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## ARTICLE

## Disease Ecology

# RHDV2 outbreak reduces survival and juvenile recruitment, causing European rabbit population collapse

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**Abstract**

Infectious diseases can cause considerable mortality in vertebrate populations, especially when a new pathogen emerges. Quantifying the impact of diseases on wild populations and dissecting the underlying mechanisms requires longitudinal individual monitoring combining demographic and epidemiologic data. Such longitudinal population studies are rare. Rabbit hemorrhagic disease (RHD) is one of the main causes of the decline in European wild rabbit (*Oryctolagus cuniculus*) populations. A new genotype of RHD virus (RHDV), called RHDV2 or GI.2, emerged in 2010, posing a new threat to previously weakened populations, particularly as this virus can infect individuals already immune to classical RHDV strains. Taking advantage of intensive monitoring from 2009 to 2014 by physical captures and microchip detections of a semi-captive population of rabbits, we finely assessed the demographic impact of an initial RHDV2 outbreak that occurred in the population and identified the most affected demographic parameters. A multi-event modeling analysis revealed decreased survival in both juveniles and adults in 2011 and 2012, suggesting an RHDV2 outbreak for two consecutive years. The short-term survival benefit of vaccination against classical RHDV strains only during these years, and the recovery of carcasses with RHDV2 detection, supported this hypothesis. Variations in population vaccination coverage also explain the difference in adult survival between the two years of the outbreak. And the transient protective effect of vaccination could explain the prolonged duration of the outbreak. A brief episode of myxomatosis in 2011 seems to have had only a limited impact on the population. During outbreak years, in individuals not recently vaccinated, monthly juvenile survival crashed (0.55), and annual adult survival was three times lower than in normal years (0.21 vs. 0.69). The combination of successive juvenile and adult survival estimates for unvaccinated rabbits during the outbreak years resulted in a very low recruitment rate in the breeding population. Finally, RHDV2 outbreaks appear to have caused

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mortalities comparable to those caused by older classical RHDV strains and may have a strong demographic impact on wild populations of European rabbit. This work highlights the importance of long-term observational and experimental studies to better understand the impact of epidemics on animal populations.

#### KEYWORDS

disease outbreak, European rabbit, GI.2, lagovirus, multi-event capture–recapture, *Oryctolagus cuniculus*, passive integrated transponder, RHDV2, survival, vaccination, virus variant

## INTRODUCTION

Infectious disease can cause considerable mortality in animal populations, thus strongly regulating their size (Morand & Deter, 2009; Tompkins et al., 2002). This is particularly true for the emergence of a novel pathogen (Cunningham et al., 2017; Dobson & Foufopoulos, 2001). In this case, the affected species or population may suffer from an immune system not yet adapted to the emerging pathogen. As a result, hosts may be highly susceptible to infection by these new pathogens, and species or populations may decline sharply (Dobson & Foufopoulos, 2001). Estimating or predicting the impact of infectious diseases on hosts' population dynamics and demographic parameters, that is, survival and recruitment, is therefore crucial for wildlife management. However, detecting diseases in wild populations, distinguishing their effect from that of other environmental factors, and quantifying their demographic impact remain difficult tasks. In particular, chronic diseases may go undetected, whereas outbreaks are generally unpredictable in both time and space. This is why the impact of diseases has often been observed by chance in longitudinal population monitoring studies, even though this was not necessarily their primary objective (Gamble et al., 2020; Garnier et al., 2016; Groner et al., 2018; Jiménez-Ruiz et al., 2023; Pigeault et al., 2018; Pioz et al., 2007). Such longitudinal population studies combining demographic monitoring and pathology data are however rare, while they could greatly improve the understanding of the demographic mechanisms involved in the impact of diseases on wild populations. It is even more rare to observe the first impact of a new pathogen on a population.

The improvement of new monitoring technologies, in particular, radio frequency identification (RFID), now allows intensive or continuous population monitoring, which enables fine-scale studies of demographic processes without the need for frequent physical recaptures or direct observations of individuals (Bonter & Bridge, 2011; Gendner et al., 2005; Kletty et al., 2019). In

parallel, the development of multi-state and multi-event capture–recapture modeling allows studying transitions of individuals between different biological states (Pradel, 2005) while considering recapture probabilities and even uncertainty in the observation. Therefore, it is now possible to finely track and model survival and transitions between states and deal with potential sources of heterogeneity in recapture probabilities. This approach is particularly suited for the longitudinal studies necessary for quantifying the impact of an epidemic on demographic parameters (Conn & Cooch, 2009; Cooch et al., 2012). In particular, it can be used to assess probabilities of survival or of infection while accounting for previous disease, serological or vaccination states of individuals (Buzdugan et al., 2017; Chambert et al., 2012; Gamble et al., 2019; Groner et al., 2018; Guitton et al., 2008; Lachish et al., 2011; Mariën et al., 2018; Pigeault et al., 2018; Rossi et al., 2011; Santoro et al., 2014; Simon et al., 2018). It can also be used to assess the impact of an epizootic event during a particular period (Garnier et al., 2016; Jiménez-Ruiz et al., 2023; Lachish et al., 2007). However, intensive and fine-scaled longitudinal monitoring is generally lacking in most epidemiological studies to concurrently dissect the related demographic processes.

The European wild rabbit (*Oryctolagus cuniculus*) provides a good model for studying the impact of diseases, and in particular new pathogens, on vertebrate populations, and better understanding the underlying demographic mechanisms. It is both a game species for hunters and an important prey for many predators in Western Europe ecosystems (Delibes-Mateos et al., 2008). Myxomatosis and rabbit hemorrhagic disease (RHD) are two highly lethal viral diseases that may affect this species in the wild and cause severe population declines (Delibes-Mateos et al., 2009; Monterroso et al., 2016; Tablado et al., 2012). Myxomatosis was caused by the introduction of myxoma virus (MV) into wild populations of European rabbit in Australia and Europe in the early 1950s. MV, which causes benign skin tumors in

American cottontail rabbits (*Sylvilagus* spp.), its original hosts, first had very high case fatality rates in European rabbits before substantial declines in mortality due to subsequent host-pathogen coevolution (Alves et al., 2019; Kerr, 2012). RHD was first reported in China in 1984 (Abrantes et al., 2012; Cooke, 2002; Müller et al., 2021; Xu & Chen, 1989), before spreading and causing severe outbreaks in Europe and Australia (Chasey & Trout, 1995; Marchandeu et al., 1998; Mutze et al., 1998; Santoro et al., 2023; Villafuerte et al., 1995). The etiological agent of the disease, the classical RHD virus (RHDV) or GI.1 according to the nomenclature of *Lagovirus europaeus* proposed by Le Pendu et al. (2017), is an RNA virus belonging to the genus *Lagovirus* (Caliciviridae). A variant of RHDV, called RHDV2 or GI.2, genetically distant from RHDV but causing a similar disease, was first detected in France in 2010 (Le Gall-Reculé et al., 2011) and then rapidly spread across Europe, replacing almost entirely RHDV strains in both wild and domestic populations (Calvete et al., 2014; Dalton et al., 2014; Le Gall-Reculé et al., 2013). First data showed that the pathogenicity of RHDV and RHDV2 differed, notably in terms of disease duration and mortality rates, and that a partial cross-protection occurred (Calvete et al., 2018; Le Gall-Reculé et al., 2013; Müller et al., 2021; O'Connor et al., 2022). In particular, under laboratory experimental conditions, the reported mortality rate for RHDV was 70%–90% and 5%–70% for RHDV2 (Lavazza & Capucci, 2018). However, RHDV2 can overcome immunity in wild rabbits previously infected by RHDV (Peacock et al., 2017). Another major difference between these two virus variants is that rabbits younger than 8 weeks of age are highly resistant to disease caused by RHDV (Abrantes et al., 2012; Morisse et al., 1991; Xu & Chen, 1989), while RHDV2 is responsible for mortalities in young animals from the age of 11 days (Calvete et al., 2018; Dalton et al., 2012, 2014; Lavazza & Capucci, 2018; Le Gall-Reculé et al., 2013; Müller et al., 2021; Neave et al., 2018).

