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Vaccine breaks: Outbreaks of myxomatosis on Spanish commercial rabbit farms

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ABSTRACT

Despite the success of vaccination against myxoma virus, myxomatosis remains a problem on rabbit farms throughout Spain and Europe.

In this study we set out to evaluate possible causes of myxoma virus (MYXV) vaccine failures addressing key issues with regard to pathogen, vaccine and vaccination strategies. This was done by genetically characterising MYXV field isolates from farm outbreaks, selecting a representative strain for which to assay its virulence and measuring the protective capability of a commercial vaccine against this strain. Finally, we compare methods (route) of vaccine administration under farm conditions and evaluate immune response in vaccinated rabbits.

The data presented here show that the vaccine tested is capable of eliciting protection in rabbits that show high levels of seroconversion. However, the number of animals failing to seroconvert following subcutaneous vaccination may leave a large number of rabbits unprotected following vaccine administration.

Successful vaccination requires the strict implication of workable, planned, on farm programs. Following this, analysis to confirm seroconversion rates may be advisable. Factors such as the wild rabbit reservoir, control of biting insects and good hygienic practices must be taken into consideration to prevent vaccine failures from occurring.

1. Introduction

In Spain the European rabbit (*Oryctolagus cuniculus*) is of major importance, both as a keystone wildlife species (Delibes-Mateos et al., 2007) and agriculturally as a staple source of meat for the Mediterranean diet.

Myxoma virus (MYXV), a member of the *Poxviridae* family, genus *Leporipoxvirus* (King et al., 2012) is the cause of myxomatosis in the European rabbit (Fenner and Ratcliffe, 1965). Prevention of myxomatosis in rabbitries relies on good hygienic practices, insect control and vaccination (for review see Arthur and Louzis, 1988). Despite the success of vaccines (Arthur and Louzis, 1988), the disease is a recurrent problem on rabbit farms throughout Spain (Rosell, 2000, 2003) and in Europe (Farsang et al., 2003; Kritas et al., 2008; Marlier et al., 2000, 2001; Belsam et al., 2010). The potential causes of vaccine breaks may include problems with vaccine, changes in the pathogen, host and environmental factors (Knight-Jones et al., 2014). More specifically, vaccine problems may stem from quality control, such as ineffective batches or lots, lapses in correct handling procedures such as failure to maintain the cold chain, or application errors such as use of inappropriate doses or the improper execution of vaccine strategies. Changes with regard to the pathogen may include the emergence or introduction of new strains which evade protective immune responses generated by vaccination or variations in doses received. Other factors include genetic resistance and health status of rabbits, population densities and exposure rates. In this study we have analyzed possible problems in vaccine and pathogen factors with regard to vaccine failures characteristic of myxomatosis outbreaks on Spanish rabbit farms. We have addressed the problem by characterizing a representative circulating MYXV strain, testing the efficacy of a commercial vaccine against it and measuring the on-farm immune response after vaccination.

Traditionally, strains of MYXV circulating in wild rabbit populations have been characterized by their virulence in experimental infections (Fenner and Marshall, 1957; Barcena

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et al., 2000). Strains are classified into 5 virulence grades (1–5 or A–E, 1 (A) being most virulent and 5 (E) the most attenuated) based on the mean survival time of rabbits after infection (Fenner and Marshall, 1957). A study characterizing virulence levels in Spanish MYXV strains circulating in wild rabbits between 1992 and 1995 detected strains of all five virulence grades (Barcena et al., 2000), with the majority (15 of 20) being of the two most virulent grades.

Due to the large nature of the MYXV genome genetic characterisation of myxoma virus has relied on RFLP analysis of complete genomes (Russell and Robbins, 1989; Saint et al., 2001; Labudovic et al., 2004; Kerr et al., 2010), or sequencing individual polymorphisms, genes or gene fragments (Kerr et al., 2003; Alda et al., 2009; Muller et al., 2010). In the most recent advance next generation sequencing of whole genomes has been used to genetically characterize a number of European and Australian strains (Kerr et al., 2012, 2013a). Haplotypes and genome types have been described (Kerr et al., 2003; Alda et al., 2009) and are useful for tracking spread (Kerr et al., 2003) but the discrimination of mutations responsible for attenuation or differences observed in virulence grades is a complicated task (Kerr et al., 2012) and as yet is not possible. The characterization of the vaccine strains SG33, Borgi and Poxlap (Petit et al., 1996; Cavadini et al., 2010; Camus-Bouclainville et al., 2011) and targeted knockout MYXV vaccines (Barrett et al., 2007a,b; Adams et al., 2008) have helped identify potential virulence factors, the deletion or mutations of which may be responsible for their attenuated phenotypes. However, the situation appears more complex for naturally occurring strains with varying levels of virulence (Barcena et al., 2000; Morales et al., 2009; Dalton et al., 2010; Kerr et al., 2012) with apparently subtle changes responsible for phenotypic variations. Despite these advances, there is currently no way to define virulence grades based on sequence analysis alone and experimentation in rabbits is still required.

