

# Rabbit Haemorrhagic Disease (RHD)

**Dr. Lorenzo Capucci**

Laboratory of Immunobiochemistry  
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini",  
Via A. Bianchi 7/9, 25124 Brescia, Italy  
Tel.: (+39 30 2290366), Fax: (+39 302290311)  
e-mail address: [capucci@bs.izs.it](mailto:capucci@bs.izs.it) website <http://www.bs.izs.it>

## Summary of general activities related to the disease

### 1. Test(s) in use/or available for the specified disease at your laboratory

#### Virological Tests

*Tests used for routine diagnostic work:*

- Sandwich ELISA test using RHDV specific Monoclonal Antibody (MAb). A similar test using specific EBHSV MAbs is used for diagnosis of EBHS.
- Sandwich ELISA test using a panel of RHDV specific MAbs. This test permits the quick detection of antigenic RHDV variants. It also includes MAbs produced and specific for the first consistent antigenic variant of RHDV (subtype RHDVa) and therefore it allows an easy and quick distinction between such variant and "classical" RHDV strains.
- Western Blot Analysis using RHDV-MAbs cross-reactive with EBHSV. It is usually performed on the few samples, which give doubtful results in ELISA test, in animals died due to the "chronic" form of the disease and in which the presence of specific antibodies interfere with the ELISAs test. The analysis is usually performed on samples previously concentrated by ultracentrifugation, both on the pellet and on the supernatant.

*Additional Tests used for particular investigations:*

- Reverse transcription Polymerase Chain Reaction (RT-PCR).
- Negative staining ImmunoElectronMicroscopy and ImmunoGold using both MAbs and rabbit and hare hyperimmune sera.
- Haemoagglutination tests

#### Serological Tests

*Tests used for routine diagnostic work:*

- Competition ELISA; two different tests, based on specific MAbs used as tracer, have been set up respectively for RHDV and EBHSV.
- ELISAs developed using antisotype MAbs to test the sera for the presence of specific anti-RHDV IgM, IgA and IgG.

*Additional Tests used for particular investigations:*

- Indirect ELISA with the purified RHDV adsorbed to the solid phase.
- Sandwich Elisa to detect IgM and IgG in liver or spleen samples already examined with the virological test. Such test is particularly useful in those animals, which die due to the "chronic" form of the disease, when the detection of the virus could be difficult. In this case, a high level of RHDV specific IgM and a low level, if any, of IgG are the unambiguous marker of positivity for RHD.

### N° of diagnostic tests performed in 2005

| Test                    | For               | Specificity | Diagnosis | Import/export | Surveillance | Total |
|-------------------------|-------------------|-------------|-----------|---------------|--------------|-------|
| ELISA                   | Antigen           | RHD         | 5         | 0             | 203          | 208   |
| ELISA (with panel mABS) | Antigen           | RHD         | 49        | 14            | 13           | 76    |
| Western Blot            | Antigen           | RHD         | 1         | 5             | 7            | 13    |
| ELISA                   | Antibody          | RHD         | 74        | 142           | 999          | 1215  |
| ELISA                   | Antibody Isotypes | RHD IgM     | 61        | 6             | 0            | 67    |
|                         |                   | RHD IgG     | 48        | 28            | 0            | 76    |
|                         |                   | RHD IgA     | 61        | 6             | 0            | 67    |
| ELISA                   | Antigen           | EBHS        | 73        | 0             | 535          | 608   |
| Western Blot            | Antigen           | EBHS        | 29        | 0             | 0            | 29    |
| ELISA                   | Antibody          | EBHS        | 51        | 0             | 907          | 958   |

## 2. Production and distribution of diagnostic reagents

- RHDV MAbs,
- EBHSV MAbs
- RHDV semipurified antigen “BS89 classical strain”
- RHDV semipurified antigen “RHDVa” variant strain
- Anti-RHDV and anti-EBHSV hyperimmune sera

Most of the Italian Istituti Zooprofilattici were provided with ELISA kits. More than 170 kits for a total of 6000 serological and 500 virological tests were produced and distributed during the last year

Diagnostic kits or as single reagents for being used in the diagnosis of RHD were delivered to: USA (4), Portugal (4), Saudi Arabia (4), Slovenia (2).

Several kits (44) for serological diagnosis of EBHS were delivered to Rumania and Hungary for controlling wild hares before exporting to western Countries where hares are released for restocking of hunting areas.

Particularly, RHDV kits for virology and serology (3000 sera) have been sent to Argentina (*Dirección de Laboratorio y Control Técnico, Dra. Verónica Torres Leedham*) in order to increase the surveillance on RHD following the identification of the disease in Uruguay at the end of 2004.

HA-negative RHDV strains have been sent on request of Prof. Wiesław Deptuła to the Department of Microbiology and Immunology University of Szczecin, Poland that is studying the pathology and immunology (cell mediated aspects) of RHD.

## Activities specifically related to the mandate of OIE Reference Laboratories

### 3. International harmonisation and standardisation of methods for diagnostic testing or the production and testing of vaccines

The objective of 2005 to prepare a serological ring test, to distribute to interested laboratory following a direct request, is still in progress and is related to the preparation of reference standard. It is foreseen that this objective will be reached within the 2006.

