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An Overview on Honeybee Colony Losses in Buenos Aires Province, Argentina

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Abstract

Honey bees (*Apis mellifera*) are essential for the ecosystem, so their loss threatens biodiversity and agriculture. Several factors have been proposed as possible causes of both massive losses and Colony Collapse Disorder. In August 2017 episodes of colony losses were registered in General Alvear, Buenos Aires province. The aim of the present study was to find possible causes of these events. The samples were screened for presence of several pathogens and the determination of maternal lineages was also performed. Seven out of ten colonies were positive for pathogens, but there was no high prevalence of any of them. It will be necessary to carry out a standardization of studies, and delineate boundaries that allow comparing cases in order to discriminate different types of mortality of colonies that occur worldwide.

Introduction

Losses of honey bees and other pollinators threat biodiversity as well as food and agricultural production (Simon-Delso et al., 2014; Watson & Stallins, 2016). The phenomenon called Colony Collapse Disorder (CCD) is expressed carrying out a complete absence of adult bees in a hive often plenty of both capped brood and food reserves (vanEngelsdorp et al., 2009). This scenario has been observed around the world during the last two decades. Some beekeepers in the United States have reported losses of up to 75% of their hives between 2006 and 2007 (Oldroyd, 2007; vanEngelsdorp et al., 2009; 2017; Ellis et al., 2010; vanEngelsdorp & Meixner, 2010). In Europe, similar phenomena have been reported (Dainat et al., 2012; Meana et al., 2017), but in those cases the symptoms would not have been the same as in the United States (Stokstad, 2007). In recent years, some cases of CCD have also been reported in Asia and South America (Farooqui, 2013; Antúnez et al., 2017) but there are not any documented cases in Africa or Oceania. In South America, particularly in Argentina, even though there are no documented cases, several records of beekeepers suggest a 30% of losses in the last years (Maggi et al., 2014). In 2017, 154 out of 170 commercial colonies nucleated in three apiaries from General Alvear (35°55′ 23.67″S, 59°57′29.58″W), Buenos Aires province showed the sudden massive loss of their bees and died at the end of summer, according CCD symptoms. The aim of the present study was to analyze the possible causes of these episodes.

Materials and Methods

Ten samples from three different apiaries located in General Alvear were collected in spring 2017 during episodes of colony losses. Particularly, four samples were from *HE* apiary, three from *CA* apiary and three from *HO* apiary. Each hive was considered a sample unit, comprising 400 live honey bees that were frozen at -32 °C. Afterwards, they were stored at -80 °C in the laboratory prior to the analysis. Bees were screened for presence of seven virus, mites (*Varroa destructor)*, bacteria (*Melisococcus plutonius* and *Paenibacillus larvae*), fungi (*Nosema* spp. and *Ascosphaera apis*), and protists (*Malpighamoeba mellificae*, *Apicystis bombi*, *Nephridiophaga* sp.) all of them already known to be present in the country (Ringuelet, 1947; Cantwell, 1970; Rossi & Carranza, 1980; Alippi, 1992a; Undeen & Vávra, 1997; Plischuk & Lange, 2010; 2011; Plischuk et al., 2011; Reynaldi et al., 2010; 2011). Bee maternal lineages were also analyzed.

The virus detection was performed according to Sguazza et al. (2013) with modifications. Ten honeybees from each sample were homogenated in a stomacher with 2 ml of sterile PBS (free of nucleases), followed by extraction and purification of viral RNA with TriZol® Reagent (Thermo Fisher Scientific) according to the manufacturer protocol. Subsequently, reverse transcription of the RNA was made using reverse transcriptase (MML-V) and random primers, in order to synthesize the complementary DNA. Then, a multiple PCR reaction was performed using specific primers for seven bee virus ABPV (*Acute Bee Paralysis Virus*), BQCV (*Black Queen Cell Virus*), CBPV (*Chronic Bee Paralysis Virus*), DWV (*Deformed Wing Virus*), KBV (*Kashmir Bee Virus*), SBV (*Sacbrood Virus*) and IAPV (*Israeli Acute Paralysis Virus*) (Table 1). The results were analyzed in a 2% agarose gel electrophoresis stained with ethidium bromide.

