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VARIATION IN ACTIVITY OF RABBIT HAEMORRHAGIC DISEASE DUE TO RABBIT POPULATION DENSITY AND A BENIGN CALICIVIRUS

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ABSTRACT: Studies commenced in 2001 to determine whether conventional rabbit control programs affected the subsequent impact of rabbit haemorrhagic disease (RHD). Eight sites were chosen in semi-arid and moist temperate areas. At each site, higher-density populations were paired with nearby populations where density was reduced by chemical/mechanical rabbit control. Rabbits were classified as having antibodies to virulent RHD, to an RHD-like non-pathogenic calicivirus (benign CV) or no antibodies (sero-negative). The prevalence of RHD antibodies was similar across regions but there were more sero-negative rabbits in semi-arid (43-54%) than temperate areas (19%), and fewer rabbits with benign CV antibodies in semi-arid (6-10%) than temperate areas (38%). This effect was evident in both juvenile and adult rabbits. Rabbit control programs reduced rabbit density, increasing the proportion of sero-negative rabbits and reducing RHD sero-prevalence in juvenile rabbits (<1300g) but not in adults (≥1300g). Hence, rabbit control slowed the circulation of RHD in juvenile rabbits but ultimately a similar proportion of rabbits were infected. Because primary infection occurs at a later age and mortality is higher in older juveniles, rabbit control does not reduce the impact of RHD. We found no effect of rabbit control on the interaction between RHD and benign CV that could be exploited to improve the effectiveness of rabbit control.

INTRODUCTION

The occurrence of RHD in Australia provides a unique opportunity to capitalise on the reduction in rabbit numbers caused by the disease. Management of rabbits can be improved by gaining a better understanding of how the virus behaves when conventional rabbit control impacts on rabbit populations. But understanding the epidemiology of RHDV is complicated by the existence of non-pathogenic or benign forms of calicivirus (Capucci et al. 1996) that were widespread in Australian rabbit populations prior to the spread of RHD in 1995 (Cooke et al. 2000). In Australia a pre-existing, benign CV is reported to provide higher levels of protection from the lethal RHD for rabbits living in cool, moist, temperate areas than to rabbits living in semi-arid or arid zones (Cooke et al. 2002). Understanding the behaviour of both virulent RHD and benign CV may enable more effective use of our conventional control techniques if those techniques affect the transmission or impact of either disease. This study examined the impact of rabbit control on antibody prevalence in the northern, semi-arid areas and southern, cool-moist, temperate areas.

METHODS

Eight study sites were monitored in South Australia and Victoria (Fig. 1). Each site consisted of two sub-sites of >5 km²: a higher-density population sub-site, paired with a nearby sub-site where rabbit population density was reduced by warren ripping, destruction of surface
harbour, baiting and fumigation. Rabbit abundance and serological status were monitored at four monthly intervals over two years.

Fig. 1. Locations of the paired monitoring sites

Indices of rabbit abundance were obtained from replicated spotlight counts of rabbits along vehicle transects (5-20km). Data were expressed as rabbits/km to allow comparison between sites with different transect lengths.

Rabbit serum and tissue samples were collected from samples of 15-30 shot or live captured rabbits at each sub-site. Sera were tested using enzyme-linked immunosorbent assays (ELISAs) for antibodies raised against RHDV. Quantitative analysis of competition ELISA and ELISAs for IgG, IgA and IgM antibody isotypes (Capucci et al. 1991a,b) enabled the classification of antibody status into three groups: sero-negative (no antibodies detected), antibodies to RHD and antibodies to a non-pathogenic or benign CV (Cooke et al. 2000). Benign CV antibodies can only be detected in rabbits that have not been infected with RHD (ie RHD antibodies mask the presence of any antibodies to the benign CV). Therefore, we considered RHD prevalence as the proportion of all rabbits tested with RHD antibodies, whereas the prevalence of benign CV immunity was taken as the proportion of RHD-negative rabbits that had benign CV antibodies.

