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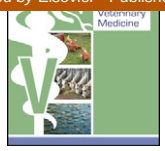

Idiopathic brood disease syndrome and queen events as precursors of colony mortality in migratory beekeeping operations in the eastern United States

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ABSTRACT

Using standard epidemiological methods, this study set out to quantify the risk associated with exposure to easily diagnosed factors on colony mortality and morbidity in three migratory beekeeping operations. Fifty-six percent of all colonies monitored during the 10-month period died. The relative risk (RR) that a colony would die over the short term (~50 days) was appreciably increased in colonies diagnosed with Idiopathic Brood Disease Syndrome (IBDS), a condition where brood of different ages appear molten on the bottom of cells (RR = 3.2), or with a "queen event" (e.g., evidence of queen replacement or failure; RR = 3.1). We also found that several risk factors—including the incidence of a poor brood pattern, chalkbood (CB), deformed wing virus (DWV), sacbrood virus (SBV), and exceeding the threshold of 5 Varroa mites per 100 bees—were differentially expressed in different beekeeping operations. Further, we found that a diagnosis of several factors were significantly more or less likely to be associated with a simultaneous diagnosis of another risk factor. These finding support the growing consensus that the causes of colony mortality are multiple and interrelated.

1. Introduction

Honey bees (*Apis mellifera* L.) play a vital role in modern agriculture. An estimated 35% of the western human diet benefits—directly or indirectly—from honey bee pollination (Klein et al., 2007). While colony numbers have increased globally over the last 60 years (Aizen et al., 2008), this increase has not kept pace with increased acreages planted with pollinator-dependent crops (Aizen and Harder, 2009). Additionally, increases in colony numbers have not been consistent across all regions, with long-term losses documented in the US and European nations (Potts et al., 2010; NRC, 2006). These trends have raised fears that demand for pollinating units will outstrip supply in the future (NRC, 2006). While some have questioned the basis of these fears (Ghazoul, 2005), researchers agree that there is a need for consistent and reliable enumeration of pollinator populations and focused research investigating the causes of mortality (Neumann and Carreck, 2010; Nguyen et al., 2010b).

In recent years, there has been increased attention paid to documenting overwintering honey bee colony mortality in North America (vanEngelsdorp et al., 2008, 2010a, 2011; Currie et al., 2010) and Europe (Potts et al., 2010; Brodschneider et al., 2010; Nguyen et al., 2010a). While these efforts have not attempted to empirically test

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the causes of mortality, most have accepted self-reports from beekeepers about which factors they believe most likely contributed to colony mortality in their particular operation (vanEngelsdorp et al., 2008, 2010a, 2011; Brodschneider et al., 2010). These factors are generally limited to those with which beekeepers are most familiar and can most readily diagnose. Some factors self-identified by survey respondents as leading causes for increased mortality, such as Varroa mite parasitism, have been corroborated by more systematic empirical surveys (Haubruge et al., 2006; Chauzat et al., 2010b; Guzmán-Novoa et al., 2010). While Varroa mites clearly contribute to colony mortality, other factors (including pesticide exposure, other bee parasites and pathogens, foraging conditions in the fall, and beekeeper management) may also negatively affect colony survival (vanEngelsdorp and Meixner, 2010). There seems little doubt that various factors can interact with one another. In Denmark, for instance, elevated losses were compounded when weather conditions in the fall prevented effective mite treatments, facilitating higher mite loads on bees that may not have had optimal pollen stores (Vejsnæs et al., 2010).

The objective of this study was to identify and quantify risk factors associated with annual colony mortality in migratory beekeeping operations in the eastern U.S. Specifically, we monitored risk factors that are readily identified during colony inspection or quantified by standard diagnostic techniques of sampled bees. These factors included clinical outbreaks of American foulbrood, European foulbrood, chalkbrood, sacbrood, deformed wing virus, queen events, brood pattern quality, and Varroa mite and Nosema spore load. Brood pattern quality is a general measure of queen and colony health and poor brood survival can be linked to reduced honey production (Woyke, 1981). A good brood pattern, as indicated by solid patches of capped brood, indicates the queen is laying viable eggs which are developing into healthy larval and pupal bees. Poor brood quality, indicated by large numbers of empty cells among capped cells, are indicative of a poor queen or disease (hygienic bees typically remove diseased larvae and pupa, leaving empty cells). In addition, we monitored Idiopathic Brood Disease Syndrome (IBDS), a syndrome first described by Shimanuki et al. (1994), but renamed here because its underlying cause it yet unknown. IBDS is diagnosed by the presence of brood at different ages that appear molten on the bottom of cells or have other symptoms reminiscent of, but not caused by, infection with American foulbrood (AFB; *Paenibacillus larvae*), European foulbrood (EFB; *Melissococcus pluton*), or sacbrood virus (SBV). Shimanuki et al. (1994) described this syndrome as part of their study of Parasitic Mite Syndrome (PMS), but unlike the symptoms of PMS in adult bees it is not believed that IBDS is caused by Varroa mites.

