillance: a tool for understanding and improving honey bee health

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Honey bee surveillance systems are increasingly used to characterize honey bee health and disease burdens of bees in different regions and/or over time. In addition to quantifying disease prevalence, surveillance systems can identify risk factors associated with colony morbidity and mortality. Surveillance systems are often observational, and prove particularly useful when searching for risk factors in real world complex systems. We review recent examples of surveillance systems with particular emphasis on how these efforts have helped increase our understanding of honey bee health.

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Surveillance in honey bees

'Observation sets the problem; experiment solves it'
Jean-Henri Fabre, (1823–1915)

Surveillance is an observation-based method of quantifying levels of 'disease' in a population. At their core, surveillance efforts quantify disease prevalence and incidence over space and time, which can help identify risk factors that contribute to disease incidence when coupled with other data. Data from surveillance efforts can identify or confirm risk factors that predict disease outcomes, and can guide the development of experimental approaches to demonstrate causation. Further, identification of risk factors can inform disease mitigation practices that can improve health at the population level [1,2••].

Health and/or disease surveillance systems exist for most human and production animal health programs. When implemented sustainably, they help mitigate and prevent important diseases in populations. Considering the importance of honey bees (*Apis mellifera*) for pollination of agricultural crops [3,2••,4,5], it is not surprising that many surveys have quantified health and disease burdens. Surveillance of non-apis species also exists, but is less developed compared to honey bees (Box 1). Surveillance system design is dictated by many factors, most importantly by the objectives of the study and availability of resources (Figure 1). Here we review examples of honey bee surveillance efforts, emphasizing their contribution toward understanding and improving honey bee health (summarized in Table 1).

Detection, characterization, quantification of disease

Monitoring is a regular, repetitive and intermittent series of measurements designed to detect changes in the health status of a defined population (see Table 1 for examples). Apiary inspections are an example of monitoring as they have long been used to estimate disease in managed honey bee populations. These inspections quantify disease prevalence and range by sampling a number of 'analytic units' (individual bees, colonies, apiaries, or operations [1]) over a defined period of time and population. Traditionally, apiary surveillance was used to identify disease outbreaks in order to enforce regulations aimed at eliminating or containing disease spread. This approach is largely credited for reducing the incidence of the bacterial disease American foulbrood (*Paenibacillus larvae*) in the US [2••]. More recently, disease surveys have expanded to include early detection of non-extant (or recently introduced) disease threats such as *Tropilaelaps clareae* mites in the US [6,7], small hive beetles (*Aethina tumida*) in Europe [8••], or *Varroa destructor* mites (*Varroa*) in Australia [9]. Determination of disease free status for particular pests has implications for trade of bees and bee products [7]. Early detection of a new organism can permit containment efforts, such as the Australian effort to contain *Apis cerana* [10]. The utility of surveillance efforts in epidemiologic studies is dependent on numerous factors, including how samples are

Box 1 Non-apis bee surveillance

Non-apis bee species are major contributors to agricultural and natural pollination systems [4,57–59]. These species are largely unmanaged and have multiple different life histories, thus requiring specialized surveillance techniques.

Recently there have been several efforts to standardize survey effort approaches that document the abundance and diversity of non-apis species [60]. Application of standardized collection methods allows for ecological network analyses to help quantify the structure of bee-plant networks in various landscapes [61–64]. When standardization is not possible (such as in the case of comparing changes in abundance and diversity over time by using historical collections), statistical analyses can help elucidate important drivers of changing populations, including changes in agricultural policy and practice [65], ecological succession [66], landscape [67] and climate change [68].

