

WOAH Reference Laboratory Reports Activities 2023

Activities in 2023

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)
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Website:	https://www.izsler.it/chi-siamo/per-chi-e-con-chi-lavoriamo/centri-direferenza/internazionali/oi-reference-laboratory-for-rabbit-haemorrhagic-disease/
Name (including Title) of Head of Laboratory (Responsible Official):	Dr. Giorgio Varisco (DVM, General Director of IZSLER)
Name (including Title and Position) of WOA Reference Expert:	Dr. Patrizia Cavadini (PhD)
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		727	278
RHDV2 Competition ELISA		820	294
EBHSV Competition ELISA		607	0
RHDV IgG Isotype ELISA		18	118
RHDV IgM Isotype ELISA		19	0
RHDV IgA Isotype ELISA		59	40
RHDV2 IgA Isotype ELISA		0	40
Direct diagnostic tests			
RHDV Sandwich ELISA		151	0
EBHSV Sandwich ELISA		201	1
RT-PCR RHDV/RHDV2		19	3
RT-PCR EBHSV		35	1
Genome sequencing		11	0
RT-PCR lagovirus		22	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	6 kits	2	POLAND, UNITED STATES OF AMERICA,
EBHSV serological kit	c-ELISA	produced	1 kit	3 kits	2	POLAND, SLOVAKIA,
RHDV2 serological kit	c-ELISA	produced	1 kit	14 kits	7	ALGERIA, FRANCE, GEORGIA, SLOVAKIA, SPAIN, UNITED STATES OF AMERICA,
RHDV/RHDV2 Differential kit	MAbs sandwich ELISA	produced	0	4 kits	12	ALGERIA, POLAND,
RHDV/EBHSV virological ki	MAbs sandwich ELISA	produced	13 kits	8 kits	3	GERMANY, POLAND, UNITED STATES OF AMERICA,
Reference positive and negative materials (liver homogenates)	RT-PCR	provided	0	1 panel	1	POLAND,
RNA from RHDV2 positive rabbit and Plasmid DNA with vp60 cloned gene	RT-PCR	produced	0	10 microliters	1	UNITED STATES OF AMERICA,

4. Did your laboratory produce vaccines?

Yes

5. Did your laboratory supply vaccines to WOA?H 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 WOA?H 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 WOA?H Standards for the designated pathogen or disease?

No

TOR4: DIAGNOSTIC TESTING FACILITIES

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

Yes

NAME OF WOA?H 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
SWEDEN	2023-11-14	cELISA RHDV2/cELISA RHDV/IgG ELISA RHDV	8	0
THE NETHERLANDS	2023-02-27	cELISA RHDV2/IgG ELISA RHDV	16	0
BELGIUM	2023-07-27	RT-PCR RHDV2	0	3
INDONESIA	2023-06-06	cELISA RHDV-RHDV2/ELISA IgG, IgA IgM RHDV	98	0

CROATIA	2023-05-11	Sandwich ELISA, RT-PCR EBHSV	0	1
FRANCE	2023-02-09	cELISA RHDV-RHDV2/ELISA IgA RHDV/ELISA IgA RHDV2	172	0

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

Yes

NAME OF THE WOA MEMBER COUNTRY RECEIVING A TECHNICAL CONSULTANCY	PURPOSE	HOW THE ADVICE WAS PROVIDED
BENIN	To give information on RHD serosurveillance	Emails
INDONESIA	To give an interpretation of the serology and RHDV sequencing results	Emails
UNITED STATES OF AMERICA	Provision of the protocol for RNA extraction and RT-PCR amplification of lagoviruses	Emails
SINGAPORE	Provision of the protocol for RNA extraction and RT-PCR amplification of lagoviruses	Emails
SOUTH AFRICA	To give an interpretation of the epidemiological meaning of serological tests for RHD	Emails
ALGERIA	Consulting on the development of a surveillance plan for lagomorph diseases in Algeria	Emails
ALGERIA	Surveillance program on hare diseases	Emails
BELGIUM	Support for the diagnosis of RHD by PCR and histopathology	Emails

TOR5: COLLABORATIVE SCIENTIFIC AND TECHNICAL STUDIES

12. Did your laboratory participate in international scientific studies in collaboration with WOA 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 - FRENCH GUIANA PORTUGAL SPAIN TUNISIA
Enfermedades infecciosas y parasitarias de una especie invasora, la liebre europea (<i>Lepus europaeus</i>), a lo largo de un gradiente ambiental y de usos del suelo en Argentina Central	2 years	To study the health status of brown hare, an invasive species, in Argentina	El Instituto de las Ciencias de la Tierra y Ambientales de La Pampa (INCITAP), Universidad Autónoma de Madrid. Universidad de Córdoba (España). Universidade Veterinaria de Vienna	ARGENTINA SPAIN
Longitudinal studies on European brown hares in	2 years	To monitor EBHSV and RHDV2 spread in hares in Catalonia, by serological approach and	Universidad Autónoma de Barcelona	SPAIN

Catalunia	perform a genomic characterization of circulating strains
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13. In exercising your activities, have you identified any regulatory research needs* relevant for WOA?H?

