Impact of RHDV2 at long-term rabbit monitoring sites

Dave Ramsey, Arthur Rylah Institute

26/4/2022

Preamble

A recent analyses of rabbit spotlight monitoring data and associated serological sampling from 18 sites located in Qld, NSW, ACT, Vic, SA and WA (Ramsey et al. 2020) revealed that the arrival of the highly pathogenic rabbit haemorrhagic disease virus – RHDV2, resulted in reductions in rabbit abundance by approximately 60%. Impacts were most pronounced in South and Western Australia and were more variable in the other eastern states.

Analysis of serological samples from rabbits for both RHDV2, RHDV1 and the non-pathogenic RCVA1 indicated that the arrival of RHDV2 around 2014/2015 resulted in rapid increases in the seroprevalence of RHDV2 and concurrent decreases in seroprevalence of both RHDV1 and RCVA1. However, the serological analysis did not support the rapid extinction of the RHDV1 strain with substantial increases in juvenile RHDV1 seroprevalence indicating that recent infections had occurred. Hence, at the end of that study in early 2018, Ramsey et al. (2020) concluded that RHDV2 and RHDV1 were currently co-existing but whether this remained true over the longer-term, was to be determined.

Following the cessation of that study, rabbit spotlight monitoring continued at six and serological sampling continued at five of the original 18 sites at Mirrabooka (NSW), Gudgenby (ACT), Coorong Scobie (SA), Nelsons and Drummonds (WA). Hence, there is an opportunity to re-assess the findings in Ramsey et al. (2020) to determine the trends in seroprevalence of RHDV2, RHDV1 and RCVA1. Analysis of spotlight data from these sites could also be used to determine whether the suppression of rabbit populations following the arrival of RHDV2 continues to be maintained now that RHDV2 is endemic.

Methods

Spotlight monitoring

Monitoring of rabbit abundance post 2018 was undertaken at 6 sites, Mirrabooka (NSW), Gudgenby (ACT), Coorong, Scobie (SA), Nelsons and Drummonds (WA). Monitoring consisted of spotlight counts conducted bi-annually, in most cases, in April (autumn) and October (spring) along transects using a hand-held spotlight from the rear of a utility vehicle driven at slow speed during the early evening. Transect lengths at each site varied from 1.0 to 17.8 km. The number of rabbits seen on the transect were recorded each night for three consecutive nights. Spotlight count data were collected up to and including January/February (summer) 2022 but were largely incomplete to various degrees for each of the 6 sites. A summary of the post Autumn 2018 monitoring record for the 6 sites is given in Table 1. Of note was that considerable historical spotlight monitoring data were now available for the Gudgenby site between 2006 and 2015. However, for these analyses, only data from 2011 were considered here so that the time series was consistent with other comparable sites.

The rabbit population dynamic state-space model detailed in Ramsey et al. (2020) was fitted to the existing data that also include the additional post 2018 data from the six sites. In particular we estimated equilibrium abundance (i.e. carrying capacity - κ) both before (κ^b) and after (κ^a) the arrival of RHDV2 (e.g. equations 3 & 4 in Ramsey et al. (2020)). Estimates of population suppression

(reductions in equilibrium abundance) were then examined for the six sites and compared to that detailed in Ramsey et al (2020).

Serology

Following the completion of spotlight counts at each site, serum samples were collected from up to 20 rabbits and screened by a series of enzyme-linked immunosorbent assays (ELISA) to determine the presence of RHDV or RHDV2 antibodies as well as antibodies to RCVA (further details of the serological testing are in (Ramsey et al. 2020) of each rabbit were also estimated using dried eye lens weight, which was accurate up to a maximum age of 500 days (Augusteyn 2007). Rabbit ages were then classified as juvenile ($\leq$ 150 days) or adult (>150 days). Due to various levels of cross-reactivity between the respective competition ELISAs, rabbits were scored as positive to RHDV (including classical RHDV1, RHDVa and K5), if the ratio of the RHDV2/RHDV cELISA reciprocal titres was <1. Similarly, rabbits were scored positive for RHDV2 if the ratio was >1 (Strive et al. 2020). For RHDV and RHDV2 cELISAs, only titres ≥1:40 were considered to be positive, while a titre of 1:20 on the blocking ELISA was considered to be positive for RCVA.

