

Activities in 2009

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). The specificity of the test can be enlarged including MAbs produced and specific for the subtype RHDVa. A similar test using specific EBHSV MAbs is used for diagnosis of EBHS.
- 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. In order to gain in sensitivity, the analysis is usually performed on samples previously concentrated 10-20 times by ultracentrifugation.

Additional Tests used for particular investigations:

- Reverse transcription Polymerase Chain Reaction (RT-PCR) : the use of this test is improving
- 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	479
ELISA	Antibody Isotypes (IgM or IgA or IgG)	RHDV	290/ 290 / 290
ELISA	Antigen	RHDV	115
Western blotting	Antigen	RHDV	5
RT-PCR	Antigen	RHDV	15
ELISA	Antibody	EBHSV	1066
ELISA	Antigen	EBHSV	365
Western blotting	Antigen	EBHSV	-
RT-PCR	Antigen	EBHDV	20

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 120 kits were supplied to various Italian Istituti Zooprofilattici Sperimentali (Roma, Torino, Perugia, Sassari Bologna, Parma), to company that imports hares from abroad, i.e. mainly Eastern European Countries (Rumania, Hungary) and to other OIE Member Countries (Germany, Poland, United States, France). Some RHDV MAbs were supplied to France.

Kit for	supplied nationally
RHD serology	10
EBHS serology	19
RHDV EBHSV virology	40
Kit for	supplied to other OIE Member Countries
RHD serology	34
EBHS serology	13
RHDV EBHSV virology	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 particularly activity this year on this item.

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

We are producing a panel of RHD reference sera according to the OIE Guide 3 (International Reference Antibody Standards for Antibody Assays). We foresee to submit the documentation for the approval of the sera as “OIE reference sera for RHD” to the OIE Standards Commission within the first half of 2010.

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

We are still working on three main areas:

- 1) surveillance on the antigenic and genetic evolution of RHDV, increasing this year the efforts also for EBHSV
- 2) looking for the presence of further rabbit calicivirus related to RHDV but not pathogenic
- 3) producing more MAbs towards IgA in order to study the mucosal RHDV immunity in comparison with serology.

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

No relevant data have been collected this year. The worldwide epidemiological situation of RHDV seems quite stable confirming the well-known utility of the vaccine as main tool for the prophylaxis of RHD, at least in farmed, laboratory and pet rabbits.

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

We received the visit of Dr Peter Kirkland from the Elizabeth Macarthur Agriculture Institute, Menangle NSW. Australia. The meeting was finalised to planning a collaboration between OIE Reference Laboratory and Australian organization in studying the pathogenic level of different worldwide RHDV isolates.

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

We did not receive any request for this item.

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 recently received some hare organs from an outbreak of EBHS occurred in Germany and gave support to evaluate serological results on survived animals

We performed virological analysis, correlated to proficiency test, following requests of a laboratory from Polonia. RHDV viral reference strain were provided to National Reference Laboratory for Poultry and Rabbit Diseases at the University of Zurich, Switzerland.

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

We are collaborating as OIE Laboratory with some Dutch, French and Australian groups mainly on the aspect of serological survey of wild rabbits. At present, we are not involved in recognized research project.

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***

Chiari M., Lanfranchi P., Zanoni M.G., Alberali L., Salogni C., Tittarelli C., Tagliabue S., Fabbi M., Lavazza A. (2009) Application of a surveillance program in wild european brown hares (*Lepus europeus*) in Brescia province, North Italy. Proceedings of the VI International Symposium on Wild Fauna. May 21-24, 2009 Paris, France

Scientific publications in peer-reviewed journals

S. R. McPheeA, K. L. Butler, J. Kovaliski, G. Mutze, L. Capucci and B. D. Cooke. Antibody status and survival of Australian wild rabbits challenged with rabbit haemorrhagic disease virus - *Wildlife Research*, 2009, **36**, 447–456

Strive T, Wright J, Kovaliski J, Botti G, Capucci L.. The non-pathogenic Australian lagovirus RCV-A1 causes a prolonged infection and elicits partial cross-protection to rabbit haemorrhagic disease virus. *Virology* 2009 Dec 22. [Epub ahead of print].

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