Analysis of the role of flies in the epidemiology of rabbit hemorrhagic disease virus *Lagovirus europaeus*/GI.2 in Portugal

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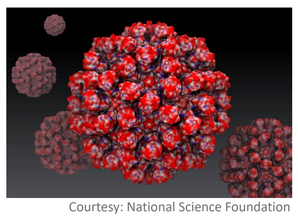
Rabbit hemorrhagic disease virus (RHDV)

- Lagovirus, family *Caliciviridae*
- RHDV2 with wider host range (e.g. *Lepus granatensis*)

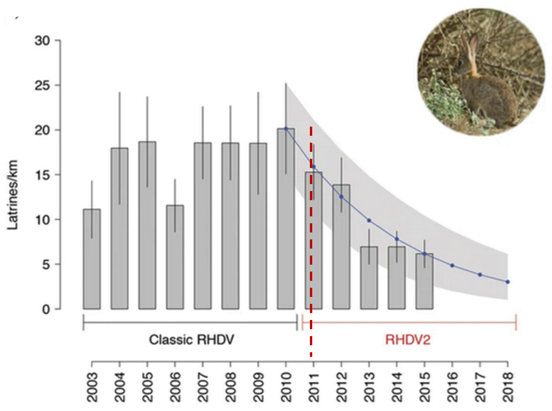
Velarde et al (2021) Transbound Emerg Dis



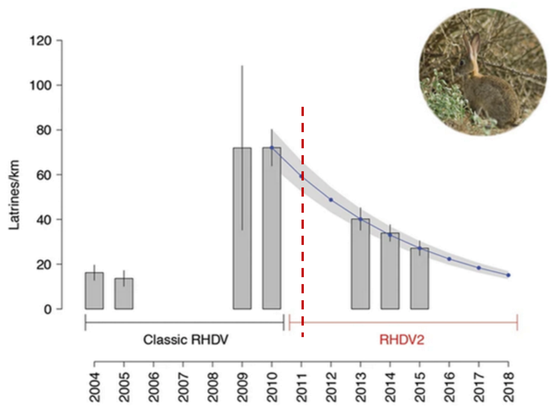
Courtesy: National Science Foundation

- The emergence of a new genotype, RHDV2, caused a huge decline in rabbit populations

Sierra de Andújar, Spain



Guadiana Valley, Portugal

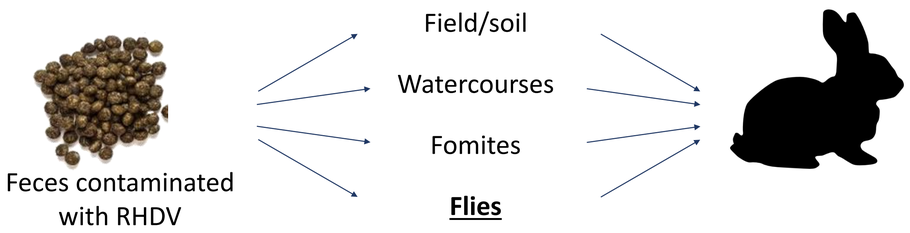


RDHV2 emergence in the Iberian Peninsula

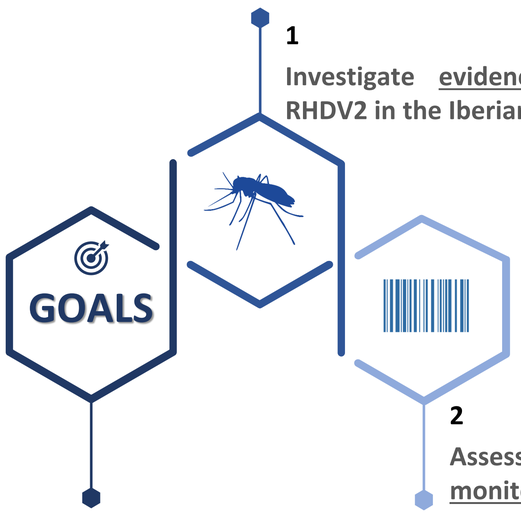
Monterroso et al (2016) Sci Rep

Rabbit hemorrhagic disease (RHD)

- Acute liver disease
- Caused by rabbit hemorrhagic disease virus (RHDV)
- Disease transmission: mainly **oro-fecal**, with **bushflies and blowflies** being known **vectors**. Mechanical transmission has been shown for carrion flies (Diptera: *Calliphoridae*) and *Aedes* mosquitoes (Diptera: *Culicidae*), among others.