Exponential growth state-space models were fitted to the time series of serological prevalence for the three strains (RCVA, RHDV and RHDV2) to examine trends in age-specific seroprevalence. Multivariate state-space models were also fitted to examine evidence for interactions between the three strains (Ramsey et al. 2020).

Table 1. Serological sampling in the period following Autumn 2018 at five sites. Su – Summer; Au – Autumn; W – Winter; Sp - Spring

Site	Year	Seasons
Mirrabooka (NSW)	2020	Su, Au, W, Sp
	2021	Su, Au, W
	2022	Su
Gudgenby (ACT)	2006-2015	
	2018	W, Sp
	2019	Su, Au, W, Sp
	2020	W, Sp
	2021	Su, A
	2022	Su
Scobie, Coorong (SA)	2020	Au, Sp
	2021	Au, Sp
Drummonds (WA)	2018	Sp
	2019	Au, Sp
	2020	Au, Sp
	2021	Su, Au
	2022	Su
Nelsons (WA)	2018	Sp
	2019	Au, Sp
	2020	Au, Sp
	2021	Su, Au, Sp
	2022	Su

Results and Discussion

Spotlight data

Population trajectories of rabbit abundance for the six sites ranged between 1 and 495 rabbits per spotlight km (Figure 1). Highest rabbit abundances were recorded at Gudgenby prior to the arrival of RHDV2 and lowest abundances were recorded at the two Western Australian sites. Estimates of equilibrium population abundance indicated that overall suppression of rabbits since the arrival of RHDV2 was 64%, similar to that estimated by Ramsey et al (2020) (Table 2). Of the six sites with post 2018 monitoring data, the highest suppression of abundance was recorded at the Gudgenby site (82%) and the lowest at Scobie (52%). However, there was high uncertainty around the estimated suppression at the Scobie site included the possibility that no suppression of rabbit abundance occurs at this site (Table 2).