Rabbit populations are severely affected by viral diseases but the related demographic processes remain imperfectly known in the wild. This is particularly the case for RHDV2. Indeed, most of the knowledge comes from laboratory analysis of domestic and wild individuals. If significant numerical declines have occasionally been documented in the wild by comparing rabbit abundance before and after RHDV2 outbreaks (Delibes-Mateos et al., 2014; Monterroso et al., 2016; Mutze et al., 2018), the actual demographic impact of RHDV2 on rabbit populations has yet to be accurately assessed at the fine scale by longitudinal individual monitoring.

In this study, we take the opportunity of a first RHDV2 outbreak in an intensively monitored semi-captive rabbit population to assess its impact on individual survival and population dynamics. More specifically, we first

(1) investigated the evolution of monthly survival over the entire study period to determine the precise period and duration of the outbreak. Next (2), we assessed the effects of season, age class, sex and RHDV vaccination status on survival rates to clearly distinguish the impact of the outbreak from potential confounding effects.

## METHODS

### Study site and rabbit population

The study was carried out from March 2009 to April 2014 in a semi-natural population of European rabbit (*O. c. cuniculus*) living in a 0.2-ha experimental pen located in northern France (Appendix S1: Figure S1). The population was founded from 41 adult rabbits (15 ♂, 26 ♀) captured in wild populations and translocated into the pen in March and April 2009. When released, all founding individuals were vaccinated against MV and classical RHDV strains. The first juveniles born in the pen were caught in June 2009. To limit the maximum population size around 80 rabbits, we occasionally released outside the pen (translocation) a random sample of the newly captured individuals born in the pen (Appendix S1: Figure S2). Conversely, we reinforced the breeding stock from 2012 to 2014 by introducing 25 adults in the pen. The pen was divided into a common grazing area, four separate warren enclosures, and a small adjacent grazing area for each warren. Rabbits could access each warren enclosure from the common grazing area through small trapdoors, but not directly from one warren to another. The four warrens served as refuges and breeding sites. The fence of the pen prevented rabbit escape and terrestrial predator intrusion. In addition to natural grass, we provided ad libitum water and supplementary food.

### Rabbit population monitoring

Rabbits were captured on three or four sessions each year (Appendix S1: Table S1). However, some individuals were occasionally able to avoid capture. Births mainly occurred from February to May. Age at first capture of juveniles could be estimated on the basis of body mass up to 4 months, with rabbits considered adult from 1 kg (Marchandeu et al., 1995). Because juveniles cannot be captured until they are 15 days old, when they begin to emerge from the burrow, we considered the 1-month age class to range from 15 to 45 days, and so on. All rabbits were marked with small metallic numbered ear tags and, progressively from November 2009, most individuals

were also marked with passive integrated transponder (PIT) tags (134 kHz Half-duplex 23 mm RFID transponders) implanted subcutaneously. The procedure for implanting a PIT tag is quick and requires no anesthesia; the minimum body mass at PIT tagging was 290 g. This enabled individual monitoring by RFID automatic detections of PIT tags. We used two TIRIS S2000 readers connected with eight RFID antennas, which equipped each trapdoor between the common grazing area and the warrens. This equipment allowed identifying the PIT number of each rabbit and recording the date and time of its passage; the detection capacity was near two identifications per second and per antenna. We could thus monitor survival of rabbits. From May 2010, we performed automatic PIT detection sessions for at least a few consecutive days each month (except in November and December 2010 due to power shortage; Appendix S1: Table S9). We also conducted monthly inspections of the pen to look for any dead individuals. Dead animals were examined for macroscopic lesions and a liver sample was collected to confirm the presence of RHDV2 by reverse transcription-polymerase chain reaction (RT-PCR) analysis and to genotype the viral strain (Le Gall-Reculé et al., 2013).

## Vaccination

From the age of 2 months, rabbits were vaccinated at least once a year against MV and classical RHDV strains, provided they had been captured. Individuals were not systematically vaccinated against the two diseases at each capture, depending on the recommended booster frequency. They were often vaccinated twice a year, but may have received up to four injections a year in the epidemic phase (Appendix S1: Table S5). In general, each year, had been vaccinated at least once against MV and RHDV. However, the proportion of vaccinated individuals in the population had varied significantly from year to year. In particular, it fell below 55% in 2011 and 2013 for MV, and in 2012 and 2013 for RHDV, respectively (Appendix S1: Table S6 and Figure S3). Immunization against RHDV occurs within 1 week and lasts up to 15 months (Lavazza & Capucci, 2018). By contrast, the RHDV vaccine protection against RHDV2 seems much more limited (Le Gall-Reculé et al., 2011). Immunization against MV is active between 4 and 12 months, depending on the vaccine used (Kerr, 2012; Varga, 2014). We used commercial vaccines at doses recommended for domestic rabbits, according to their age class (Appendix S1). Only from April 2014, that is after the end of study period, we started to use the new vaccine against both RHDV and RHDV2 strains.

## Capture–mark–recapture data

We considered data from June 2009 to April 2014 (58 monthly intervals or sessions), that is after founding rabbits had begun to breed and before beginning to use the new vaccine against the RHDV2 strain. In this way, we avoid any confounding effect on survival due to the type of RHDV vaccine. Likewise, to avoid the mortality phenomena associated with arrival in a new environment (Letty et al., 2007), we excluded from the survival analysis the 11 founding adults no longer known to be alive after June 2009, and the 25 individuals introduced in the pen from 2012 to 2014. We also excluded 14 other individuals whose sex was not determined. Thus, the data used to model survival involved a total of 198 rabbits (97 ♂, 101 ♀), among which 152 were marked with a PIT tag (71 ♂, 81 ♀). Apart from the 30 founding adults still known to be alive in June 2009, the 168 other rabbits were born in the pen and captured as young or adult from 2009 to 2013. We combined data from physical captures and PIT detections. For the 20 months including a physical capture session, we only considered capture data, while we ruled out possible concomitant PIT detections. On the other hand, PIT detections were included in the dataset for the 31 months without physical capture session (Appendix S1: Table S9). This approach enabled us to model both the probability of capture or automatic detection and, in the event of physical capture, the possible transitions for individuals newly marked with a PIT tag or vaccinated against RHDV.

## Multi-event capture–mark–recapture modeling

We estimated monthly survival probabilities by performing analyses under the general framework of multi-event capture–mark–recapture models (MECMR) accounting for uncertainties about states of individuals, and for heterogeneity in capture rates (Pradel, 2005). MECMR are defined by their events (observations), their states and three kinds of parameters: initial state probabilities ( $I$ ) of individuals being in specific states when first encountered, conditional probabilities of state transition ( $\Phi$ ) of individuals between two successive occasions, survival being one of the transition parameters between states, and event probabilities ( $B$ ) of individuals being observed in different underlying biological states (Pradel, 2005). Specifically, events correspond to states in which individuals can be observed or captured, as some states may not be observable.

We performed a multi-state analysis of monthly MECMR data according to sex, age class, and individuals'

