Presence, load and distribution of seven honey bee viruses in the South of Spain and relationship with colony weakening

Vicente-Rubiano, M.[a]*, Kukielka, D.[b] Martínez-López, B.[a,c], Sánchez-Vizcaíno, J.M.[a]

[a] VISAVET Center and Animal Health Department. Veterinary Faculty. Complutense University of Madrid, Spain.
[b] Biology Department and Microbiology area. Pharmacy Faculty. CEU San Pablo University, Madrid, Spain
[c] IREC (CSIC-UCLM-JCCM), Ciudad Real, Spain
* Corresponding author: E-mail address: **marina@sanidadanimal.info**

INTRODUCTION

The western honey bee *Apis mellifera* has suffered important losses in the last years [1]. As colonies are maintained in environmental-dependent conditions, pathogens, specially honey bee viruses, are though to be a key factor in combination with certain environmental factors in the colony losses. The objective of this study was to describe the presence, load and distribution of seven important honey bee viruses in Spain as well as to evaluate the effects that beekeeping managing and environmental factors have in the Spanish colonies. The community of Andalucía, situated in the south of Spain, was used as an example for other similar Spanish and European regions.

MATERIALS AND METHODS

Andalucía was selected to perform an extensive honey bee sampling, as is the first Spanish Community in hive census and the second one in honey production. Ninety-six colonies of different regions and beekeeping practices were sampled and epidemiologically surveyed in 'spring-summer' and 'fall-winter' seasons. Samples were analyzed by RT-qPCR for the presence and viral load of acute and chronic bee paralysis virus (ABPV, CBPV), Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), black queen cell virus (BQCV), sacbrood virus (SBV) and deformed wing virus (DWV). Colonies were classified in 'weak' and 'healthy' depending on the presence or not of diseases in the colony at the moment of sampling. Comparisons between seasons and groups were performed using Mann-Whitney (MW) test. A logistic regression model was performed in R language (the R project) to identify the risk factors potentially associated with the weakening of the colonies. Environmental variables and variables intrinsic to the colony were included in the logistic regression model.

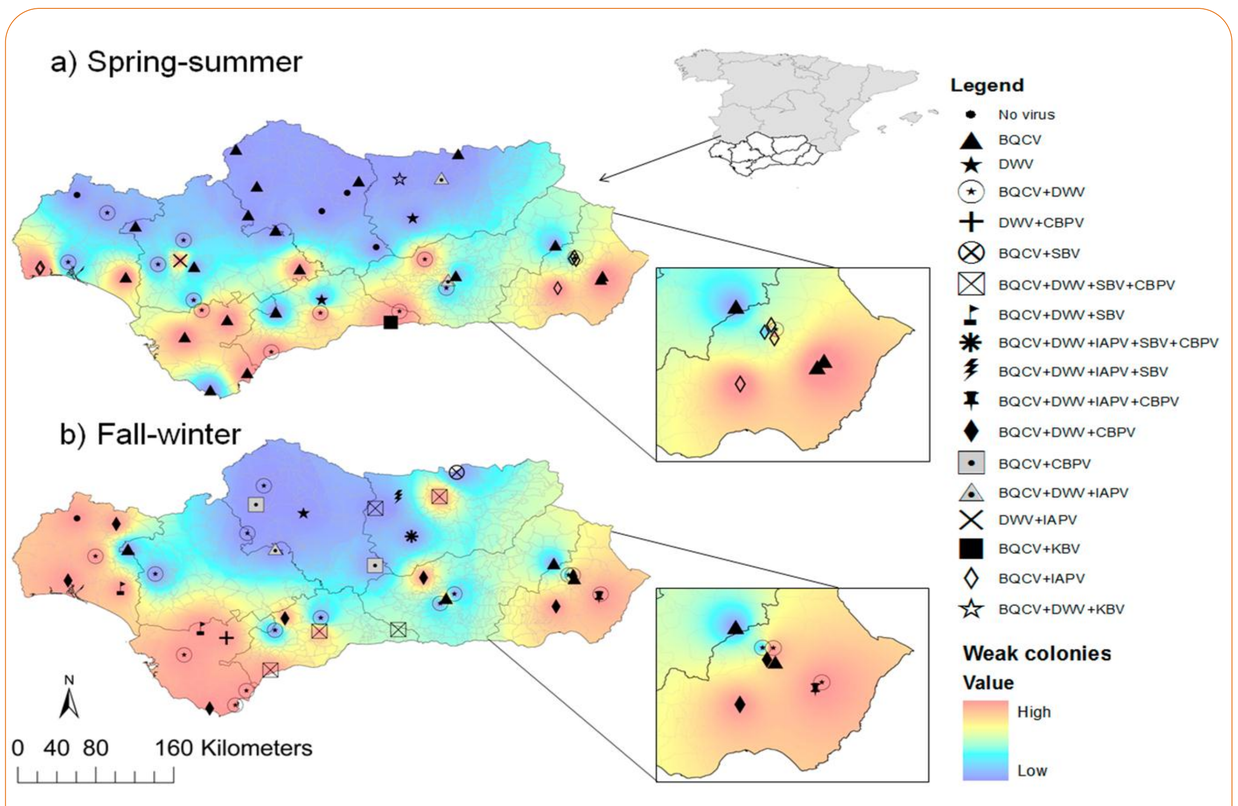

Figure 1. Distribution of honey bee viruses and weak colonies in the study region. (a) Spring-summer season (b) fall-winter season. Red and blue areas indicate higher and lower values of inverse distance weighted interpolation of presence of weak and healthy colonies, respectively.