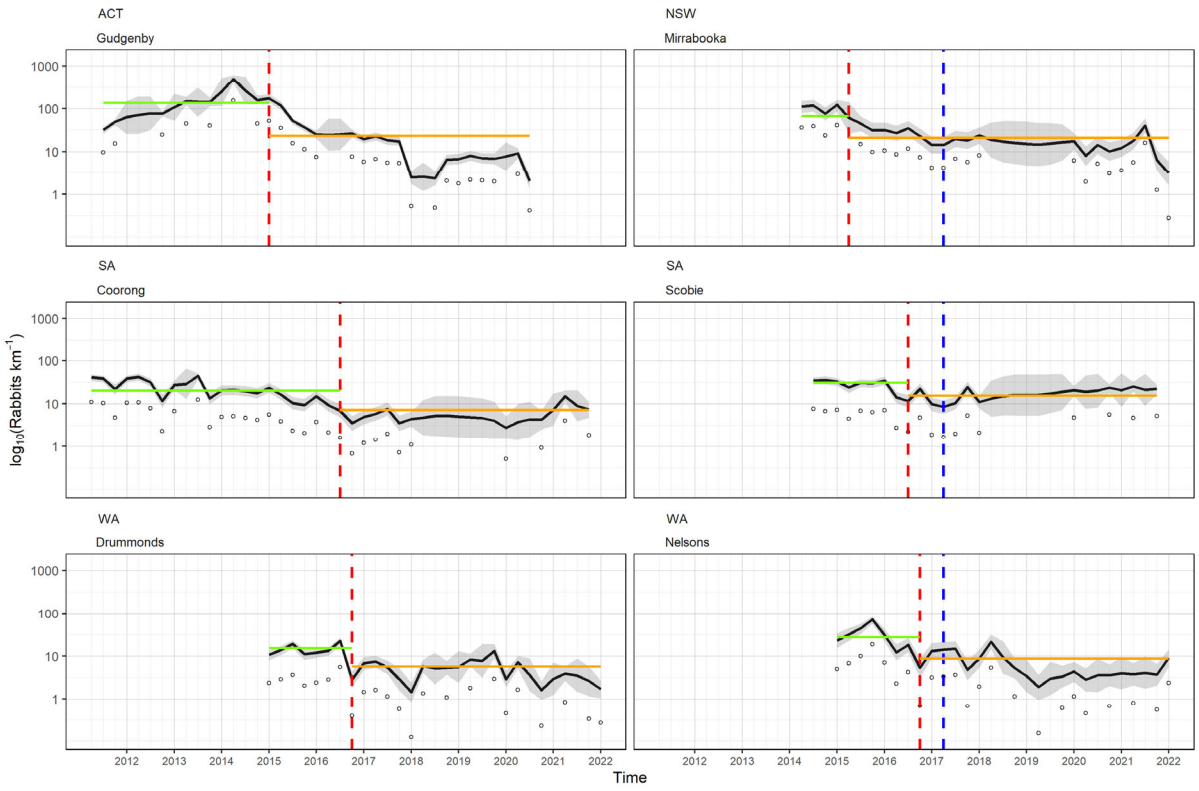

Figure 1. Predicted abundances (line) and observed counts (circles) of rabbits (rabbits/spotlight km) at each of the six sites monitored between 2011 and 2022. Solid lines are posterior medians, and shaded polygons are the 95% credible intervals of expected abundances. Dashed red vertical line indicates the arrival time of RHDV2 at the site and the dashed vertical blue line indicates the release of K5. Horizontal green and light blue lines give the estimates of κ^b and κ^a, respectively, for sites with at least one year of monitoring data prior to the arrival of RHDV2. y-axes are $\log_{10}$ transformed.

Table 2. Estimates of equilibrium rabbit abundances (κ) during winter-spring (rabbits/km) for the period prior to ($\hat{\kappa}^b$) and following ($\hat{\kappa}^a$) the arrival of RHDV2, and the proportional change in the equilibrium abundance (Δ). Estimates are presented for the six sites with post 2018 monitoring data. The overall estimate includes all sites with at least one year of monitoring data prior to the estimated arrival of RHDV2. se – standard error; LCL – lower 95% credible interval; UCL – upper 95% credible interval.

Site	$\hat{\kappa}^b$	se(κ_b)	$\hat{\kappa}^a$	se(κ_a)	Δ	LCL(Δ)	UCL(Δ)
Coorong	20.3	4.7	7.3	2.4	**-0.64**	-0.85	-0.34
Drummonds	15.4	5	5.8	1.9	**-0.61**	-0.86	-0.32
Gudgenby	137.3	59.2	23.6	11.3	**-0.82**	-0.96	-0.55
Mirrabooka	66.5	32.9	21.1	8	**-0.68**	-0.92	-0.24
Nelsons	27.5	10.5	8.7	3.6	**-0.67**	-0.89	-0.33
Scobie	31.2	11	15.6	6	**-0.52**	-0.8	0.17
Overall	39.9	30.2	15.4	11.3	**-0.64**	-0.91	-0.01

Serological data

Average trends in seroprevalence from the five sites with post-2018 serum samples revealed that RHDV seroprevalence continues to decline, especially in adult aged rabbits (Figure 1). Although seroprevalence in juvenile RHDV seroprevalence has also continued to decline, there is some evidence of recent RHDV infections, especially at the SA and WA sites (Figure 3). In contrast, RHDV2 seroprevalence continues to increase, with an average adult seroprevalence of approximate 60 - 70% and juvenile seroprevalence of approximately 30 - 40% (Figures 1 & 4).

In contrast to RHDV, the seroprevalence of RCVA, while declining initially, appears to be making a recovery with average RCVA seroprevalence in both juveniles and adults increasing over the last two years (Figure 1). Estimates of the interaction between strains now indicates that RCVA seroprevalence in the previous quarter has a stronger negative effect on RHDV2 seroprevalence and the reciprocal effect of RHDV2 on RCVA seroprevalence is weaker (Table 2). This contrasts with the findings in Ramsey et al. (2020) where there was a strong effect of RHDV2 on RCVA seroprevalence and a weak reciprocal effect of RVCA on RHDV2 seroprevalence. Hence, this indicates evidence that the reciprocal strain competition between RCVA and RHDV2 is now more balanced suggesting that there is firmer evidence for the coexistence between these two strains. In contrast RHDV continues to be at a competitive disadvantage due to continued competition with RHDV2. However, although RHDV seroprevalence continues to decline, the serological data still does not yet indicate extinction of RHDV strains.