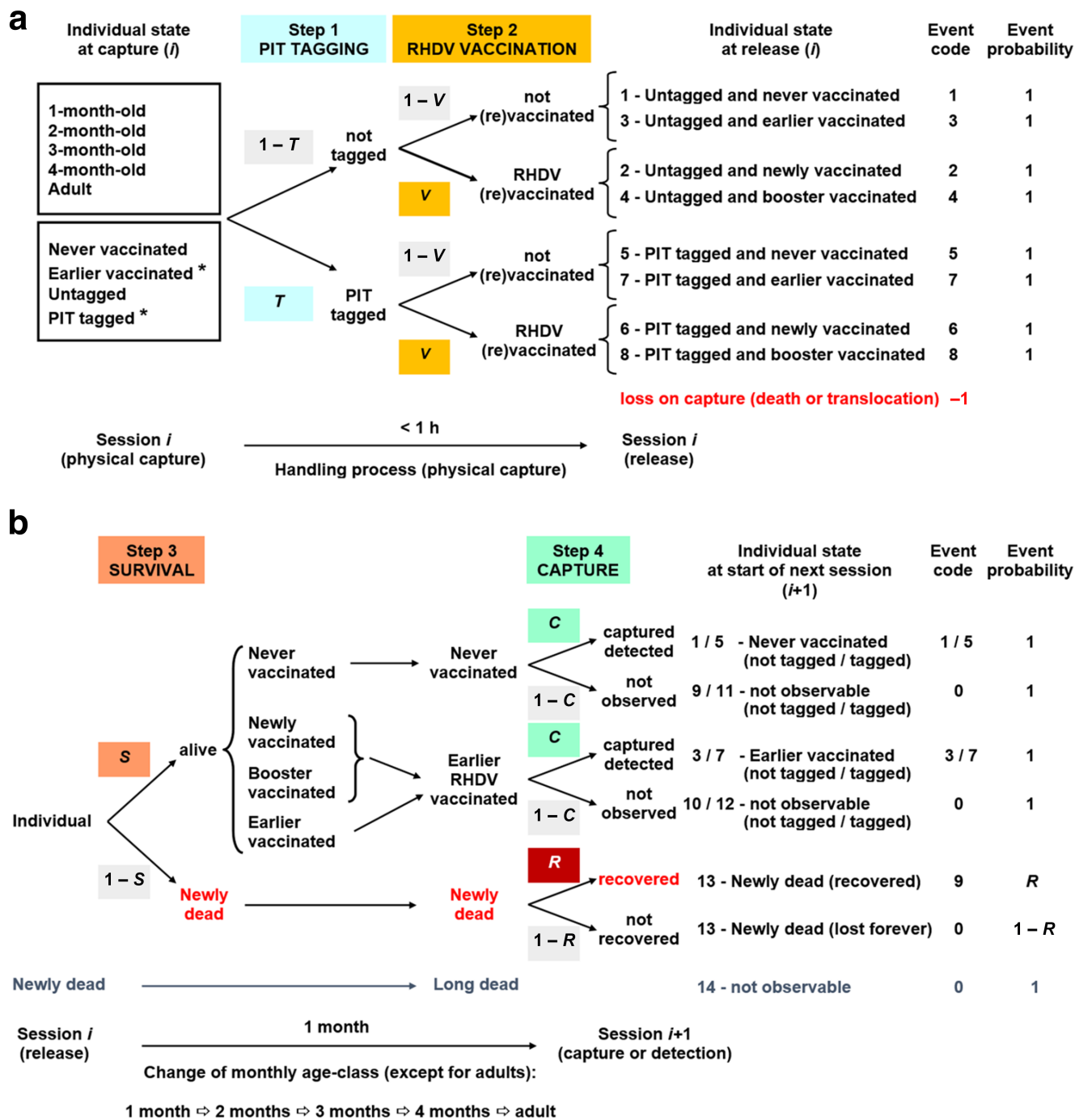


RHDV vaccination and PIT tagging status. We considered the state of each individual on the first day of each month. The initial state vector was modeled as constant with years, all rabbits being first encountered as unvaccinated and untagged (except founding rabbits vaccinated before June 2009). Thereafter, each individual could evolve among 14 possible states (Appendix S2) depending on its history (captured or detected/not captured or not detected, PIT tagged/not PIT tagged, never RHDV vaccinated/newly vaccinated/earlier vaccinated/booster vaccinated, alive/dead/long dead).

Concerning the transition from one state to another between consecutive monthly sessions, we divided it into four subsequent steps: conditional on physical capture on session  $i$ , (1) PIT tagging probability ( $T$ ), and (2) RHDV vaccination probability ( $V$ ), (3) monthly survival probability ( $S$ ) between sessions and, conditional on survival, (4) recapture (physical capture or PIT detection) probability ( $C$ ) on session  $i + 1$  (Appendix S2). That is, we modeled the whole physical capture process, including PIT tagging and RHDV vaccination, as a three-step transition (Figure 1). Individuals may have been already tagged or vaccinated, and may change state during the handling process between physical capture and release: permanent PIT tagging for untagged individuals, first or booster RHDV vaccination (depending on whether or not the individual has been previously vaccinated against RHDV). PIT tagging was considered as a non-reversible state change since there was no tag loss. We modeled the “newly vaccinated” and “booster vaccinated” states as transient states lasting only one month, with all these vaccinated individuals then switching to a permanent “earlier vaccinated” state (until a later booster vaccination). This assumption limits the complexity of the model, although the actual long-term immune status is likely to differ depending on whether the individual has received a first vaccination or a booster. However, we could test common or different effects on survival among the different vaccinated states: “never vaccinated,” “newly vaccinated” (first vaccination) and “booster vaccinated” (any later revaccination), both states being considered as “recently vaccinated,” and “earlier vaccinated” (for more than 1 month). Individuals not physically recaptured did not change their tag state (“untagged” or “tagged”) and could not be (re)vaccinated. In particular, this was the case during months without physical capture sessions (no monitoring or only PIT detection sessions). As dead rabbits could be recovered during the monitoring, we also considered the states “newly dead” and, for subsequent occasions, “long dead” (Choquet, 2008; Duriez et al., 2009; Juillet et al., 2011). In addition, we included sex and age as group covariates. The model used five monthly age classes (from 1 to 5 months and more).

Depending on their age at first capture, individuals change age class every month until they reach the last class. However, following preliminary analyses and given that rabbits can reach sexual maturity at 3 or 4 months old (Tablado et al., 2009), we considered that survival and capture parameters were identical between the first three age classes (juveniles) and the last two (subadults and adults), respectively. We therefore modeled a “juvenile” age group (Juv3) for individuals aged between 1 and 3 months, and an “adult” age group (Ad4) for individuals aged 4 months and over. It should also be noted that we did not simultaneously consider MV vaccination status, in order to limit the number of possible states and the complexity of the modeling. Furthermore, as we did not collect individual blood samples, the actual underlying MV and RHDV serological statuses of individuals remained uncertain and unknown, and we therefore did not model them.

We separately modeled physical recapture probability for months including physical capture sessions and detection probability for months including only PIT detection sessions, but we did not model together physical recapture and detection probabilities for months with both physical capture and PIT detection sessions. This solution appeared to be the best compromise in terms of modeling complexity. This was also possible because capture sessions were usually regularly spaced, alternating with detection sessions (Appendix S1: Table S9), and as both recapture and detection probabilities were expected to be high. In particular, a tagged individual that was not detected in a given month was most likely dead, and if it died without having been captured in a month with physical capture, its death would only appear as almost certain in the dataset in the next month with detection data, that is, a month later in most cases. Because the capture-detection process was modeled as a state transition, the single observational event probability remaining to assess was the recovery of newly dead individuals ( $R$ ). Events (observable states) during physical capture and PIT detection sessions were: (0) not encountered; (1) never vaccinated and untagged; (2) newly vaccinated and untagged; (3) earlier vaccinated and untagged; (4) booster vaccinated and untagged; (5) never vaccinated and PIT tagged; (6) newly vaccinated and PIT tagged; (7) earlier vaccinated and PIT tagged; (8) booster vaccinated and PIT tagged. Dead recovery (9) could only occur at capture or detection (Figure 1 and Appendix S2). A coding of  $(-1)$  was used at release in case of translocation or of loss on capture (in that case, the modeling of the capture history stops at the last non-trivial event). PIT tagging, RHDV vaccination, and recovery probabilities were modeled as constant regardless of time and individual categories because they depended only on study



**FIGURE 1** Fate diagrams illustrating conditional transitions between states and related events used in the multi-event models. State transitions related to the handling process during physical capture at session *i* (a): passive integrated transponder (PIT) tagging for untagged individuals ( $T$ ) and rabbit hemorrhagic disease virus (RHDV) vaccination or booster ( $V$ ); \* non-observable initial states (except for previously vaccinated founder rabbits). Monthly transitions from session *i* to session *i* + 1 (b): survival ( $S$ ) and capture or detection ( $C$ ) probabilities. PIT tagging and vaccination or booster are only possible at the time of physical capture. PIT tagging is a non-reversible state change. Rabbits just vaccinated for the first time or those just boosted move respectively into the “newly vaccinated” and “booster vaccinated” states for 1 month, before switching to the “earlier vaccinated” state (until a later booster vaccination). The vaccination and PIT tagging states of the individuals are known with certainty ( $p$  Event = 1), with only the probability of dead recovery ( $R$ ) of newly dead individuals to be estimated. After 1 month, newly dead individuals enter the definitive “long dead” state. The numbers refer to the coding of states and events used in the transition and event matrices (Appendix S2).

protocol. In particular, the recovery probability was considered as identical between types of monitoring occasion (physical capture and PIT detection sessions). We set as null the probabilities of PIT tagging and RHDV vaccination outside of physical captures, as well as the probabilities of capture, detection, and recovery for the 8 months without monitoring (Appendix S1: Table S9). Survival was modeled for all intervals.

