

Disease name

RABBIT HAEMORRHAGIC DISEASE

Reference Expert name
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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.

Test	For	Specificity	Total
ELISA	Antibody	RHDV	2214
ELISA	Antibody Isotypes (IgM or IgA or IgG)	RHDV	1644
ELISA	Antigen	RHDV	420
Western blotting	Antigen	RHDV	23
ELISA	Antigen	EBHSV	622
ELISA	Antibody	EBHSV	1334
Western blotting	Antigen	EBHSV	8

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 141 kits (117 on 2006) were supplied to various Italian Istituti Zooprofilattici Sperimentali (Roma, Torino, Palermo, Padova, Treviso, Perugia, Sassari, Bologna, Parma, Napoli), to company that imports hares from abroad, i.e. mainly Eastern European Countries (Rumania, Hungary) and to other OIE Member Countries (Poland, Slovakia, United States, Australia, New Zealand, Hungary)

Kit for	supplied nationally
Serology RHD	21
Serology EBHS	24
Virology RHD/EBHS	49
Kit for	supplied to other OIE Member Countries
Serology RHD	27
Serology EBHS	16
Virology RHD/EBHS	4

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

No activity on this item.

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

Nowadays the laboratory does not prepare or posses internationally recognised standard RHD rabbit sera but it prepares and supplies own panel of reference RHD sera to use as standard in the evaluation of diagnostic test. Answering to request received at the end of 2006, we sent two panels of sera to two laboratories of OIE Member Countries that will use the sera to standardize their own methods. This should be the first step towards the production of internationally recognised standard reference sera, likely within next two years.

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

The Italian Ministry of Health supports a research program on RHDV devoted to the standardization of a set of different PCR (different targets and methods including nested and real time PCR) to use to improve the diagnosis and in studies of molecular epidemiology and pathogenesis. The research is in the course and the final results are expected for the beginning of 2008.

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

In the course of 2007, we had not registered any significant epidemiological or virological data different from those of previous years.

Considering the reduced commercial interest on rabbits and the success of vaccine in the control of the disease, few countries carry out a minimum of surveillance on RHD and therefore we almost did not receive any samples for characterization from abroad.

Some more interest is on the wild rabbits, particularly in Australia where the goal is to keep RHDV active as much as possible or, for opposite reasons, in country where rabbits are among the main hunting target and/or object of conservation issues (i.e. Spain, France, The Netherlands, Greece).

From a scientific point of view, the main data came from the use of high sensitive PCR (real time or nested) in different experimental and epidemiological situations. Using these methods, the RHDV or RHDV-like genome is often found also in animal not apparently involved in RHD outbreaks. These findings, due probably to the presence of viruses/strains RHDV related but not pathogen, may create problems in the diagnosis and control of RHD and therefore must be clarified coupling high sensitive PCR with well designed and reproducible experimental project.

We have continued to study the distribution and diffusion of the RHDVa variant in Italy and we found that 60,9% of the cases of RHD occurred in the last year were caused by such variant.

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

No activity on this item this year.

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

The laboratory took in for one weeks a post doc from Argentina (SENASA Buenos Aires) in order to improve her knowledge on RHDV diagnosis and research methods. The guest had the opportunity to learn on and use the main ELISA methods and to train with the basic PCR principles and methods.

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

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 French, Greece, The Netherlands and Australia (a study on hare sera to look for presence of RHD related antibodies).

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.

A similar collaborative study is in the course with French laboratories 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 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*

None

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

1. MCINTOSH M.T., BEHAN S.C., MOHAMED F.M., LU Z.Q., MORAN K.E., BURRAGE T.G., NEILAN J.G., WARD G.B., BOTTI G., CAPUCCI L. and METWALLY S.A. (2007) A pandemic strain of calicivirus threatens rabbit industries in the Americas. *Virology Journal* 2007, 4: 96-109.
2. PACI G., LAVAZZA A., FERRETTI, BAGLIACCA M., (2007) Relationship between habitat, densities and metabolic profile in brown hares (*Lepus europaeus* Pallas). *Italian Journal of Animal Science*, 6 (3), 241-255.

■ ***Other communications***

Chapter of books

1. FROLICH K, LAVAZZA A (2007). "European Brown hare Syndrome" in P.C Alves, N. Ferrand, K. Hacklaender (eds) "Lagomorph Biology", Springer, Berlin, pp. 253-262.
 2. LAVAZZA A., CAPUCCI L. (2007). "How many caliciviruses are there in rabbits? A review on RHDV and correlated viruses" in P.C Alves, N. Ferrand, K.Hacklaender (eds) "Lagomorph Biology", Springer, Berlin, pp. 253-262.
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