We used basic epidemiological methods to calculate and compare the relative risk associated with exposure to these easily-quantified putative risk factors. This risk-factor approach is commonly used in human studies to inform future hypothesis-driven analytical studies designed to elucidate causes of disease and mortality (Koepsell and Weiss, 2003). Just as in human studies, we intend for the results of this study to highlight areas for future research intended to aid us in understanding and mitigating colony losses.

2. Materials and methods

2.1. Colony selection

This cohort study monitored honey bee, *A. mellifera*, colonies in three migratory beekeeping operations (OP1, $n = 20$; OP2, $n = 24$; OP3, $n = 18$). The study was conducted during a 10-month period (mean = 300 days) between March 2007 and January 2008. The selected operations were considered representative of East coast migratory operations, as the beekeepers transported their beehives north and south within the eastern United States to pollinate and/or produce honey on a diverse variety of crops and natural vegetation (Fig. 1). All colonies travelled from the state of Florida to New Jersey or Maine and back to Florida within the year, pollinating various crops en route. Colonies were selected randomly from an apiary within each beekeepers operation that contained colonies that had survived the previous year and were likely to be of similar lineages. Selected colonies were tagged with individually numbered cattle ear tags for tracking purposes. Upon first inspection, each colony's queen was located, marked, and had one of her wings clipped to help monitor queen replacement. Wing clipping is not thought to have a negative impact on queen longevity and was done in addition to marking queens because queen markings are commonly removed from marked queens by her attending workers (Laidlaw and Page, 1997). Surviving colonies were inspected at intervals that varied depending on the frequency that the colonies were moved (Fig. 1).

2.2. Colony measurements

During each inspection, the condition of monitored colonies was first noted. Colonies were considered to be dead when they were found completely depopulated of live adult bees. The strength of surviving colonies was assessed by separately estimating the number of frames covered with adult bees and containing capped brood (DeGrandi-Hoffman et al., 2008). The quality of brood was also assessed by averaging the number of empty brood cells in four randomly-selected patches of contiguous capped brood (100 brood cells per patch). When the four patches had an average of $\geq$20% empty cells, the brood pattern was considered to be 'poor'.

During inspection, clinical symptoms of disease were noted, including chalkbrood (CB) *Ascosphaera apis*; European foulbrood (EFB), *M. pluton*; American foulbrood (AFB), *P. larvae*; sacbrood virus (SBV); and deformed wing virus (DWV). Also clinical symptoms of Idiopathic Brood Disease Syndrome (IBDS) were noted, as described above and by Shimanuki et al. (1994).

During each inspection, the condition of a colony's queen was also assessed. Attempts were always made to find the original marked and clipped queen. In cases where the marked queen was not found (12.5% of the time), it was assumed that she was present if eggs were found in the brood nest. A colony was diagnosed as having experienced