## Goodness-of-fit tests

Formal goodness-of-fit (GOF) tests are not available for multi-event models (Pradel et al., 2003, 2005). Therefore, we used ad hoc procedures by running available GOF tests on partial recapture data alone. We used U-CARE 2.3.2 (Choquet, Lebreton, et al., 2009) to test the fit to the Cormack–Jolly–Seber (CJS) model that accounts only for time variation in parameters. For each analysis, we defined 10 groups according to age class at first capture and sex. The global GOF test did not indicate a lack of fit of the CJS model ( $\chi^2 = 35.1604$ ,  $p = 1$ ,  $df = 92$ ). There was neither transience nor trap-dependence effects in any of the classes of rabbits. Therefore, we did not use an overdispersion coefficient during the model selection.

## Model selection

We used the program E-SURGE V 2.0.2 for model selection and parameter estimation (Choquet & Nogué, 2011; Choquet, Rouan, & Pradel, 2009). We modeled survival and recapture probabilities according to age, sex and RHDV vaccination and PIT tagging state of each individual, but also to the effect of time (year, period of outbreak, and season). We grouped months into seasons to compare breeding (March–August) and overwinter (September–February) seasons. Because we had detected an outbreak of RHDV2 during the study, we looked for the best pattern of time accounting for an expected difference in survival among years or periods. Then we tested simplified time patterns by grouping calendar years or annual biological cycles (March–February of the following year) to compare the “standard” and “outbreak” periods. With the model combining the best age and time structures for recapture and survival probabilities, we focused analyses on factors that could affect juvenile and adult survival. In particular, to prove that an RHDV2 outbreak caused a survival decline, we looked for a positive short-term effect of RHDV vaccination on rabbit survival. Indeed, when RHDV2 emerged in 2010, the mortalities reported in vaccinated rabbits suggested that RHDV vaccination may give only partial protection against RHDV2

(Le Gall-Reculé et al., 2011). For this reason, we evaluated survival models including a protective effect of RHDV vaccination (initial or booster) limited to 1 month (vac-1mo). One may therefore expect a higher survival in recently vaccinated rabbits if an RHDV2 outbreak occurred. Beforehand, we also assessed models including other modalities of effect of RHDV vaccination on survival. We modeled the effect of RHDV vaccination on survival for each age group separately. As an episode of myxomatosis had occurred during autumn 2011, we specifically tested a long-lasting effect of MV vaccination on survival in 2011, by adding to the model a group covariate (vac-MV) indicating whether the individual had been vaccinated against MV a few months earlier. In parallel, we carried out a separate analysis using the same model structure but only data on MV vaccination status to test for a short-term protective effect of MV vaccination during the different periods.

We performed the model selection by using the Akaike information criterion, corrected for lack of fit and small sample size (QAIC<sub>c</sub>) (Burnham & Anderson, 2002). Models with the lowest QAIC<sub>c</sub> values and comprised within a range of  $\Delta\text{QAIC}_c \leq 2$  were considered as good candidates. As a last criterion between two competing models, following the principle of parsimony, we chose to select the model with the smallest number of parameters. In the case of nested models, we used the likelihood ratio test (LRT) for model selection (Lebreton et al., 1992).

## RESULTS

### Rabbit population dynamic and RHDV2 outbreak

From the foundation of the population in March–April 2009 with 41 rabbits, and despite the early disappearance of many of them (apparent or supposed mortality: 11 rabbits until June, and 26 in total in 2009), the growth of the population started rapidly. Indeed, 63 individuals were born in the pen through year 2009, and the breeding population reached around 60 adults in 2010 and 80 adults in early 2011. However, the adult population size steadily decreased thereafter, before stabilizing around 20 individuals from 2015 (Appendix S1: Figure S2). The population recruitment crashed at the same time. As an illustration, when including rabbits translocated out of the pen, the total number of newly captured individuals decreased significantly from 201 in 2010 to 39 in 2011. Thereafter, the juvenile recruitment remained low until 2017, except for two rebounds in 2012 and 2015. But the population did not recover from this decline, even after 2014, when the



new vaccine specifically adapted to RHDV2 strains started to be used. While we cannot fully explain the population dynamics, an outbreak of RHDV2 was suspected to be the cause of the decline that occurred approximately from early 2011 to 2013. In particular, we found five dead rabbits, including two juveniles less than 8 weeks old, in or near the pen between March and May 2011, whose necropsies revealed lesions suggestive of death caused by an RHD virus (Appendix S1: Table S7). Precisely, two of these rabbits were found in the pen: a three- or four-week-old juvenile never vaccinated and a two-year-old male for which the last RHDV vaccination occurred in January 2011. Molecular analysis (RT-PCR) confirmed the presence of RHDV2 in the livers of these individuals, a sign of viral multiplication and infection. In parallel, despite regular campaigns of vaccination against MV, we recorded in September 2011 three cases of myxomatosis in adults. Among them, a one-year old male vaccinated 6 months before was known to have subsequently recovered from the disease. The two other adults, newly captured, were never again known to be alive. This episode of myxomatosis in autumn 2011 actually coincided with a drop in the vaccination coverage of the population, since in 2011 MV vaccination was only carried out in March, and only for 50% of individuals known to be alive that year (Appendix S1: Figure S3).

### Physical recapture and PIT detection probabilities

Model selection led to a general recapture model combining physical recapture and PIT detection probabilities (Appendix S3: Table S1). This general recapture model was used for subsequent survival model selection. For months including physical capture sessions, the recapture probability depended on PIT tagging status (untagged vs. tagged), age (juveniles vs. adults), season (breeding vs. overwinter) and period (standard years [2009, 2010, 2014] vs. outbreak years [2011, 2012, 2013]). Overall, physical recapture estimates ranged from 0.11 to 0.99 for juveniles and from 0.40 to 0.99 for adults (Appendix S3: Figure S1). Parameters for recapture probability of untagged individuals could not be estimated outside the breeding season in standard years, probably due to the reduction in sample size as more and more rabbits were progressively PIT tagged. For months including only PIT detection sessions, the detection probability of PIT-tagged individuals was very high and depended on sex (females: 0.88 [0.85; 0.91]; males: 0.93 [0.90; 0.96]; Appendix S3: Figure S1). Estimates of average rates of PIT tagging and RHDV vaccination during physical capture sessions were 0.37 (0.33; 0.42) and 0.55

(0.52; 0.59), respectively. Additionally, the overall recovery rate for dead individuals was 0.21 (0.15; 0.28).

