TITULO DEL PROYECTO: BPRACTICES. New indicators and on-farm practices to improve honeybee health in the *Aethina tumida* era in Europe.

Entidades participantes: Instituto Zooprofilattico Sperimentale del Lazio e della Toscana, University of Namik Kemal, Agricultural Institute of Slovenia, Austrian Agency for Healt & Food Safety, Mississippi State University, Instituto Zooprofilattico Sperimentale delle Venezie, Centro de Investigación Apícola y Agroambiental de Marchamalo CIAPA-IRIAF

Investigador Coordinador (OPI al que pertenece): Giovanni Formato. Instituto Zooprofilattico Sperimentale del Lazio e della Toscana. Subproyecto 4: Mariano Higes Pascual, CIAPA-IRIAF.

TÍTULO DEL SUBPROYECTO PARTICIPADO POR EL IRIAF: WP3. Nosema research.

Organismo Financiador: European research area on sustainable animal production.
Duración: desde: 01/02/2017hasta: 31/01/2020
Nº de Proyecto: ERA-NET SUSAN Proposal 83/PCIN-2017-096
Financiación: Total: 678.000 € / Subproyecto IRIAF: 94.000 €

PERSONAL INVESTIGADOR DEL SUBPROYECTO PARTICIPADO POR EL IRIAF:

EQUIPO PARTICIPANTE	SITUACIÓN ADMINIST. (*)	DEDICACIÓN (UNICA O COMPARTIDA)	CENTRO
INVESTIGADOR PRINCIPAL: Mariano Higes Pascual	Funcionario	Compartida	CIAPA
PERSONAL INVESTIGADOR: Raquel Martín Hernández	Contratada	Compartidia	Investigadora INCRECYT en CIAPA

OBJETIVOS

1. Establecer directrices para establecer buenas prácticas de apicultura (GBP), armonizadas dentro de los países socios en el proyecto.
2. Establecer directrices sobre métodos de diagnóstico de laboratorio innovadores, armonizados entre los socios del proyecto, con la colaboración del Laboratorio de Referencia de la Unión Europea para la Salud de las Abejas (ANSES).
3. Establecer directrices sostenibles para el control de las enfermedades de las abejas melíferas con respecto al bienestar de las abejas y la calidad de los productos de la colmena.
4. Realizar un estudio económico sobre el impacto de la aplicación de las medidas innovadoras del sistema GBP.
5. Difusión de resultados y asistencia técnica / capacitación, que se beneficiarán de la participación transnacional de Apimondia (http://apimondia.com/) y la plataforma FAO TECA (http://teca.fao.org/)

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y el lanzamiento de una aplicación web gratuita para actuar como un sistema dinámico de vigilancia del estado de salud de las colonias y una capacitación y capacitación continua para los apicultores.

Objetivos suproyecto CIAPA:

WP 3 - "Nosema research". Socio 4. La nosemosis es la patología más común que afecta a las abejas melíferas adultas y está asociada a una vida útil reducida y a la mortalidad invernal. El WP 3 del proyecto BPRACTICES desarrollará nuevas GBPs para prevenir y controlar la enfermedad a nivel del colmenar con el objetivo de mejorar el desarrollo y reducir la infección de la colonia. Estas GBP incluirán: muestreos y cuantificación de esporas de Nosema spp en abejas melíferas adultas; análisis de laboratorio (recuentos de microscopía óptica o PCR); tratamientos con compuestos orgánicos (p. ej., extractos de plantas) así como establecer futuras líneas de investigación.

RESULTADOS FINALES

The project has been carried out following the guidelines set out in the scientific report. In our case, we lead WP3 and have participated in the planned shared tasks.

The definitive list of Good Beekeeping Practices - GBPs (general) and Biosecurity Measures in Beekeeping- BMBs (disease specific) for Nosema diseases.

http://www.izslt.it/bpractices/2019/12/31/good-beekeeping-practices-gbp-the-bpractices-guidelines)

The definitive list of harmonized laboratory methods for Nosema detection.

Due to the absence of specific clinical signs, a proper laboratory diagnosis should be made by determining the presence of spores and therefore confirming the infection. One of the most used methods to confirm the presence of spores is by microscopy. This analysis should be done on the older bees in a colony, since this is the most infected population. So, collect forager bees at the hive entrance (or adult bees from a frame with no brood when foragers are not available), and at least analyse 60 bees (to detect 5% of sick bees with 95% confidence, Fries, 1993). Take whole abdomens or the digestive tract and macerate them in water. Examine the solution on a slide under a cover (x 400 magnifications) in a light field or preferably in a phase contrast microscope (Cantwell, 1970). Spores are refractory, with a well-defined dark edge. The spores of *N. ceranae* are smaller than those of *N. apis* which are oval. Fluorescence analysis has been also proposed to detect Nosema spp. spores (Snow 2016). However, mixed infections are frequent in colonies, and differentiating both species might be difficult. To confirm Nosema species use molecular tools as PCR, RT-PCR, or transmission electron microscopy.

