PROCEEDINGS OF THE 11th WORLD RABBIT CONGRESS
Qingdao (China) - June 15-18, 2016
ISSN 2308-1910

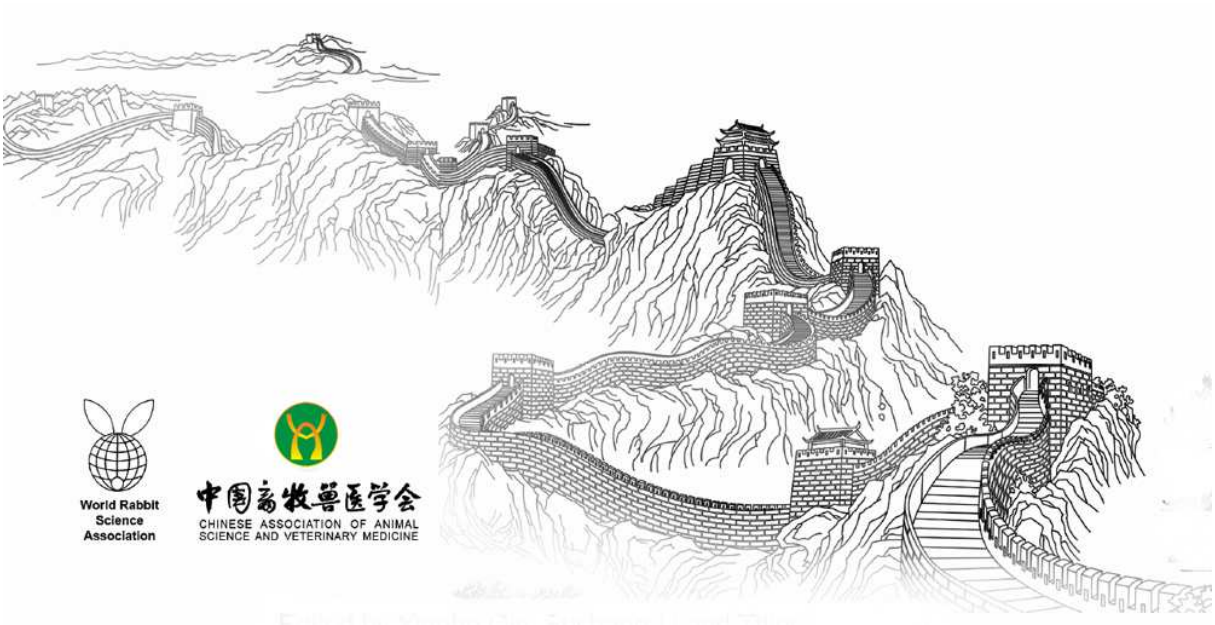
Session
Pathology and Hygiene

Valls L., Sánchez-Matamoros A., Padrell M., Maldonado J.

TEMPORAL EVOLUTION OF RABBIT HAEMORRHAGIC DISEASE VIRUS (RHDV) AND IMPACT OF VACCINATION DURING THE RHD EPIDEMIC IN SPAIN 2013-2015.

Full text of the communication

How to cite this paper :
Valls L., Sánchez-Matamoros A., Padrell M., Maldonado J., 2016 - Temporal evolution of rabbit haemorrhagic disease virus (RHDV) and impact of vaccination during the RHD epidemic in Spain 2013-2015. *Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 603-606.*



TEMPORAL EVOLUTION OF RABBIT HAEMORRHAGIC DISEASE VIRUS (RHDV) AND IMPACT OF VACCINATION DURING THE RHD EPIDEMIC IN SPAIN 2013-2015

Valls L.[1+], Sánchez-Matamoros A.[1+], Padrell M.[1+]*, Maldonado J.[1+]

[1]HIPRA, Av. de la Selva 135, 17170, Amer, Spain
[+]Equal contribution to this work
*Corresponding authors: maria.padrell@hipra.com

ABSTRACT

In 2011, the emergence in Spain of the new variant of the rabbit haemorrhagic disease virus (RHDV), named RHDV-2, sparked an emergency response that included an active surveillance program and the provisional licencing of RHDV-2 vaccines. Better knowledge of the virus epidemiology was needed, particularly when emergency vaccination was implemented because both classical and RHDV-2 strains of the virus were co-circulating in commercial rabbitries. Therefore, the authors evaluated the molecular epidemiology and temporal evolution of RHDV, along with the effect of vaccination as a control measure in Spain during 2013-2015. Tissue samples from suspected RHD cases were tested for RHDV by haemagglutination and RT-PCR. Positive samples were partially sequenced and compared to classical RHDV and RHDV-2 strains by phylogenetic analysis. Samples from 134 suspected cases occurring in 104 farms located in 17 Spanish provinces with a high density of commercial rabbit populations were analysed. A total of 65 cases were attributed to the RHDV-2 virus alone. A positive relation between the reduction of suspected cases and the implementation of control programs including RHDV-2 vaccination was observed. We observed a geographic and temporal correlation between outbreaks regardless of the implementation of RHDV-2 vaccination program. These results are consistent with the fast spread of the virus as previously described, as well as with the replacement of classical strains by the RHDV2 as the main aetiological agent of RHD outbreaks. Finally, this study provides new insights into the role of vaccination in the control of the RHD in commercial rabbit farms.