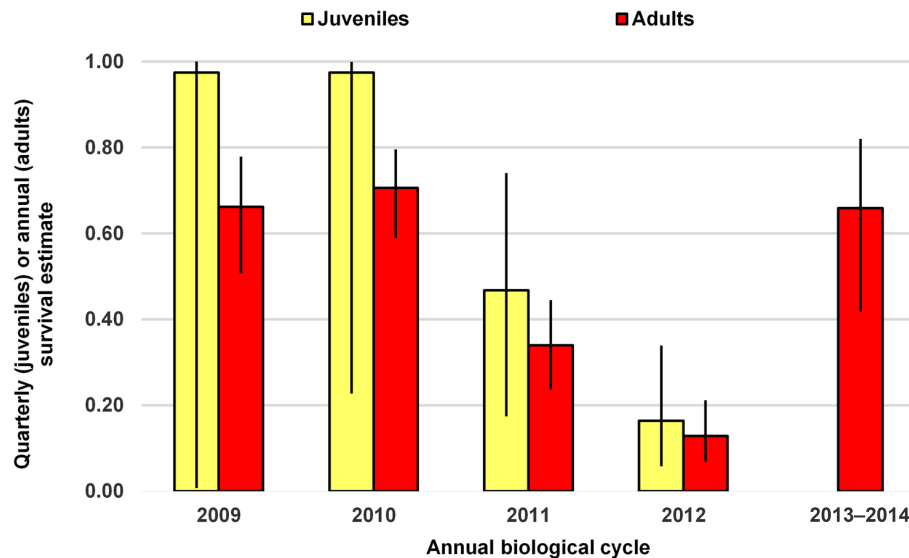
### Survival period pattern and related RHDV2 outbreak

We modeled survival on the basis of annual biological cycles, from March to February of the following year, as this fitted the data better than modeling calendar years (Appendix S4: Table S1). In a first step, we performed exploratory modeling to estimate survival in juveniles and adults per biological cycle. The results showed a sharp drop in juvenile and adult survival during the 2011 and 2012 biological cycles compared with the other cycles (Figure 2). The start of the decline in survival coincides with the detection of RHDV2 in the population. Furthermore, survival appeared to be lower in 2012 than in 2011.

These preliminary analyses suggested a two-period pattern, with an RHDV2 outbreak period spanning 2 years, from March 2011 to February 2013 (cycles 2011 and 2012), other cycles being considered as the standard period (2009, 2010, and 2013–2014). However, model selection led to a survival model including an interaction between a three-period pattern and age (Appendix S4: Table S1). That is, within the suspected RHDV2 outbreak period, survival proved to be significantly different between cycles 2011 and 2012. This difference in survival between cycles was concomitant with a drop in RHDV vaccination coverage in the population (Appendix S1: Figure S3).

### Demonstration of an occurrence of RHD outbreak

We detected a temporary positive effect of RHDV vaccination on survival in both age groups. In juveniles, the small and unbalanced numbers of individuals between the RHDV vaccinated and unvaccinated groups during the 2011 and 2012 cycles (Appendix S1: Table S1) did not allow a separate assessment of the effect of RHDV vaccination during each outbreak cycle. Among competing models, modeling an effect of RHDV vaccination limited to the two-year outbreak period appeared to be the most relevant and biologically supported (Table 1 and Appendix S4: Table S2). In adults, the best models included an effect of RHDV vaccination on survival in addition to the period (Table 1 and Appendix S4: Table S3). Precisely, the two-period model, when including a vaccination effect limited to the 2011 cycle, outperformed the three-period model. This indicates that



**FIGURE 2** Rabbit survival estimates for juveniles and adults per biological cycle (March to February of the following year). Quarterly estimates for juveniles and annual estimates for adults are extrapolated from the survival model “age · yearbio5” (Appendix S4: Table S1); 2013–2014: Data from March to April 2014 pooled with the 2013 cycle. Juvenile survival cannot be estimated for the 2013–2014 cycle, as no juveniles were monitored. CIs are indicated.

the difference in survival between the 2011 and 2012 outbreak cycles could be explained by the absence of a significant effect of RHDV vaccination in 2012.

In adults, modeling also revealed an additional difference in survival in 2011 between autumn, when the myxomatosis episode occurred, and other seasons. However, the model including a long-lasting effect of MV vaccination on adult survival in autumn 2011 was not retained, indicating similar survival between adult rabbits previously vaccinated against MV and those not (Appendix S4: Table S3 and Figure S1). In juveniles, the data did not allow us to assess the effect of MV vaccination on survival in 2011. Furthermore, we found no short-term effect of MV vaccination on survival, regardless of age group or time period (Appendix S4: Figure S2).

### Estimation of survival rates during the outbreak

According to the parameter estimates of the selected model, monthly survival appeared nearly maximal (0.99 [0.67; 1.00]) in juveniles during the standard period, irrespective of their RHDV vaccination status. However, during the outbreak period, monthly survival crashed to 0.55 (0.41; 0.68) in unvaccinated juveniles, whereas it was still nearly maximal (1.00 [NA; NA]) in those vaccinated against RHDV (Figure 3a). By calculating survival estimates over 3 months to match the duration of the juvenile stage, this would have resulted during the outbreak

period in a near-maximal survival (1.00 [NA; NA]) in RHDV-vaccinated juveniles and only a survival of 0.16 (0.07; 0.32) in unvaccinated ones. There was no effect of sex on juvenile survival.

In adults, excluding autumn, monthly survival in 2011 was 0.98 (0.93; 0.99) for those recently vaccinated against RHDV and 0.92 (0.88; 0.94) for those not recently vaccinated. Monthly survival was lower in autumn 2011: 0.96 (0.86; 0.99) for those recently vaccinated and 0.83 (0.74; 0.90) for those not. In 2012, monthly survival was 0.84 (0.80; 0.88) for all adults (Figure 3b). Averaged monthly survival in 2011 for those recently vaccinated against RHDV (0.98 [0.91; 0.99]) did not significantly differ from monthly survival in standard years for all adults, recently vaccinated or not (0.97 [0.96; 0.98]). Adult annual survival during the standard period calculated from monthly estimates was 0.68 (0.60; 0.75). In 2011, the first year of the outbreak, it was 0.74 (0.33; 0.92) for recently vaccinated adults, but only 0.26 (0.13; 0.42) for others. Annual survival then fell to 0.13 (0.07; 0.21) for all adults in 2012. Finally, considering the outbreak period as a whole, and modeling an average effect of RHDV vaccination over cycles 2011 and 2012, the annual survival during the outbreak period would have been 0.21 (0.15; 0.29) in adults not recently vaccinated. Compared with annual survival over the standard period for adults not recently vaccinated (0.69 [0.59; 0.76]), the RHDV2 outbreak appears to have led to a threefold reduction in adult survival (Figure 4).

**TABLE 1** Summary of model selection for survival probabilities of juvenile and adult rabbits.

Juvenile survival model (Juv3)		Adult survival model (Ad4)	<i>k</i>	Deviance	QAIC <sub>c</sub>
<b>outbio + yroutbio · vac-1mo</b>	<b>outbio + yroutbio1 · [vac-1mo + aut/others] + yrnormbio · seas</b>		<b>22</b>	<b>6231,61</b>	<b>6275,89</b>
outbio + vac-1mo	outbio + yroutbio1 · [vac-1mo + aut/others] + yrnormbio · seas		22	6231,29	<b>6275,56</b>
outbio · vac-1mo	outbio + yroutbio1 · [vac-1mo + aut/others] + yrnormbio · seas		23	6231,29	<b>6277,59</b>
outbio + yroutbio1 · vac-1mo	outbio + yroutbio1 · [vac-1mo + aut/others] + yrnormbio · seas		23	6231,61	<b>6277,91</b>
outbioB + yroutbio · vac-1mo	outbio + yroutbio1 · [vac-1mo + aut/others] + yrnormbio · seas		23	6231,61	<b>6277,91</b>
outbioB	outbio + yroutbio1 · [vac-1mo + aut/others] + yrnormbio · seas		22	6239,93	6284,20
outbio	outbio + yroutbio1 · [vac-1mo + aut/others] + yrnormbio · seas		21	6243,15	6285,40
vac-1mo	outbio + yroutbio1 · [vac-1mo + aut/others] + yrnormbio · seas		21	6270,56	6312,81
outbio + yroutbio · vac-1mo	outbio + yroutbio1 · [vac-1mo + aut/others] + yrnormbio · seas + sex		23	6228,01	<b>6274,31</b>
outbio + yroutbio · vac-1mo	outbio + yroutbio1 · [vac-1mo + autumn · vac-MV] + yrnormbio · seas		23	6231,00	<b>6277,30</b>
outbio + yroutbio · vac-1mo	outbio + yroutbio1 · vac-1mo · [aut/others] + yrnormbio · seas		23	6231,11	<b>6277,40</b>
outbio + yroutbio · vac-1mo	outbio + yroutbio1 · vac-1mo + yrnormbio · seas		21	6236,46	6278,71
outbio + yroutbio · vac-1mo	outbio + yroutbio1 [aut/others] + yrnormbio · seas		21	6239,77	6282,02
outbio + yroutbio · vac-1mo	outbio + yroutbio1 · vac-1mo		20	6242,22	6282,45
outbio + yroutbio · vac-1mo	outbio + yroutbio1 + vac-1mo + yrnormbio · seas		21	6241,70	6283,95
outbio + yroutbio · vac-1mo	outbioB		19	6249,01	6287,22
outbio + yroutbio · vac-1mo	outbioB + yroutbio · vac-1mo		20	6247,23	6287,46
outbio + yroutbio · vac-1mo	outbio + yroutbio · vac-1mo + yrnormbio · seas		20	6250,89	6291,11
outbio + yroutbio · vac-1mo	outbio		18	6258,91	6295,09
outbio + yroutbio · vac-1mo	outbio · vac-1mo		20	6256,61	6296,84
outbio + yroutbio · vac-1mo	vac-1mo · seas		20	6318,18	6358,41