Table 1. Frequency of virus presence in the sampled colonies in Andalucía.

Season	Health/ weak	N	BQCV (%)	DWV (%)	SBV (%)	CBPV (%)	IAPV (%)	KBV (%)	Mean ± SD of viruses
Spring-summer	Healthy	68	63.24	27.94	0	0	7.35	2.94	1.01±0.78
	Weak	28	75	21.43	0	0	28.57	0	1.25±0.65
	Total	96	66.67	26.04	0	0	13.54	2.08	1.08±0.75
Fall-winter	Healthy	45	84.44	51.11	26.67	22.22	8.89	0	1.93±1.03
	Weak	42	85.71	78.57	21.43	28.57	2.38	2.38	2.17±1.05
	Total	92	85.06	64.37	24.14	26.44	5.75	1.15	2.05±1.05

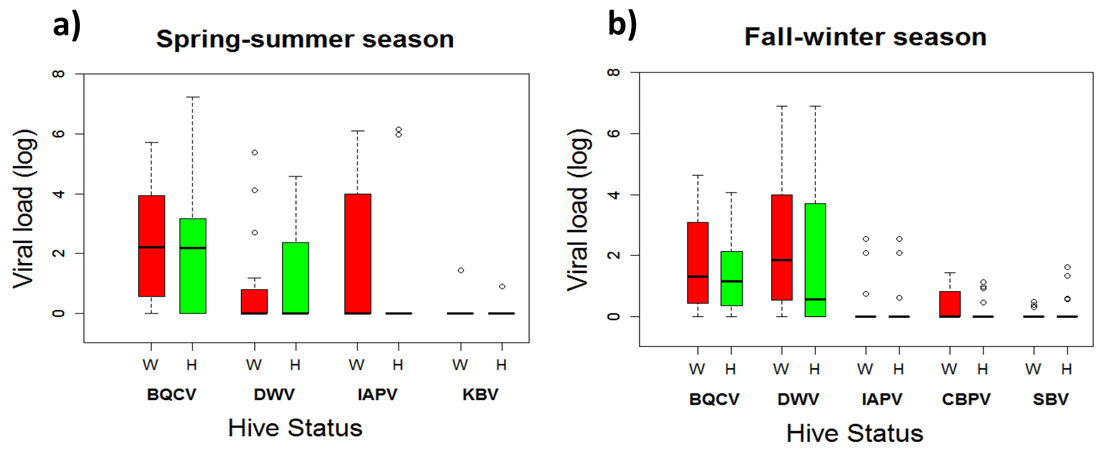

Figure 2. Viral load found in the sampled colonies in Andalucía. (a) Spring-summer season (b) fall-winter season. W: weak colony; H: healthy colony.

RESULTS

Viruses BQCV, DWV, SBV, CBPV, IAPV and KBV were found in the collected samples whereas ABPV was not detected. Distribution of bee viruses showed that viruses are widespread along the study region. Weak colonies were mainly distributed in the southern area and co-infections were frequent **(Figures 1a and 1b)**. The most frequent viruses were BQCV (66.67% in spring-summer; 85.06% in fall-winter) followed by DWV. In general, weak colonies had the highest virus frequencies and the higher number of viruses per colony **(Table 1).** Regarding differences in viral load between seasons, BQCV and IAPV loads were higher during the spring-summer season compared to fall-winter season **(Figure 2)**. However, we found the opposite tendency for DWV, with higher load in the fall-winter season compared to spring-summer (MW $P<0.05$). No significant differences in viral load were detected between the 'weak' and 'healthy' groups during the spring-summer and fall-winter seasons, except for CBPV loads, which were significantly higher in the 'weak' group during the fall-winter season. Although not significant, during fall-winter BQCV and DWV loads tended to be higher in the 'weak' colonies **(Figure 2)**. One logistic regression model was used for each season **(Table 2)**. Environmental and beekeeping factors were the risk factors for colony weakening in spring-summer, whereas sanitary factors such as presence of DWV and application of a single treatment against *Varroa destructor* were also found as risk factors in the fall-winter season (**Figure 3)**.

Table 2. Variables included in the final logistic regression models.

