1st ICECVM – Athens, Greece, 26th-27th Setembre 2019

PROJECT FIGHT 2

Development of an Edible Bait Vaccine to Control Rabbit Haemorrhagic Disease Virus 2 (RHDV2) in Wild Rabbits

Carvalho CL [1], Monteiro M[1], Carvalho P[1], Mendonça P[1], Correia J[2], São Brás B[2], Peleteiro C[2], Duarte E[3], Mira A[3], Branco S[3], Roldão A[4], Duarte MD[1,2]

[1]Instituto Nacional de Investigação Agrária e Veterinária (INIAV I.P.), Av. da República, Quinta do Marquês 2780-157 Oeiras, Portugal
[2]Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária (FMV-UTL), Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal
[3]Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Instituto de Formação e Investigação Avançada (IIFA), Universidade de Évora, (UÉ) Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal
[4]Instituto de Biologia Experimental e Tecnológica (iBET), Avenida da República, Estação Agronómica, 2780-157 Oeiras, Portugal

Context of the study

RHDV2, a ***Calicivirus*** of the genus ***Lagovirus***, causes **rabbit haemorrhagic disease (RHD)**, an often-lethal systemic infection in the European rabbit (***Oryctolagus cuniculus***)[1,2]. Since its emergence in 2010 in France[2], RHDV2 replaced the classical RHDV genogroups (G1-G6) that circulated previously[1,3,4]. Currently, RHDV2 is one main factor underlying the **wild rabbits' decline**, which is a key-stone species in the Mediterranean ecosystems of the Iberian Peninsula. RHDV2 affects adult and juvenile animals, hampering the recruitment of new individuals to wild populations compromising their dynamics, indirectly impacting on several **endangered predator species**[5].

RHD cannot be eradicated due to the high environment resistance of the virus and easy spread by insects, rodents, birds of prey or anthropogenic actions. Also, **disease control** is difficult despite in the industry vaccination, good management practices and biosecurity measures are effective[6].

Commercial RHDV2 vaccines currently available are **inactivated**, obtained from infected animal liver extracts and the route of administration is usually subcutaneous, requiring handling of the animals. Further than the risks associated with **incomplete virus inactivation** and the inadvertent release of infectious virus in the field, these vaccines are **not suitable for wild rabbits**, requiring capture for inoculation which causes great stress. The immunity induced by these vaccines is short and, hence, the protection transient. The previous commercial RHDV vaccines, most also inactivated, were shown to be **ineffective** in conferring cross protection against RHDV2[6].

Main objectives

FIGHT-TWO (PTDC/CVT-CVT/29062/2017) strategic framework is the development and **production of an edible and innocuous (pathogen- and genome-free) RHDV2 vaccine**, based in Virus-Like Particles (VLPs), to be distributed in the field as bait or in dry feed. This oral vaccine overcomes the need of capture and manipulation of the animals, unfeasible in wild populations, and will potentially protect a broad proportion of the rabbit populations, crucial to abrogate virus transmission leading to the control the infection.

VP60 (major capsid protein) -VLPs are protein cages that mimic the overall structure of the native virions harbouring **no genetic material**[7], although able to induce a protective immune response when administered parenterally[8] or orally[9]. The **oral immunogenicity** of VP60 in rabbits has been described more than two decades ago[9-13], however this strategy was never implemented due to cost/benefit ratios. Currently, VLP-based vaccine technologies have the potential of producing higher concentration of VLPs in a much-reduced time-frame. The VLP purification process required for rabbit immunization is expected to be simpler therefore **less expensive**[14,15]. The recombinant VP60 based-VLPs RHDV2-vaccine, will be **updated** according to the virus evolution in an progressive ***modular system***, as it is the case of Influenza vaccines[16] [**Figure 1**].