Key words: RHD, RHDV-2, phylogenetic analysis, control, RHD vaccine

INTRODUCTION

Rabbit haemorrhagic disease (RHD) is a fatal and highly infectious disease of the European rabbit (*Oryctolagus cuniculus*), first documented in China in 1984 (Liu *et al*., 1984). It causes high mortality in rabbits older than 6-8 weeks, and it is currently endemic in Europe where it causes large economic losses in commercial rabbitries. The development of efficient commercial vaccines in the late 1980s made effective control of the disease possible. However, in 2010 atypical outbreaks of the disease causing mortality at all ages were reported in France (Le Gall-Reculé *et al*., 2011) and soon after in Spain, Italy, Portugal, Germany and in the United Kingdom (Dalton, 2015). The aetiological agent was isolated and characterized as genetically and antigenically different from the classical strains, and it was named RHDV-2 (Dalton *et al*., 2012; Le Gall-Reculé *et al*., 2013).

Since 2011, many RHDV-2 outbreaks were reported in Spain, causing important losses in commercial rabbitries (Maldonado, 2012). Furthermore, classical RHD vaccines did not show complete cross-protection against the disease. In response, the Spanish authorities implemented a surveillance program (MAGRAMA, 2013 and 2014), and the Spanish Medicines Agency (AEMPS) provisionally authorised monovalent RHDV-2 vaccines such as Cunipravac RHDV-2 Variant (July 2014) to control the spread of the disease (AEMPS, 2014).

The aim of this study was to investigate the temporal evolution of RHDV and the impact of vaccination as a control measure during the RHD epidemic that occurred in Spain between 2013 and 2015.

MATERIAL AND METHODS

This study examines two different periods in RHDV-2 control, before and after HIPRA vaccine authorization (AEMPS, 2014). The pre-authorisation period covers the months between October 2013 and July 2014; the post-authorization period is from July 2014 to August 2015. Samples were collected by veterinary practitioners from different rabbit producing areas in Spain and they were sent to the diagnostic laboratory at HIPRA headquarters (Diagnos, Amer, Girona, Spain). Each sample was collected from liver, spleen, lung and kidney tissue of one or more young and adult rabbits which had apparently been affected by RHDV.

Upon arrival, tissue samples were mechanically disrupted in PBS to get a 10% w/v homogenate. The presence of the RHDV was screened by haemagglutination (HA) (OIE, 2010). Samples with HA titres ≤1/160 were confirmed by an end-point RHD generic RT-PCR (OIE, 2010). Samples that were positive either in the HA or in the generic RT-PCR were recorded as RHDV positive. Further characterisation of positive specimens was accomplished by a specific RT-PCR targeting the VP60 gene (OIE members, Personal communication), followed by sequencing and phylogenetic analysis using Geneious Pro and Mega software's.

Comparisons were made with available RHDV sequences (Genbank accession numbers: Classical RHDV (AJ319594, FR823355, AJ535092, AM884395, EF558575, KP129400, X87607, JX886001, U54983, EF558578, EF558585, EU003579, EU003580, AF295785, GU373618, Z49271, DQ189078, AF402614); RHDVa variant (EU003578, DQ205345, KF270630, EU250330, EF558583) and RHDV-2 (HE800529, FR819781, HE800530, HE819400, HE800531, HE800532, KF442964, KM115714, KP090976, JX133161, KM979445, KP129396, KC741409, JQ929052, JQ627641, KP129398, KC345612)).

RESULTS AND DISCUSSION

A total of 134 samples from suspected RHDV outbreaks occurring in 104 commercial rabbit farms distributed across 17 Spanish provinces were analysed. Each farm sent 1 to 4 samples during the study period, which were collected from young and/or adult rabbits showing serious illness or sudden death. The distribution of suspected cases and the age of affected animals were as expected during the epidemic, since rabbits at all ages were reported to be susceptible to RHDV-2 infection (Le Gall-Reculé *et al*. 2011, Dalton *et al*., 2012) and RHDV-2 rapidly spreads over long distances (OIE, 2015).