In this study we set out to evaluate vaccine efficiency against a MYXV field isolate. Firstly, by genetically characterizing MYXV field isolates from farm outbreaks and selecting a representative strain for which to assay its virulence. Using this strain to challenge vaccinated individuals we determined if a current vaccine is protective under laboratory conditions. Finally we compare methods (route) of vaccine administration under farm conditions and evaluate immune response in vaccinated rabbits.

2. Materials and methods

2.1. Samples

Table 1 shows a summary of the samples obtained from myxomatosis outbreaks. Eyelid or lung tissue was removed post-mortem by an on-site veterinary surgeon and sent directly to the laboratory. The year and month of sampling, province, isolate identification, vaccination policy of farm (i.e. whether or not farmer routinely vaccinates against MYXV) are shown. We analyzed multiple samples (between two and seven) from a small number of individual farms (4 farms). The sequences of all virus isolates from an individual farm resulted identical for a given mutation; we therefore chose to sequence one sample per farm as representative of the virus causing a particular outbreak. Samples were identified by letters representing the province of origin, the month and year of sampling and a number. If multiple samples were received from the same province in the same month the final number in the identification code was used to discriminate outbreaks/samples.

Table 1
Myxomatosis samples analyzed between 2008 and 2014.

Date	Province	Isolate identification	On-Farm vaccination[a]
Feb, 2014	La Rioja	LaR02/14-1	Yes
Jan, 2014	La Rioja	LaR01/14-1	Yes
Dec, 2013	Leon	Leo12/13-1	Yes
Oct, 2013	Segovia	Seg10/13-1	Yes
Oct, 2013	Barcelona	Bar10/13-1	Yes
Oct, 2013	Lleida	Ler10/13-1	No
Jul, 2013	Tarragona	Tar07/13-1	Yes
Mar, 2013	Huesca	Hue03/13-1	Yes
Oct, 2012	Soria	Sor10/12-1	No info.
Jul, 2012	Barcelona	Bar07/12-1	No
May, 2012	Barcelona	Bar05/12-1	No info.
May, 2012	Tarragona	Tar05/12-1	No info.
Feb, 2012	Navarra	Nav02/12-1	No
Feb, 2012	Guipúzcoa	Gip02/12-1	Yes
Jan, 2012	Guipúzcoa	Gip01/12-1	No info.
Dec, 2011	Navarra	Nav12/11-3	Yes
Dec, 2011	Navarra	Nav12/11-5	Yes
Oct, 2011	León	Leo10/11-2	Yes
Oct, 2011	Gerona	Ger10/11-1	Yes
Oct, 2011	Barcelona	Bar10/11-2	No
Oct, 2011	Barcelona	Bar10/11-3	No
Apr, 2011	León	Leo04/11-1	Yes
Jun, 2010	Albacete	Alb06/10-2	Yes
Jun, 2010	Albacete	Alb06/10-1	Yes
May, 2010	Valencia	Val05/10-1	Yes
Feb, 2010	Huesca	Hue02/10-1	Yes
Oct, 2009	Navarra	Nav10/09-1	Yes
Sep, 2009	Teruel	Ter09/09-1	Yes
Sep, 2009	Lleida	Ler09/09-1	Yes
Aug, 2009	Lleida	Ler08/09-1	No
Sep, 2009	Huesca	Hue09/09-2	Yes
Sep, 2009	Huesca	Hue09/09-1	Yes
Jul, 2009	Cuenca	Cue07/09-1	Yes
Jun, 2009	Tarragona	Tar06/09-2	Yes
Jun, 2009	Tarragona	Tar06/09-1	Yes
May, 2009	Albacete	Alb05/09-1	Yes
Mar, 2009	Portugal	Por03/09-1	Yes
May, 2009	Granada	Gran05/09	Yes
Apr, 2009	Soria	Sor04/09-4	Yes
Nov, 2008	Navarra	Nav11/08-1	Yes

[a] The column titled Farm vaccination indicates whether or not the rabbitry vaccinated as a routine measure of control against myxoma virus, regardless of the vaccination status of the animal from which the sample was taken.

2.2. Virus and cells

Myxoma virus isolates were generated and titered in RK13 cells in DMEM (Gibco, Carlsbad, CA) containing 10% foetal calf serum (FCS – previously PAA laboratories, UK, currently GE Healthcare, USA) and gentamycin (40 mg/L, Gibco, Carlsbad, CA). as described in Dalton et al. (2010).

The MYXV strain used in the virulence study was isolated from eyelid samples received from a rabbit farm in 2009. The isolate is identified by its region of origin, the month and year of isolation and a unique number for the particular outbreak – Granada May 2009-1 (Gran05/09-1).

2.2.1. Virus isolation

A tissue extract was prepared as follows: 10% w/v suspension of diced eyelid or lung tissue was prepared in PBS containing a penicillin /streptomycin mix (100 U/mL and 100 μg/mL, respectively) and frozen and thawed (−80 °C / 37 °C) three times. Extracts were clarified by centrifugation at 750×*g* for 10 min. Duplicate flasks of subconfluent RK13 cells were infected, one with filtered extract (0.22 μm) and one with non-filtered extract, using 1 mL tissue extract for 1 h at 37 °C. Following this incubation, the cell extract was removed and replaced with growth media. Mock-inoculated (media only) flasks of cells were used as negative controls. Cells were observed every 24 h for cytopathic effect for up