Both detection and quantification of *V. destructor* were performed according to the validated World Organization

Table 1. Analyses of *A. mellifera* from General Alvear, Buenos Aires, Argentina: Primers used for pathogens and maternal lineages detection. [ABPV = *Acute Bee Paralysis Virus*; BQCV = *Black Queen Cell Virus*; CBPV = *Chronic Bee Paralysis Virus*; DWV = *Deformed Wing Virus*; IAPV = *Israeli Acute Paralysis Virus*; KBV = *Kashmir Bee Virus*; *M. plutonius* = *Melissococcus plutonius*; *N. apis* = *Nosema apis*; *N. ceranae* = *Nosema ceranae*; *P. larvae* = *Paenibacillus larvae*; SBV = *Sacbrood Virus*].

Primer	Virus	Nucleotide sequence (5'-3')	Product length (bp)	Reference
AIVf	IAPV	GGTGCCCTATTTAGGGTGAGGA	158	Sguazza *et al.* (2013)
IAPVr		GGGAGTATTGCTTTCTTGTTGTG		
DWVf	DWV	TGGTCAATTACAAGCTACTTGG	269	Sguazza *et al.* (2013)
DWVr		TAGTTGGACCAGTAGCACTCAT		
SBVf	SBV	CGTAATTGCGGAGTGGAAAGATT	342	Sguazza *et al*. (2013)
SBVr		AGATTCCTTCGAGGGTACCTCATC		
AIVf	ABPV	GGTGCCCTATTTAGGGTGAGGA	460	Sguazza *et al.* (2013)
ABPVr		ACTACAGAAGGCAATGTCCAAGA		
BQCVf	BQCV	CTTTATCGAGGAGGAGTTCGAGT	536	Sguazza *et al.* (2013)
BQCVr		GCAATAGATAAAGTGAGCCCTCC		
CBPVf	CBPV	AACCTGCCTCAACACAGGCAAC	774	Sguazza *et al.* (2013)
CBPVr		ACATCTCTTCTTCGGTGTCAGCC		
Primer	**Bacteria**	**Nucleotide sequences (5'-3')**	**Product length (bp)**	**Reference**
MeliFOR	*M. plutonius*	GTTAAAAGGCGCTTTCGGGT	281	Garrido Bailón *et al.* (2013)
MeliREV		GAGGAAAACAGTTACTCTTTCCCCTA		
Primer 1	*P. larvae*	AAGTCGAGCGGACCTTGTGTTTC	973	Govan *et al.* (1999)
Primer 2		TCTATCTCAAAACCGGTCAGAGG		
Primer	**Fungi**	**Nucleotide sequence (5'-3')**	**Product length (bp)**	**Reference**
AscosFOR	*A. apis*	TGTGTCTGTGCGGCTAGGTG	136	Garrido Bailón *et al.* (2013)
AscosREV		GCTAGCCAGGGGGGAACTAA		
N. ceranae Sense	*N. ceranae*	CGGATAAAAGAGTCCGTTACC	250	Chen *et al.* (2009)
N. ceranae antisense		TGAGCAGGGTTCTAGGGAT		
N. apis Sense	*N. apis*	CCATTGCCGGATAAGAGAGT	269	Chen *et al*. (2009)
N. apis antisense		CCACCAAAAACTCCCAAGAG		
Primer	**Maternal Lineage**	**Nucleotide sequence (5'-3')**	**Product length (bp)**	**Reference**
Cytb f	Cytochrome b	TATGTACTACCATGAGGACAAATATC	485	Crozier *et al.* (1991)
Cytb r		ATTACACCTCCTAATTTATTAGGAAT		

for Animal Health method (World Organization for Animal Health [OIE], 2008) with modifications. Briefly, three hundred bees per hive were treated with surfactants to allow separation from mites. Then a double sieve was used, the upper one retained the bees and the lower one the mites. Mites were counted and the percentage of infection was estimated by multiplying by 100 the number of mites per bee (number of mites/number of bees) in each sample.