Schematic representation of the oro-fecal transmission for RHDV



1

Investigate evidence of mechanical transmission of RHDV2 in the Iberian Peninsula

Especially important in the field:
- Low cost
- Easier sample collection – rabbit carcasses are frequently inaccessible (e.g. rapid removal by scavengers)

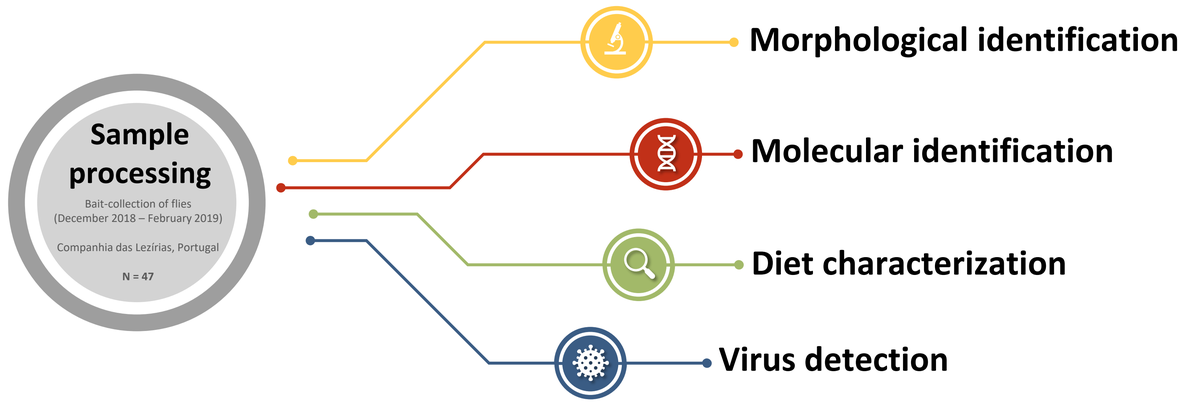
2

Assess the potential of the methodology as a tool for virus monitoring in wild populations from the Iberian Peninsula

Useful to prepare pools of insects: increased probability of detecting RHDV2

Take home messages

- **Molecular identification** complementary to the **morphological identification** (not shown)
- **Diet characterization**: large percentage of non-identified material. Improve PCR?
- **Virus detection**:
  - ✓ timeline coincident with our results
  - ✓ In Portugal, RHDV genetic material was found in other insect families: Ceratopogonidae, Staphylinidae and Simuliidae *Abade dos Santos (2018)*
  - ✓ Samples may not contain enough infectious viral particles to induce disease in susceptible rabbits *McColl et al (2002) Epidemiol Infect*
  - ✓ Increase sampling/testing and quantify viral particles