No

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 aimed at achieving 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, participation in international projects and established collaborations, also characterized by the exchange of reagents and materials, contribute to acquiring and exchanging 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:

4

1) Domanico M, Cavadini P, Nardini R, Cecca D, Mastrandrea G, Eleni C, Galiotta V, Attili L, Pizzarelli A, Onorati R, Amoruso C, Stilli D, Pacchiarotti G, Merzoni F, Caprioli A, Ricci I, Battisti A, Lavazza A, Scicluna MT. Pathological and virological insights from an outbreak of European brown hare syndrome in the Italian hare (*Lepus corsicanus*). 2023. *Front Microbiol.* Oct 19;14:1250787. doi: 10.3389/fmicb.2023.1250787. PMID: 37928681; PMCID: PMC10622795.

2) Cavadini P, Trogu T, Velarde R, Lavazza A, Capucci L. Recombination between non-structural and structural genes as a mechanism of selection in lagoviruses: The evolutionary dead-end of an RHDV2 isolated from European hare. 2023. *Virus Research*, Volume 339, 2024,199257, <https://doi.org/10.1016/j.virusres.199257>.

3) Faehndrich M, Woelfling B, Klink JC, Roller M, Baumgärtner W, Wohlsein P, Raue K, Strube C, Ewers C, Prenger-Berninghoff E, Verspohl J, Lavazza A, Capucci L, Tomaso H, Siebert U. Findings and Infectious Diseases in Selected European Brown Hare (*Lepus europaeus* Pallas, 1778) Populations from Schleswig-Holstein, Germany. *Pathogens*. 2023 Nov 5;12(11):1317. doi: 10.3390/pathogens12111317.

4) Faehndrich M, Klink JC, Roller M, Wohlsein P, Raue K, Strube C, Prenger-Berninghoff E, Ewers C, Capucci L, Lavazza A, Tomaso H, Schnitzler JG, Siebert U Status of Infectious Diseases in Free-Ranging European Brown Hares (*Lepus europaeus*) Found Dead between 2017 and 2020 in Schleswig-Holstein, Germany. *Pathogens*. 2023 Feb 2;12(2):239. doi: 10.3390/pathogens12020239.

b) International conferences:

1

1) Milk from rabbit mothers vaccinated against RHD contains a relevant level of specific IgA and IgG. Vittoria Di Giovanni, Francesco Gratta, Antonio Lavazza, Patrizia Cavadini, Lorenzo Capucci. *EPIZONE 15th Annual Meeting, New Perspectives for the New Era*, 26-28 April 2023 Novi Sad, Serbia.

c) National conferences:

3

1) Cavadini Patrizia, Giovanni Campisi, Alice Vismara, Vittoria Di Giovanni, Tiziana Trogu, Antonio Lavazza e Lorenzo Capucci. Recombination between viral genomes as a

potential mechanism for lagoviruses evolution. 7th National Congress of the Italian Society for Virology. One Virology One Health. 25-27 June 2023, Brescia, Italy
 2) Di Giovanni Vittoria, Giulia Pezzoni, Roberto Benevenia, Antonio Lavazza, Lorenzo Capucci, Patrizia Cavadini. Molecular mapping of antigenic determinants of RHDV2. 7th National Congress of the Italian Society for Virology. One Virology One Health. 25-27 June 2023, Brescia, Italy
 3) Di Giovanni V., R. Soldati, G. Pezzoni, A. Lavazza, L. Capucci, P. Cavadini. Produzione di anticorpi monoclonali specifici verso le IgA di lepre. SIDiLV XXII Congresso Nazionale 11-13 Ottobre 2023 Brescia, Italia.

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

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 : 3

b) Seminars : 2

c) Hands-on training courses: 0

d) Internships (>1 month) 0

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	ALGERIA	2
A	INDONESIA	1
B	CUBA	30
B	CHINA (PEOPLE'S REP. OF)	20
A	SPAIN	3

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-148-L-rev.6.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, 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 WAOH 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 WOAHP?

No

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

No

TOR10: NETWORK WITH WOAHP REFERENCE LABORATORIES

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

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

24. Do you network (collaborate or share information) with other WOAHP Reference Laboratories designated for the same pathogen?

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

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

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

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

Not applicable (Only WOAHP 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 WOAHP Reference Laboratories for the same pathogen?

Yes

Purpose for inter-laboratory test comparisons ¹	Role of your reference laboratory (organizer/participant)	No. participating laboratories	Name of the Test	WOAHP Member Countries
We prepared and distributed a panel of positive samples (antigen positive extracts) to be used by recipients for the validation of their analytical performances	Organizer	1	ELISA and RT-qPCR	POLAND,

TOR12: EXPERT CONSULTANTS

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

Yes

KIND OF CONSULTANCY	Location	SUBJECT (FACULTATIVE)
Last review of the the Chapter of the Manual was completed on 2022 and approved and published on 2023.	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 2023 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 for RHDV2.

Following the epidemic occurrence of RHDV2 in North and Central America, we continued to collaborate and support American colleagues in 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 WOAHP member countries.