RFA Research Proposal - Monitoring RHDV in Flies

Background to the research proposal

A growing body of evidence suggests that flies are the main vectors that facilitate the rapid spread of emergent new RHDV variants around Australia.

The capacity of flies to spread RHDV has been recognized for a long time. Even before RHDV was introduced to Australia, Gehrmann and Kretzschmar (1991) showed that iridescent flies (Phormia sp.) were able to transmit the disease under laboratory conditions. Asgari et al. (1998) demonstrated that fly spots containing faecal and oral egesta are infective when collected from surfaces in the vicinity of blow-flies that had fed on RHDV-contaminated liver, and Berman (unpublished) showed that grass collected from near (but not in contact with) carcases on which blow-flies had been feeding became contaminated with RHDV, and was infective when fed to susceptible rabbits. Flies were implicated in the initial escape of RHDV from Wardang Island (Cooke 1996) and its subsequent rapid spread (Kovaliski 1998).

Amy Ianella (unpublished current PhD project, University of Adelaide) monitored flies at the sites of five small rabbit populations in the broad vicinity of Turretfield, SA. The initial purpose was to determine whether it was possible to detect the source/route of virus being carried in to the Turretfield site to initiate annual outbreaks. Flies carrying RHDV were trapped at every site where rabbit carcases were recovered that contained RHDV. At those sites, contaminated flies were trapped before RHDV was first detected in a dead rabbit and after RHDV was last detected in a dead rabbit. Flies carrying RHDV were also trapped at every site where no rabbit carcases were recovered.

These data confirm the key role of flies in RHDV transmission and explain how novel RHDV strains can circulate rapidly between distant rabbit populations. In particular they highlight the potential for detection and identification of RHDV strains in areas where it is often difficult to obtain carcases of dead rabbits for virus extraction, the only currently available method for obtaining RHDV samples.

Despite this evidence, flies have not been used for routine monitoring of RHDV because of a lack of suitable method for handling bulk samples of captured flies. Research to date has required expensive and time-consuming analysis of multiple small sub-samples, many of which contain no virus, in order to detect virus in the small number of RHDV-positive flies in a bulk sample.

Two new RHDV variants have arrived in Australia from unknown sources in the past 3 years: an RHDV strain genetically similar to some isolated in China; and an RHDV2 strain similar to one isolated in Portugal. A third new strain from Korea, brought in for testing by the Invasive Animals Co-operative Research Centre in the RHD Boost program, is scheduled for release in March 2017. These strains are commonly referred to as field strain RHDV1, Chinese RHDV1, RHDV2, and K5-RHDV1, respectively, where the 1 and 2 denominations identify groups that provide complete cross-immunity between strains within the group (i.e. RHDV2 can infect and kill rabbits immune to the other viruses, but K5-RHDV1 cannot infect rabbits previously infected by a field strain RHDV1).

If an efficient analytical method is developed, fly sample collection from remote areas may allow far more complete analysis of how these RHDV strains interact and compete than is possible from the few carcases of RHDV-killed rabbits that are found and submitted for analysis. However, the logistics of fly sampling itself may remain an obstacle to monitoring RHDV. Fly sampling is relatively quick and easy, but travel costs and the time spent (1-3 days) waiting for flies to enter traps are still prohibitive for routine sampling in remote areas unless collaborative projects can be established with groups operating in remote areas for other purposes.

RFA Research Proposal – Monitoring RHDV in Flies: sample testing

Purpose of the research: what do we want to find out?

The primary purpose of the proposed research is to develop a method for optimum extraction of RHDV viral RNA from bulk samples of flies that can subsequently be applied to process fly samples collected from regional and remote areas, in order to answer 5 research questions:

1. What is the geographic spread of RHDV2?
2. What is the persistence and geographic spread of K5-RHDV1 after release?
3. What is the relative prevalence of RHDV2, Chinese RHDV1, K5-RHDV1 and field strains of RHDV1 derived from the original release in 1995?
4. How can we best identify genetic changes in RHDV strains: mutations and recombinants?
5. Which fly species are important vectors?

Questions 1 & 2 are the priorities, 3 & 4 are highly desirable spinoffs and 5 is a possible bonus.

