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Asymptomatic presence of *Nosema* spp. in Spanish commercial apiaries

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ABSTRACT

Nosemosis is caused by intracellular parasites (*Nosema apis* and *Nosema ceranae*) that infect the midgut epithelial cells in adult honey bees. Recent studies relate *N. ceranae* to Colony Collapse Disorder and there is some suggestion that *Nosema* spp., especially *N. ceranae*, induces high mortality in honey bees, a fact that is considered as a serious threat for colony survival.

604 samples of adult honey bees for *Nosema* spp. analysis were collected from beekeeping colonies across Spain and were analysed using PCR with capillary electrophoresis. We also monitored 77 Andalusian apiaries for 2 years; the sampled hives were standard healthy colonies, without any special disease symptoms.

We found 100% presence of *Nosema* spp. in some locations, indicating that this parasite was widespread throughout the country. The two year monitoring indicated that 87% of the hives with *Nosema* spp. remained viable, with normal honey production and biological development during this period of time. The results of these trials indicated that both *N. ceranae* and *N. apis* could be present in these beehives without causing disease symptom and that there is no evidence for the replacement of *N. apis* by *N. ceranae*, supporting the hypothesis that nosemosis is not the main reason of the collapse and death of beehives.

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1. Introduction

Nosemosis is a parasitic disease that attacks all adult forms of honey bees: workers, queens and drones (Bailey, 1955; Alaux et al., 2011; Traver and Fell, 2011). The disease is caused by the microsporidia *Nosema apis* and/or *Nosema ceranae* (Bailey, 1962; Fries et al., 1996). As a mechanism of infection, microsporidia are mainly characterised by their ability to disperse among their host as spores. Inside the host the spores germinate and the sporoplasm enter the host cell via the polar filament which is injected into the host cell (Kudo, 1954; Fries et al., 1996, 2006; Lee et al., 2008). There are other microsporidia species that can parasites insects (Avery and Anthony, 1983; Bomar et al., 1993), reptiles (Jacobson et al. 1998), birds (Dorrestein and van der Hage, 1999), fishes (Lom and Nilsen, 2003) and mammals (Percy and Barthold, 1993; Wasson and Peper, 2000), including humans (Shadduck et al., 1990; Cali, 1991; Didier, 2005).

Both *N. apis* and *N. ceranae* develop inside the epithelial cells of the bee's ventriculus, interfering with the absorption of proteins (Liu, 1984; Puerta et al., 2001; Chen et al., 2009). Although this parasite is essentially distributed worldwide (Matheson, 1996; Chen et al., 2007; Higes et al., 2007; Klee et al., 2007; Liu et al., 2008; Williams et al., 2008), the virulence of both species is controversial (Paxton, 2010), and it was also suggested that *N. ceranae* could cause colony collapse (Cox-Foster et al., 2007; Higes et al., 2008, 2009b).

Over the last decade, Colony Collapse Disorder (CCD) is having a massive impact in Spain, with approximately 30% of the Spanish hives being lost in recent years (unofficial data from beekeepers' associations). Spain has the largest number of beehives (2,300,000 hives) in the European Union, and Andalusia is the Spanish region that has the largest number of hives (442,466 hives). Due to this large amount of beehives, CCD has become more important in this country in which the income of 80% of professional beekeepers (having more than 150 hives) depends heavily on beekeeping (General Register of Livestock Farms. Ministry of the Environment, Rural and Marine Affairs, 2008).

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In response to the increasing concern over CCD, we monitored samples of honey bee hives in the main Spanish areas where beekeeping is practised professionally to clarify the role of nosemosis in CCD and to determinate whether *N. ceranae* causes this disorder.

2. Materials and methods

2.1. Sampling

Samples were collected and monitored by the beekeeping technicians of the different livestock organisations and health advisory associations. Each sample consisted of 100 adult bees collected from the outer honey frame of the same hive. The samples were stored in a freezer at −20 °C until analysis.

The hives chosen did not show any clinical symptoms and did not receive any antifungal treatment, but they were treated with authorised *Varroa destructor* products at least once a year. The hives were randomly selected and identified for further sample collection for follow up of the hive's status over time. In addition, the beekeeper completed a questionnaire.

