Azorean Honeybees versus viruses: who wins?

Ana R. Lopes[1], Joachim de Miranda[2], Raquel Martín-Hernandez[3,4], M. Alice Pinto[1]

[1] Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus Sta Apolónia, 5300-253, Bragança, Portugal; [2] Ekologicentrum, Swedish University of Agricultural Sciences, Uppsala, Sweden; [3] Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla La Mancha, CIAPA de Marchamalo (Guadalajara, Spain); [4] Instituto de Recursos Humanos para la Ciencia y la Tecnología (INCRECYT-FEDER), Fundación Parque Científico y Tecnológico de Castilla—La Mancha, 02006 Albacete, Spain

FCT Fundação para a Ciência e a Tecnologia

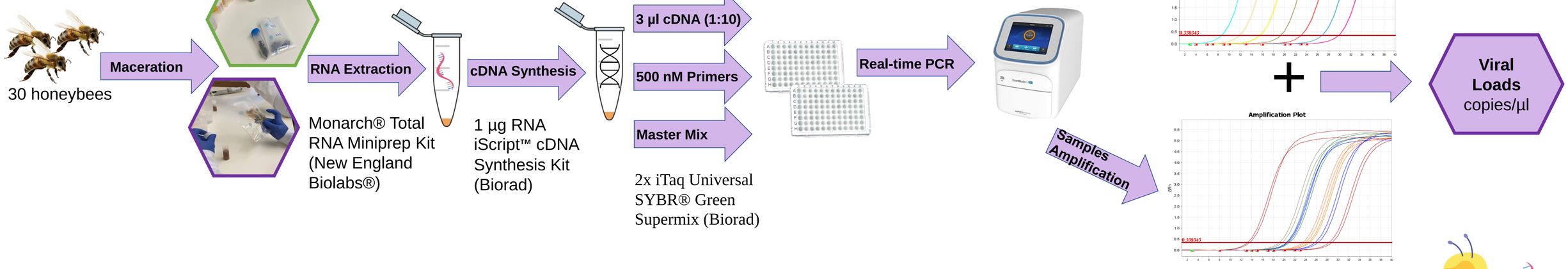
Centro de Investigação de Montanha (CIMO) — ipb Instituto Politécnico de Bragança — encontro CIÊNCIA '21

Honeybee pollination services and hive products are at stake due to increasing worldwide colony losses. Many drivers of colony losses have been proposed, among which the ectoparasitic mite *Varroa destructor* (Vd), and its numerous vectored viruses (Francis et al., 2013; de Miranda et al., 2013), and the Microsporidia *Nosema ceranae* (Nc) play a prominent role. There are only a few Vd-free and possibly even fewer Nc-free honeybee sanctuaries in the World, with the Azores being one. This archipelago not only has islands with and without Vd (Figure 1) and Nc but it also harbors genetically complex honeybee populations, enabling unprecedented analyses of changes in epidemiological patterns in response to both invasions. Here we show the first survey on viruses ever performed in the Azores. We used RT-qPCR to examine prevalence and viral loads of the most common viruses, namely: Black Queen Cell (BQCV), Sacbrood (SBV), Chronic Bee Paralysis (CBPV), Deformed Wing (DWV), Lake Sinai (LSV), Bee Makula-Like (BeeMLV) and the complex AKI (Acute Paralysis, Kashmir Bee, Israelis Acute Bee Paralysis).

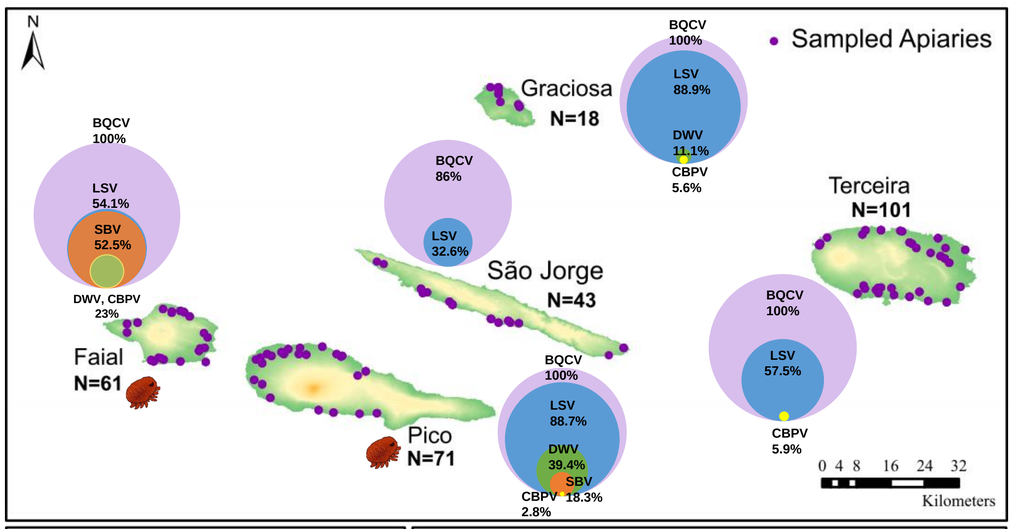
Sampling and Molecular Analysis

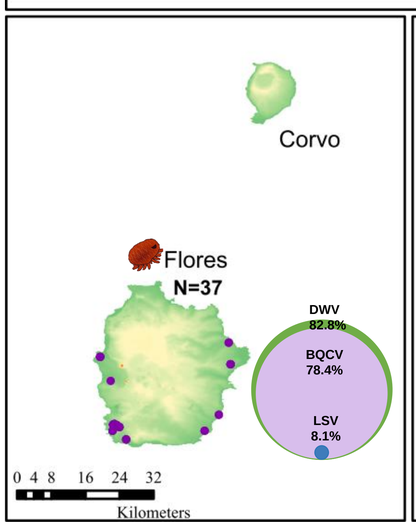
✓ Sampling took place in 2014, 2015, and 2020 and comprised 495 colonies (3 colonies/apiary) distributed across 8 Islands (Figure 1).