A review of the best low environmental impact methods for Nosema control.

After confirmation of nosemosis, the health status of the bee colony should then be evaluated (if there is a normal and correctly structured population and which clinical signs are present) and the period of the year in which the infection has been detected. The prognosis is different according to the moment when the infection has been detected and for the same level of bees infected, the prognosis is worse when detected during autumn-winter, to one detected during spring or summer. In the wintering period, the colony has no capacity to raise new bees to compensate those bees lost because the infection. On the contrary during the productive period, the colony is able to compensate for the premature death of infected bees by raising new young bees that balance the colony (maintaining colony homeostasis).For that reason, In the case of weak colonies in autumn and winter,

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it would be necessary to apply a pro-duct that prevents the percentages of parasitized bees continue to increase during the winter brood stop, which would cause their collapse during the winter or at the beginning of the following spring. However, when the parasite is detected in spring or summer, it would be more convenient to enhance the growth of the bee colony through appropriate beekeeping techniques, and then, after the end of the productive period, perform the application of any of the products available in the market to ensure the maintenance of low parasitic percentages (below 40%) during wintering. Consequently, the application of a treatment, is therefore essential before the winter stop or at the end of the winter. Spring treatments should only be applied if the colony shows obvious symptoms of depopulation and weakness. Regarding the beekeeping practices that should be applied in the apiaries, we would highlight the annual or biannual renewal of queens, avoid the nutritional deficiencies of the colony (use of food of known composition and free of pathogens), annual renewal of wax from brood combs (if possible with pesticide-free wax), cleaning and disinfection of beekeeping material and beehives, as well as proper location of the hives.

Laboratory analysis guidelines. Interlaboratory comparison (ILC)/Test performance study (TPS), "ring trial"

The ILC conducted in the frame of BPRACTICES was designed as TPS for the molecular detection of *P. larvae* and M. plutonius. The aim was to estimate the diagnostic sensitivity/specificity of different methods for detecting *P. larvae/M. plutonius* from debris, with the main focus on sensitivity. Six partner laboratories participated in the TPS: IZSLT, CIAPA, AIS, NKU, AGES, and EU-RL.

Method	Estimation	Confidence interval
Real-time PCR Dainat *M. plutonius*	97.6%	[86.6%, 100.0%]
Real-time PCR Dainat *P. larvae*	92.9%	[80.3%, 98.2%]
PCR Bakonyi	92.9%	[80.3%, 98.2%]
PCR Govan	83.3%	[69.1%, 92.0%]
PCR Kilwinski	97.6%	[86.6%, 100.0%]
Real-time PCR Roetschi	97.6%	[86.6%, 100.0%]

The harmonized laboratory analysis guidelines as output of the Ring Tests results

1.Performance study for Hive debris (ring tests) to diagnosis *Aethina tumida* (SHB) and *K. ohmeri.*

a) *Aethina tumida* results: 97,0% general sensibility and a 84,4% general specificity.
b) *K. omeri* results: 100% general sensibility and a 92% general specificity.

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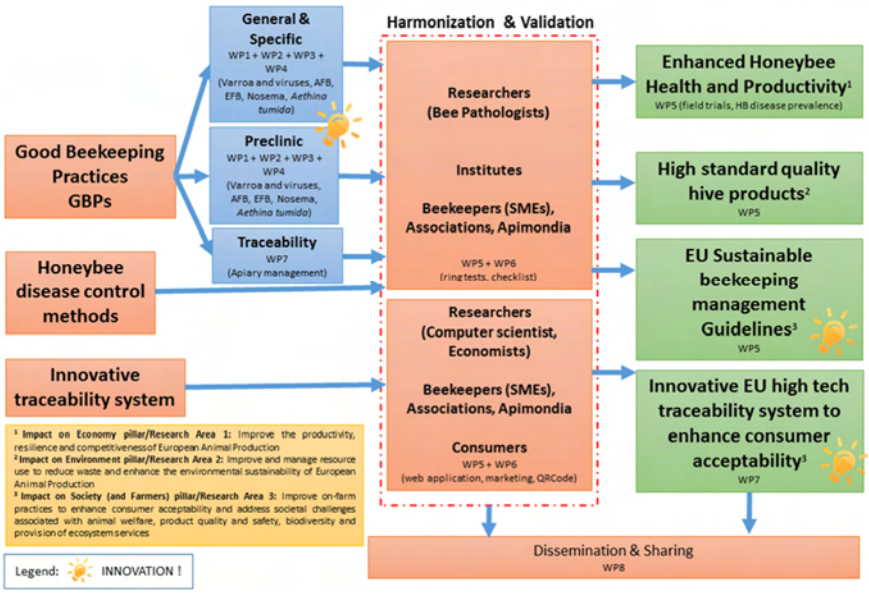

FORMACIÓN DE PERSONAL EN RELACIÓN AL PROYECTO.