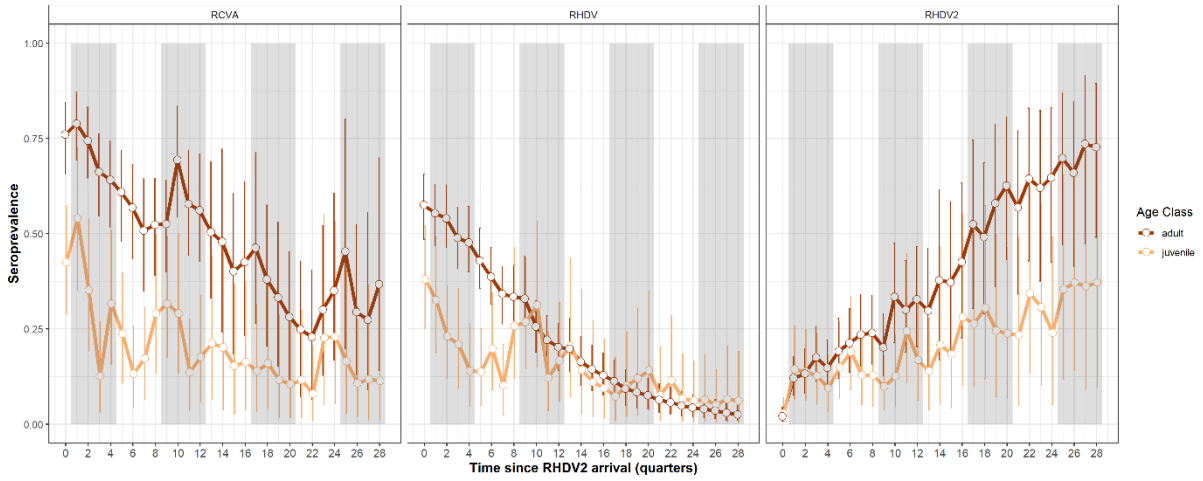

Figure 1. Estimated average trends in the seroprevalence of RCVA, RHDV and RHDV2 for juvenile (≤ 150 days old) and adult (> 150 days) rabbits following the arrival of RHDV2 at each site.

Table 2. Table of parameter estimates describing the effect of antibody prevalence for a strain at time *t*-1 (columns) on the change in antibody prevalence for a strain at time *t* (rows). Values in bold indicate interactions between strains that have 95% credible intervals that do not include zero. Diagonal entries describe self-effects of a strain at time *t*-1 on the same strain at time *t*.

Strain	RCVA	RHDV1	RHDV2
RCVA	0.285 [0.162, 0.406]	0.021 [-0.156, 0.193]	**-0.215 [-0.319, -0.119]**
RHDV1	0.128 [0.023, 0.235]	0.112 [-0.051, 0.278]	**-0.164 [-0.251, -0.082]**
RHDV2	**-0.166 [-0.295, -0.048]**	**0.271 [0.103, 0.45]**	0.749 [0.649, 0.844]

