

WOAH Reference Laboratory Reports Activities 2022

Activities in 2022

This report has been submitted : February 1, 2023 10:35 AM

Laboratory Information

Name of disease (or topic) for which you are a designated WOA Reference Laboratory:	RABBIT HEMORRHAGIC DISEASE (RHD)
Address of laboratory:	via Antonio Bianchi 7/9, 25124 Brescia (Italy)
Tel.:	+39 030 2290617
E-mail address:	lorenzo.capucci@izsler.it
Website:	https://www.izsler.it/chi-siamo/per-chi-e-con-chi-lavoriamo/centri-di-riferenza/internazionali/oie-reference-laboratory-for-rabbit-haemorrhagic-disease/
Name (including Title) of Head of Laboratory (Responsible Official):	Dr. Piero FRAZZI (DVM, General Director of IZSLER)
Name (including Title and Position) of WOA Reference Expert:	Dr. Lorenzo Capucci (BSc)
Which of the following defines your laboratory? Check all that apply:	Governmental

TOR1: DIAGNOSTIC METHODS

1. Did your laboratory perform diagnostic tests for the specified disease/topic for purposes such as disease diagnosis, screening of animals for export, surveillance, etc.? (Not for quality control, proficiency testing or staff training)

Yes

Diagnostic Test	Indicated in WOA Manual (Yes/No)	Total number of test performed last year	
		Nationally	Internationally
Indirect diagnostic tests			
RHDV Competition ELISA	YES	458	325

RHDV2 Competition ELISA	YES	967	547
EBHSV Competition ELISA	YES	389	150
RHDV IgA Isotype ELISA	YES	67	0
RHDV IgG Isotype ELISA	YES	35	23
RHDV IgM Isotype ELISA	YES	68	0
Direct diagnostic tests		Nationally	Internationally
RHDV Sandwich ELISA	YES	301	1
EBHSV Sandwich ELISA	YES	186	0
RT-PCR RHDV/RHDV2	YES	15	3
RT-PCR EBHSV	YES	25	0
Genome sequencing	YES	29	0
RT-PCR lagovirus	YES	97	0

TOR2: REFERENCE MATERIAL

2. Did your laboratory produce or supply imported standard reference reagents officially recognised by WOA?H?

No

3. Did your laboratory supply standard reference reagents (nonWOAH-approved) and/or other diagnostic reagents to WOA?H Members?

Yes

TYPE OF REAGENT AVAILABLE	RELATED DIAGNOSTIC TEST	PRODUCED/ PROVIDE	AMOUNT SUPPLIED NATIONALLY (ML, MG)	AMOUNT SUPPLIED INTERNATIONALLY (ML, MG)	NO. OF RECIPIENT WOA?H MEMBER COUNTRIES	COUNTRY OF RECIPIENTS
RHDV serological kit	c-ELISA	produced	1 kit	10 kits	4	America Europe
EBHSV serological kit	c-ELISA	produced	2 kits	15 kits	3	Europe
RHDV/EBHSV virological ki	MAbs sandwich ELISA	produced	14 kits	11 kits	3	America Europe
RHDV2 serological kit	c-ELISA	produced	1 kit	16 kits	6	America Europe
RHDV/RHDV2 Differential kit	MAbs sandwich ELISA	produced	0	4 kits	1	Europe
Monoclonal antibodies	Immunohisto-chemistry and ELISA	produced	0	2mg/2ml	1	Europe
Reference positive and negative materials (liver homogenates)	PCR	provided	0	1 panel	2	America MiddleEast
Positive and negative reference sera	Serological ELISA	provided	0	1 panel	2	Asia and Pacific Europe

Positive RHDV control	RT_PCR	provided	0	1	1	America
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4. Did your laboratory produce vaccines?