Season	Variable	Result	Season	Variable	Result
Spring-summer	Mean number of hives per apiary	Protective factor	Fall-winter	Absence of winter artificial feeding	Protective factor
	Percentage of the year feeding in cultures	Risk factor		Percentage of hives moved in transhumance	Protective factor
	Absence of winter artificial feeding	Protective factor		Absence of correction of bees' drift	Risk factor
	Temperature range (maximum-minimum) in the previous month	Protective factor		Application of a single treatment against V. destructor	Risk factor
	Days of frost in the previous month	Risk factor		Positive to DWV	Risk factor
	Mean of wind speed in the previous month	Risk factor			

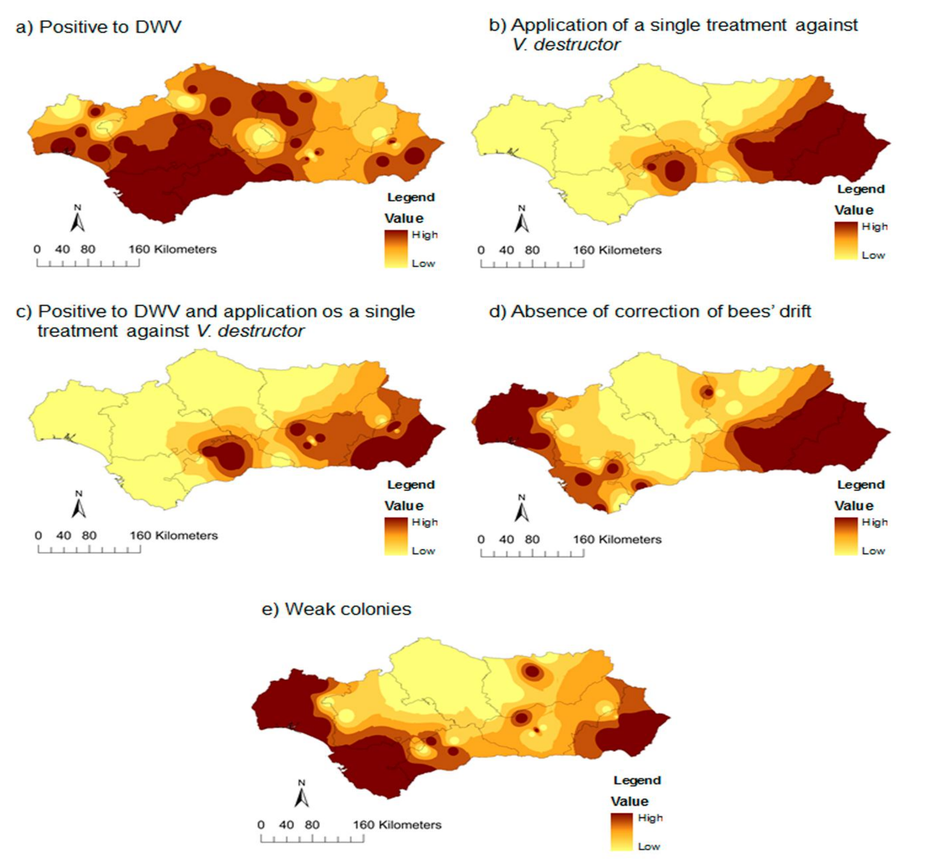

Figure 3. Spatial distribution of risk factors associated with weak colonies in the fall-winter season. Inverse distance weighted interpolation has been used to show the spatial distribution of the risk factors in the region of study.

DISCUSSION AND CONCLUSIONS

This study has described the frequencies, load and distribution of seven honey bee viruses and its seasonal variations, together with the epidemiological factors that may influence in the health status of the colonies. Viruses are widely distributed along the region of study and have viral loads of 10^7 even in healthy colonies. In general , weak colonies have higher viral loads, and these differences are evidenced mainly in the fall-winter season. Risk factors associated with weakening of colonies are different in each season. Particularly during the fall winter season DWV was associated with application of a single treatment of *Varroa destructor*, agreeing with previous studies [2-3], and suggesting that this virus, combined with the mite may be implicated in the weakening of the colonies during fall-winter.

The tendencies observed here may guide the design of future research to elucidate the main causes of the colony losses and this study may also help in the implementation of surveillance and control measures in honey bee viral diseases.

ACKNOWLEDGEMENTS

Funding: National Beekeeping Program (MAGRAMA). Grants: MVR -FPU grant. Contracts: BML -Juan de la Cierva (JCI-2011-10724). Thanks to Andalusian beekeepers, Reference Beekeeping Centre of Andalucía and University of Córdoba (CERA), Coordinator of Farmers Associations (COAG) and SUAT research group .

REFERENCES

[1] VanEngelsdorp D. and Meixner M. D., 2010. Invertebr. Pathol. 103, S80-S95.
[2] Dainat B. et al., 2012. Appl. Environ. Microbiol. 78, 981-987.
[3] Dainat B. and Neumann P., 2013. J. Invertebr. Pathol. 112, 278-280.