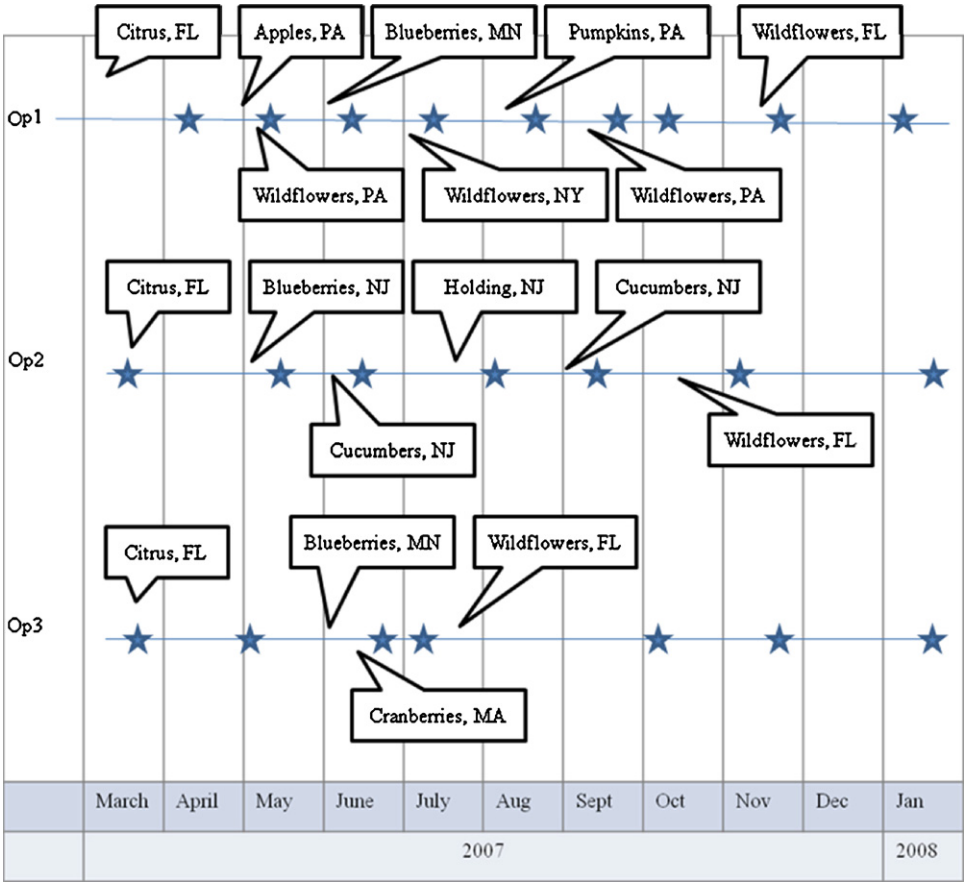

Fig. 1. Timeline of colony inspection over the course of the study. Placement of colonies on various floral sources is indicated (boxes), as are the times colonies were inspected (starbursts). Data from inspections collected at times indicated by solid star bursts were considered for calculating all case incidence rates. However, in an attempt to equalize "exposure time", only data from inspection periods indicated by large starbursts were included for calculating relative risk variables and for comparing colony measures and disease prevalence over time. The period of time between these inspections is indicated within the starbursts and represent days between inspections (see text for details).

a "queen event" if the colony was found to (1) have emergency or supersedure queen cells, (2) contain a virgin or replacement (unmarked) queen, or (3) was apparently queenless with no eggs and larvae. When found, replacement queens had their wings clipped.

During each inspection, colonies had samples of adult bees removed from a frame containing capped and uncapped brood (when available) and stored in 70% ethanol (~320 bees). These bees were used to determine the mean abundance of Varroa mites in adult bees (Rinderer et al., 2004). Moreover, a sample of 30 worker bees from this same sample was macerated in 30 ml of water to determine *Nosema* spp. spore loads (after Cantwell, 1970).

2.3. Analysis

Survivorship was calculated for each group of colonies at the time of each inspection. To calculate mortality rate (percentage of colonies dying per month), colonies found dead during an inspection were parsimoniously assumed to have died midway between the last inspection (when they were found alive) and the inspection in which they were found dead.

In this study, we sought to quantify the risk of colony mortality associated with exposure to different easily-diagnosed risk factors using standard epidemiologic methods (Koepsell and Weiss, 2003). In most cases in this study, we considered the prevalence of different diseases the risk factors of interest while colony mortality was the outcome of interest. Disease prevalence was therefore calculated for each risk factor measured over the course of study. For Varroa and Nosema where some level of exposure was present ubiquitously (or nearly so), we considered colonies to have been exposed only when these parasites surpassed a predetermined threshold. We defined this threshold for Varroa mites to be greater than 5 mites per 100 adult bees (Genersch et al., 2010) and for Nosema to be greater than 1 million spores per bee (E. Mussen, personal communication).

A common method to quantify risk from exposure is by calculating the relative risk (RR) of mortality. RR quantifies the magnitude of excess risk that a colony will result in an outcome after an exposure. In order to examine

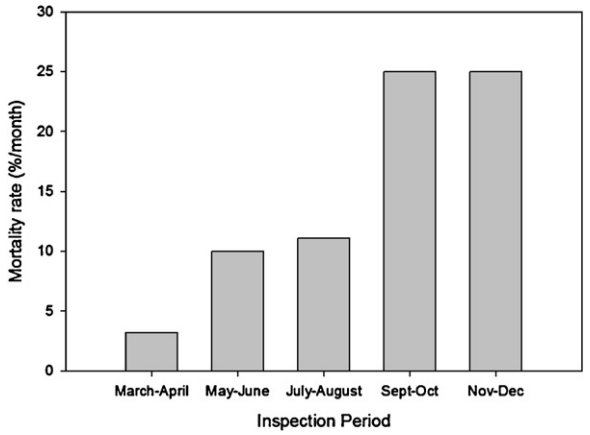

Fig. 2. Rate of colony mortality over the course of the study was not equal. As rates of mortality were not different among operations (see text), mean rates of mortality for all colonies are presented.