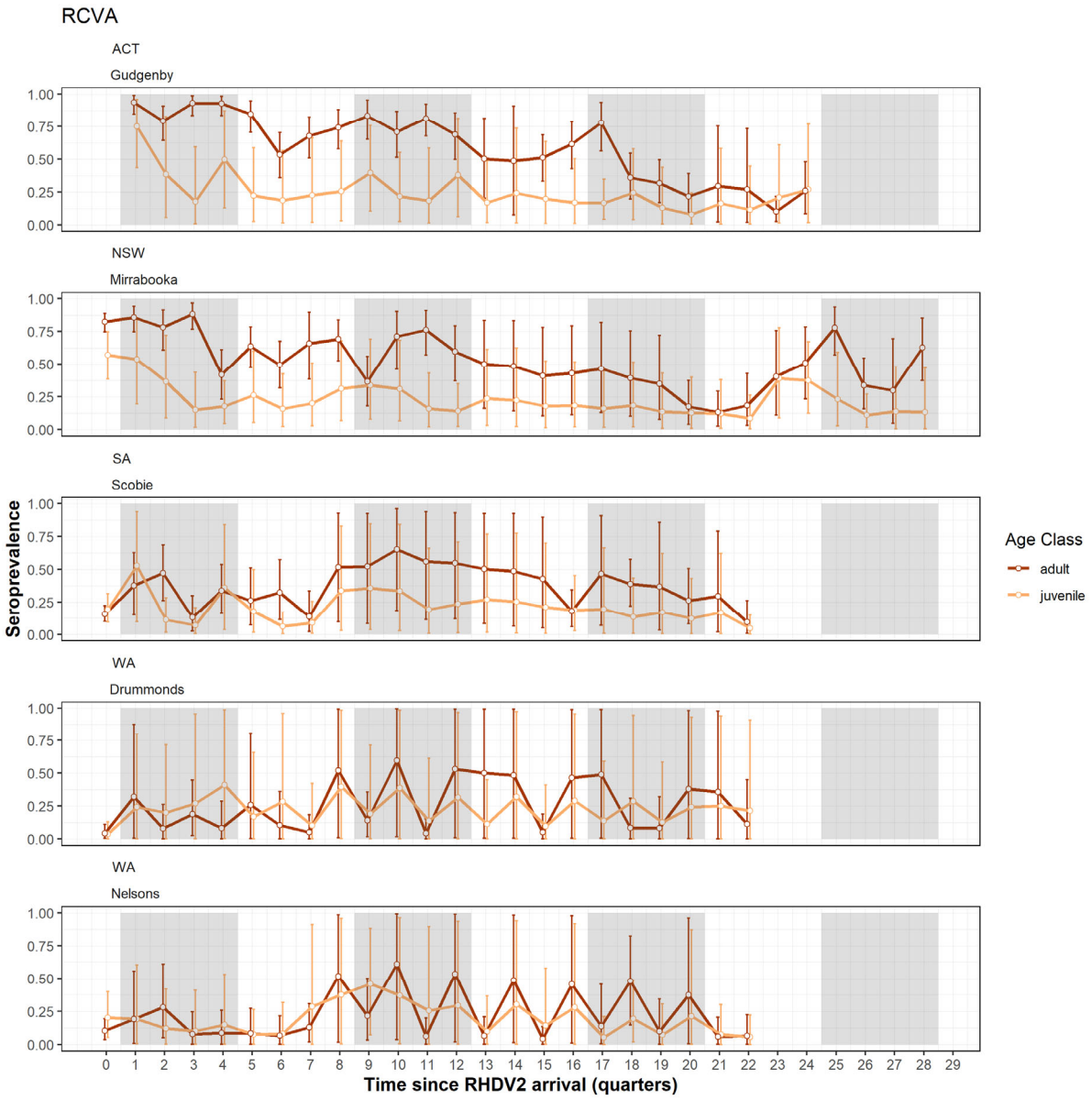

Figure 2. Estimated average trends in the seroprevalence of RCVA for juvenile (≤ 150 days old) and adult (> 150 days) rabbits following the arrival of RHDV2 at the five sites monitored since 2018.