The detection of *M. plutonius, P. larvae* and *A. apis* was carried out by the homogenization and subsequent DNA extraction of ten individuals per sample with DNAzol® Reagent (Thermo Fisher Scientific). Subsequently, multiple PCR amplification was performed following Garrido-Bailón et al. (2013) (Table 1).

The presence of *Nosema* spp. as well as protists was determined by individual homogenization in 1 ml of bidistilled water and observation of a drop in a phase contrast microscope (x400; x1000). For prevalence estimation, 100 individuals per sample were scrutinized. Each positive homogenate was filtered and infective stages were quantified using an Improved Neubauer chamber (Cantwell, 1970). A PCR analysis was performed according to Chen et al. (2009) in order to determine the infective species of *Nosema* spp. (Table 1).

The determination of maternal lineages was performed from total DNA extraction. The DNA was extracted from the thorax of one individual per sample using DNAzol® Reagent (Thermo Fisher Scientific). PCR-RFLP technique was carried out to discriminate introgression of African genes in the samples (Pinto et al., 2003, with modifications) (Table 1). The variation in the 485 base pair fragment restriction patterns was obtained and analyzed in 1% agarose gel electrophoresis stained with GelRed™ (Biotium).

Results

The results of the detection of honey bee diseases and the genetic determination of maternal lineages are shown in Table 2. Samples have exhibited the presence of virus in three of the ten hives studied, five hives presented variable percentages of *V. destructor*, and in three hives (one of each apiary) bees with low spore loads (<10,000 spores/bee) of *Nosema* spp. were detected. All of them were determined as *Nosema ceranae*. None of the hives exhibited the presence of *M. plutonius*, *P. larvae*, *A. apis*, or any protist. All the individuals analyzed belonged to European maternal lineages.

Table 2. Analyses of *A. mellifera* from General Alvear, Buenos Aires, Argentina: Presence of pathogens and maternal lineage determination. Results are expressed as (+) for those pathogens present in the sample and (-) for those that did not exhibit presence. Maternal lineage is indicated with (x). [*A. bombi* = *Apicystis bombi*; ABPV = *Acute Bee Paralysis Virus*; BQCV = *Black Queen Cell Virus*; CBPV = *Chronic Bee Paralysis Virus*; DWV = *Deformed Wing Virus*; IAPV = *Israeli Acute Paralysis Virus*; KBV = *Kashmir Bee Virus*; *M. mellificae* = *Malpighamoeba mellificae*; *M. plutonius* = *Melissococcus plutonius*; *N. apis* = *Nosema apis*; *N. ceranae* = *Nosema ceranae*; *P. larvae* = *Paenibacillus larvae*; SBV = *Sacbrood Virus*; *V. destructor* = *Varroa destructor*].

		Virus							Mites	Bacteria		Fungi		Protists			Maternal Lineage	
Apiary	Hive	ABPV	BQCV	CBPV	DWV	KBV	SBV	IAPV	*V. destructor.*	*M. plutonius*	*P. larvae*	*Nosema spp.*	*A. apis*	*M. mellificae*	*A. bombi*	*Nephridiophaga* sp.	European	African
HE	I	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	X	-
	II	-	-	-	-	-	-	-	1.5%	-	-	-	-	-	-	-	X	-
	III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-
	IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-
CA	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-
	II	-	-	-	-	-	+	-	2.0%	-	-	+	-	-	-	-	X	-
	III	-	-	-	-	-	-	-	1.0%	-	-	-	-	-	-	-	X	-
HO	I	-	-	-	-	-	+	+	0.3%	-	-	-	-	-	-	-	X	-
	II	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	X	-
	III	-	-	-	-	-	-	-	1.3%	-	-	-	-	-	-	-	X	-