At each sub-site, at all observation periods the proportion of animals within immune classes, log10 transformed number of rabbits/spotlight km and the proportion of rabbits <1300g were calculated. Then for each sub-site the average over observation periods of the calculated values was used as data in the analysis, but excluding times when less than five rabbits were captured. Sub-site means were then analysed as a three, region by two treatment factorial analysis of variance with two error terms. The region main effects were compared to a between site error term. The treatment main effect and interaction between region and treatment where compared to a between sub-site within site error term. Since no interactions between region and treatment were found (P>0.05) only main effects are presented.

RESULTS

Estimates of rabbit abundance and the proportions of rabbits within different immune classes appeared to cluster around three regions: the semi-arid northern region, (Orarparinna,
Yednalue and Venus Bay), the central region (Hattah and the Coorong) and the southern, higher rainfall region of central Victoria (Maryborough, Bendigo and Bacchus Marsh) (Fig. 1).

1. Rabbit abundance
There were more rabbits in the southern temperate region (8.5 rabbits/spotlight km) than in the central (1.3/km) or semi-arid northern regions (2.4/km) (P=0.017, using log10 transformed values of the arithmetic means). Control efforts were successfully applied; reducing rabbit populations by 76% on log-transformed values (P<0.00025, 95% CI 64-84). On a numeric scale, this equated to a mean reduction from 6.82/km in the uncontrolled areas to 1.97 rabbits/spotlight km in controlled areas. There were no significant regional differences in the proportional reduction achieved by control efforts (P=0.146).

2. Antibody status of rabbits

Influence of region
For both juvenile and adult rabbits, the proportions of populations classified with virulent RHD antibodies were similar for all regions (Fig 2). There were more rabbits classified as sero-negative in northern (54%) and central (43%) regions than in southern areas (19%), at the expense of fewer rabbits classified with the benign CV antibodies in northern (6%) and central (10%) regions than in southern areas (38%).

Fig. 2. The proportion juvenile (a) and adult (b) rabbits with no antibodies (sero-negative) or with antibodies to benign CV or RHD, in northern, central and southern regions.

Influence of rabbit control
Rabbit control significantly reduced the proportion of young rabbits classified with virulent RHD antibodies and increased the proportion of young rabbits that remained sero-negative. The overall proportion of young rabbits classified with antibodies to benign CV appeared to be little affected by rabbit control. However when the effect of control was restricted to the southern region, where benign CV was common, there was an indication (P=0.06) of a relatively large decrease in incidence of the benign CV classification in juvenile rabbits. Overall, there was no significant effect of rabbit control on the antibody status of adults (P>0.1, Table 1). However in two cases where seroprevalence of RHD antibodies in non-controlled populations was already low, RHD was much less prevalent in adults from controlled lower-density populations.
**Seasonal effects**

At all sites, RHD showed distinct seasonal patterns of activity that were generally consistent with previous studies. Antibody prevalence increased to almost 100% during some outbreaks and declined when young susceptible animals were recruited into the populations. In the arid and central regions, outbreaks of RHD occurred in sero-negative rabbits during late winter/spring. Virus activity was very low in summer but significant disease activity also occurred during autumn and early winter in arid pastoral areas. In the higher rainfall districts of southern Victoria, distinct outbreaks of RHD occurred in spring and summer but most animals infected were already carrying antibodies from infection with benign CV earlier in the season.

