Appearance of RHDV2 – investigating a new disease variant

Author: Cheryl Ramsbottom

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ABSTRACT

Rabbits should be annually vaccinated against two potentially fatal viral infections – myxomatosis and viral haemorrhagic disease. However, a new variant of the latter has emerged over the past few years.

This article aims to offer an overview of the disease, as well as suspected methods of transmission, symptoms and diagnosis. Ways to reduce exposure will also be briefly covered.

Keywords: rabbits, RVHD1, RVHD2, diagnosis, vaccination

Viral haemorrhagic disease (VHD) – also known as rabbit calicivirus disease or rabbit viral haemorrhagic disease – is highly infectious and shows a high mortality rate in both wild and domestic rabbits.

The infectious agent responsible for the disease is rabbit calicivirus, otherwise known as rabbit haemorrhagic disease virus (RHDV). In the UK, two distinct strains of the latter now exist – RHDV1 and the new RHDV2.

RHDV1
RHDV1 was first reported in 1984 in China, then two years later in Europe. To date, it has been reported in more than 40 countries in Africa, the Americas, Asia, Europe and Oceania, and is endemic in most parts of the world (World Organisation for Animal Health; OIE, 2015).

RHDV1 is endemic in the UK and controlled by local isolation and vaccination.

**RHDV2**

An outbreak of disease occurred in a rabbitry in France in 2010, which resulted in a 25% mortality of its vaccinated rabbits (Le Call-Reculé, 2013).

Once the dust settled, this was identified as a new variant of VHD from DNA sequencing and genetic analysis. It has also historically been present in other parts of Europe since mid 2010 (Westcott et al, 2014).

Moredun Research Institute in Scotland also confirmed it had diagnosed more than 10 positive cases of RHDV2 from PCR testing across England, Scotland and Wales since January 2015 (Rocchi, 2016).

RHDV2 differs from the original RHDV1 in a number of ways. Rabbits infected with RHDV2 virus typically do not show the symptoms that commonly occur with RHDV1 infection.

Diagnosis is also challenging due to RHDV2 being less fatal, with the rabbit being able to carry the disease for a longer period of time without any visible signs of infection. Unlike the original strain of RHDV1, rabbits younger than eight weeks of age are suspected to have no natural immunity to RHDV2 (Table 1).

**Spread**

<table>
<thead>
<tr>
<th></th>
<th>RHDV1</th>
<th>RHDV2</th>
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</thead>
<tbody>
<tr>
<td>Incubation period</td>
<td>1-4 days</td>
<td>3-9 days</td>
</tr>
<tr>
<td>Period of illness</td>
<td>3-4 days</td>
<td>Up to 5 days</td>
</tr>
<tr>
<td>Age affected</td>
<td>&gt;6-8 weeks old</td>
<td>Rabbits of any age</td>
</tr>
</tbody>
</table>

Data based on a collaboration of opinion from numerous references.
A great deal is still to be learned about this virus. It is thought to spread the same way as RHDV1, directly from rabbit to rabbit, through fomites – including cage material and feeds – as well as via biting insects or by inhalation of viral particles (Rosenwax, 2013; Figure 1).

Henning et al (2005) conducted a study to investigate the persistence of RHDV1 in the environment and found the virus in animal tissues, such as rabbit carcases, can survive for at least three months in the field.

VHD is protected in tissues and can survive for more than seven months in organ suspensions stored at 4°C – that is, the refrigerator. It can also survive at room temperature in a dried state for at least three months on towels or blankets (OIE, 2015) and RHDV2 is assumed to have similar persistence.

Survival of RHDV in the tissues of dead animals could, therefore, provide a persistent reservoir of the virus, which could initiate new outbreaks of the disease after extended delays (Henning et al, 2005).

**Diagnosis**

In most cases, no specific symptoms exist and some cases show no symptoms at all. Any seen may include:

- loss of appetite
- lethargy
- fever
During an outbreak, a small percentage of rabbits may show chronic or subclinical evolution of the disease, which is characterised by severe and generalised jaundice, loss of weight and lethargy. These animals often die one to two weeks later, most likely due to liver dysfunction (OIE, 2015).

In 2015, the OIE stated it was “unknown” how long rabbits may remain infectious after recovering from RHD.

It said: “A low level of serum antibodies is sufficient to protect rabbits from the disease, but infection at the intestinal level could occur with shedding of the virus in the faeces. High sensitivity PCR demonstrates a long-term persistence – up to two months – of the viral RNA in recovered or in vaccinated and then infected rabbits.”