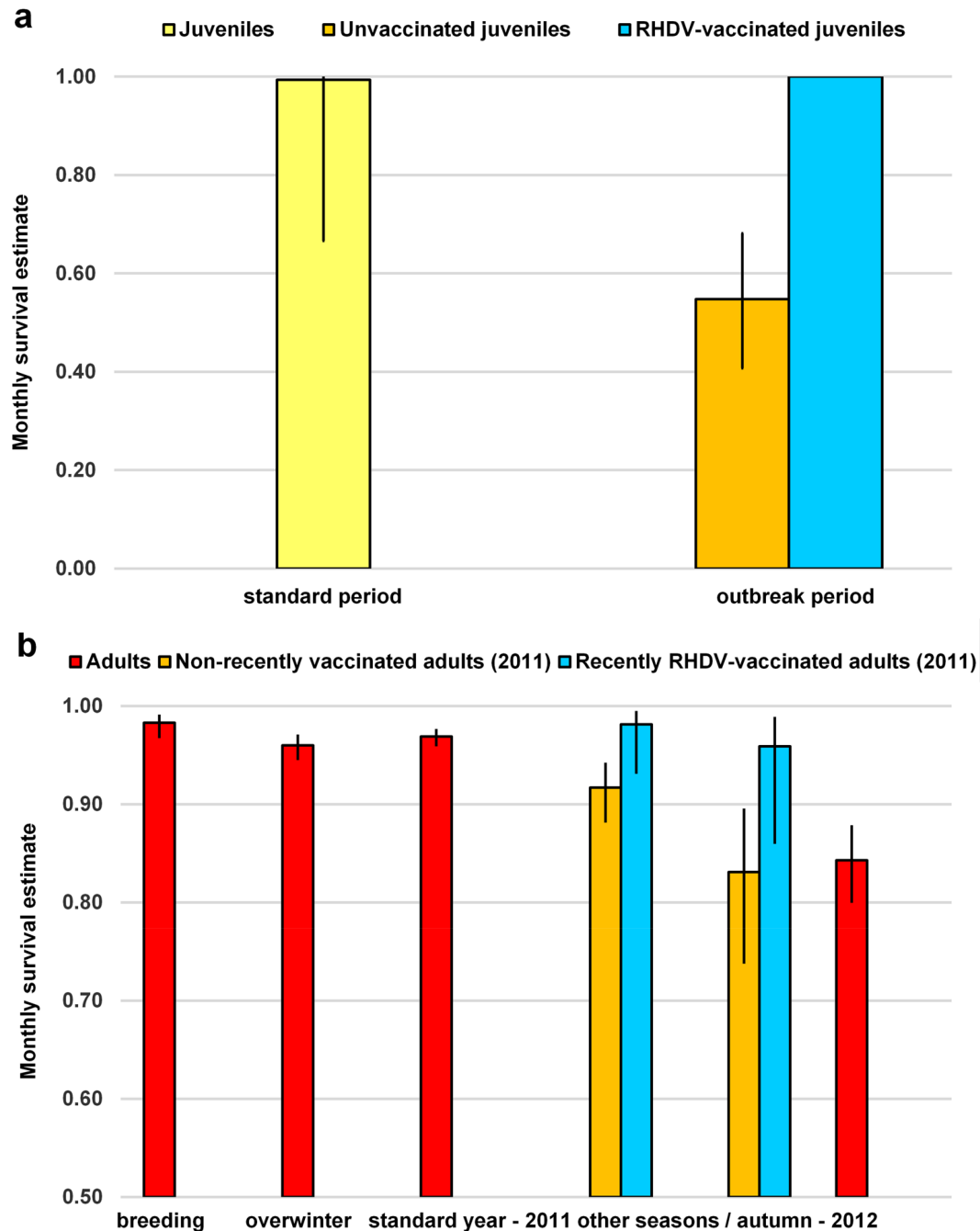
Note: Number of estimated parameters (*k*), deviance, and corrected Akaike information criterion (QAIC<sub>c</sub>) are indicated. Juv3, juvenile rabbits (age ≤ 3 months); Ad4, adult rabbits (age ≥ 4 months); sex, females versus males; vac-1mo, individuals vaccinated against rabbit hemorrhagic disease virus (RHDV) the previous month versus others; vac-MV, individuals vaccinated against MV in 2011 versus others; yrnormbio, standard annual biological cycles (2009, 2010, and 2013–2014); yroutbio, outbreak biological cycles (2011 and 2012, from March 2011 to February 2013); yroutbio1, first outbreak cycle (2011, from March 2011 to February 2012); outbio, standard cycles versus outbreak cycles; outbioB, additional difference between outbreak cycles (2011 ≠ 2012); seas, breeding season (spring and summer) versus overwinter (autumn and winter); autumn, autumn; aut/others, autumn versus other seasons. The selected model is at the top of the table and competing models ( $\Delta\text{QAIC}_c \leq 2$ ) for juvenile and adult survival are indicated by a bold QAIC<sub>c</sub> value. Model selection is detailed in Appendix S4: Tables S2 and S3.

In adults, the selected model also included a season effect during standard cycles, but not during the outbreak period (Appendix S4: Table S3). Standard 6-month adult survival during breeding season was 0.90 (0.82; 0.95), whereas it was 0.78 (0.71; 0.84) during overwinter. There was no significant effect of sex on adult survival (Appendix S4: Table S3), although survival tended to be slightly lower in males than in females. Additionally, PIT tagging had a marginal short-term deleterious effect on adult survival, but not on juvenile survival, indicating that this effect of PIT tagging should not be strong anyway (Appendix S5).

## DISCUSSION

Our study reveals what likely occurred in the wild when the RHDV2 virus first spread in populations of European

rabbit. Moreover, it provides one of the very first fine-scaled description of the overall impact of an RHDV2 outbreak on survival and demography in a rabbit population, and of the mechanism potentially leading to population collapse. The intensive and continuous individual longitudinal monitoring over 5 years and the high recapture and detection probabilities of individuals guarantee the robustness of our results. This has enabled us to monitor survival on a monthly scale and detect transient effects. We thus detected a short-term protective effect of RHDV2 vaccination on survival, making it possible to link the prolonged decline in rabbit survival to the occurrence of an RHDV2 outbreak in the population. Indeed, the aim of the study was not to quantify the survival gain provided by vaccination, but rather to use it as evidence of the RHDV2 outbreak. In addition, the detection of RHDV2 in the livers of dead rabbits showing lesions suggestive of RHD proved that they had indeed