The National Institute of Agrarian and Veterinarian Diseases (INIAV I.P.) that harbours the Nacional Reference Laboratory for Animal Diseases, and the **Instituto de Biologia Experimental e Tecnológica (iBET),** a private institute with vast experience in animal and human vaccine production, coordinated the project. The direct partnership includes two Portuguese Veterinary Universities - **Universidade de Évora (UÉ) and Faculdade de Medicina Veterinária de Lisboa (FMV)**. Other institutions are considered indirect partners of the consortium. The project aims to mobilize several other layers of society including the hunting sector **[Figure 2**].

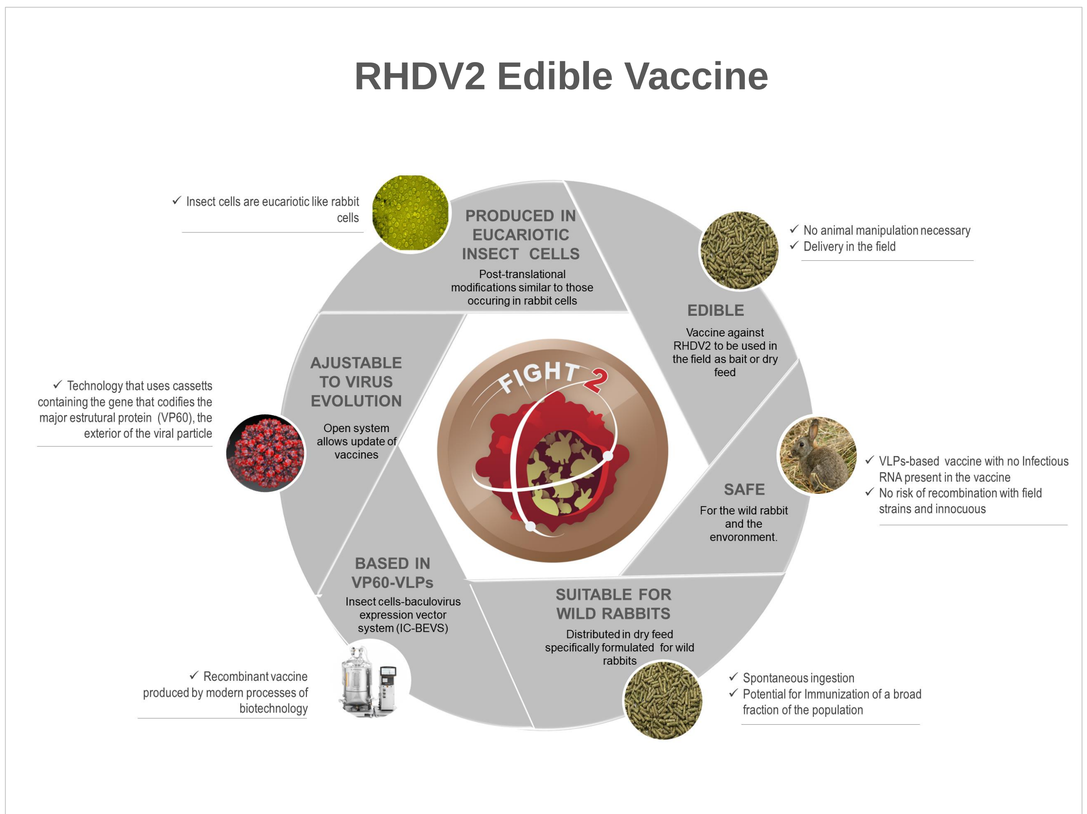
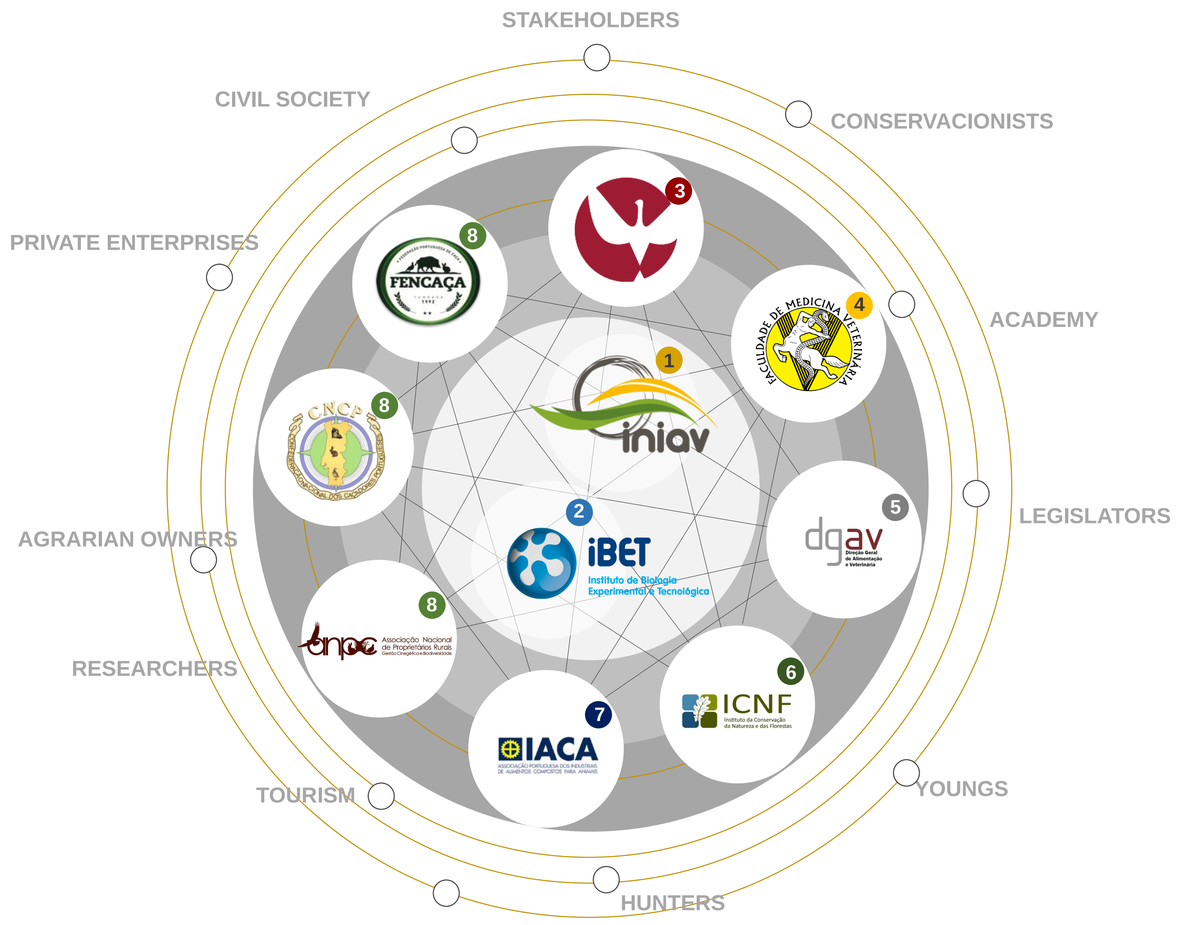