The average number of samples per month was 5.15. Pre-vaccination samples were 8.3 per month but post-vaccination dropped to 3.5 samples per month. This substantial decrease seems to indicate that the use of vaccination targeting RHDV-2 as a preventive measure could have driven the reduction of RHD clinical signs and mortality, and hence the number of samples submitted. It is worth noting that, in comparison with classical RHD, clinical diagnosis of RHDV-2 at the beginning of the epidemic was more complex in terms of interpretation of gross lesions so laboratory confirmation of the presence of the disease was essential. This fact could also be a possible explanation for the reduction of sent samples in the post-vaccination period, although we encouraged veterinary practitioners to confirm suspected cases by laboratory analysis.

The HA detected 54 positive samples, whereas the RT-PCR detected 65, which represented an increase of 17%. This apparent higher sensitivity of the RT-PCR may be rooted in methodological limitations since the HA assay is highly dependent on assay conditions like incubation temperature, source of the erythrocytes, and the pH level (Capucci *et al*. 1996).

Positive samples represented a total of 48.5% of the received samples and belonged to animals of different ages. Despite the fact that the number of submissions decreased after the vaccine was authorized, no

differences between the percentage of positive samples pre- and post-vaccination were detected (Table 1). A plausible explanation for this observation is that veterinarians were actively submitting samples from cases that they considered to be caused by RHDV-2. This may also explain the reason why the percentage of positive samples post--authorisation in this study was higher than the percentage registered in the official surveillance program (MAGRAMA, 2013 and 2014), which clearly shows a reduction in RHDV-2 cases after the implementation of vaccination programs.

Table 1: Percentage of positive or negative samples per studied period

	Percentage of positive samples	Percentage of negative samples
All period	48,51	51,49
Pre-authorisation period	52,00	48,00
Post-authorisation period	44,07	55,93

Similar geographic distribution of positive cases pre- and post-authorisation period was observed. Samples came from Lleida, Teruel, Barcelona, Girona, Tarragona, Castellón, Segovia, Burgos, Pontevedra and Valencia. These provinces are located in the most important areas for rabbit production in Spain, which represent 80.1% of the Spanish rabbit production (MAGRAMA, 2015).

The analysis of the VP60 sequences from newly generated and previously available RHDV-2 described in Spain, France, Italy, and Portugal, revealed nucleotide similarities in the range of 93.9-98.9%. On the other hand, the comparison with sequences from described genogroups of classic RHDV strains (G1-G5) showed similarities in the range of 79.4-82.0% for G1, 80.1-83.1% for G2, 79.3-82.2% for RHDVa, and 80.1-82.6% for G3-G5.

On the basis of branch grouping on the phylogenetic tree, it was confirmed that all viruses detected in this study belonged to the RHDV-2 cluster, and that all but one (47183/Spain.Bar/2013) were closely related to each other. The phylogenetic tree also showed a close relationship among almost all the analysed viruses and Spanish and Portuguese RHDV-2 isolates, while French and Italian RHDV-2 isolates showed the highest divergence. This could also be an indication of geographic association among outbreaks. On the other hand, strain 47183/Spain.Bar/2013 diverged from the others between 6.4% and 4.5% in nucleotide composition, and it was more closely related to the French isolates (2.6% divergence in average). This could be associated with live animal importations or geographic location, as detailed in Dalton *et al.* 2014 for other strains coming from Gerona province. Finally, we did not detect temporal clusters in the phylogenetic tree. However, a temporal divergence of the RHDV-2 isolates has been observed between the studied viruses (2013 to 2015) and the Spanish viruses (2011 to 2012) (data not shown). Nevertheless, further studies are needed to confirm these observations.

In terms of the limitations of this study, one concern is the small number of samples tested, since it was a passive diagnostic exercise rather than a comprehensive surveillance program. However, as described above, samples came from the main rabbit production areas in Spain which effectively represent the national rabbit industry. This approach is consistent with the sample distribution mentioned by other authors in similar studies (Dalton *et al*, 2014).

These results confirm that RHDV-2 was the aetiological agent of all RHD outbreaks in commercial rabbit farms in Spain from 2013 onwards. These data, along with other recent studies (MAGRAMA, 2014, Dalton *et al.* 2014) support the fast spread of the virus, and the displacement of classic RHDV strains by RHDV-2 strains in Spain and other regions of Europe (Le Gall-Reculé *et al.*, 2013, Lopes *et al.*, 2015a ; Lopes *et al.*, 2015b).