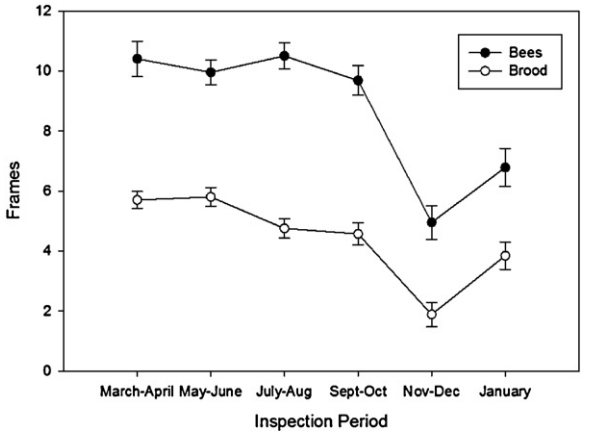

Fig. 3. Mean number of frames of bees and brood in surviving colonies over the course of study.

possible associations between risk factors odd ratios were also calculated.

2.4. Statistics

All statistical analyses were performed using JMP statistical package (SAS, 2007). We used a Chi-square test to compare mortality rates among the different operations. We compared colony size measures (frames of bees and brood) and parasite loads (Varroa and Nosema) using a multivariable analysis where operation and inspection period were set as random effects. The prevalence of risk factors was also explored using multivariate analysis where operation and inspection period were used as fixed effects. Prevalences were compared between populations of colonies that died verses those which survived over the duration of the study. For each risk factor, we calculated the relative risk and 95% CI of colony mortality during the interval between the occurrence of a risk factor and the subsequent inspection. Similarly RR was calculated for repeat diagnosis with a risk factor between one inspection and the next. Not all inspection data were used to calculate RR, data from inspections conducted at 50-day intervals were used to help ensure similar time periods for RR calculations. The statistical significance ($P < 0.05$) of relative risk and odds ratios were determined using the Chi-square test, unless fewer than 5 expected or observed cases were noted, in which case Fisher's exact test was used.

3. Results

3.1. Mortality rate

Fifty-six percent of the colonies died during the 10 months of this study ($n = 62$). This represents an average mortality rate of $14.9 \pm 4.28\%$ (mean $\pm$ SE) per 2-month period. This rate was not constant, with the rate of mortality increasing as the study progressed (Fig. 2; $P = 0.017$). The mortality rate did not differ among the three operations ($P = 0.42$).

3.2. Colony size and parasite loads

Both the number of frames of bees and frames of brood differed between operations and over time ($P < 0.0001$; $P < 0.0001$, respectively). Generally, OP3 had colonies with the largest number of frames of bees, while OP1 had colonies that had the fewest frames of brood (Table 1). Colonies were largest during the first half of the study and were smallest in November and December (Fig. 3).

Similarly, the average mean abundance of Varroa mites and Nosema spore counts differed between operations and over the course of study ($P < 0.0001$ and $P = 0.007$, respectively). Varroa levels where lowest in OP1 while Nosema levels were highest in OP3.Varroa mites reached a peak in the September/October inspection period (Fig. 4), and Nosema spore counts peaked during the May/June inspection period (Fig. 5).

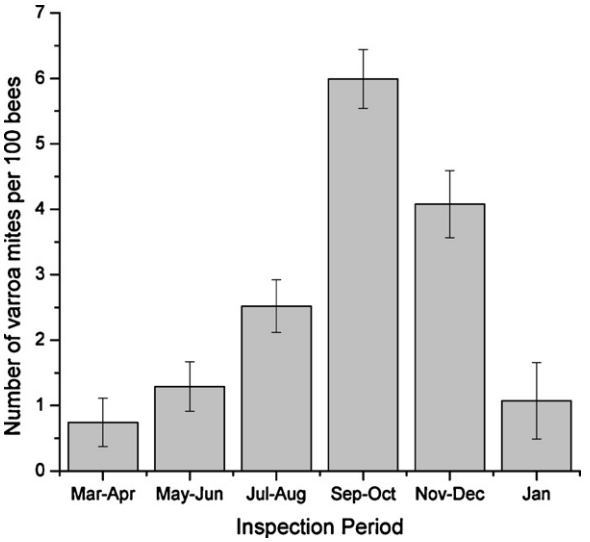

Fig. 4. Average Varroa mite infestation in monitored colonies over the course of study.

Table 1
Average colony size (frames of bees and brood) and parasite load (Varroa mite per 100 bees and Nosema spore count per bee × 10^6) in differed between operations in a study on colony mortality in migratory beekeeping operations in the Eastern United States (2007–2008). Differences between operations are indicated by different letter in each column.