No se han desarrollado Tesis Doctorales en relación al proyecto.
La becaria predoctoral María Benito Murcia ha colaborado en algunas tareas del presente proyecto, concretamente en las pruebas de PCR y qPCR, así como en el manejo de métodos y reactivos relacionados con los trabajos de laboratorio.

INFORMACIÓN CIENTÍFICA Y TÉCNICA PROPORCIONADA POR EL PROYECTO.

Artículos científicos:
1.- Good Beekeeping Practices (GBPs) and disease prevention, in "Apimondia. Working for the benefit of bees and apiculture". World Bee (May, 2018) , Rivera-Gomis j., Bubnic J., Cersini A., Chabert M., Chauzat MP., Eggenhoeffner R., Erat S., Gregorc A., Haefeker W., Higes M., Jannoni-Sebastianini R., Lietaer C., McCabe P., Moosbeckhofer R., Muz D., Necati Muz M., Ozdemir N., Pietropaoli M., Ravarotto L., Ribarits A., Riviere MP, Smodis Skerl M.I., Søgaard Jørgensen A., Formato G.

2.- Biosecurity measures in European beekeeping. Revue scientifique et technique (International Office of Epizootics), January 2020. M. Pietropaoli, A. Ribarits, R. Moosbeckhofer, H. Köglberger, O. Alber, A. Gregorc, M.I. Smodis Skerl, J. Presern, J. Bubnic, M. Necati Muz, M. Higes, B. Tiozzo, R. Jannoni-Sebastianini, J. Lubroth, J. Cazier, C. Lietaer, M. Bagni, R. Zilli & G. Formato.

Difusión de resultados:

1.- http://www.izslt.it/bpractices/dissemination/
2.-http://www.izslt.it/bpractices/2019/12/31/good-beekeeping-practices-gbp-the-bpractices-guidelines/

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3.- https://www.oie.int/fileadmin/Home/eng/Publications_%26_Documentation/docs/pdf/revue_plurithematique/2019/11122019-00160-EN_Rivera-Gomis-Formato_ANG.pdf

4.- Nouveaux indicateurs et pratiques apicoles en Europe pour améliorer la santé des abeilles mellifères dans le domaine de la recherche européenne à l'ère d'Aethina tumida. La Santè de l'Abeille. Maggio-Giugno n. 285 pag. 223-228. Della Marta U., Leto A., Pietropaoli M., Belardo V., Rivera-Gomis J., Cersini A., Chabert M., Chauzat MP, Eggenhoeffner R., Erat S., Gregorc A., Higes M., Moosbeckhofer R., Muz D., Necati Muz M., Ozdemir N., Ribarits A., Riviere MP, Vejsnæs F., Kilpinen O., Bagni M., Ravarotto L., Tiozzo B., Ruzza M., Smodis Skerl M., Lietaer C., Mccabe P., Jannoni-Sebastianini R., Haefeker W., Formato G.

5.- La proposta del progetto europeo B-PRACTICES. Argomenti. Numero 2/2018 pag. 66-69. Della Marta U., Leto A., Pietropaoli M., Belardo V., Rivera-Gomis J., Cersini A., Chabert M., Chauzat MP, Eggenhoeffner R., Erat S., Gregorc A., Higes M., Moosbeckhofer R., Muz D., Necati Muz M., Ozdemir N., Ribarits A., Riviere MP, Vejsnæs F., Kilpinen O., Bagni M., Ravarotto L., Tiozzo B., Ruzza M., Smodis Skerl M., Lietaer C., Mccabe P., Jannoni-Sebastianini R., Haefeker W., Crovato S., Mascarello G., Mantovani C., Formato G.

6.- Good Farming Practices in Apiculture (Good Beekeeping Practices GBPs). Proceedings of Honey Bee Health Symposium 2019, Rome, page 75 – 76. Bubnic, A. Ribarits, R. Moosbeckhofer O. Alber, P. Kozmus, R. Jannoni Sebastianini, W. Haefeker, H. Koeglberger, M. I. Smodis Skerl, B. Tiozzo, M. Pietropaoli, J. Lubroth, E. Raizman, C. Lietaer, R. Zilli, R. Eggenhoeffner, M. Higes, M. N. Muz, C. D'Ascenzi, M. P. Riviere, A. Gregorc, J. Cazier, E. Hassler, J. Wilkes, G. Formato.

7.- Biosecurity Measures in Beekeeping. Proceedings of Honey Bee Health Symposium 2019, page 76 – 77. R., Kozmus, P., Jannoni-Sebastianini, R., Haefeker, W., Koeglberger, H., Smodis Skerl, M. I., Tiozzo, B., Pietropaoli, M., Lubroth, J., Zilli, R, Eggenhoeffner, R., Higes, M., Muz, M. N., D'Ascenzi, C., Riviere, M. P., Chauzat, M. P., Gregorc, A., Formato, G.

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