CAN INSECTS TRANSMIT RABBIT HAEMORRHAGIC DISEASE VIRUS?

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ABSTRACT

A field experiment in Otago, New Zealand, investigated whether healthy rabbits, *Oryctolagus cuniculus*, exposed to insects in an area where rabbit haemorrhagic disease (RHD) was present could be infected with the disease. Thirty rabbits were placed individually in cages, half of which were covered with fine mesh. All rabbits in the mesh covered cages survived. In the 'open' cages, four rabbits died, three of which tested positively for RHD in an ELISA test, and serum samples from surviving rabbits suggested that two may have received a sub-lethal dose of virus. Of the insects trapped in the cages, *Hybopygia varia* (Walker) (Sarcophagidae) was considered the most likely vector, and in some cases tested positively for RHDV.

Keywords: rabbit calicivirus disease, rabbit haemorrhagic disease, insect vectors, disease transmission, *Hybopygia varia*.

INTRODUCTION

Rabbit haemorrhagic disease (RHD), otherwise known as rabbit calicivirus disease has been found only in the European rabbit, *Oryctolagus cuniculus* (L.). RHD was first reported in China in 1984, and in Europe in 1986. It has spread quickly throughout Asia, Africa and the Americas, having been reported in 40 countries (Robertshaw 1992). The virus was illegally imported and released in New Zealand in August 1997, after an application to import the virus was rejected by the Ministry of Agriculture and Fisheries in July 1997 on the grounds that insufficient was known about the epidemiology of the disease.

Little is known about the mechanisms of transmission of the virus, and whether insects play a role in disease spread. In the event that the disease might be accidentally or deliberately released into New Zealand, a report was commissioned by MAF Policy on potential vectors of rabbit haemorrhagic disease virus (RHDV). In this report, eight species of Diptera were identified as potential vectors (Crosby and McLennan 1996). These authors distinguished between direct-contact (transferring virus from infected to uninfected rabbits) and indirect-contact vectors (transferring virus via rabbit forage) and indicated that three fly-strike species of blow flies, *Lucilia cuprina* (Wiedemann), *L. sericata* (Meigen), and *Calliphora stygia* (F.) would be likely to transmit virus to healthy rabbits by both methods. *Hybopygia varia* (Walker), was also considered likely to be a vector, as well as some carrion-feeding species. Based on Australian studies reported in Dugdale and Clarkson (1995), which indicated that virus loses its viability after a few hours in bloodsucking insects, Crosby and McLennan (1996) concluded that mosquitoes and blackflies were unlikely to be vectors of RHD.

Heath (1998) reported on the species of flies visiting rabbit carrion at two sites in Central Otago since the introduction of RHD. Seven species of Calliphoridae were trapped, with *C. vicina* being the most numerous, followed by *L. sericata*. *H. varia* was very rare, with only three specimens in a total dipteran catch of >6000, despite this species being common in baited bottle traps in the same area. RHDV was found in the

four most commonly-trapped blowflies, *C. vicina*, *C. stygia*, *C. quadrimaculata* (Svederus) and *L. sericata* with the latter more frequently positive for RHDV.

In Australia, flies and mosquitos (Cooke 1997), and fleas (Sinclair *et al.* 1998), have been shown capable of carrying RHDV. Studies have shown that virus persisted in the gut of flies fed on infected rabbit liver and emerged in faeces in a viable condition. It was therefore proposed that the virus could be spread indirectly by contamination of plants on which rabbits graze (B. Cooke pers. comm.). Lugton (1998) demonstrated an association between rabbit population reduction and high flea infestation. The role of insects in the direct transmission of the disease to live rabbits has not been demonstrated.

The field experiment reported here was a preliminary investigation of the potential for insects to transmit RHD directly to healthy rabbits in an area where RHD was present in the local population.

MATERIALS AND METHODS

On 3 March 1998, 30 rabbits were caught in the field near Whangarei, Northland, an area where RHD was not present. The rabbits were flown to Mosgiel and immediately placed with food in individual plastic containers with gauze-covered holes in the lids. Blood samples were taken from one ear of each rabbit on 5 March, the samples centrifuged, and RHD competitive antibody ELISA tests carried out on 1:10 and 1:40 sample dilutions of the serum by the MAF Qual Central Animal Health Laboratory, Wallaceville (Lavazza and Cappucci 1996). On 6 March the rabbits were transported in their plastic containers to a farm at Dunback (North Otago) in an area where recent rabbit deaths from RHD had been recorded (Otago Regional Council, pers. comm.). The site used was a grass pasture containing no stock when the experiment was first established, but four cattle gained access during the trial. The paddock was bounded by a small plantation of trees on one side, a scrub-filled gully on two sides and pasture on the other.

While in the field, the rabbits were housed in wire cages 64 x 32 x 35cm high (Figure 1). Particle board was used to cover the roof of each cage for protection from rain and sun. A hole approximately 15 x 10cm was cut in the centre of the roof, a piece of sticky insect trap film (AgriSense-BCS Ltd., Pontypridd, UK.) stapled to the underside, and 12mm galvanised wire mesh stapled to the upper side to cover the hole.