Figure 1. Characteristics of the VP60-VLPs based edible vaccine against RHDV2.



Direct Partners

1 INSTITUTO NACIONAL DE INVESTIGAÇÃO AGRÁRIA E VETERINÁRIA I.P. (INIAV)
National Reference Laboratory for Animal Diseases; Investigation supporting public policies

2 INSTITUTO DE BIOLOGIA EXPERIMENTAL E TECNOLÓGICA (IBET)
Experiência no Desenvolvimento de Vacinas Baseadas em VLPs

3 UNIVERSIDADE DE ÉVORA (UÉ)
Pathology and Animal Experimentation

4 FACULDADE DE MEDICINA VETERINÁRIA DE LISBOA (FMV-UTL)
Pathology and Animal Experimentation

Indirect Partners

5 DIREÇÃO GERAL DE VETERINÁRIA (DGAV)
National Veterinary and Sanitary Authority

6 INSTITUTO NACIONAL DE CONSERVAÇÃO DA NATUREZA (ICNF)
Nacional Authority for Nature's Conservation and Utilization of Hunting Resources

7 ASSOCAÇÃO PORTUGUESA DOS INDUSTRIAIS DE ALIMENTOS COMPOSTOS PARA ANIMAIS (IACA)
Develpment of dry feed specifically formulated for the wild rabbit

8 ORGANIZAÇÕES DO SETOR DA CAÇA (OSCS) DE 1º NÍVEL (FIRST LEVEL HUNTING ORGANIZATIONS - FENCAÇA, CNCP, ANCP)
Observers in the field, participants in trials; Vaccine users

Figure 2. Project Figth-two partnership.

Materials and Methods

The insect cells-baculovirus expression vector system (IC-BEVS) will be used to produce this novel vaccine.

Results and Conclusions

A nucleotide bank of RHDV2 *vp60* sequences is being obtained to support the selection of a subset of representative strains to be **included in the vaccine**. The *vp60* gene of those strains will be **cloned and used to construct the recombinant baculoviruses**.

FIGHT-TWO will allow to proceed with one of 12 measures specified in a **National Action Plan for the Control of Rabbit Haemorrhagic Viral Disease in Rabbits** (Dispatch 4757/17 of 31 May, Portuguese Ministry of Agriculture).

Project FIGHT-TWO supports other generalist management policies towards the **recovery of wild rabbit populations and RHD control**, the recovery of ecosystems where the rabbit is keystone and the reactivation of hunting activities in Portugal.

Funding: Project Fight-two (**PTDC/CVT-CVT/29062/2017**, PT2020), is financed by the **Portuguese Foundation for Science and Technology (FCT**).

Acknowledgments: Project **+Coelho1**: "*Avaliação Ecossanitária das Populações Naturais de Coelho-Bravo Visando o Controlo da Doença Hemorrágica Viral"* and Project **+Coelho2**: "*Desenvolvimento e implementação de medidas práticas impulsionadoras da recuperação dos leporídeos silvestres em Portugal"*, financed by the **Fundo Florestal Permanente (FFP), Portuguese Ministry of Agriculture**.

References:

[1]Le Gall-Reculé *et al.*, 2013. Vet. Res. 44.
[2]Le Gall-Reculé *et al.*, 2011. Vet. Rec. 168, 137–8.
[3]Lopes *et al.*, 2015. Viruses 7, 27–36. doi:10.3390/v7010027
[4]Mahar *et al.*, 2018. . J Virol. 2018 Jan 2;92(2). pii: e01374-17
[5]Delibes-Mateos *et al.*, 2014. Emerg. Infect. Dis. 20, 2166-8.
[6]Carvalho *et al.*, 2017. World Rabbit Sci. 25: 73-85.
[7]Crisci *et al.*, 2012. Vet Immunol Immunopathol. 148(3-4):211-25
[8]Müller *et al.*, 2019. Arch Virol. 164(1):137-148.
[9]Plana-Duran *et al.*, 1996. Arch Virol. 141(8):1423-36.
[10]Bárcena *et al.*, 2000. J Virol. 74(3):1114-23
[11]Torres *et al.*, 2000. Vaccine.19(2-3):174-82
[12]Martín-Alonso *et al.*, 2003. Transgenic Res. 12(1):127-30
[13]Gil *et al.*, 2006. Transgenic Res. 12(1):127-30
[14]Vicente *et al.*, 2011. J Invertebr Pathol. 2011 Jul;107 Suppl:S42-8
[15]Peixoto *et al.*, 2007. J Biotechnol. 10;127(3):452-61
[16]Sequeira *et al.*, 2017. Vaccine. pii: S0264-410X(17)30246-3