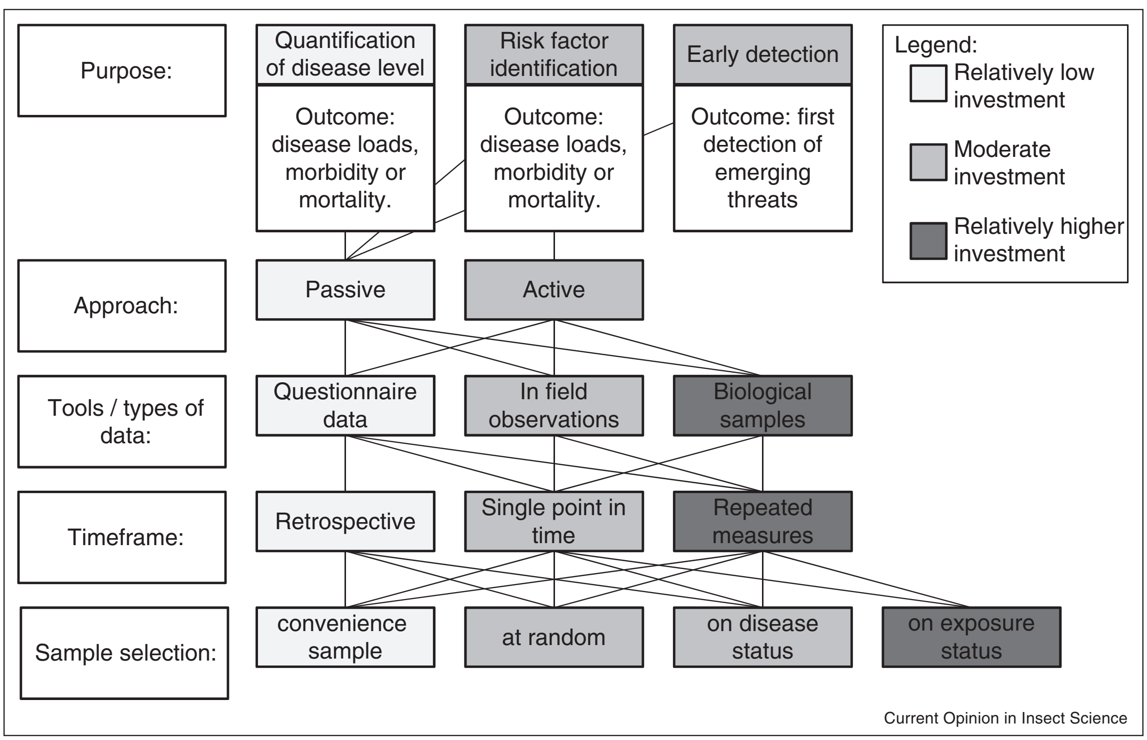
Surveys of non-apis bee populations have been conducted to identify disease loads in populations [69–72], although generally these studies have concentrated on possible disease spillover from honey bees. Further surveillance on non-apis bees and their diseases is much needed.

selected, number of analytic units sampled, specificity of the diagnostic test, and sample collection methodologies [2••]; all of which are constrained by the pragmatic reality of limited resources.

A notable monitoring program quantified disease load and colony mortality by inspecting randomly selected apiaries in 17 different European countries [8••]. By randomly selecting colonies and implementing a standardized inspection approach, the resulting data avoided selection biases inherent with many survey efforts. The ability to randomly select colonies from a known population is a central tenant of good survey design, but in practice is problematic as random sampling requires a near-complete description and access to the honey bee population, which is often difficult to attain or create.

Modified apiary inspections can be used to perform more directed surveillance for the discovery and characteriza-

Figure 1



Describes different approaches to honey bee surveillance and the corresponding relative degree of investment (time and monetary). *Purpose*: objective of the surveillance program. *Outcome*: measure of health under surveillance. *Passive*: approach: no intervention imposed on the regular management of the colonies under surveillance. *Active*: approach: implicates manipulation of the conditions experienced by (at least part of) the colonies under surveillance. *Questionnaire data*: interview or self-reported recollection from the stakeholder. *In field observations*: the overt symptoms expressed in the colonies under surveillance. *Biological samples*: clinical diagnostics from a physical sample collected from colonies under surveillance. *Retrospective*: collection of data regards exclusively past events. *Single point in time*: cross-sectional design where the collection of data (exposures and outcomes) are made at the same unique point in time. *Repeated measures*: the same colonies under surveillance are assessed repeatedly through time. *Convenience sample*: sample from the target population is only determined by the availability and willingness of the stakeholders. *At random*: selection of the sample from the target population is completely randomized, meaning all individuals from the target population have the same probability of being sampled. *Selection on disease status*: case–control studies comparing individuals classified as 'diseased' versus individuals classified as 'disease-free' for the disease of interest. *Selection on exposure status*: cohort studies comparing individuals classified as 'exposed' to individuals classified as 'non-exposed' for the risk factor of interest.

Table 1

Surveillance types. Without judging for the individual designs potential precision level, biases and confounding effects, we classified recent honey bee research according to the type of surveillance design to identify methodologies underrepresented in current publication trends.

