Veterinary Microbiology 198 (2017) 43–50

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Challenges in the rabbit haemorrhagic disease 2 (RHDV2) molecular diagnosis of vaccinated rabbits

C.L. Carvalho[a], E.L. Duarte[a,b], M. Monteiro[c], A. Botelho[c], T. Albuquerque[c], M. Fevereiro[c], A.M. Henriques[c], S.S. Barros[c], Margarida Dias Duarte[c,*]

[a] Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Instituto de Investigação e Formação Avançada (IIFA), Universidade de Évora, Núcleo da Mitra, 7000, Évora, Portugal
[b] Departamento de Medicina Veterinária, Universidade de Évora, Núcleo da Mitra, 7000, Évora, Portugal
[c] Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal

ARTICLE INFO

Article history:
Received 3 April 2016
Received in revised form 29 November 2016
Accepted 3 December 2016

Keywords:
Rabbit haemorrhagic disease virus
RHDV2
RT-qPCR
RHDV2 vaccines
Vaccinated rabbits
Cq values
Molecular diagnosis

ABSTRACT

Molecular methods are fundamental tools for the diagnosis of viral infections. While interpretation of results is straightforward for unvaccinated animals, where positivity represents ongoing or past infections, the presence of vaccine virus in the tissues of recently vaccinated animals may mislead diagnosis.

In this study, we investigated the interference of RHDV2 vaccination in the results of a RT-qPCR for RHDV2 detection, and possible associations between mean Cq values of five animal groups differing in age, vaccination status and origin (domestic/wild).

Viral sequences from vaccinated rabbits that died of RHDV2 infection (n = 14) were compared with the sequences from the commercial vaccines used in those animals. Group Cq means were compared through Independent *t*-test and One-way ANOVA.

We proved that RHDV2 vaccine-RNA is not detected by the RT-qPCR as early as 15 days post-vaccination, an important fact in assisting results interpretation for diagnosis.

Cq values of vaccinated and non-vaccinated infected domestic adults showed a statistically significant difference ($p < 0.05$), demonstrating that vaccination-induced immunity reduces viral loads and delays disease progression. Contrarily, in vaccinated young rabbits higher viral loads were registered compared to non-vaccinated kittens. No significant variation ($p = 0.3824$) was observed between viral loads of non-vaccinated domestic and wild RHDV2-victimised rabbits. Although the reduced number of vaccinated young animals analysed hampered a robust statistical analysis, this occurrence suggests that passively acquired maternal antibodies may inhibit the active immune response to vaccination, delaying protection and favouring disease progression.

Our finding emphasises the importance of adapting kitten RHDV2 vaccination schedules to circumvent this interference phenomenon.

1. Introduction

Six years after its emergence in Europe, rabbit haemorrhagic disease virus 2 (RHDV2) continues to provoke severe economic losses in the industry, to cause great concerns on the conservation of diminished wild rabbit populations and dependent endangered carnivore species, and to affect deeply the cinegetic activity and tourism associated income of some countries.

RHDV2, reported for the first time in 2010 (Le Gall-Reculé et al., 2011a), is classified within the *Lagovirus* genus (Le Gall-Reculé et al., 2011a) along with the close genetically related RHDV, European brown hare syndrome virus (EBHSV) and non-pathogentic lagoviruses (Le Gall-Reculé et al., 2011b). Since its emergence in France (Le Gall-Reculé et al., 2011a), RHDV2 quickly spread throughout neighbouring European countries (Dalton et al., 2012; Abrantes et al., 2013; Le Gall-Reculé et al., 2013; Baily et al., 2014; Westcott et al., 2014)(information on the FLI, 10|21|2013), replacing the previously circulating classical strains (Lopes et al., 2015). RHDV2 was registered outside Europe in Australia (Hall et al., 2015).

* Corresponding author.
E-mail address: margarida.duarte@iniav.pt (M.D. Duarte).

http://dx.doi.org/10.1016/j.vetmic.2016.12.006
0378-1135/