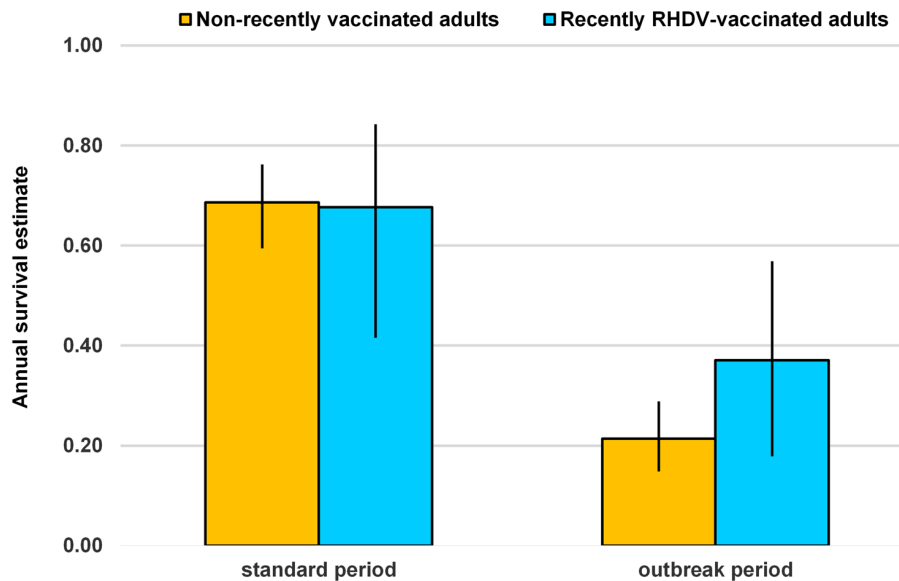


**FIGURE 3** Rabbit monthly survival estimates for juveniles (a) and adults (b) according to time period (standard cycles [2009, 2010, 2013–2014] vs. outbreak cycles [2011, 2012]), season and rabbit hemorrhagic disease virus (RHDV) vaccination status. Estimates are derived from the selected model (survival model “ $Juv3 \cdot [outbio + yroutbio \cdot vac-1mo] + Ad4 \cdot [outbio + yroutbio1 \cdot [vac-1mo + aut/others] + yrnormbio \cdot seas]$ ”; Table 1). CIs are indicated (not estimable for RHDV-vaccinated juveniles during the outbreak period).

been infected by RHDV2. Thus, given the infectivity of this virus and the very low or non-existent immunity to the new genotype, the most likely scenario is that the population has been affected by an RHDV2 outbreak. Moreover, the partial and very short-term protection conferred by RHDV vaccination totally rules out the hypothesis of a classical RHDV outbreak, as the survival rate of vaccinated individuals would have been much higher in

this case. Furthermore, RHDV vaccination had no effect on survival outside the presumed RHDV2 outbreak period, when rabbit survival was high. However, the absence of serological data on vaccine or natural antibodies made it impossible to determine whether vaccination had induced antibody titers considered protective, or to reconstruct the actual course of the outbreak. Finally, while the use of different types of commercial vaccines





**FIGURE 4** Adult rabbit annual survival estimates according to time period (standard cycles [2009, 2010, 2013–2014] vs. outbreak cycles [2011, 2012]) and rabbit hemorrhagic disease virus (RHDV) vaccination status. Annual values are extrapolated from monthly estimates (survival model “ $Juv3 \cdot [outbio + yroutbio \cdot vac-1mo] + Ad4 \cdot [outbio \cdot vac1-mo]$ ”; Appendix S4: Table S3). CIs are indicated.

throughout the study may have been a source of variability in vaccine protection, it seems impractical to assess the effect of each vaccine. And here, this question seems to concern mainly myxomatosis, due to the use of live attenuated homologous and heterologous vaccines at different times.

## Virulence and demographic impact of RHDV2

When RHDV2 emerged, experimental infection of laboratory rabbits showed that mortality rates induced by this virus were lower than those induced by RHDV. Indeed, with RHDV2, Le Gall-Reculé et al. (2013) recorded a mortality of 9%–46%, depending on the viral strain, and Le Minor et al. (2013) recorded a mortality of 48% when mortalities caused by RHDV is generally 80%–100% (Bertagnoli et al., 1996; Cancellotti & Renzi, 1991). These experiments were performed with RHDV2 strains that circulated in 2010–2012. However, Capucci et al. (2017) showed a subsequent increase in the pathogenicity of RHDV2 from 2014. In our semi-natural conditions, the overall annual mortality rate during the RHDV2 outbreak amounted to 79% in non-recently vaccinated adults. By comparison, Jiménez-Ruiz et al. (2023) estimated 77% mortality in seronegative rabbits during an RHDV2 epidemic peak that lasted only 5–6 weeks, indicating higher pathogenicity in this case. The estimated annual mortality among unvaccinated adults also appeared slightly lower during this RHDV2 outbreak

than during former RHDV outbreaks (88% in Marchandea et al., 1998).

Our study shows that RHDV2 outbreaks can reduce the annual survival probability of adults in natural populations by three. Furthermore, by combining the successive survival probabilities of unvaccinated juveniles and adults over a year during the RHDV2 outbreak, we can estimate that only 5%–10% of juveniles survived to the start of the next breeding season and were thus able to be recruited into the breeding population. Thus, similar to the RHDV virus, the overall demographic impact of RHDV2 outbreaks in wild rabbit populations proves to be very high as initially estimated when this novel virus strain was first detected in 2010 (Le Gall-Reculé et al., 2011). Our data are consistent with most field observations throughout the world. In Spain, RHDV2 outbreaks caused 60%–80% declines in natural rabbit populations (Delibes-Mateos et al., 2014; Monterroso et al., 2016). Similarly, in Australia, Mutze et al. (2018) reported rabbit numbers reduced by approximately 80% following the arrival of RHDV2 in two study sites. Our study enables understanding the demographic mechanisms leading to such impacts with moderately virulent strains. Indeed, in addition to the decreased survival of adult breeders, juvenile recruitment may almost entirely collapse during an RHDV2 outbreak, which may strongly affect population dynamics. Furthermore, after rabbit populations have been weakened by such outbreaks, population decline may be durably prolonged by other factors of the environment like predation, shooting, or various diseases (Marchandea et al., 2000).

Thus, RHDV2 appears as a major threat to wild rabbit populations.

### Prolonged duration of epizootic

The apparent prolonged impact of the RHDV2 outbreak over two consecutive years was a striking fact in our study. Molecular analyses proved the presence of the RHDV2 virus in the population in 2011 when survival significantly decreased. In 2012, the higher overall mortality in both adults and juveniles suggests that the virus was still present in the population. But it is not clear whether the population experienced one outbreak that lasted 2 years, or two separate outbreaks in 2011 and 2012. Several field observations showed that, when RHDV2 emerged in 2010–2012, some populations were exposed to epidemics that lasted several months with regular mortalities detected (unpublished data). More recently, Jiménez-Ruiz et al. (2023) recorded continuous RHDV2 circulation in their study population for several months after an initial epidemic peak. With the RHDV virus, such prolonged or recurrent outbreaks over successive seasons or years have also been observed (Cooke et al., 2000; Marchandeu et al., 2000; Wells et al., 2015). Several hypotheses may explain this epidemiology. By providing some transient immunity, RHDV immunity caused by RHDV vaccination in our case or by previous exposure to RHDV in wild populations (Abrantes et al., 2012; Cooke, 2002; Müller et al., 2021; Peacock et al., 2017) may have lengthened the progress of RHDV2 outbreaks. However, in the absence of RHDV vaccine immunity in the population, the RHDV2 outbreak could have been more intense and rapid, as reported by Jiménez-Ruiz et al. (2023), but ultimately with a comparable overall demographic impact. Similarly, the maternally derived immunity may contribute to delay RHDV exposure in the young, thereby slowing the progress of outbreaks or even temporarily stopping them (Cooke et al., 2000). But in our case, the recent emergence of RHDV2 must have considerably reduced the potential protective role of maternal antibodies. In addition, like Matthaëi et al. (2014) for RHDV, we also do not favor the hypothesis of prolonged RHDV2 infection in juveniles, which would represent a reservoir of virus between outbreaks. The persistence of the virus in infected carcasses inside warrens is another factor likely to contribute to the lengthening of outbreaks over successive months (McColl et al., 2002). A further explanation could be a continuous circulation of the virus on a regional scale causing subsequent distinct outbreaks among rabbit subpopulations interconnected by virus-carrying insect vectors (Asgari et al., 1998), as suggested in Australia (Kovaliski et al., 2014; Schwensow et al., 2014).