Survey design (definitions from [73])	Samples (S)/ questionnaires (Q)	Transversal (T)/ longitudinal (L)	References	Objectives		
				Early warning surveillance	Monitoring (colony, disease prevalence)	Identification of risk factors
Monitoring	S/Q	L	[8••]	X	X	X
	S	L	[11,24,26,54]		X	
	S	T	[12]		X	X
	S	L	[13•,25]		X	X
	S	T	[17•]		X	
	Q	L	[28,30,31,32,33, 34,35,36,38]		X	X
	S/Q	L	[45]		X	X
	S/Q	T	[43•,44••]		X	X
	S/Q	L	[53••]		X	
Cross-sectional	S	T	[14,15•]		X	
Case studies	S	T	[20]			X
Case–control	S/Q	T	[21]			X
	S	T	[22]			X
Cohort	S/Q	L	[46••]			X
	S	L	[47,48,49•,50•,51,52••]			X

T (transversal), collected at one point in time.
L (longitudinal), more than one observation per replicate over time.

tion of potential new diseases and/or pathogens, including virulence and distribution. Identifying new cases helps direct future monitoring or research to better understand if they contribute to colony mortality and morbidity. Several surveillance programs have utilized new molecular tools to discover new, possibly pathogenic, honey bee viruses, including Aphid Lethal Paralysis virus strain Brookings, Big Sioux River virus, four strains of the Lake Sinai virus, and the tobacco ringspot virus [11,12,13•].

Surveillance of known pathogens can shed light on the etiology of disease and support a hypothesis that is difficult to test experimentally. Examining the relationship between disease and other variables of interest at a single time point in a defined population can be done using cross-sectional studies (Table 1). In a cross-sectional study of Hawaiian colonies, Deformed Wing Virus (DWV) prevalence was correlated with the number of years *Varroa* was present on the island [14]. The observed DWV strain diversity was greatest in samples from *Varroa* free islands, while a single DWV strain replaced all others when *Varroa* was present for over three years. These findings imply that mite-mediated transmission of DWV favors certain, possibly more virulent, DWV strains [14]. This hypothesis is supported by survey results that demonstrate *Varroa* dramatically changes the viral complex in infested honey bee populations [15•], and experimental research showing one virulent DWV strain benefits from the direct injection route mediated by *Varroa* [16].

Generally, understanding the dynamics of 'new' host–parasite/pathogen interactions does not lend itself well to direct hypothesis testing, but benefits from surveillance efforts. A survey in Kenya documented the prevalence of *Varroa*, *Nosema* spp., DWV, Black Queen Cell Virus (BQCV), and Acute Bee Paralysis Virus (ABPV) [17•]. The surveyed pathogens did not appear to affect colony strength suggesting the presence of more benign diseases in the region, a more resistant host, or a combination of both. Data from a Swedish effort that looked at viral levels in '*Varroa* tolerant' colonies seems to support the concept of host-based tolerance, as the study population has increased virus tolerance [18•] and an ability to reduce mite fitness [19].

Identification of risk factors

Surveillance can be used to investigate putative causes of unexplained disease states. Analyses of a limited number of disease samples can be reported as a case study: a detailed description and analysis of the occurrence of a particular health problem, its development and its outcome (Table 1). Although widely used in human and other animal health fields, case study reporting is rare for honey bees. A recent exception described efforts to determine the putative cause of two collapsed colonies [20], with the clinical disease symptoms ascribed to *Nosema ceranae*. On its own, a case study has limited utility in explaining population level health. However, if findings from other case studies make similar conclusions, the results can identify associated risks.

In case–control studies, colonies are first selected based on whether or not they have the disease/health status of interest, and then their exposure histories are obtained and compared to indentify correlations between different risk factors (Table 1). One case–control study found apiaries with high rates of loss were less likely to have been treated for *Varroa* than apiaries with low rates of loss [21]. Another found higher pathogen loads and a different gut microbe community in collapsing colonies as compared to apparently healthy colonies [22].

Surveillance efforts can also help validate experimentally identified risk factors. An experiment that measured effects of temperature on spore viability of *Nosema apis* and *N. ceranae*, found *N. ceranae* spores were more tolerant of higher temperatures while *N. apis* spores were more tolerant of colder temperatures [23]. This suggests *N. apis* should be more prevalent in colder locations, a finding documented by surveillance programs in Sweden, Germany, and Taiwan [24–26].