FIGURE 1: Diagram of field cage (not to scale).

This allowed a sample of the flying insects which entered the cage to be collected as they orientated up towards the light and became trapped. The floor of the cage was covered with particle board so that the rabbit had no contact with the ground. One rabbit was placed in each cage and supplied with commercial rabbit pellets, fresh carrots and water. Fifteen cages were constructed as above, allowing flying insects access to the rabbits (open cages). A further 15 cages were similarly assembled but with fly-screen mesh (1x1mm holes) taped and stapled to the sides to prevent the access of insects. A fine mesh bag was tied over the end of the cage that opened, to further preclude insect entry (mesh cages). Black plastic sheeting was used to cover one end of each cage to afford further shelter for the rabbits.

Three open and three mesh cages were tested at the site before the experiment by placing liver as a fly attractant in a plastic container inside each cage. The cages were left for three days. No flies, eggs or fly larvae were found in the mesh cages, whereas in the open cages, the liver container contained flies, eggs and fly larvae. The insect-proofing was therefore considered adequate.

A pair of cages from each treatment was placed about 1m apart and 1m from the perimeter of a 45cm high wire mesh (4cm) enclosure, which prevented local rabbits from directly contacting the caged animals. The 15 pairs of cages were located at 50m intervals around the perimeter of the paddock. A fly trap constructed from a 1 litre plastic drink bottle containing fresh liver bait (Wools of New Zealand 1997) was placed 10m from each pair of cages. These were used to monitor the fly species which were active 10 days before and during the period of the field experiment. Flies from the bottle traps were identified to species and counted.

The rabbits were left in the field for 10 days. They were checked daily for the first 3 days and then every 2-4 days and food and water supplies maintained. Any tears found in the insect mesh (caused by the rabbits) were either repaired with tape or additional mesh was added. Three cages with damage to the mesh were not repaired as indicated in Table 2. The spleen and liver were removed from each rabbit that died during the experiment and assayed using an RHD antigen ELISA test by the MAF Qual Central Animal Health Laboratory, Wallaceville (Lavazza and Cappucci 1996; Motha and Clark 1998). The remainder of the carcasses were kept in deep freeze. Live rabbits were returned to the laboratory and blood tests taken the following day. The samples were processed as above using RHD competitive antibody ELISA tests at 1:10, 1:40, 1:160 and 1:640 dilutions.

The sticky trap samples were removed from the cages, placed in individual plastic bags, and frozen at -20°C. Flies were identified to family and other insects to order. Since *H. varia* (the only larger dipteran species trapped) was considered the most likely vector of those collected, they were removed from each sticky trap using forceps sterilised in 10% Virkon. All *H. varia* from each individual sticky trap were placed in 20ml sterilised universal bottles and processed by the MAF Qual Central Animal Health Laboratory, Wallaceville using a RT (reverse transcriptase) PCR procedure to identify RHDV (Tham *et al.* submitted). *H. varia* were also sorted from the bottle trap catches (2-10 March period only) and processed as above for RHDV.

RESULTS AND DISCUSSION

Rabbits

The results of the serum RHD competitive antibody ELISA tests taken before and after the field experiment are shown in Table 1. Data are percent inhibition of optical density at a 1:40 dilution of the sample. The higher the value, the greater the antibody reaction.

Four rabbits died during the field experiment, all of which were in open cages, and three of these tested positively in the tissue sample ELISA tests (Table 1). Histological examination of the rabbit that tested negative in the ELISA test by the MAF Qual Invermay Animal Health Laboratory was unable to confirm whether there was any indication of the disease because freezing had badly disrupted the tissues. One of the dead rabbits that was positive in the RHD antigen ELISA test was found dead the day after the experiment was established in the field (open cage 15). Serum from this rabbit

had tested negative for virus in the RHD competitive antibody ELISA test carried out two days previously (Table 1). The other two rabbits died after 5-10 days field exposure.

The serum antibody ELISA tests taken the day after the field experiment gave a high (93%) inhibition level for one of the rabbits and higher than average (41%) for another (Table 1), suggesting that these rabbits may have received a sub-lethal viral dose, or that the virus transmitted was no longer viable. If it is conservatively estimated that four rabbits became infected during the field experiment (open cages at sites 6, 7, 11 and 15), using a simple binomial analysis, the proportion infected in the open cages was significantly higher than would be expected from a random infection of rabbits between treatments ($P < 0.05$).

TABLE 1: Percent inhibition of optical density data at 1:40 serum sample dilution for RHD antibody ELISA tests before and after the field trial, and RHD antigen detection ELISA test results for liver/spleen samples of rabbits that died in the field.