CONCLUSIONS

This study provides new insights into the displacement of the classical RHDV by the RHDV-2 variant, and the role of vaccination in the virus spread during the RHD epidemic in Spain. A geographical and temporal association has been observed regardless of the vaccination program that was established. It is necessary to

continue implementing surveillance and vaccination programs in the face of the need to control the epidemiological evolution and reduce the economic consequences of this disease.

ACKNOWLEDGEMENTS

The authors thank members of the OIE for providing us with the HA and RT-PCR protocols and DIAGNOS technicians for their technical assistance and dedication to this study.

REFERENCES

AEMPS. 2014. Spanish Medicines Agency, Resolución por la que se autoriza la utilización provisional de la vacuna contra la nueva variante de la enfermedad vírica hemorrágica del conejo, de acuerdo con el artículo 29 del Real Decreto 1246/2008, de 18 de Julio, por el que se regula el procedimiento de autorización, registro y farmacovigilancia de los medicamentos veterinarios fabricados industrialmente. Madrid, 10 July.

Capucci L., Chasey D., Lavazza A., Westcott D. 1996. Preliminary characterization of a non-haemagglutinating strain of rabbit haemorrhagic disease virus from the United Kingdom. *J. Vet. Med., 43, 245-250*.

Dalton K.P., Nicieza I., Balseiro A., Muguerza M.A., Rosell J.M., Casais R., Alvarez A.L., Parra F. 2012. Variant rabbit haemorrhagic disease virus in young rabbits, Spain. *Emerg. Infect. Dis., 18, 2009-2012*.

Dalton K.P., Nicieza I., Abrantes J., Esteves P.J., Parra F. 2014. Spread of new variant RHDV in domestic rabbits on the Iberian Peninsula. *Vet Microbiol., 21, 169(1-2), 67-73*.

Dalton K.P., Abrantes J., Lopes A.M., Nicieza I., Álvarez Á.L., Esteves P.J., Parra F. 2015. Complete genome sequence of two rabbit hemorrhagic disease virus variant b isolates detected on the Iberian Peninsula. *Arch. Virol., 160, 877-881*.

Le Gall-Reculé G., Zwingelstein F., Boucher S., Le Normand B., Plassiart G., Portejoie Y., Decors A., Bertagnoli S., Guérin J.L., Marchandeau S. 2011. Detection of a new variant of rabbit haemorrhagic disease virus in France. *Vet Rec., 168, 137-138*.

Le Gall-Reculé G., Lavazza A., Marchandeau S., Bertagnoli S., Zwingelstein F., Cavadini P., Martinelli N., Lombardi G., Guérin J.L., Lemaitre E., Decors A., Boucher S., Le Normand B., Capucci L. 2013. Emergence of a new lagovirus related to Rabbit Haemorrhagic Disease Virus. *Vet. Res., 44, 81*.

Liu S.J., Xue H.P., Pu B.Q., Qian N.H. 1984. A newviral disease in rabbits. *Anim. Husb. Vet. Med., 16, 253-255*.

Lopes A.M., Correia J., Abrantes J., Melo P., Ramada M., Magalhães M.J., Alves P.C., Esteves P.J. 2015a. Is the New Variant RHDV Replacing Genogroup 1 in Portuguese Wild Rabbit Populations? *Viruses, 7, 27-36*.

Lopes A.M., Dalton K.P., Magalhaes M.J., Parra F., Esteves P.J., Holmes E.C., Abrantes J. 2015b. Full genomic analysis of new variant rabbit hemorrhagic disease virus revealed multiple recombination events. J. Gen. Virol., 96, 1309-1319.

MAGRAMA. 2013. Spanish Ministry of Agriculture, "Informe de resultados del plan de vigilancia de la enfermedad hemorrágica del conejo".

MAGRAMA. 2014. Spanish Ministry of Agriculture, "Informe de resultados del plan de vigilancia 2014 de la mixomatosis y la enfermedad hemorrágica del conejo".

MAGRAMA. 2015. Spanish Ministry of Agriculture, "El sector de la carne de conejos en cifras. Principales indicadores económicos en 2014".

Maldonado J. 2012. Paradigms and new challenges in the diagnosis of the viral hemorrhagic disease of rabbits. 37th Symposium on Cuniculture, ASESCU. Barbastro, Spain, 24-25 May.

OIE. 2010. Terrestrial Manual, chapter 2.6.2. Rabbit haemorrhagic disease. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.06.02_RHD.pdf. Accessed 24 November 2015.

OIE. 2015. OIE Technical Disease Cards. Rabbit haemorrhagic disease. Last updated July 2015. http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/RHD.pdf

==========