We have presented a development project in order to improve the application of molecular diagnostic methods to RHD.

### 4. Preparation and supply of international reference standards for diagnostic tests or vaccines

The set up of Reference Standards sera is in the course. It is foreseen to have the complete collection of the sera (negative, positive at different titres also in relation to the main antibodies class – IgM, IgA and IgG) within the 2006.

### 5. Research and development of new procedures for diagnosis and control

RHD is known as an overt disease caused by a high pathogenic calicivirus and, as consequence, immunological methods have showed enough sensibility and specificity to be used as main tools in the laboratory diagnosis. At the same times, the presence of at least a virus highly related to RHDV but not pathogenic is well accepted among more research groups. However, recent scientific publications based mainly, if not only, on molecular phylogenetic studies of RHDV genome belonging to not characterized viruses, seems to put in discussion more aspects to day's RHDV knowledge. In order to directly compare these results and knowledge of the “old virology” with those of the “new virology”, and to improve the laboratory diagnostics methods for RHD, we are starting with a research project that use molecular methods (PRC real time and nested PCR followed by sequencing). We hope to have first consistent results of the research within the end of 2006.

After having completed the laboratory assessment (immune response and experimental challenge) a field trial is in due course for evaluating the potency of a RHD inactivated vaccine prepared using both the classical and the variant strain of RHDV. This vaccine was developed in order to confer a stronger level of protection to rabbits since the epidemiological and diagnostic results are indicating that most (around 65%) of the RHD outbreaks are caused by variant strains.

**6. Collection, analysis and dissemination of epizootiological data relevant to international disease control**

- We have continued to study the distribution and diffusion of the RHDVa variant in Italy and we found that almost 65% of the cases of RHD occurred in the last three years were caused by such variants.
- Indeed some other strains with minor genomic and antigenic changes were found, thus indicating that RHDV, as many other ssRNA virus, is prone to easily mutate and change its characteristics.
- One notification, concerning the occurrence of RHD for the first time in Uruguay was sent to OIE.

**7. Provision of consultant expertise to OIE or to OIE Member Countries**

- We have recently completed the revision of the CHAPTER 2.8.3."Rabbit Haemorrhagic Disease", that will be published in the 6<sup>o</sup> edition of the "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals".
- We have supplied Argentina with the ELISA RHDV serological test in order to set up a surveillance programs towards RHD.

**8. Provision of scientific and technical training to personnel from other OIE Member Countries**

Two people from Slovakia and one people from Poland for execution and interpretation of diagnostic test for serological diagnosis of EBHS and RHDV.

Two people from France, for discussion on epidemiology of RHD in free ranging rabbits and the use of diagnostic methods for detection non pathogenic RHDV-like viruses.

**9. Provision of diagnostic testing facilities to other OIE Member Countries**

In just the case of samples coming from Uruguay (at the beginning of 2005) we reported the information to the OIE Central Bureau. This decision was taken since the disease was considered exotic for that country (first report). In that occasion we received the sample for confirmation and for antigenic and genomic characterization.

**10. Organisation of international scientific meetings on behalf of OIE or other international bodies**

None

**11. Participation in international scientific collaborative studies**

None

**12. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences)**■ ***Presentations at international conferences and meetings***

None

■ ***Scientific publications in peer-reviewed journals***

MARCHANDEAU S., LE GALL-RECULE G., BERTAGNOLI S., AUBINEAU J., BOTTI G., LAVAZZA A. (2005) Serological evidence for a non-protective RHDV-like virus. *Veterinary Research* 36: 53-62.

■ ***Other communications***

*Giornate di Coniglicoltura ASIC 2005 Forlì, 30 settembre – 1 ottobre 2005*

- LAVAZZA A., G. PERUGINI, M. CERIOLO, A. CERRONE, G. BOTTI, L. CAPUCCI. Risultati di una indagine sieroepidemiologica sulla diffusione del calicivirus apatogeno del coniglio (RCV) in animali alla macellazione.
- CERIOLO M., R. BRIVIO, C. SALOGNI, G. GRILLI, A. LAVAZZA. Valutazione dello stato zoonosanitario e immunitario per la individuazione di parametri “in campo” del benessere del coniglio allevato.

*Workshop nazionale di virologia veterinaria “Diagnostica ed epidemiologia delle infezioni virali degli animali”. Istituto Superiore di Sanità. Roma, 28-29 novembre 2005.*

- CAPUCCI L., LAVAZZA A.. Quali e quanti sono i calicivirus dei lagomorfi? Una rassegna dei recenti dati su RHDV, EBHSV e virus correlati.
- LAVAZZA A., CERRONE A., AGNOLETTI F., PERUGINI G., FIORETTI A., CAPUCCI L.. Prevalenza e diffusione della variante patogena del virus della malattia virale emorragica del coniglio (RHDVA) negli allevamenti cunicoli italiani.
- LAVAZZA A., PERUGINI G., CERIOLO M., CERRONE A., BOTTI G., CAPUCCI L.. Risultati di una indagine sieroepidemiologica sulla diffusione del calicivirus apatogeno del coniglio (RCV) in animali alla macellazione.