Operation	Colony size		Parasite load	
	Frames of bees (Mean ± SE)	Frames of brood (Mean ± SE)	Varroa mite (Mean ± SE)	Nosema spores (Mean ± SE)
OP1	8.45 ± 0.32 b	2.96 ± 0.20 b	1.99 ± 0.36 b	0.95 ± 0.14 b
OP2	8.81 ± 0.29 b	5.31 ± 0.16 a	3.45 ± 0.31 a	0.81 ± 0.12 b
OP3	11.22 ± 0.37 a	5.13 ± 0.22 a	3.77 ± 0.40 a	1.42 ± 0.16 a

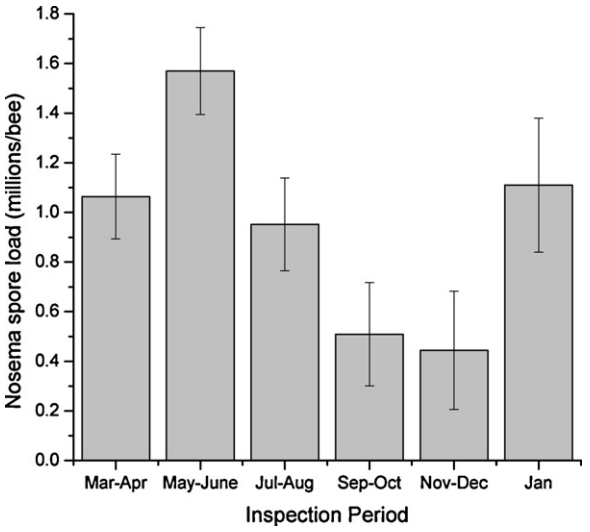

Fig. 5. Average Nosema spore load in colonies over the course of study.

Table 2
A comparison of the prevalence of risk factors in surviving and non-surviving colonies in a study on colony mortality in migratory beekeeping operations in the Eastern United States (2007–2008). Differences in prevelence are indicated by different letters in the same row ($P < 0.05$).

	Prevalence × (95% CI)	
	Surviving colonies	Non-surviving colonies
n	27	35
Risk factor		
Brood condition/disease		
Pattern†	31.4 (4.9–21.5)	26.8 (4.3–18.1)
Chalkbrood	11.9 (4.3–19.7)	8.4 (1.7–15.1)
Sacbrood	5.7 (0.0–13.30) b	22.2 (15.5–29.0) a
IBDS	3.9 (0.0–8.40)	7.1 (0.3–11.1)
Adult bee disease		
DWV	10.1 (0.06–14.1) a	1.1 (0.0–4.7) b
Varroa‡	9.4 (6.8–11.9) a	1.1 (0.0–3.2) b
Nosema spore$^{\beta}$	35.2 (26.9–43.5)	35.1 (27.9–42.4)
Queen		
Queen event	7.9 (0.1–15.1) b	21.2 (15.0–27.5) a

$^{\beta}$ Nosema spore load of 1 million spores per bee.
† Poor brood pattern as indicated by ≥20% of capped cells missing.
‡ Varroa mite threshold of 5 mites per adult bee exceeded.

3.3. Prevalence of risk factor and colony survival

Clinical signs of two brood diseases, AFB and EFB, were detected only once over the course of study. AFB was found in one colony during the initial inspection period while EFB was found in another colony during the last inspection in January 2008. Due to their low incidence, these diseases were not considered for further analysis.

The prevalence of DWV and Varroa exceeding a threshold of 5 mites per 100 bees differed between operations (Table 3) and between colonies that survived the entire length of study compared to those which did not (Table 2; DWV: $P = 0.001$ and Varroa: $P < 0.0001$). Poor brood pattern differed between operations (Table 3: $P < 0.0001$) but not between surviving verses non-surviving colonies. Prevalence was not different among operations for any other factor measured. Non-surviving colonies had over twice the number of queen events when compared to surviving colonies (21.2 vs. 7.9 cases per 100 colonies inspected; $P = 0.007$; Table 2). Non-surviving colonies had close to 4 times the prevalence of clinical SBV infections (22.2 cases per 100 colonies inspected) as compared to surviving colonies (5.7 cases per 100 colonies inspected; $P = 0.0018$). The prevalence of IBDS (5.7 per 100 colonies inspected; 95% CI = 2.7–8.7), and exceeding the Nosema spore threshold of one million spores per bee (3.5 per 100 colonies inspected; 95% CI = 29.8–40.6), was not different between surviving and non-surviving colonies ($P > 0.05$).

3.4. Relative risk (RR) of mortality after risk factor exposure

Colonies diagnosed with IBDS were more than 3.2 times (RR = 3.2; 95% CI = 1.5–7.0) as likely to die by the next

Table 3
Prevelance of risk factors which differed ($P < 0.05$) between operations in a study on colony mortality in migratory beekeeping operations in the Eastern United States (2007–2008). Differences between operations are indicated by different letters within the same column.