Developing the sample testing method

Laboratory research will use various PCR approaches to:

- Optimize method for extraction of viral RNA from bulk fly samples
- Determine standard method for routine screening to identify what virus is where
- Explore advanced techniques for extending the value to answer other questions (including but not necessarily restricted to those above).

Dr Adam Croxford is a University of Adelaide post-doctoral research associate currently based at the Waite Campus, Urrbrae. He has access to MiSeq NGS sequencing facilities necessary for developing state of the art methodology and will be in Adelaide until June 2017 co-supervising students, but has no current project salary in 2017. He is keen to undertake developmental work on RHDV and flies. He comes highly recommended for his skill and value for money in recent project work by fellow researchers at the Waite, Dr David Taggart and Dr Brad Page.

Note that Dr Robyn Hall from CSIRO is currently working on a similar project in Canberra.

Given the recent escape and spread of RHDV2 and imminent release of K5, there is an urgent need to make progress in this area and considerable benefit in having different laboratories testing different approaches for a common goal. This would also be an opportunity to train the PIRSA technician, John Kovaliski, who has been responsible for basic PCR analyses of RHDV and who may then be able to conduct routine analyses on an ongoing basis. It would also provide a means to continue research directions of Dr Nina Schwensow, a former recipient of RFA research funding support, and keep her engaged in a collaborative manner.

Field sampling of flies for detection of RHDV

RFA has discussed joint projects with Bush Heritage Australia and Australian Wildlife Conservancy to address rabbit control. Both organizations operate permanently-staffed conservation reserves in some rabbit-prone remote areas of Australia that could be invaluable contributors if included in a national RHDV/fly monitoring network.

The Centre for Invasive Species Solution is a new collaborative research institute being established from July 2017 to conduct research projects aimed at reducing the impact of feral animals, including biological control of rabbits. One of the research aims is to determine where and how the various strains of RHDV present in Australia are interacting in field situations, and whether that can be manipulated by virus releases to improve rabbit control. Fly sampling is a novel solution to identifying which viruses are present where in the environment.

RFA Research Proposal – Monitoring RHDV in Flies: sample collection

Because travel costs and the time spent (1-3 days) waiting for flies to enter traps are prohibitive for routine sampling in remote areas, the major difficulty with this approach is finding staff who are permanently located in field areas and can assist with fly sampling as a minor part of their normal work routine. Flies can be trapped by many simple funnel-type traps; the simplest being manufactured from an empty coke bottle with the top cut off and inverted, more complex versions having wind-oriented mounting and separate capture and bait chambers for ease of use. In general, traps are set for a day or two, then the flies removed and frozen for storage.

Method

How many traps are needed, and where?

- Probably 1-3 and bulk the samples from any multiple traps. Can be reasonably close to homestead/research centres particularly if there are any rabbits around the area. Flies move long distances but we are particularly interested in species that are attracted to areas with rabbits.

How many days per trapping period?

- 1-3 consecutive days. It is unlikely to matter so long as you catch a sample of flies.
- What volume of flies constitutes a good sample: possibly 100 ml or more

What is the time interval between trapping periods?

- Open to negotiation, but possibly biannual, quarterly, or monthly, or concentrate sample collections more around spring when RHDV is usually more actively spreading (maybe Aug, Oct, Dec with 1 in March/April if only 4 per year are feasible)
- There would be considerable benefit in getting the first fly samples collected before release of K5-RHDV in early March 2017. If any sites are within 20-50 km of proposed K5 release sites, sampling 3 and 8 weeks after releases would be valuable to test K5 persistence and spread

What bait and traps can be use?

- Doesn't matter so long as they catch flies and are cleaned adequately (or replaced) between trips
- Cheap option for short-term (coke bottle). Sturdy/convenient/efficient option for longer term sites (wind oriented). Smelly meat, or a mixture of dung, meat and sodium sulphide are effective baits.

Sample storage

- Initially zip-lock bags or 200 ml plastic jars in a standard freezer.
- Subsequent collection to be arranged and storage (-80C) at a central laboratory facility.

Project establishment

If landholders wish to participate, PIRSA would commit some time and resources to get the trapping programs established.