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NOTES AND COMMENTS

Advances in *Paenibacillus larvae* and American foulbrood monitoring in honey bee colonies from Argentinean apiaries

Natalia Jorgelina Fernández[1,2*], Liesel Brenda Gende[1,2] and Martín Javier Eguaras[1,2]

[1]Arthropods Laboratory, School of Natural and Exact Sciences, Universidad Nacional de Mar del Plata, Funes, 3350. 7600, Mar del Plata, Argentina.
[2]CONICET [National Council for Scientific and Technical Research], Rivadavia 1917. C1033AJ, Buenos Aires, Argentina.

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*Corresponding author: Email: njfernan@mdp.edu.ar

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American foulbrood (AFB), an infectious disease affecting the honey bee *Apis mellifera* L., is caused by the spore-forming bacterium *Paenibacillus larvae.* Being able to kill larvae and pupae, AFB leaves adult bees unaffected, which thus become asymptomatic carriers (Lindström, 2008). Given the fact that AFB can only be detected visually, the diagnosis of this disease is usually complex and late. The development of a monitoring tool capable of detecting this disease has therefore become critical. Several works on *P. larvae* spore transmission and distribution at the colony and apiary level have been published (Lindström *et al.*, 2008; Lindström, 2008); yet none of them have focussed on the number of spores in relation to the degree of disease, and so allowed the establishment of a damage threshold regarding the spores: bee ratio. Studies of such a relationship could, however, become useful monitoring tools, capable of assessing the sanitary behaviour of the colonies based on the spore loads registered, and so prevent American foulbrood development. The aim of our study was to establish a relationship between the number of spores per bee and the extent of disease development in the colony. It was further to establish a minimum number of spores (threshold) from which the clinical symptoms of AFB start to appear in the colonies.

A total of 69 colonies exhibiting AFB clinical signs kept in Langstroth hives in four apiaries in Buenos Aires province, Argentina, were inspected from September 2007 to January 2008. All frames from each colony were examined, and the number of infected cells, along with the scales contained, was quantified. Both stages were classified as diseased larvae (DL). Each hive was then classified with respect to the infection level, applying the following classification: Healthy (0 DL); Low (1 to 10 DL); Moderate (11 to 50 DL); and Severe (more than 50 DL) per colony, respectively (Table 1). Samples of nurse bees were collected from each hive (Nordström *et al.*, 2002). Thirty bees from each sample were prepared according to Hornitzky and Karlovskis (1989). Three plates were prepared with MYPGP agar containing nalidixic acid (9 µg ml^{-1}) and incubated at 35-37ºC in microaerophilia (5-10% of CO_2) for between four and seven days. Once the incubation period was completed, *P. larvae* colonies were identified by macroscopic and microscopic observation and catalase tests, and bacterial colonies were counted. These counts were then mathematically converted as a function of dilutions and cultured volume to spores per bee.

The Kruskal-Wallis test (K-W) was used to ascertain whether significant differences existed in the number of spores (CFU) per bee between the Healthy and Low cluster and the Healthy and Diseased cluster (Low, Moderate and Severe included), respectively. The same statistical analysis and classification criteria (i.e., Healthy and Diseased) were utilized to analyze data in each apiary on a separate basis. The Pearson Correlation Coefficient was used in each apiary to assess the relationship between spore load and disease stage. Statistical analyses were carried out using SPSS 11.5 (1998).

Apiary 1 produced the largest number of CFU per bee. The hives classified as Healthy yielded variable spore loads and the spore load was notably high for all clusters. The high count value detected could be ascribed to the continuous use of antibiotics, which may retard or mask the onset of the disease. It is well established that the use of antibiotics such as oxytetracycline can bring about recurrence (Alippi, 1996). Apiary 2, in contrast, was the only site from which negative cultures were observed from bee hives classified in the field as Diseased, and in which oxytetracycline had been applied to control the disease, the spore load being lower than that found in Apiary 1. This apiary also yielded high levels of *Varroa destructor* prevalence. A clear cut difference was noticed in the number of CFU per bee in Healthy and Diseased colonies in Apiaries 3 and 4. In both cases, no antibiotics were indicated to treat the disease (Table 1).

Table 1. Characteristics and geographic coordinates of apiaries. Results of correlation coefficients and significance values for Kruskal-Wallis test.

Apiary No.	**Apiary features**	**Nº of bee hives**	**N° of bee hives in each group**	**Coordinates**	**Correlation Coefficient**	**Kruskal-Wallis *p* Value**
Total apiaries	Healthy *vs*. Low	69	41 *vs.* 6	----------	----------	0.156 (+)
Total apiaries	Healthy *vs.* Diseased		41 *vs.* 28		-0.063	0.001***
Total apiaries	Use of antibiotics *vs.* no use of antibiotics		36 *vs.* 33		----------	0.040***
1	Prior outbreaks. Use of antibiotics	23	H:18 L:2 M:1 S:2	37°27'S 57°44'W	0.946	0.000***
2	Presence of V*arroa destructor*. Use of antibiotics	*13*	H:8 L:1 M:1 *S:3*	*38º10´06´´S 57º 38´10´´W*	0.476	0.129 (+)
3	Prior outbreaks. Prompt removal of infected material. No use of antibiotics	20	H:16 L:1 M:0 S:3	37°28'S 45°38'W	0.946	0.014***
4	First outbreak. No use of antibiotics	13	H:9 L:2 M:0 S:2	37°53'S 58°15'W	0.939	0.000***

(***) Statistically significant differences between Healthy and Diseased clusters. (+) No significant differences detected (*p<0.05).*
H: Healthy; **L:** Low; **M:** Moderate; and **S:** Severe.

Paenibacillus larvae spores may be detected even when no AFB symptoms are visible in the hive (Lindström *et al.,* 2008). Samples from adult bees could therefore provide reliable information regarding the actual health status of the colony (Lindström *et al*., 2008). The spore detection method from adult bees allows the identification of contaminated colonies at the subclinical stage of the disease (Basualdo *et al*., 2008). In our study, 42% of the total bee hives inspected were found to be infected with AFB at a subclinical level. This result accounts for the highly predictive value of adult bee samples in determining the risk of the development of clinical symptoms (Nordström, *et al*., 2002; Lindström *et al*., 2008).

Lindström (2008) and Lindström *et al.* (2008) focused their research on *P. larvae* spore distribution as a function of bee samples collected from different areas within the hive (brood chamber, super) and at an apiary level. The aim of the present work was to individually determine the infectious stage of the colonies and to analyze the spores to bee relationship at different stages of the disease. The analysis of the spores to bee relationship in bee hives exhibiting different disease stages and coming from apiaries with different AFB history explains the complexity entailed in monitoring AFB. Nonetheless, based on the apiary history i.e., on outbreaks prior to the study (number and severity), environmental factors, drifting of contaminated bees among hives, presence of other pathologies and prior antibiotic use, the status of the apiaries in relation to AFB can be inferred.

The basis of an Integrated Pest Management programme lies in disease monitoring, prevention and control. The relevance of a tool capable of detecting AFB occurrence before its clinical signs become apparent is therefore worth investigating.

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