There were two types of sampling:

(a) Monitoring of 77 Andalusian apiaries (one hive per apiary) during 2006–2007. Samples were collected twice a year in the spring and autumn. If any of the beehives died during the sampling period they were randomly replaced by other disease symptom-free hives belonging to the same apiary for future studies.

(b) To determine the presence of *Nosema* spp., five different beehives from each of 118 apiaries around the country were also sampled during 2007.

2.2. DNA extraction

Fifteen bee abdomens of each sample were macerated in 10 ml of Milli-Q ultrapure water and filtered through a sieve of 0.036 mm in diameter. The filtered suspension was processed using the DNeasy Plant Mini Kit (Quiagen®).

2.3. DNA amplification

The amplified regions belonged to a gene sequence of the 16S ribosomal RNA subunit: the *N. apis* region is 242 bp. and *N. ceranae* region is 254 bp. This size difference allowed the identification of both species by PCR.

The primers used for the amplification reaction were described by Higes et al. (2006). Our research team used the same NOS-REV primer and changed a pair of bases of the NOS-FOR primer; two bases were added to the 5′ end of the sequence that appeared in the database, and the last two bases of the 3′ end were removed because there were differences in these nucleotides between the *N. apis* and *N. ceranae* (U26534, GI857487; U26533, GI857489) sequences. The primer sequence used was 5′-TATGCCGACGATGTGATATG-3′.

These primers were used to sequence the products obtained by PCR and to confirm the sequences in both species. Sequencing was performed with the BigDye Terminator v 3.1 Cycle Sequencing kit

Table 1
Detection of *Nosema* spp. in asymptomatic colonies in Andalusia (Southern Spain) during 2006 and 2007.

Andalusia										
Period of sampling	Province	N° samples	*N. ceranae*		*N. apis*		Coinfection (*N. apis* + *N. ceranae*)		Not detection	
			No. samples	(%)	No. samples	(%)	No. samples	(%)	No. samples	(%)
Spring 2006	Almeria	10	5	50	1	10	4	40	–	–
	Cadiz	6	4	66.6	1	16.7	1	16.7	–	–
	Cordova	10	7	70	–	–	3	30	–	–
	Granada	10	7	70	–	–	3	30	–	–
	Huelva	8	7	87.5	–	–	1	12.5	–	–
	Jaen	7	6	85.7	–	–	1	14.3	–	–
	Malaga	11	9	81.8	–	–	2	18.2	–	–
	Seville	8	8	100	–	–	–	–	–	–
Autumn 2006	Almeria	10	7	70	–	–	3	30	–	–
	Cadiz	8	6	75	–	–	2	25	–	–
	Cordova	9	6	66.6	–	–	3	33.3	–	–
	Granada	9	6	66.6	–	–	3	33.3	–	–
	Huelva	5	5	100	–	–	–	–	–	–
	Jaen	6	6	100	–	–	–	–	–	–
	Malaga	1	1	100	–	–	–	–	–	–
	Seville	6	6	100	–	–	–	–	–	–
Spring 2007	Almeria	35	15	43	5	14	12	34	3	9
	Cadiz	32	27	85	1	3	3	9	1	3
	Cordova	34	34	100	–	–	–	–	–	-
	Granada	35	19	54	2	6	13	37	1	3
	Huelva	35	34	97	–	–	–	–	1	3
	Jaen	33	33	100	–	–	–	–	–	–
	Malaga	34	24	71	–	–	9	26	1	3
	Seville	33	25	76	–	–	7	21	1	3
Autumn 2007	Almeria	9	7	78	1	11	1	11	–	–
	Cadiz	8	5	62	–	–	1	13	2	25
	Cordova	6	6	100	–	–	–	–	–	–
	Granada	3	2	67	–	–	–	–	1	33
	Huelva	8	8	100	–	–	–	–	–	–
	Jaen	9	9	100	–	–	–	–	–	–
	Malaga	5	5	100	–	–	–	–	–	–
	Seville	3	3	100	–	–	–	–	–	–
Total andalusia		446	352	78.9	11	2.5	72	16.1	11	2.5

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