Yes

5. Did your laboratory supply vaccines to WOAHA Members?

No

TOR3: NEW PROCEDURES

6. Did your laboratory develop new diagnostic methods for the designated pathogen or disease?

No

7. Did your laboratory validate diagnostic methods according to WOAHA Standards for the designated pathogen or disease?

No

8. Did your laboratory develop new vaccines for the designated pathogen or disease?

No

9. Did your laboratory validate vaccines according to WOAHA Standards for the designated pathogen or disease?

No

TOR4: DIAGNOSTIC TESTING FACILITIES

10. Did your laboratory carry out diagnostic testing for other WOAHA Members?

Yes

NAME OF WOAHA MEMBER COUNTRY SEEKING ASSISTANCE	DATE	WHICH DIAGNOSTIC TEST USED	NO. SAMPLES RECEIVED FOR PROVISION OF DIAGNOSTIC SUPPORT	NO. SAMPLES RECEIVED FOR PROVISION OF CONFIRMATORY DIAGNOSES
NORWAY	2022-06-14	cELISA RHDV/EBHSV - Virological ELISA	40	0
SPAIN	2022-02-18	RT-PCR	0	2
THE NETHERLANDS	2022-06-01	cELISA RHDV2 7 IgG ELISA RHDV2	15	0
THE NETHERLANDS	2022-02-11	cELISA RHDV2	44	0
SWEDEN	2022-04-05	cElisa RHDV2 IgG ELISA RHDV2	4	0

11. Did your laboratory provide expert advice in technical consultancies on the request of an WOAHA Member?

Yes

NAME OF THE WOAHA MEMBER COUNTRY RECEIVING A TECHNICAL CONSULTANCY	PURPOSE	HOW THE ADVICE WAS PROVIDED
NORWAY	To give information on the epidemiology and diagnosis of RHDV/EBHS and related viruses	Email and analyses of samples
PHILIPPINES	Provision of the protocol for RNA extraction and RT-PCR	Emails

	amplification of lagoviruses	
TUNISIA	revision of the protocol for RNA extraction and RT-PCR amplification of lagoviruses	Emails
MEXICO	Provision of the protocol for RNA extraction and RT-PCR amplification of lagoviruses	Emails
INDONESIA	Provision of the protocol for RNA extraction and RT-PCR amplification of lagoviruses	Emails
GERMANY	To give interpretation of the epidemiological meaning of serological and virological tests for RHD and EBHS	Emails

TOR5: COLLABORATIVE SCIENTIFIC AND TECHNICAL STUDIES

12. Did your laboratory participate in international scientific studies in collaboration with WOAHO Members other than the own?

Yes

Title of the study	Duration	PURPOSE OF THE STUDY	PARTNERS (INSTITUTIONS)	WOAH MEMBER COUNTRIES INVOLVED OTHER THAN YOUR COUNTRY
PRIMA - LAGMED "Improvement of preventive actions to emerging LAGoviruses in the MEDiterranean basin: development and optimisation of methodologies for pathogen detection and control"	3 years (protracted)	i) To monitor RHD epidemiology in the Mediterranean basin and perform a genomic characterization of circulating strains, ii) To test and apply biosecurity measures to prevent outbreaks and better contain the disease in the field and in rabbit-production systems, particularly in countries located south to the Mediterranean basin. iii) To advise and train stakeholders and partners in Africa on disease diagnosis and prophylaxis, and technical management.	1.CIBIO/InBIO-UP Portugal 2.INIA Spain 3.Universidad de Córdoba Spain 4.ANSES France 5.ONCFS France 6.INRA-ENVT France 7.ENMV de Sidi Thabet Tunisia 8.ENSV d'Alger Algeria	ALGERIA FRANCE PORTUGAL SPAIN TUNISIA
Study of the presence of lagoviruses in micromammals	2 years	To verify the possibility that lagovirus can infect other species than lagomorphs	CITA, Zaragoza, SPAIN	SPAIN
Enfermedades infecciosas y parasitarias de una especie invasora, la liebre			El Instituto de las Ciencias de la Tierra y Ambientales de La Pampa	

<p>europaea (<i>Lepus europaeus</i>), a lo largo de un gradiente ambiental y de usos del suelo en Argentina Central</p>	<p>2 years</p>	<p>To study the health status of brown hare, an invasive species, in Argentina</p>	<p>(INCITAP), Universidad Autónoma de Madrid. Universidad de Córdoba (España). Universidad de Veterinaria de Vienna</p>	<p>ARGENTINA SPAIN</p>
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TOR6: EPIZOOLOGICAL DATA