### Effect of RHDV vaccination against RHDV2

Although the actual serological statuses of individuals were unknown, we detected a transient protection, over at least 1 month, of RHDV vaccination in both juveniles and adults during the outbreak. In adults, the fact that this transient protective effect of RHDV vaccination was only demonstrated in 2011 but not in 2012 (the presumed second year of the RHDV2 outbreak), may be explained by lower and less regular population vaccination coverage in 2012 than in 2011; the same RHDV vaccine was used for both years (Appendix S1: Tables S1, S5, and S6). In 2011, the high frequency of booster vaccinations against RHDV among adults would have temporarily slowed the progress of the RHDV2 outbreak. This was no longer the case in 2012, as a large part of vaccinations took place in the second half of the year, perhaps after or at the very end of the outbreak. In addition, given the low number of RHDV vaccinations in 2012, a difference in survival between recently vaccinated and unvaccinated adults would have been difficult to detect due to a lack of statistical power. Vaccination against myxomatosis or RHDV can be ineffective when a population is already naturally sufficiently immune to the specifically targeted viruses, or in the absence of outbreaks caused by them (Rouco et al., 2016). Nevertheless, low or transient vaccine effectiveness is also likely when faced with a variant of the virus. And, despite rabbits immunized against RHDV possibly dying from a subsequent RHDV2 infection (Peacock et al., 2017), it was already known that vaccination against RHDV could confer some transient protection against RHDV2 (Dalton et al., 2014; Le Gall-Reculé et al., 2011; Müller et al., 2021). Thus, the short-term benefit of RHDV vaccination on survival that we observed supports the hypothesis of an RHDV2 outbreak causing the population decline.

### Part of mortality imputable to myxomatosis

Could a part of mortality be imputable to myxomatosis? In parallel with the RHDV2 outbreak, we also recorded a myxomatosis episode in autumn 2011. No further episodes of myxomatosis were detected during the study period, and we found no short-term effect of MV vaccination on survival, regardless of age or period. As often happens in the wild, myxomatosis occurred at the end of the breeding season when there are many susceptible individuals in the population, particularly among juveniles (Tablado et al., 2012). This was the case in late summer 2011 when the relative low population vaccination coverage enabled myxomatosis to spread. The decline in adult

survival recorded in autumn 2011 suggests concomitant mortality caused by myxomatosis. The fact that MV vaccination had no effect on adult survival could be explained by insufficiently protective vaccine immunity when the myxomatosis episode occurred in September 2011, 6 months after vaccination. In any case, the positive short-term effect of RHDV2 vaccination on adult survival strongly suggests that RHDV2 was the main cause of mortality in 2011, and that the myxomatosis episode had only a moderate impact on the population. Most of the excess mortality in 2011 therefore seems to be attributable either to RHDV2 or to an interaction between the two diseases, with myxomatosis increasing RHDV2 mortality. Furthermore, the spread of MV in the population in autumn 2011 may have led to a subsequent increase in adult mortality in 2012, even though myxomatosis was no longer observed in the population. Several studies have shown an interaction between myxomatosis and other diseases. For instance, the immunosuppressive effect of MV (Jeklova et al., 2008) may be responsible for an increase of the severity of helminth infections (Boag et al., 2013). Serological data also suggest a relationship between the risk of infection with MV and RHDV2 (Marchandeau et al., 2004) and, in a case of coinfection by MV and RHDV2, Carvalho et al. (2020) suggested that previous MV infection likely favored subsequent RHDV2 infection. In particular, previous exposure to MV has been shown to reduce rabbit survival by around 10% when subsequently infected with RHDV2 (Barnett et al., 2018; Wells et al., 2018); but Jiménez-Ruiz et al. (2023) found contradictory results. Moreover, Wells et al. (2018) suggested that a previous infection by MV might reduce the future survival rate of individuals, despite recovery, possibly due to negative long-term effects such as fat reserves depletion, digestive disorders or some immunodeficiency caused by MV.

### Are semi-captive conditions representative of epidemiological dynamics in the wild?

Despite the study being conducted in semi-natural conditions, the results appear representative of the first spread of the RHDV2 virus in small populations of wild rabbits. However, we could not finely investigate the chronological spread of RHDV2 within and among social groups or related warrens. In our study, viruses could probably be spread easily, by close contact or indirect transmission (Abrantes et al., 2012; Asgari et al., 1998; Lavazza & Capucci, 2018), among social groups due to the small size of the breeding pen and its design. Indeed, rabbits from the four warrens could feed together in the common grazing area, and we also detected some exploratory or

dispersal events, juvenile and adult males being more prone to change of social group (J. Letty, personal observation). So, as suspected in wild conditions, dispersal of subadult rabbits between warrens may play a critical role in disease transmission because they are less likely to belong to separate social groups (Jennings & Mutze, 2017). In addition, we might also have unintentionally increased virus spread among individuals or warrens when handling rabbits during grouped capture sessions.

Of course, baseline survival is expected to be higher in semi-captivity than in the wild due to some prophylactic management and the absence of terrestrial predators. This may partly explain the higher survival estimated here than during an RHDV2 outbreak in a wild population (Marchandeau et al., 1998). In addition, out of outbreak periods, the standard survival estimates in both juveniles and adults appeared to be higher in our semi-captive population than in free-ranging ones (Guitton et al., 2008; Tablado et al., 2012). In other respects, survival of semi-captive rabbits did not differ significantly between sexes in either juveniles or adults, but adult survival appeared to be lower during the overwinter than during the breeding season. This seasonal difference in adult survival may result not only from seasonal weather conditions but also from higher population density and the resulting social stress after the breeding season (Rödel et al., 2004).

### Value of longitudinal studies of host–pathogen dynamics in the wild

Beyond the specific case of the demographic impact of RHDV2 outbreaks on European rabbit populations, this work emphasizes the value of longitudinal field studies to investigate epidemiological dynamics in the wild. However, such opportunities may be relatively rare. They are first made possible by the long-term monitoring already underway before or during the spread of the disease (Gamble et al., 2019; Garnier et al., 2016; Lachish et al., 2007; Marchandeau et al., 1998; Mutze et al., 2002; Pigeault et al., 2018; Pioz et al., 2007). Of course, such monitoring programs greatly benefit from technological development. Second, to establish the link between disease and host population dynamics, it is essential to be able to identify the pathogen infecting the host species and quantify its demographic impact. To this end, biological tests, such as serology or genetic analysis, can be used to detect the pathogen (Chambert et al., 2012; Gamble et al., 2019; Jiménez-Ruiz et al., 2023; Lachish et al., 2011; Mariën et al., 2018; Pigeault et al., 2018; Rossi et al., 2011; Santoro et al., 2014). However, when

the underlying biological status of individuals cannot be determined, vaccination and other veterinary treatments may be an attractive alternative to study the effect of the pathogen on the host by comparing the survival or fecundity of individuals according to the treatment they received (Guitton et al., 2008; Newey & Thirgood, 2004). In our study, the combination of long-term monitoring and preventive vaccination allowed revealing the demographic impact of an underlying outbreak. This emphasizes how essential are long-term observational and experimental studies to understand which ecological and demographic mechanisms drive the impact of diseases on wild populations. This should help improve the capacity to predict and manage host–pathogen interactions in the wild.

### AUTHOR CONTRIBUTIONS

Jérôme Letty and Stéphane Marchandeau designed the study and coordinated field data sampling. Gilles Holé, Jérôme Letty, Yves Léonard, and Régis Vannesson captured and handled the animals. Régis Vannesson managed the semi-captive population. Nicolas Chatelain designed and installed the RFID system. Aurélien Besnard and Rémi Choquet designed the modeling analysis. Jérôme Letty conducted the modeling analysis and prepared the initial draft of the manuscript. Aurélien Besnard, Nicolas Chatelain, Rémi Choquet, and Stéphane Marchandeau contributed to the writing and critically revised the manuscript. All authors approved the final version of the manuscript.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

### DATA AVAILABILITY STATEMENT

Data and code (Letty et al., 2024) are available from Figshare: <https://doi.org/10.6084/m9.figshare.20481441>.

### ETHICS STATEMENT

Animal care complied with French institutional guidelines and regulations. All captures and manipulations of

individuals took place before the entry into force of the Nagoya protocol in France.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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