Operation	*n*	Prevalence × (95% CI)				
		Pattern†	CB	DWV	SBV	Varroa‡
OP1	20	52.3 (43.4–61.3) a	21.1 (13.9–29.1) a	0.0 (0.0–4.7) b	29.6 (21.5–37.7) a	0.8 (0–3.7) b
OP2	24	20.0 (11.9–28.1) b	0.0 (0–7.3) b	10.6 (5.7–14.4) a	1.9 (0.0–9.3) c	9.7 (7.1–12.3) a
OP3	18	14.4 (5.0–23.8) b	10.8 (0.2–19.3) ab	4.1 (0.0–9.1) ab	2.2 (0–5.4) b	2.2 (0–5.2) b

† Poor brood pattern as indicated by ≥20% of capped cells missing.
‡ Varroa mite threshold of 5 mites per adult bee exceeded.

Table 4
Relative risk of mortality preceding (~50 days) a diagnosis of various colony risk factors in a study on colony mortality in migratory beekeeping operations in the Eastern United States (2007–2008).

	Relative risk
n	
Risk factor	
Brood condition/disease	
Pattern[†]	1.6 (0.77–3.23)
Chalkbrood	1.1 (0.41–2.97)
Sacbrood	0.9 (0.85–0.92)
IBDS	3.2 (1.15–6.97)[*]
Adult bee disease	
DWV	0.6 (0.08–3.95)
Varroa[‡]	1.0 (0.42–2.19)
Nosema spore[β]	1.4 (0.73–2.56)
Queen	
Queen event	3.1 (1.67–5.80)[*]

β Nosema spore load of 1 million spores per bee.
† Poor brood pattern as indicated by ≥20% of capped cells missing.
‡ Varroa mite threshold of 5 mites per adult bee exceeded.
* Indicates significant RR ($P < 0.05$).

inspection period (50 days later, on average) when compared to colonies without the condition ($P = 0.013$; Table 4). Colonies diagnosed with a queen event were more than three times (RR = 3.1; 95% CI = 1.67–5.80) as likely to die by the next inspection when compared to those without evidence of a queen event ($P = 0.0017$; Table 4).

3.5. Odds ratio (OR) of simultaneous diagnosis of two risk factors

Diagnosis with a poor brood pattern was associated with clinical infections of chalkbrood and sacbrood, as well as exceeding a Nosema threshold of 1 million spores per bee (Table 5). Chalkbrood was also found to have a positive association with sacbrood and IBDS, while having a negative association with Varroa mite as indicated by an odds ratio of less than 1.0. Sacbrood had a strong association with IBDS. Varroa mite and phenotypic DWV infection also had a positive association. No other risk factors were found to have a significant association (Table 5).

3.6. Relative risk of remaining diagnosed with a risk factor after exposure in the previous inspection period

Diagnosis of a poor brood pattern significantly elevated the risk that the colony would have a poor brood pattern in subsequent inspection (RR = 2.5 (95%CI: 1.7–3.5); $P < 0.001$). Similarly, colonies showing clinical signs of CB disease had an increased risk of being diagnosed CB (RR = 3.7 (95% CI 2.0–6.7); $P < 0.0001$) during the next inspection.

Colonies in which clinical signs of DWV were observed were more likely to have DWV re-diagnosed (RR = 6.6 (95% CI: 2.9–14.7); $P = 0.003$) when next inspected.

4. Discussion

We quantified the impact of easily-defined measures of risk on colony mortality, in migratory honey bee colonies using epidemiological methods. To that end, we found

Table 5
Odds ratio of simultaneous diagnosis of two different risk factors during an inspection factors in a study on colony mortality in migratory beekeeping operations in the Eastern United States (2007–2008).

	Odds ratio (OR (95% CI)) for diagnosis during next inspection							
	Disease							
	Pattern[†]	Chalkbrood	Sacbrood	IBDS	DWV	Varroa[‡]	Nosema[β]	Queen event
Brood condition/disease								
Pattern[†]	–	6.2 (2.89–13.6)[*]	4.3 (1.0–17.4)[*]	2.4 (0.9–6.12)	1.2 (0.4–3.12)	0.9 (0.5–1.8)	1.7 (1.1–2.8)[*]	0.7 (0.3–2.2)
Chalkbrood	–	–	10.7 (2.7–41.9)[*]	10.7 (2.7–41.9)[*]	0.4 (0.05–3.3)	0.12 (0.02–0.9)[*]	1.1 (0.5–2.3)	1.3 (0.4–4.1)
Sacbrood	–	–	–	14.7 (3.6–60.1)[*]	2.0 (0.4–4.2)	0.6 (0.1–2.5)	1.7 (0.7–4.3)	1.5 (0.5–5.1)
IBDS	–	–	–	–	2.7 (0.9–8.1)	0.6 (0.1–2.5)	1.7 (0.7–0.4)	1.7 (0.7–4.3)
Adult bee disease								
DWV	–	–	–	–	–	3.1 (1.9–8.3)[*]	0.3 (0.1–1.03)	0.6 (0.2–2.4)
Varroa[‡]	–	–	–	–	–	–	1.7 (0.7–4.3)	1.6 (0.5–5.5)
Nosema[β]	–	–	–	–	–	–	–	1.4 (0.2–8.4)