Discussion

Interactions between multiple drivers or risk factors could be the most probable explanation for elevated mortality rates in honey bee colonies (Potts et al., 2010; Meana et al., 2017), but the reasons that trigger CCD are still in debate (Stockstad, 2007; Williams et al., 2010;Stavely et al., 2014). Several factors have been proposed as possible causes of massive losses related to CCD. Some hypotheses suggested that pathogens like *N. ceranae*, *V. destructor,* bacteria, and several viruses could be responsible of these losses (Cox-Foster et al., 2007; McMenamin & Genersch, 2015; Brutscher et al., 2016; Meana et al., 2017), as well as pesticides (Chauzat et al., 2006). Unfavorable weather conditions and consequent lack of available food, large-scale transhumance practices, nutrition, genetic, or even a combination of several factors are considered some of other potential drivers (Stokstad, 2007; vanEngelsdorp et al., 2009; Ellis et al., 2010; Potts et al., 2010; Ratnieks & Carreck, 2010; Huang, 2012; Francis et al., 2014; Watson & Stallins, 2016; Maggi et al., 2016; Richardson, 2017). Moreover since both honey bee host and pathogens (if involved) are genetically diverse, symptoms and causes of colony losses may well change in different regions (Neumann & Carreck, 2010). On the other hand, some authors proposed that extensive colony losses are not unusual and have occurred repeatedly over decades and regions (Oldroyd, 2007; Ratnieks & Carreck, 2010).

The loss of colonies is well documented in the northern hemisphere (Oldroyd, 2007, vanEngelsdorp et al., 2009; 2017; vanEngelsdorp & Meixner, 2010; Neumann & Carreck, 2010; Ellis et al., 2010; Dainat et al., 2012; Meana et al., 2017) but case studies in the southern hemisphere are almost nonexistent. According to Antúnez et al. (2017) 28% of annual losses were estimated in Uruguay. In Argentina, even though there are no documented cases, several records of beekeepers suggest a 30% of losses in the last years (Maggi et al., 2014). Particularly in Buenos Aires province, a survey over 200.000 colonies showed that 54% of producers had less than 10% of dead hives, 33% between 10-20%, and 13% more than 20% (Reynaldi & Guardia López, 2011). This scenario seems to alert about the need of document these cases and to find out the possible mortality causes as well as to compare regional cases with others worldwide.

In this study, seven out of ten colonies harbored different pathogens. Three of them presented coinfections between virus–fungi, mites–virus–fungi, and virus-mites. However, pathogens varied between hives and, at the same time, the coinfections did not occur among the same pathogens. Even though other studies support the hypothesis of pathogens as the main cause of losses (Cox-Foster et al., 2007; McMenamin & Genersch, 2015; Meana et al., 2017), our results suggest that their presence could not explain the losses by themselves. Not only because there was not a high prevalence of any pathogen, but also because the identity and coinfections were not repeated between hives. Regarding a possible effect of agrochemicals, the studied apiaries were sited 20-30 km away from General Alvear city and no extensive crop cultivation exist in these area, making unlikely the situation of intoxication or weakening by agrochemicals.

Until now there is no single documented cause for CCD in Argentina. Instead, many causes arise from the hypothesis that this is a multifactorial complex syndrome. Reframing discussions in a pluralistic way is needed, but reductionism should not be rejected outright (Watson & Stallins, 2016). A clearer separation that delineates the boundaries between the different cases of bee mortality is necessary to make estimations and comparisons between them and to be able to define CCD causes.

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Author's Contribution

ML Genchi García, FJ Reynaldi and S Plischuk conceived the study, analyzed the samples and wrote the manuscript; CM Bravi contributed to genetic analysis and revised the final version of the manuscript.

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