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**Figure 2.** Rabbits Pumpkin and Treacle waiting for their health checks and RHD vaccinations.

Definitive diagnosis of RHDV2 is difficult in a live animal; as with RHDV1, no validating blood test exists and postmortem is advocated for any unexplained or sudden deaths in rabbits, with owners’ consent.

On postmortem, liver changes may be seen and, sometimes, bloodstained fluid in the body cavity may be present, but this is not always the case (Raftery, 2016).

Moredun Research Institute believes the best sample for PCR testing is a liver sample, which can be sent frozen or in viral transport media.

It is best to avoid weekend shipment, however, and postage should follow guidelines for UN3373.
Biological Substances Category B (Rocchi, 2016).

According to the OIE, fresh liver, spleen, blood or formalin-fixed samples of any organ can be used to aid diagnosis; however, some may have a higher chance of false negatives. The liver is, therefore, the organ of choice as it has the highest viral titre in acute or peracute cases.

Moredun Research Institute’s PCR test on liver samples has been validated to provide an accurate diagnosis and it might be possible to develop a test for live cases as serum may contain high levels of the virus (OIE, 2015); however, this has not been confirmed or validated. For more information and a sample submission form, visit www.moredun.org.uk/virus-surveillance-unit/rhdv

**Vaccination**

Research continues into RHDV2, but the most effective way of prevention is vaccination. The Avian and Exotic Animal Clinic at Ashleigh Veterinary Centre in Manchester imported a vaccine directly from the manufacturer in France after completing the relevant paperwork and has been administering it to domestic rabbits since May 2016.

No adverse affects have been reported, except the occasional hard swelling at the subcutaneous injection site, which can persist for up to two months (Figure 2). Other veterinary practices could also source this vaccine.

Another vaccine, developed in Germany, requires two injections three weeks apart to protect against RHDV1 and RHDV2. However, stock of this vaccine was quickly used up in neighbouring countries from January 2016 onwards.

**Figure 3.** Treacle the rabbit being handled as an example of barrier nursing.

The French-manufactured vaccine, meanwhile, is available in 1, 5, 10 and 50 dose vials, and provides protection from RHDV1 and RHDV2. The manufacturer recommends a single subcutaneous injection for rabbits older than 10 weeks old, with an annual booster.
The datasheet, meanwhile, states protection is reached seven days post-administration and the vaccine must be given three weeks apart from other vaccinations, such as the annual combination vaccine for RHDV1 and myxomatosis.

As with other vaccines, it needs to be kept refrigerated between 4°C and 8°C. Once the multidose vials are breached and mixed, the vaccine is viable for up to two hours.

No results exist from trials on pregnant rabbits; however, according to the manufacturer, experience of use in commercial rabbits has not revealed any problems in vaccinating them.

The VMD requires veterinary practices to complete an online import certificate before allowing receipt of the vaccine. Some of the national wholesalers already have it in stock.

**Husbandry**

It is unknown how long the virus survives in the environment. Therefore, for suspected or confirmed cases, barrier nursing is required (**Figure 3**). Deep cleaning of the environment for positive cases with Defra-approved product is highly recommended.

While no research has been carried out, as of yet, on the effects of disinfectants against RHDV1 or RHDV2 calicivirus specifically, it is advisable all veterinary practices contact their disinfectant manufacturers to confirm adequate efficiency against caliciviruses in general.

For owners, meanwhile, it is advisable they minimise their rabbit(s) contact with wild rabbits and try to prevent them from coming into contact with biting insects – especially on warm, damp and cloudy days when insects are more active. Some owners may wish to bring their rabbits indoors if they are not vaccinated (**Figure 4**).
Owners should also consider disinfection of clothing and footwear after passing through areas where wild rabbits may roam. RHDV1 can survive heat of 50°C for about an hour (OIE, 2015), so low-temperature washing machine cycles may not neutralise the virus. However, this is yet to be formally confirmed for RHDV2.

A risk may also exist with wild plants – acting as fomites – causing infection, as many owners forage for their rabbits. However, isolation or quarantine is advocated for a minimum of an eight-week period for any new rabbit entering a collection.

Other considerations to minimise the spread of the disease include regular cleaning and disinfection of rabbit hutches and runs, correct storage of food/hay and proper hygiene controls between rabbits.

**Acknowledgements**

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**References**
Raftery A (2016). Personal communication.