β Nosema spore load of 1 million spores per bee.
* Indicates significant ($P < 0.05$) odds ratio.
† Poor brood pattern as indicated by ≥20% of capped cells missing.
‡ Varroa mite threshold of 5 mites per adult bee exceeded.

that, over the short term (~50 days), the presence of IBDS (a generally unknown syndrome of brood) increased the risk of colony mortality by 3.8 times. In addition, evidence of queen replacement or failure increased the risk that a colony would die by 3.1 times. We also found that exposure to several different factors were associated with one another as indicated by significant odds ratios (Table 5).

The complexity of these results supports a growing consensus that causes of honey bee colony mortality and morbidity are multiple and interrelated (Genersch and Evans, 2010; Genersch et al., 2010; Neumann and Carreck, 2010; vanEngelsdorp et al., 2010b). However, this study is the first that quantifies mortality with exposure to certain risk factors using epidemiological methods.

Colonies diagnosed with IBDS were nearly four times more likely to die by the next inspection period compared to colonies without this condition. This was the most pronounced measure of mortality risk recorded in this study. As outlined above, our case definition for determining "exposure" to this condition was based on the work of Shimanuki et al. (1994); specifically, the presence of brood at different ages that appear molten on the bottom of cells or may have other symptoms reminiscent of infection with AFB, EFB, or SBV. We considered this brood syndrome to be unassociated with those symptoms of PMS in adult bees, which are thought to be directly caused by Varroa mites. Our findings, as they relate to the symptoms in brood (called here IBDS), do not support an association with Varroa mites, as evidenced by a non-significant odds ratio for the two factors. Indeed, even the reports that initially described PMS suggested that the role of mites in the symptoms in brood was likely secondary to the symptoms described (Shimanuki et al., 1994; Hung et al., 1996). However, mites may play a role in creating IBDS by acting as a vector for a causative agent or because mite feeding somehow activates asymptomatic infections (Hung et al., 1996). Attempts to isolate a single causative agent in symptomatic brood removed from PMS suffering colonies have failed, although viruses such as acute bee paralysis virus (ABPV) and Kashmir bee virus (KBV) have been implicated (Hung et al., 1996). If IBDS is indeed a symptom of viral infection, its persistence in infected colonies with low Varroa mite pressure is not surprising considering that viruses are able to persist in colonies even when mite levels remain low or after they have been controlled with chemical treatments (Highfield et al., 2009; Martin et al., 2010).

This study also identified "queen events" as a leading factor in colony mortality. Not only was the relative risk of mortality increased in colonies diagnosed with a queen event significant, the incidence rate of queen events was nearly twice as high in colonies that died by the end of the study when compared to those colonies that had not died (Table 1). Beekeepers themselves have consistently self-identified queen failure as a leading cause of winter mortality in recent winter loss surveys (vanEngelsdorp et al., 2010a; Brodschneider et al., 2010), and this study corroborates beekeeper suspicions. Additional studies are necessary to elucidate the underlying causes of queen events and the mechanism that governs its apparent association with increased colony mortality.

Poor brood pattern (e.g., more than 20% of capped brood missing) indicated that colonies were more likely to be re-diagnosed with a poor brood pattern in a subsequent inspection and was found to be associated with the brood diseases (chalkbrood and sacbrood) and Nosema levels in excess of 1 million spores per bee (Table 5). There are many potential causes of a spotty brood pattern, including cannibalism of diploid drone larvae because of homozygosity at the *csd* locus (see Tarpy and Page, 2002), larval death due to pesticide poisoning (Pettis et al., submitted for publication), and worker bee hygienic behavior which removes diseased or dead larvae (Gilliam et al., 1983; Boecking and Spivak, 1999). It may be that colonies diagnosed with a poor brood pattern are successfully removing diseased larvae infected with the agents causing disease (e.g., CB and SBV), and thus in some cases poor brood patterns may simply indicate that colonies are able to keep infections below detection thresholds for clinical infections to be diagnosed. *Nosema* spp. infection in queen honey bees can lead to rapid supercedure (Furgala, 1962) and it has been proposed that failing queens reduce egg laying and, thus, brood levels in advance of supercedure (Pettis et al., 1997). Therefore, *Nosema* spp. infection could also indirectly lead to poor brood patterns. Comparing the prevalence of risk factors among different groups of colonies also proved insightful. First, the difference in incidence rates in the three different beekeeping operations (Table 3) was notable. Many causes for these differences are possible, including differences in management practices, colony genetics, risk-factor exposure, and the environment. It should be noted that while we did not record specific management practices of the beekeepers, all reported treating for Varroa during the same treatment window (October–November). While our study was not designed to identify the causes for these differences, this finding does support the growing consensus that many factors contribute to colony mortality and that studies that investigate these causes may need to be equally diverse (vanEngelsdorp et al., 2011; Williams et al., 2010).