14. Did your Laboratory collect epidemiological data relevant to international disease control?

Yes

IF THE ANSWER IS YES, PLEASE PROVIDE DETAILS OF THE DATA COLLECTED:

We analyse the genomic and antigenic characteristics of the different pathogenic and non-pathogenic lagovirus strains identified in rabbits, hares and cottontails, mainly in European countries but also in some other parts of the world. The studies are pointed to achieve data on the presence and distribution of lagovirus strains infecting lagomorph species as target species or even as spillover hosts.

15. Did your laboratory disseminate epidemiological data that had been processed and analysed?

Yes

IF THE ANSWER IS YES, PLEASE PROVIDE DETAILS OF THE DATA COLLECTED:

Epidemiological data obtained from the examination of samples received from member countries are usually organized and elaborated in communications at meetings and conferences and for the preparation of scientific papers. Indeed, the participation in international projects and in established collaborations, also characterized by the exchange of reagents and materials contribute to acquire and exchange data and information.

16. What method of dissemination of information is most often used by your laboratory? (Indicate in the appropriate box the number by category and list the details in the box)

a) Articles published in peer-reviewed journals:

5

- 1) ASIN JAVIER, DANIEL REJMANEK, DEANA L. CLIFFORD, ANDREA B. MIKOLON, EILEEN E. HENDERSON, AKINYI C. NYAOKE, MELISSA MACÍAS-RIOSECO, NICOLAS STREITENBERGER, JULIANN BEINGESSER, LESLIE H. WOODS, ANTONIO LAVAZZA, LORENZO CAPUCCI, BEATE CROSSLEY, FRANCISCO A. UZAL (2022). Early circulation of rabbit hemorrhagic disease virus type 2 (RHDV2) in domestic and wild lagomorphs in southern California, USA (2020-2021). *Transbound Emerg Dis*, 69(4) 394-405. <https://doi.org/10.1111/tbed.14315>
- 2) CALVETE C, CAPUCCI L, LAVAZZA A, SARTO MP, CALVO AJ, MONROY F, CALVO JH (2022). Changes in European wild rabbit population dynamics and the epidemiology of rabbit haemorrhagic disease in response to artificially increased viral transmission *Transbound Emerg Dis*. 69(5) 2682-2696. <https://doi.org/10.1111/tbed.14421>
- 3) CAPUCCI L, CAVADINI P, LAVAZZA A Viral haemorrhagic disease: RHDV type 2, ten years later. (2022). *World Rabbit Congress World Rabbit Sci* 30(1) 1-11. <https://doi.org/10.4995/wrs.2022.16505>
- 4) MOHAMED, F., GIDLEWSKI, T., BERNINGER, M. L., PETROWSKI, H. M., BRACHT, A. J., DE RUEDA, C. B., BARRETTE, R. W., GRADY, M.,

O'HEARN, E. S., LEWIS, C. E., MORAN, K. E., STURGILL, T. L., CAPUCCI, L., & ROOT, J. J. (2022). Comparative susceptibility of eastern cottontails and New Zealand white rabbits to classical rabbit haemorrhagic disease virus (RHDV) and RHDV2. *Transboundary and Emerging Diseases*, 69, e968– e978. <https://doi.org/10.1111/tbed.14381>

5) ALBINI S, HETZEL U, CAVADINI P, VOGLER BR (2022). Inconspicuous post-mortem findings in rabbits from Switzerland naturally infected with Rabbit Haemorrhagic Disease Virus 2. *Schweiz Arch Tierheilkd* 164(5) 375-383. <https://doi.org/10.17236/sat00354>

b) International conferences:

6

1) BARATELLI M., MOLIST-BADIOLA J., PUIGREDON-FONTANET A., PASCUAL M., BOIX O., MORA-IGUAL F.X., WOODWARD M., LAVAZZA A., CAPUCCI L, 2021. Characterization of the maternal derived antibody immunity against RHDV-2 after administration in breeding does of an inactivated vaccine. 12th World Rabbit Congress - November 3-5, 2021 - Nantes, France, Communication P-05, p.99.