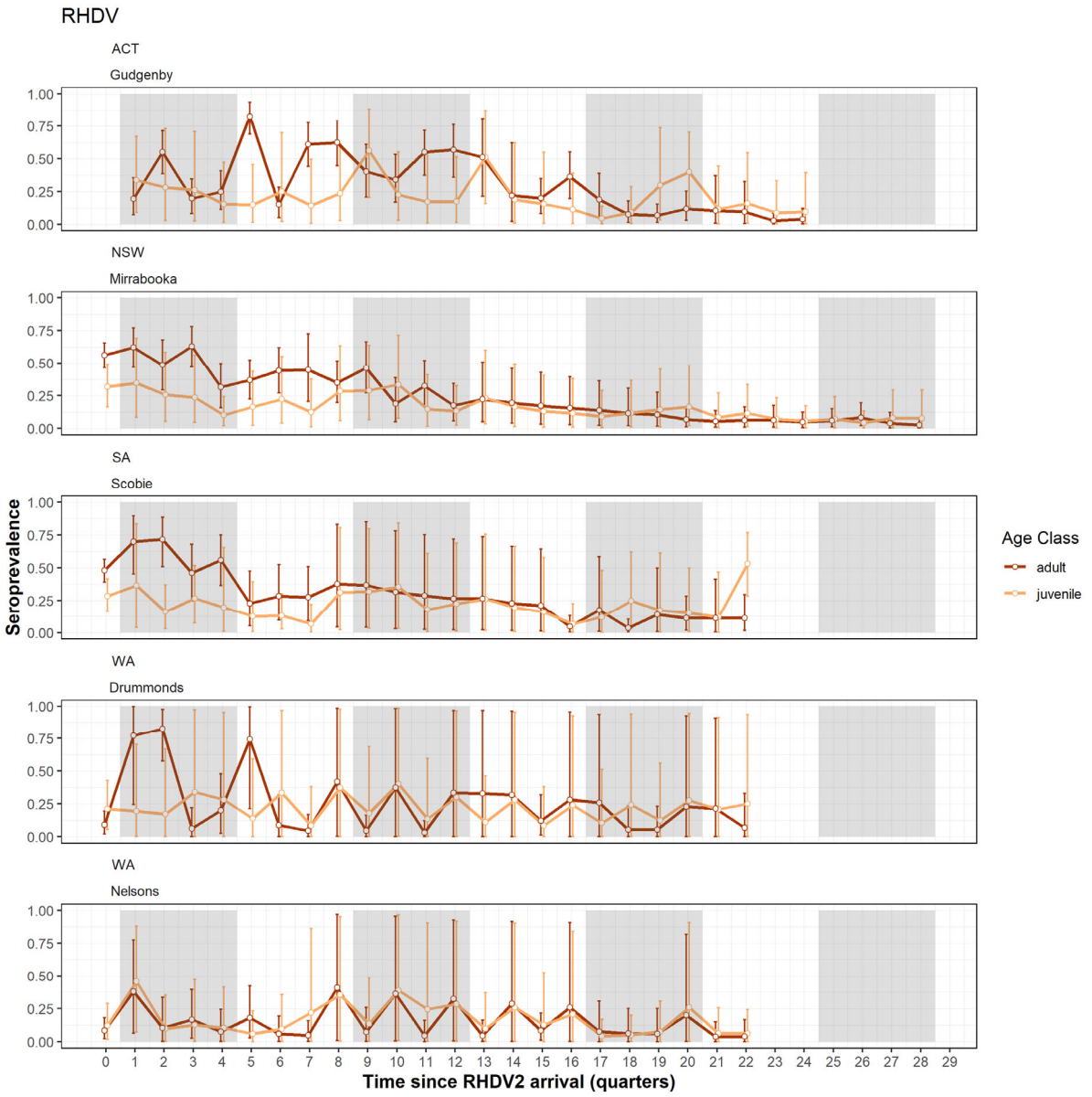

Figure 3. Estimated average trends in the seroprevalence of RHDV for juvenile (≤ 150 days old) and adult (> 150 days) rabbits following the arrival of RHDV2 at the five sites monitored since 2018.