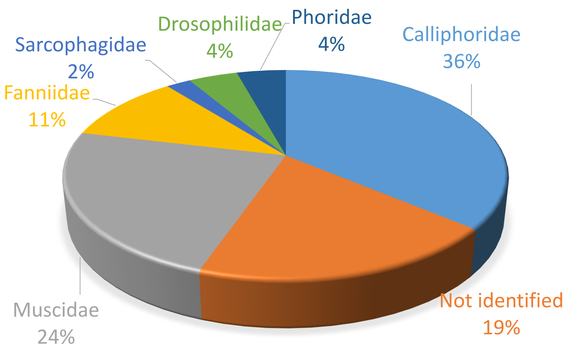


Molecular identification

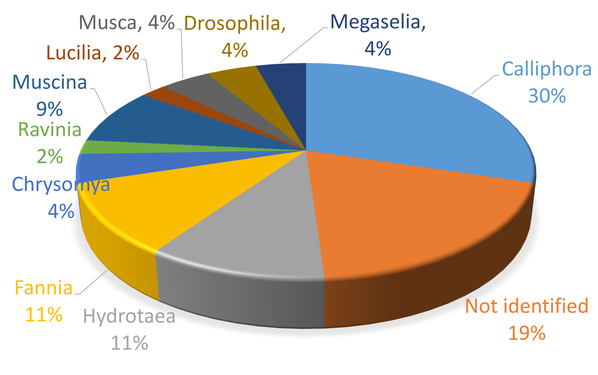
- DNA extraction
- PCR with COI universal primers LCO1490 and HCO2198

Folmer et al (1994) Mol Mar Biol Biotechnol

- Identification at the family level:



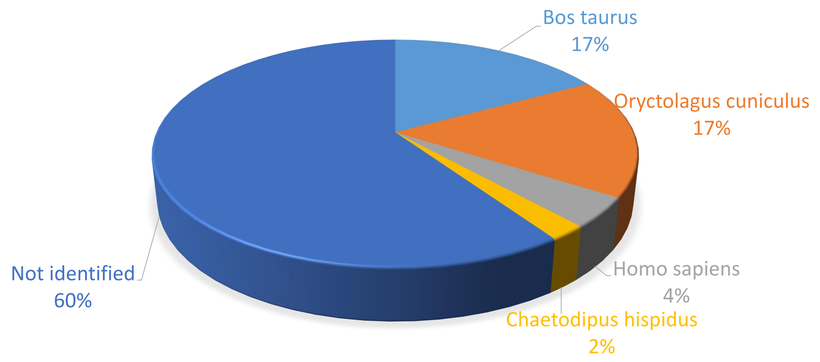
- Identification at the genus level:



Diet characterization

- DNA extraction
- PCR with CYTB universal primers cyt_bb1 and cyt_bb2

González et al (2015) Acta Tropica

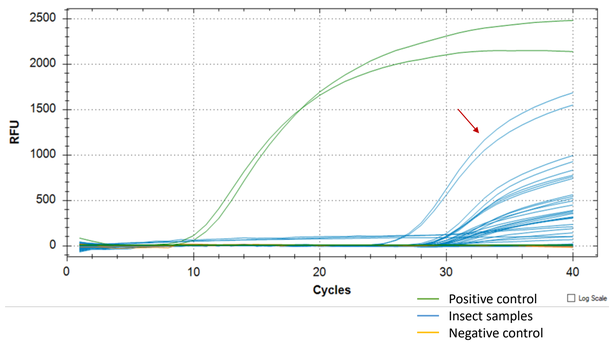


Virus detection

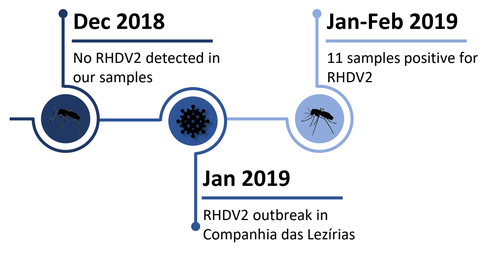
- RNA extraction
- RT-PCR with conserved primers (~300 bp) *Lopes et al (2013) Arch Virol*

No RHDV2 detected:
- **Low viral loads**?
- Degraded viral particles?
- Wrong species?

- qRT-PCR with conserved primers (~130 bp) *Duarte et al (2015) J Virol Methods*



RHDV2 detected:
- **More sensitive PCR**
- One sample with **Ct = 23**
  - ✓ Muscidae
- 10 samples with 26 < Ct < 30
  - ✓ Calliphoridae
  - ✓ Fanniidae
  - ✓ Muscidae
  - ✓ Drosophilidae



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