2) CAPUCCI LORENZO, CAVADINI PATRIZIA, LAVAZZA ANTONIO - Viral haemorrhagic disease: RHDV type 2, ten years later (Invited paper). WRSA - 12th World Rabbit Congress - November 3-5, 2021 - Nantes, France P-00, p.98.

3) CAVADINI P, CAMPISI G., VISMARA A., LAVAZZA A., CAPUCCI L., 2021. Study of genetic evolution of RHDV-2 in Italy from 2011 to 2019. 12th World Rabbit Congress - November 3-5, 2021 - Nantes, France, Communication P-11, p. 100.

4) CAVADINI P, TROGU T, VELARDE NIETO R, LAVAZZA A, CAPUCCI L Recombination between viral genomes as a potential mechanism for speciation: the example of rabbit haemorrhagic disease type 2 (RHDV2) - BG12. 6th World Lagomorph Conference, Montpellier, France, July 4-8, 2022, organized by OFB Office Francais de la Biodiversite, Lagomorph Specialist Group, World Lagomorph Society. p 89

5) CAVADINI PATRIZIA, DAVIDE MUGETTI, ALICE VISMARRA, ANTONIO LAVAZZA, LORENZO CAPUCCI. Occurrence and characterization of Rabbit Calicivirus (RCV) strains in Italy over 20 years". 12th International ESVV Congress ESVV Ghent 20-23 September 2022

6) DI GIOVANNI VITTORIA, PATRIZIA CAVADINI, ROBERTO BONAVEGNA, GIULIA PEZZONI, ANTONIO LAVAZZA, LORENZO CAPUCCI "Molecular mapping of antigenic determinants of RHDV2" 12th International ESVV Congress ESVV Ghent 20-23 September 2022

c) National conferences:

0

d) Other (Provide website address or link to appropriate information):

0

TOR7: SCIENTIFIC AND TECHNICAL TRAINING

17. Did your laboratory provide scientific and technical training to laboratory personnel from other WOAHA Members?

Yes

a) Technical visit : 2

b) Seminars : 1

c) Hands-on training courses: 0

d) Internships (>1 month) 1

Type of technical training provided (a, b, c or d)	Country of origin of the expert(s) provided with training	No. participants from the corresponding country
a	TUNISIA	1
a	ALGERIA	3
d	PORTUGAL/SPAIN	1

b	PHILIPPINES	30
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TOR8: QUALITY ASSURANCE

18. Does your laboratory have a Quality Management System?

Yes

Quality management system adopted	Certificate scan (PDF, JPG, PNG format)	
UNI CEI ENISO/IEC 1702	PDF	CERTIFICATO-DI-ACCREDITAMENTO-2.pdf

19. Is your quality management system accredited?

Yes

Test for which your laboratory is accredited	Accreditation body
RT_PCR	ILAC MRA, ACCREDIA
Serological Competitive RHDV-ELISA ILAC MRA	ILAC MRA, ACCREDIA
Serological Competitive RHDV2-ELISA	ILAC MRA, ACCREDIA
Virological sandwich MAbs RHDV/EBHSV-ELISA	ILAC MRA, ACCREDIA
Immunohistochemistry	ILAC MRA, ACCREDIA
Electron Microscopy negative staining methods	ILAC MRA, ACCREDIA

20. Does your laboratory maintain a "biorisk management system" for the pathogen and the disease concerned?

Yes

The laboratory works according to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 1.1.4 and the WHO Laboratory Biosafety Manual. A risk analysis approach was adopted to manage the biological risks of specific agents aimed at biosecurity in veterinary laboratories and animal facilities. As a result of this process, the assignment of RHDV to the risk group (BLS2) relevant to the country was defined and the consequent steps were taken to work in laboratory facilities defined by containment levels appropriate to the types of risks identified.