**Table 1.** The effect of rabbit control on the proportions of young and adult rabbits with CV antibody classes: sero-negative (no antibodies), antibodies to benign CV or RHD.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>No control</th>
<th>Control</th>
<th>SED</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young rabbits &lt;1300g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sero-negative (%)</td>
<td>38</td>
<td>54</td>
<td>4.6</td>
<td><strong>0.019</strong></td>
</tr>
<tr>
<td>Benign CV (%)</td>
<td>19</td>
<td>17</td>
<td>3.6</td>
<td>0.503</td>
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<tr>
<td>Southern Region only</td>
<td>45</td>
<td>31</td>
<td>5.8</td>
<td>0.060</td>
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<tr>
<td>Other regions</td>
<td>4</td>
<td>8</td>
<td>4.5</td>
<td>0.381</td>
</tr>
<tr>
<td>RHD (%)</td>
<td>43</td>
<td>29</td>
<td>4.8</td>
<td><strong>0.040</strong></td>
</tr>
<tr>
<td><strong>Adult rabbits ≥1300g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sero-negative (%)</td>
<td>29.2</td>
<td>35.7</td>
<td>4.2</td>
<td>0.182</td>
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<td>Benign CV (%)</td>
<td>18.1</td>
<td>19.1</td>
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<td>0.741</td>
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<tr>
<td>RHD (%)</td>
<td>52.7</td>
<td>45.2</td>
<td>4.1</td>
<td>0.124</td>
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</tbody>
</table>

**DISCUSSION**

In this study, conventional rabbit control programs reduced the proportion of young rabbits with protective RHD antibodies, but had no consistent effect on the antibody status of adults. From this we conclude that lowering rabbit density delays infection with RHD, (i.e. increases the mean age at which juvenile rabbits are first infected) but that the virus continues to circulate in lower-density populations and ultimately infects approximately the same proportion of the population. Because young sero-negative rabbits are more likely to survive infection than older sero-negative juveniles or sero-negative adults (Robinson *et al.* 2002), it is likely that conventional rabbit control increases the overall mortality rates from subsequent RHD outbreaks, albeit by slightly increasing the life-span of the individuals before infection. Hence rabbit control slows the circulation of RHD but does not reduce its effectiveness. Exceptions to this generalisation may occur where transmission of RHD in the area is already poor.

In the southern region there are also indications that rabbit control reduced the proportion of rabbits with benign CV antibodies. Failure to detect a more general effect may have been because so few rabbits had benign CV antibodies in the central and northern regions. Antibodies to the benign CV can currently be detected only by an indirect method: they react strongly to only one of four ELISAs that were developed to test for antibodies raised against RHDV. This leaves open the possibility of misclassification of some rabbits with low antibody titres so that results must be viewed cautiously where the prevalence of benign CV
antibodies is very low. Nevertheless, as previously observed by Cooke et al. (2002), there was a significantly higher incidence of benign CV antibodies in rabbits from moist temperate areas than those from drier areas. The level of protection offered by benign CV from RHD infection is unknown, but recent trials suggest that they may reduce mortality in the field by as much as 50% in some circumstances (G. Mutze and R. Sinclair, unpublished data), which would greatly reduce the impact of RHD.

At the commencement of this study, we believed that if rabbit control (reduced density) affected one virus more than the other there would be implications for optimum timing and frequency of control. For example, rabbit control might leave recovering populations largely susceptible to both diseases and epidemic RHD might then spread back in more quickly than benign CV. We found no such effect of rabbit control on the interaction between RHD and benign CV that could be exploited to improve the effectiveness of rabbit control. Prevalence of both viruses appeared to be reduced amongst young rabbits in lower density populations, but each virus was affected to a similar extent.

Our results suggest that current rabbit control operations during summer, in South Australia and north-western Victoria, make good use of the seasonal reduction of rabbit populations by RHD during spring. However, in the higher rainfall districts of southern Victoria, RHD activity often occurred in summer, so rabbit control should be conducted only in late summer or early autumn, after RHD outbreaks but before the breeding season commences. Finally, our results show that from a very young age the majority of rabbits in southern Victoria have antibodies to either or both of the benign CV and RHD. Despite the limited impact of RHD for rabbit control in this area, the potential for improvement from further releases of RHDV appears to be limited.

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REFERENCES


