

# Activities in 2008

## Disease name **RABBIT HAEMORRHAGIC DISEASE**

Reference Expert name  
**Lorenzo Capucci**

Address of laboratory  
Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna  
Via Bianchi 9, 25124 Brescia – Italy  
Tel.: (0039 030 2290 617), Fax: (0039 030 2290 559)  
e-mail [lorenzo.capucci@bs.izs.it](mailto:lorenzo.capucci@bs.izs.it), website/[www.bs.izs.it/Referenza/CREMAVILA/CREMAVILA](http://www.bs.izs.it/Referenza/CREMAVILA/CREMAVILA)

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### 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 isotypes.

*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.

<b>Test</b>	<b>For</b>	<b>Specificity</b>	<b>Total</b>
ELISA	Antibody	RHDV	796
ELISA	Antibody Isotypes (IgM or IgA or IgG)	RHDV	659 / 785 / 646
ELISA	Antigen	RHDV	194
Western blotting	Antigen	RHDV	8
ELISA	Antigen	EBHSV	747
ELISA	Antibody	EBHSV	800
Western blotting	Antigen	EBHSV	7

## 2. Production and distribution of diagnostic reagents

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

ELISA reagents for virologic and serological diagnosis of RHD and European Brown hare syndrome (EBHS), including internal ELISA controls (negative and positive) sent as “kit”. A total of 151 kits (141 on 2007 and 117 on 2006) were supplied to various Italian Istituti Zooprofilattici Sperimentali (Roma, Torino, Palermo, Padova, Treviso, Perugia,, Bologna, Parma,.) to company that imports hares from abroad, i.e. mainly Eastern European Countries (Rumania, Hungary) and to other OIE Member Countries (Germany, Sweden, Poland, United States, Australia, New Zealand, France, Scotland)

<b>Kit for</b>	<b>supplied nationally</b>
RHD serology	19
EBHS serology	26
RHDV_EBHSV virology	63
<b>Kit for</b>	<b>supplied to other OIE Member Countries</b>
RHD serology	24
EBHS serology	18
RHDV_EBHSV virology	9

### **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

A continuous collaboration with Australian main laboratory for RHD serology (CISRO – Adelaide), that use our reagents and methods, is in the course.

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

Nowadays the laboratory does not prepare internationally recognised standard RHD rabbit sera but it produces and supplies a panel of reference RHD sera to use as standard in the evaluation of diagnostic test.

We are planning to produce a panel of RHD reference sera according to the OIE Guide 3 (International Reference Antibody Standards for Antibody Assays) within 2009.

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

We received founding from our Health Ministry to carry out a project to study the epidemiology of RCV (the non pathogenic virus related to RHDV) in rabbits’ industrial farms of different Italian regions. In addition we are using RCV as model to study immunological response to infection and the clearance of the virus and its genome from the body. During these studies, still in course, we improved the serological and virological methods, particularly using new PCRs protocols. The main results, that we are still to be fully confirmed include:

1) the isolation of new hypothetical RCV strains (farms with RHD serology positive but no history and/or signs of disease). The sequence of the VP60 gene confirms that this isolate are related to RCV but also to the isolate identified in UK by Gould et al.

2) RCV, that is an enteric virus, is present in the stools of rabbit after few days post-infection. Specific RCV antibodies are also detectable within few days, starting from IgM and IgA with IgG as last. Interestingly, in some cases the virus is still identifiable in the stools even in presence of high level of serum antibodies.

These results will be published in the course of this year.

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

With respect to previous years, the worldwide RHD epidemiological situation during 2008 was stable and it was confirmed the presence in the field of two main subtypes: RHDV, the virus originally isolated in Europe since 1984, and RHDVa, the virus identified in Europe since 1996 and now reported worldwide. The few data available suggest that the two subtypes are equivalent as regards their epizootiological characteristics. During 2008 there was not report on the failure on the use of the vaccine based on the RHDV virus and this support the laboratories data that this vaccine induce an sufficient immunity to protect rabbits also in case of RHDVa infections.

The most important scientific data published during 2008 certainly consist in the identification of a new non-pathogenic rabbit calicivirus in the Australian wild rabbit population (T. Strive et al, Virology - December 2008). The finding confirms the serological data collected since 1996, using the methods and reagents supplied by our OIE reference laboratory, that suggested the presence of this further rabbit calicivirus with a reduced genetic relation with RHD. In fact, the phylogenetic analysis confirms the presence in the rabbits of three genetic lineages: one pathogenic (RHDV) and two non pathogenic (enteric viruses) one identified by us in Europe (RCV) and one in Australia (RCV-A). Considering that RCV-A is probably present also in other rabbits populations (i.e. European one), this report is important in helping to better explain the serological results.

These data have been discussed and disseminated at national level during a day – meeting held in Brescia with Tanja Strive from CISRO and Brian Cooke (University of Canberra) as speakers.

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

We have sent, just few weeks ago, a document to Prof. Steven Edwards, President of the OIE Biological Standards Commission, in relay to his question on the optimal methods to be indicated in the OIE Code for the RHD serological diagnosis.

We received a request from Dr Abrahão Buchatsky, Coordinator of CGAL/SDA/MAPA Ministério da Agricultura, Pecuária e Abastecimento - Brasil to be their reference laboratory for RHDV. Of course, our answer has been positive considering that is the main goal of an OIE Reference laboratory.

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

Dr.ssa Tanja Strive (CISRO – Canberra) spent more than one month during last spring at the OIE laboratory here in Brescia. During this period she learnt the basic concept of ELISA test in serological analysis and the application of the results in epidemiological studies. During the same period we received the visit of Dr. Brian Cooke of Canberra University in order to discuss of the Australian situation at the light of the worldwide situation.

We also received a short visit of a Dutch PhD student (Stefan de Klepper), who has prepared his dissertation thesis on rabbit population dynamics in The Netherlands and for that he wanted to study RHD serology

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

We did not receive any kind of request for the direct RHD diagnosis (virological identification or confirmation). We performed serological analysis, correlated to epidemiological studies, following requests of laboratories from The Netherlands and Australia.

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

No activity on this item..

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

A collaborative study is in the course with Australian laboratories in order to improve the knowledge on the epidemiological and virological evolution of the RHD in the country.

Another collaborative study is in the course with a Dutch university in order to improve the knowledge on the epidemiological presence and diffusion of RHDV and RHDV-like non-pathogenic viruses among wild rabbits in The Netherlands.

**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*

- LAVAZZA A., CAPUCCI L. Invited lecture "Viral infection of rabbits" in G. Xiccato, A. Trocino e S. Lukehart (eds) "Proceedings of the 9° World Rabbit Congress" – Verona, 10-13 june 2008 pag 879-894 e "Quaderno n° 72 - Fondazione Iniziative Zooprofilattiche e Zootecniche" Brescia, Italy pp. 247-267.

*Scientific publications in peer-reviewed journals*

- CAPUCCI L. LAVAZZA A. (2008) CHAPTER 2.6.2."Rabbit Haemorrhagic Disease", in "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals". 6° edizione. OIE, Paris pp pp.947-961.

■ *Other communications*

**13. Inscription of diagnostic kits on the OIE Register**

- i) **Did you participate in expert panels for the validation of candidate kits for inscription on the OIE Register? If yes, for which kits?**

No

- ii) **Did you submit to the OIE candidate kits for inscription on the OIE Register? If yes, for which kits?**

No

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