Site	Mesh cages			Open cages		
	RHD antibody ELISA (% inhibition of optical density)		Tissue antigen ELISA	RHD antibody ELISA (% inhibition of optical density)		Tissue antigen ELISA
	Pre-field	Post field		Pre-field	Post field	
1	15	18	-	19	16	-
2	-4	-3	-	-5	41	-
3	26	19	-	-3	19	-
4 ¹	-8	5	-	5	5	-
5	5	-24	-	2	6	-
6	3	15	-	19	93	-
7	11	13	-	-3	died	+ve
8	19	6	-	2	16	-
9	29	22	-	-4	died	-ve
10 ¹	-11	-1	-	13	16	-
11	36	no sample ²	-	-15	died	+ve
12	5	-5	-	19	27	-
13	7	13	-	-2	-6	-
14 ¹	-4	-12	-	1	10	-
15	12	12	-	-19	died	+ve

¹ mesh damaged; ² insufficient blood extracted

Insects

Thirteen families of flies (Diptera) were represented in the sticky trap collections. The most abundant were Sciaridae (41%), Sphaeroceridae (14%), Cecidomyiidae (17%) and Sarcophagidae (15%). The latter was represented by only one species, *H. varia*, which breeds in ruminant dung. It is the size of a blow fly and was the dipteran caught on the sticky traps that was considered most likely to have vector potential as it is attracted to animal body fluids, eg. human sweat, and therefore possibly the mucous membranes of rabbits. *H. varia* was a potential RHD vector identified by Crosby and McLennan (1996). A European study found that conjunctival infection of rabbits was possible with about 100 virus particles, and that *Phormia* spp. (Diptera: Calliphoridae) transmitted RHD to susceptible rabbits seven hours after contamination with virus material from dead rabbits (Gehrmann and Kretzschmar 1991). These authors concluded that disease transmission by flies is of epidemiological importance.

The number of individuals of the four main insect families collected on the sticky traps on the underside of the roof of the cages is shown in Table 2. Clearly, the mesh cages did not exclude all flies, allowing access by individuals of very small species. However, for all dipteran groups, the mean number per cage was significantly lower for the mesh cages than the open cages ($P < 0.05$). One individual of *H. varia* was found in one of the three cages where damage to the mesh had not been repaired. Other insect orders represented on sticky traps were Coleoptera, Hymenoptera, Thysanoptera, Collembola and Hemiptera, but these were present in very small numbers compared with the dipteran catch.

TABLE 2: Mean number of Diptera trapped per cage of the four major families collected in the mesh and open cages; total numbers are shown in brackets.

Dipteran family	Mean number per cage $\pm$ SE	
	Mesh cages	Open cages
Sarcophagidae (<i>Hybopygia varia</i>)	0.07 $\pm$ 0.07 (1)	1.67 $\pm$ 1.0 (25)
Sphaeroceridae	0.29 $\pm$ 0.13 (4)	1.33 $\pm$ 0.65 (20)
Sciaridae	1.10 $\pm$ 0.22 (15)	3.73 $\pm$ 0.78 (56)
Cecidomyiidae	0.21 $\pm$ 0.21 (3)	1.80 $\pm$ 0.68 (27)

In the baited bottle traps, a total of 102, 536 and 399 flies were trapped during the trapping periods 25 Feb - 2 Mar; 2-10 Mar and 10-17 Mar respectively. The dipteran species most commonly trapped were *H. varia*, *C. stygia*, *C. vicina* Robineau-Desvoidy and *L. sericata*. These species represented over 95% of the catch on all three collection dates. Other than *H. varia*, none of these species were found in the sticky traps in the rabbit cages.

The results of the RT PCR tests on *H. varia* from sticky trap samples showed that flies were positive for RHDV in open cages at sites 6 and 7, and negative for the open cages at sites 9, 13, and 11, and mesh cage at site 10. The single individual from the open cage at site 12 was not tested. The *H. varia* sorted from the bottle trap samples were positive for RHDV at 8 sites (2, 6, 7, 8, 9, 10, 11, and 13) and negative for the other 7 sites.

Other insects collected from sticky traps have been retained, but since knowledge of their biology indicates that they have less potential to be effective vectors of RHDV, they have not been analysed for virus at this stage.

CONCLUSION

The results of this preliminary field experiment indicate that exposure of healthy rabbits to insects may have resulted in the transmission of a fatal dose of RHD to three, and a sub-lethal dose to at least one other of the animals in the open cages. The mode of transmission remains unclear but may have occurred following direct contact of the rabbits with flies, probably in this case the sarcophagid *H. varia*, some of which tested positive for RHDV. Further research is required to: determine more accurately the mode of transmission; compare the efficacy of viral transmission by insects via direct and indirect means; confirm the role of *H. varia* as a vector, and therefore the potential for enhancement of rabbit biological control with RHD by stock management to provide dung breeding sites for these flies. Fly monitoring is being used in Otago to determine seasonal patterns of activity for fly species, and eventually it is anticipated that information on optimum periods for disease transmission can be identified, which will further assist in optimising the timing of biological control management for rabbits.

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