Also notable were the significant difference in the prevalence of queen events, SBV, DWV, and colonies exceeding the threshold for Varroa mites between surviving and non-surviving colonies. That queen events occurred 2.6 times more frequently in non-surviving colonies is not surprising considering the significant relative risk associated with this condition and the relative frequency of its diagnosis. The potential causes for queen failure are multiple, and include pathogen load (Loskotova et al., 1980; Camazine et al., 1998), pesticide exposure (Pettis et al., 2004), and number of mates (Tarpy et al. unpublished data; see also Richard et al., 2007). Considering the pronounced effect queen events have on colony survivorship, studies specifically designed to measure and address possible interactions between risk factors and queen replacement are urgently needed.

Over the long term, non-surviving colonies had nearly four times the incident rate of SBV when compared to surviving colonies. Unlike queen events, however, we did not observe increased risk of mortality in colonies over the short term (as indicated by a non-significant RR; Table 1). This suggests that the effects of SBV on colony health may be sub-lethal or that clinical infection with SBV may be

a consequence of some other sub-lethal factor that compromises colony health (e.g., pesticide exposure or poor nutrition, both of which are known to contribute to compromised bee immune systems (Alaux et al., 2010a,b)). It is important to note that this study was not designed to detect sub-lethal effects (i.e., decreased productivity) of SBV or other risk factors measured. Therefore, our inability to detect negative consequences of exposure to certain risk factors is not equivocal to saying these risk factors had no effect on colony health.

The incident rate of DWV infection occurred at over nine times the rate in surviving colonies as compared to non-surviving colonies, while Varroa mites exceeded the threshold at over eight times the rate in surviving colonies as compared to non-surviving colonies. At first glance, these findings are counter intuitive, as the prevalence of both DWV and Varroa exceeding threshold were markedly higher in surviving colonies, suggesting these factors may be associated with colony health. This is unlikely to be the case, however, as there is broad and compelling evidence that both Varroa mites and DWV have negative effects on both individual bee and colony health (Korpela et al., 1992; Highfield et al., 2009; Chauzat et al., 2010a,b; Guzmán-Novoa et al., 2010; Genersch et al., 2010; Martin et al., 2010; Le Conte et al., 2010; Schäfer et al., 2010). A more probable explanation for higher prevalence of these two factors in surviving colonies can be inferred after considering the population dynamics of Varroa mites in observed colonies (Fig. 4). Average Varroa mite populations were highest in the September/October sampling period, and by this time 40% of the colonies that would die in the study had already died. Thus, many non-surviving colonies simply had not survived long enough to have mite levels surpass the threshold. The same explanation potentially explains increased DWV rates as well. Both this and other studies have shown a linkage between high Varroa mite levels and DWV infection (Table 3; Highfield et al., 2009; Bowen-Walker et al., 1999). Indeed, in this study, we diagnosed 27 cases of colonies with clinical infections of DWV. However, only five of these cases were diagnosed before the September/October inspection period (data not shown). Again, only 60% of those colonies that would eventually die were left in the study population during this period. Had this study been larger, or had it continued for a longer duration, the negative effects of these factors may have been detected.

5. Conclusion

This study used epidemiological methods to quantify the risk of exposure from several easily diagnosed factors on colony mortality and morbidity in migratory beekeeping operations in the eastern United States. Systematic epidemiologic methods have long been used to quantify risk for human and domestic animal mortality. As demonstrated by this study, these methods hold promise for understanding the risk of managed honey bee populations as well. Specifically, this study identified two risk factors that were predictive of colony mortality over the short term: queen events, and IBDS. The ease at which these conditions can be accurately diagnosed in colonies makes them excellent monitoring tools for beekeepers attempting to assess the health of the bees in their operations. Unfortunately, the underlying causes of these conditions are poorly understood, and our findings suggest that previous assumptions—such as the putative role of Varroa mites in IBDS—may be incorrect. These results add to the growing body of work that suggests that the causes of colony mortality and morbidity are multiple and complex. While further epidemiological studies are needed to help verify these findings, hypothesis-driven research specifically aimed at trying to understand the causes of queen failure and IBDS should be prioritized.

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