Surveillance efforts need not require field visits. Owner or caretaker observations can capture information about a population's health, and have been used extensively to document colony losses [1,27]. Repeated surveys have shown that losses are highly variable between regions and over time [28–30], which may be explained by subgroups within a population. A consistent finding within US loss data is that beekeepers in northern states lose more colonies than those in southern states [28,31–36], suggesting winter temperature may explain some variability in loss rates. Indeed, winter loss rates in Pennsylvanian beekeeping operations were correlated to average winter temperature [31].

Although questionnaires are relatively easy to conduct, they are prone to biases: respondents may not be representative of the population, have poor recall, among others [27]. Further, when comparing results among different questionnaire-based surveillance efforts, consideration of different methodologies and definitions is essential. For instance, the timeframe for 'winter' loss calculations can be a set date [1,37] or self-determined by the responding beekeeper [30], making direct comparisons of loss rates difficult to interpret.

Properly designed questionnaires increase the ability to identify management practices correlated with increased survivorship. A consistent finding in several loss and management surveys has linked application of *Varroa* treatments with increased winter survival [30,38,39]. Despite the consistency of these findings across different surveys, this relationship remains correlative and should not be interpreted as causative unless data from experimental testing is considered [40–42]. However, resource availability can limit the ability to conduct experimental studies. In these cases, a combination of questionnaires and field surveys can be informative. An Argentinian study paired monitoring of *Varroa* loads with retrospective management survey data to identify factors that were predictive of above threshold *Varroa* populations at the time of sampling [43•]. Another cross-sectional study, which also combined field sampling and questionnaires, found that acaricide treatments were 4.9 times more likely to fail when applied to colonies with *Varroa* infestations over a 3 mites per 100 bees threshold [44••]. Both these and other cases demonstrate a strong link between *Varroa* levels and colony mortality [45] and validate this method as a cost effective surrogate approach to more intensive surveillance.

Longitudinal surveillance (repeated sampling of the same colonies over time) is a powerful tool for connecting 'risk factor' exposure with disease outcomes. This approach can quantify the association between a risk factor and likelihood that a disease outcome will result in the future. Cohort studies are a form of longitudinal monitoring that compares the incidence of a particular health outcome between subsets of defined populations selected for having experienced a common exposure status (Table 1). For example, a US cohort study followed colonies in different migratory beekeeping operations and identified a close association with the occurrence of a queen event or the presence of 'Idiopathic Brood Syndrome' with subsequent colony mortality [46••]. Similar cohort studies have found relationships between *Varroa* (or its control), DWV, or a combination as being predictive of mortality [47,48,49•,50•].

Longitudinal studies can also identify and quantify non-biotic factors that may predict disease outcomes. European researchers recently published a multi-year study conducted in 21 apiaries in 11 countries identifying effects of bee genotype and environmental factors on mortality and morbidity [51,52••]. They demonstrated that location strongly influenced autumn mite loads and viral (ABPV and DWV) prevalence. Location effects had a more pronounced affect on disease outcome than host genotype, suggesting disease thresholds likely differ by geography [52••]. Colonies in locations with a shorter active season (i.e. temperate regions) have longer lived winter bees compared to colonies with a longer active season [51], suggesting colonies managed in temperate regions require more vigilant pathogen control. Longitudinal data has also been paired with landscape data to develop a model, EcoBEE, that predicts optimal apiary sites [53••].

Multi-year longitudinal monitoring of colony health can identify region specific risk factors associated with colony mortality. Once identified, these factors can inform management and research priorities. Longitudinal trends in disease and/or risk factor prevalence may help predict future morbidity and mortality. Unfortunately, few of these long-term monitoring efforts exist, with some notable exceptions based in Europe [45,52••,54] and more recently in the US [39].

Conclusions and further directions

Honey bee health surveillance efforts quantify disease and disease risk factors in managed honey bee populations. Over time, these efforts provide data that can identify emerging threats and place disease measures in context by establishing baseline metrics. As surveillance based studies are becoming more common, increased efforts to standardize approaches [55••] would foster greater comparison among studies and increase potential benefits. To maximize benefits and allow for comparison of studies across time and regions, several challenges need to be addressed: designs that ensure representative data are obtained, better coordination among efforts, and standardization of approaches [56]. The value of continuing and initiating other long-term surveillance efforts cannot be over stated. Surveillance data can be used to guide disease intervention methods and policy, hypothesis driven research efforts focused on discovering causes of disease, and, most importantly, measure the impact the application of this knowledge has on improving bee health.

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