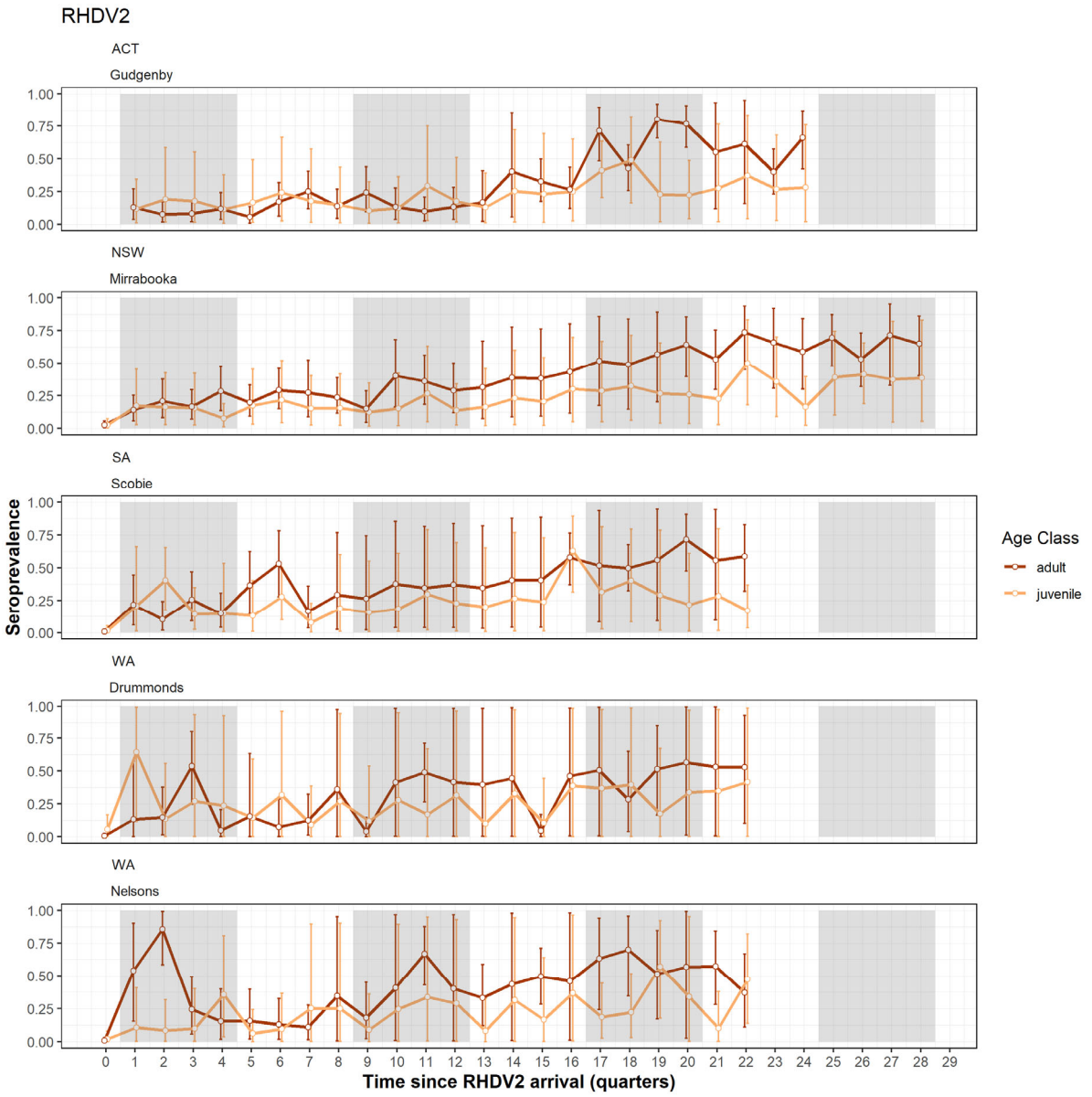

Figure 4. Estimated average trends in the seroprevalence of RHDV2 for juvenile (≤ 150 days old) and adult (> 150 days) rabbits following the arrival of RHDV2 at the five sites monitored since 2018.

Conclusions

Analysis of additional spotlight monitoring and serological data from rabbit populations at 6 of the original 18 long-term monitoring sites has revealed additional insights into the population dynamics of rabbits and the effects of RCVA/RVDV/RHDV2 infections and their effects on rabbit populations. Rabbit populations at all monitored sites, with the exception of Scobie, continue to show suppression of abundance since the arrival of RHDV2, averaging 64%. Hence, we can conclude that the majority of rabbit populations at monitored sites continue to be suppressed from RHDV2 epidemics. RHDV2 is now the dominant strain in rabbit populations with a seroprevalence of 60-70% in adult rabbits. Although seroprevalence of RCVA was initially depressed by competition with RHDV2, this strain now shows stronger evidence of coexistence with RHDV2. This could be due to reduced competition for infection of juvenile rabbits from RHDV2.

The seroprevalence of RHDV1 continues to decline at monitored sites approaching zero prevalence in adult rabbits. Hence, it is unlikely that RHDV1 strains now play a major role in the regulation of rabbit abundance. Some residual infections in juvenile rabbits suggest that the RHDV1 strains continue to be extant in places, especially Western Australia. However, this must be tempered by the fact that sample size of juvenile rabbits subject to serological testing was low, which was evidenced by the high uncertainty in prevalence estimates for RHDV1, especially in the Western Australian sites.

To determine whether RHDV2 continues to suppress rabbit population over the longer term, it would be essential that monitoring continues at the six sites currently being monitored. Improved estimates of abundance and serological parameters could be obtained with the addition of data from some of the other discontinued sites from Ramsey et al (2020), notably from Victoria (i.e. Sunbury) and Qld (i.e. Wallangarra East), but this must be balanced with the fact that these sites now have not been monitored for at least 5 years, resulting is a severe loss of continuity leading to less robust inferences from these sites.

References

Augusteyn, R. C. 2007. On the relationship between rabbit age and lens dry weight: Improved determination of the age of rabbits in the wild. Molecular Vision 13:2030–2034.

Ramsey, D. S. L., T. Cox, T. Strive, D. M. Forsyth, I. Stuart, R. Hall, P. Elsworth, and S. Campbell. 2020. Emerging RHDV2 suppresses the impact of endemic and novel strains of RHDV on wild rabbit populations. Journal of Applied Ecology 57:630–641.

Strive, T., M. Piper, N. Huang, R. Mourant, J. Kovaliski, L. Capucci, T. E. Cox, and I. Smith. 2020. Retrospective serological analysis reveals presence of the emerging lagovirus RHDV2 in Australia in wild rabbits at least five months prior to its first detection. Transboundary and Emerging Diseases 67:822–833.