Molecular Analysis Workflow



Results





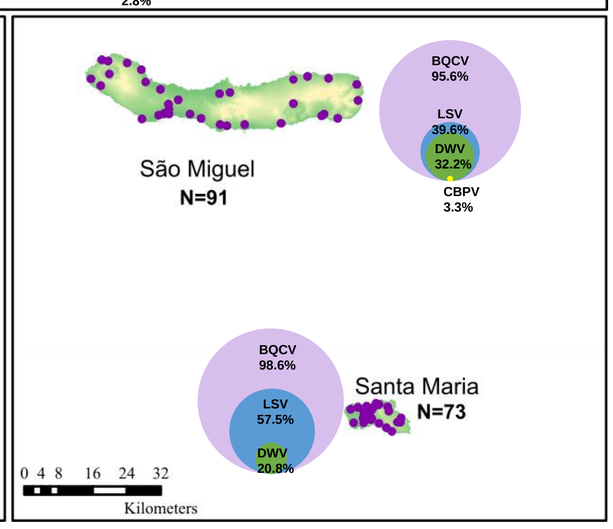


Figure 1. Sampling and viral prevalence at the population level. Islands with V. destructor are denoted by .

Prevalence Data (Figure 1)

- ✓ **AKI** complex and **BeeMLV**: **not detected**.
- ✓ **BQCV:** widespread distribution with Faial, Graciosa, Pico and Terceira having 100% of prevalence and Flores with only 78.4%.
- ✓ **SBV:** in **Faial** (50%) and **Pico** (18.3%).
- ✓ **CBPV:** in Faial (23%), Terceira (5.9%), Graciosa (5.6%), São Miguel (3.3%), Pico (2.8%), with generally low prevalence.
- ✓ **LSV**: found in every island, with prevalence ranging from 8.1 % to 88.9%.
- ✓ **DWV:** in 6 islands (prevalence varied between 11.1 to 82.8%)**. São Jorge** and **Terceira** are **DWV-free Islands.**

Viral Load Data (Table 1)

- ✓ **BQCV** and **LSV** are disseminated across the Azores**.** However, the highest viral loads were observed for **LSV** (4 of the 8 islands with 10^6 copies/µl) (Table 1 - red circles).
- ✓ **CBPV** has the lowest prevalence, although positive colonies harbor high viral loads.
- ✓ The maximum number of viruses with a high/moderate viral load found by island was 5 and 4 in Faial and Pico (Vd – islands), respectively.

Table 1. Summary of mean viral loads/Island. Red circles – high viral loads; Orange to Yellow circles – moderate viral loads; Green – low viral loads. Islands with V. destructor are denoted by .

	N	BQCV (copies/µl)	SBV (copies/µl)	CBPV (copies/µl)	LSV (copies/µl)	DWV (copies/µl)
Faial	61	6.62E+04	2.87E+05	3.09E+06	9.89E+05	4.92E+05
Flores	29	1.23E+05			1.43E+03	3.21E+05
Graciosa	18	1.41E+05		1.26E+01	1.02E+06	2.53E+01
Pico	71	5.76E+04	1.14E+06	1.79E+01	1.79E+06	6.02E+04
São Jorge	37	2.39E+05			2.29E+04	
São Miguel	87	2.77E+04		1.76E+06	1.73E+06	2.67E+05
Santa Maria	72	7.44E+04			3.44E+05	1.17E+05
Terceira	101	3.07E+05		1.05E+05	1.28E+06	

Summary

- ✓ **Azores** is an **important reservoir of virus-free honeybees** with islands like São Jorge and Terceira without DWV and Flores, Graciosa, São Jorge, São Miguel, Santa Maria e Terceira without SBV.
- ✓ Although Varroa-DWV relationships are well known, viral loads were found with a high level for most of the viruses independently of Vd-presence or absence.
- ✓ Nevertheless**,** it is crucial to protect the honeybees of **São Jorge** and **Terceira** since these islands harbor stocks without two of the most problematic honeybee stressors: DWV and Vd.

References

- Francis et al. (2013). Varroa-virus interaction in collapsing honey bee colonies. PloS one, 8(3). doi: 10.1371/journal.pone.0057540
- de Miranda et al. (2013). Standard methods for virus research in Apis mellifera. *Journal of Apicultural Research, 52*(4), 1-56. doi:10.3896/IBRA.1.52.4.22

Acknowledgements

We are grateful to all veterinarians and technicians of the "Direção Regional da Agricultura e Desenvolvimento Rural dos Açores" who collected all the samples across the Azores, namely: Frank Aguiar, Nuno Salvador, Janyne Sousa, Ivan castro, Célia Mesquita, Ana Jorge, José Dias, Paulo Rico, Pedro Leal, Vagner Paulos, Luis Xavier, Luís Silva, Martins Silva, Carlos Gouveia, Ana Carina Coimbra, João Ramos, João Arruda, Edgardo Melo, João Luís and Moniz da Ponte. Special thanks are due to Paula Vieira for the coordination of the sampling. This work was funded by the research project BEEHAPPY (POCI-01-0145-FEDER-029871. Ana R. Lopes acknowledges the PhD scholarship funded by the FCT (SFRH/BD/143627/2019).