TOR9: SCIENTIFIC MEETINGS

21. Did your laboratory organise scientific meetings related to the pathogen in question on behalf of WOA?H?

No

22. Did your laboratory participate in scientific meetings related to the pathogen in question on behalf of WOA?H?

Yes

Title of event	Date (mm/yy)	Location	Role (speaker, presenting poster, short communications)	Title of the work presented
Meeting between Philippines Bureau of Animal Industry (BAI) and OIE RHD Reference Laboratory IZSLER Brescia: "Rabbit haemorrhagic disease and its impact on	2022-01-28	VDC on line	Speaker	1. Lagovirus, immune response, diagnosis and available vaccines. (L. Capucci) 2.RABBIT HAEMORRAGIC DISEASE – RHDclinical signs, epidemiology, vaccination

rabbit breeding"				and management of outbreaks (A.Lavazza)
Technical questions on rabbit hemorrhagic disease and presentation at a stakeholder forum	2022-03-10	VDC on line	Speaker	1.RABBIT HAEMORRAGIC DISEASE – RHD clinical signs, epidemiology, vaccination and management of outbreaks (A.Lavazza) 2. RHD vaccines: what they are and why they are effective in protecting the rabbit from RHD (if properly used)! (L. Capucci)

TOR10: NETWORK WITH WOAHA REFERENCE LABORATORIES

23. Did your laboratory exchange information with other WOAHA Reference Laboratories designated for the same pathogen or disease?

Not applicable (only WOAHA Reference Laboratory designated for the disease)

24. Are you a member of a network of WOAHA Reference Laboratories designated for the same pathogen?

Not applicable (Only WOAHA Reference Laboratory designated for the disease)

25. Did you organise or participate in inter-laboratory proficiency tests with WOAHA Reference Laboratories designated for the same pathogen?

Not applicable (Only WOAHA Reference Laboratory designated for the disease)

26. Did your laboratory collaborate with other WOAHA Reference Laboratories for the same disease on scientific research projects for the diagnosis or control of the pathogen of interest?

Not applicable (Only WOAHA Reference Laboratory designated for the disease)

TOR11: OTHER INTERLABORATORY PROFICIENCY TESTING

27. Did your laboratory organise or participate in inter-laboratory proficiency tests with laboratories other than WOAHA Reference Laboratories for the same pathogen?

Yes

Purpose for inter-laboratory test comparisons ¹	Role of your reference laboratory (organizer/participant)	No. participating laboratories	Region(s) of participating WOAHA Member Countries
We prepared and distributed a panel of positive samples (both antigen positive extracts and sera) to be used by recipients for the validation of their analytical performances	Organizer	3	America Europe

TOR12: EXPERT CONSULTANTS

28. Did your laboratory place expert consultants at the disposal of WOAHA?

Yes

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KIND OF CONSULTANCY	Location	SUBJECT (FACULTATIVE)
Preparation of the OIE Case Definition for Rabbit Hemorrhagic Disease	On site, by email	Contribution to the preparation of technical report with the case definition attached.
Revision of OIE Standards. Chapter 3.7.2 of the WOAHP Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2022	On site, by email	We changes table 1 to make it coherent with the "RHD case definition". Then we included some other changes in the chapter originating from the latest data and information from scientific literature i.e., species susceptibility to RHD/RHDV2 and new biotechnological vaccines.

29. Additional comments regarding your report:

Yes

During 2022 the laboratory has applied both nationally and internationally the specific direct and indirect tests (MAbsELISA and RT PCR for antigen detection, cELISA and isotype_ELISAs for antibodies detection) specifically developed developed for RHDV2.

Following the epidemic occurrence of RHDV2 in North and Central America we still continued to collaborate and support American colleagues for the detection of the virus in wild and domestic lagomorphs.

Even more information on pathogenic and non-pathogenic lagoviruses' spread, host susceptibility, and antigenic and genomic characteristics were acquired thanks to the scientific collaboration and research projects with colleagues from various member Countries. A technical support, including training and visiting period, mainly on diagnostic activity and providing of reagents and materials was given to different OIE member countries.