



Short Communication

Detection of *Spiroplasma melliferum* in honey bee colonies in the USHuo-Qing Zheng^{a,b}, Yan Ping Chen^{b,*}^a College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, PR China^b USDA-ARS Bee Research Laboratory, Beltsville, MD 20705, USA

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ABSTRACT

Spiroplasma infections in honey bees have been reported in Europe and Asia quite recently, due to intensive studies on the epidemiology of honey bee diseases. The situation in the US is less well analyzed. Here, we examined the honey bee colonies in Beltsville, MD, where *Spiroplasma melliferum* was originally reported and found *S. melliferum* infection in honey bees. Our data showed high variation of *S. melliferum* infection in honey bees with a peak prevalence in May during the course of one-year study period. The colony prevalence increased from 5% in February to 68% in May and then decreased to 25% in June and 22% in July. Despite that pathogenicity of spiroplasmas in honey bee colonies remains to be determined, our results indicated that spiroplasma infections need to be included for the consideration of the impacts on honey bee health.

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1. Introduction

Spiroplasmas are small, helical, motile Eubacteria and are descendants of gram positive bacteria that lack a cell wall (Regassa and Gasparich, 2006). *Spiroplasma melliferum* and *Spiroplasma apis* are two pathogens that have been identified in Western honey bees, *Apis mellifera* (Clark, 1977; Mouches et al., 1982).

S. melliferum was first reported in honey bees during the course of an examination of honey bees for pathogenic microorganisms in Beltsville, MD, USA in 1976 (Clark, 1977, 1978; Clark et al., 1985). *S. apis* was abundantly detected in honey bees from colonies showing symptoms of “May disease” in France in early 1980s (Mouches et al., 1982, 1984, 1983). *S. apis* strain B31 was found to cause death in honey bees when injected and strain B39 when fed (Mouches et al., 1982). *S. melliferum* caused similar disease symptoms in bees when fed, but was less pathogenic at the colony level (Clark, 1978). However, current knowledge concerning the pathogenesis of spiroplasmas in infected honeybee colonies is limited and no significant bee colony losses were observed to be linked to the spiroplasma infection (Clark, 1978; Neumann and Carreck, 2010).

The recent large-scale losses of honey bee colonies in America and Europe have attracted extensive research on the epidemiology

and pathogenesis of pathogenic microorganisms that cause serious diseases in honey bees (Cox-Foster et al., 2007; Neumann and Carreck, 2010). In recent years, spiroplasma infection in honey bees has been documented in China (Hui et al., 2010; Li et al., 2012), Korea (Ahn et al., 2012) and Belgium (Ravoet et al., 2013). However, the spiroplasma infection in honey bees was not reported in the past three decades in North America, with the exception of a recent work presented in a meeting (Schwarz and Evans, 2012). Here, by taking advantage of a recently published PCR detection method (Meeus et al., 2012), we examined the honey bee colonies in Beltsville, MD for spiroplasma infection.

2. Methods and materials

For colony level prevalence, ten colonies were randomly selected from an apiary and sampled in September, October, November of 2012 and January, February, May, June and July of 2013 from the experimental apiary in the Bee Research Lab of USDA-ARS, Beltsville, MD. Three additional colonies were included to replace colonies died during the study. Additional bee samples were collected from 30 colonies in February and 40 colonies in May and July of 2013 from another three experimental apiaries. The four sampling apiaries were 1–2 km away from each other and underwent the same routine beekeeping management by the same beekeepers. Thirty worker bees were randomly collected from the inner lids of hives for each colony. Only half of the colonies sampled in February were sampled in May and July due

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to the high winter losses. Sample sizes of 10, 30, 40 and 50 gave

As-636. This was not surprising since *S. melliferum*, but not *S. apis* was found in the same area by Clark and colleagues (Clark, 1977; Clark et al., 1985).

Although the negative detection in samples collected in September, October, November, and January could be due to the lower sample size in these months (see M & M for confidence level of sample size), our results, with much higher sample size in February, May, and July, showed a variation in the occurrence of *S. melliferum* infection in honey bee colonies in the sampling area. While only 5% of colonies were found to be positive for spiroplasma in February, 68% of colonies were positive in May. This result was in accordance with previous finding that spiroplasma disease occurred mainly from late May to early July and was commonly called “May disease” (Mouches et al., 1982). A previous study (Raju et al., 1981) showed that *S. melliferum* was found in the feces deposited on the surface of flowering plants by infected bees. It is presumably that bees would get infected while they were foraging on the contaminated nectar and pollen and then bring the pathogen to their hives. Further studies are warranted to investigate the correlation between seasonal variation in spiroplasma prevalence and honey bee foraging activities.

The higher prevalence of *S. melliferum* in diseased bees than in randomly collected bees in a diseased colony, suggested the correlation of *S. melliferum* infection to the death of the colony in one month. However, despite that typical symptom of spiroplasma disease could be observed, it was unclear that whether *S. melliferum* or other disease agents caused the death. The fact that most of the infected colonies recovered after May suggested low or short-term impact of *S. melliferum*, which was also suggested by Clark (1977) showing no productivity losses at colony level, or high resistance of honey bees to this infection.

The high variation with low or no occurrence at most time around the year and the low impact at colony level of *S. melliferum* infection, provided explanations to why it was not reported in previous studies in the past decades, regardless of the technique obstacles. However, in an era when honey bees are facing numerous threats (De la Rúa et al., 2009; Genersch, 2010; Mullin et al., 2010), the effects of spiroplasmas infections need to be considered. *Spiroplasmas* infections may only singly add to the pathology burden of honey bees or increase the vulnerability of honey bees by interaction or combination with other factors like parasites, viruses, poor nutrition and chemical residues.

Besides in honey bees, *S. melliferum* has also been found in the hemolymph of bumble bees, leafcutter bees, and robber flies as well as in the intestinal tract of sweet bees, digger bees, bumble bees, and butterflies (Alexeev et al., 2012). However, the degree of its pathogenicity has not yet been clear. The physiology of *S. melliferum* and its mechanisms of interaction with hosts remain poorly studied except for studies of its motility. The recent assembly of the *S. melliferum* KC3 genome and its proteogenomic annotation provided new molecular tools on these aspects (Alexeev et al., 2012).

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