World Organisation for Animal Health

Anna Toffan - - ITALY

WOAH Reference Laboratory Reports Activities 2024

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LABORATORY INFORMATION

***Name of disease (or topic) for which you are a designated WOAH Reference Laboratory:**	Viral encephalopathy and retinopathy
***Address of laboratory:**	WOAH Reference Laboratory for Viral Encephalopathy and Retinopathy of Marine Fish, Fish virology Dep., Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) Viale dell'Università, 10 35020 Legnaro (Padova), Italy
***Tel:**	+39-049 808 43 88
***E-mail address:**	atoffan@izsvenezie.it
Website:	https://www.izsvenezie.it/
***Name (including Title) of Head of Laboratory (Responsible Official):**	Calogero Terregino, Director of the Specialized Virology and Experimental Research Unit/Acting Director of the Research and Development Department (IZSVe)
***Name (including Title and Position) of WOAH Reference Expert:**	Anna Toffan DVM, PhD, ECAAH, Head of the Fish Virology Department
***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 WOAH Manual (Yes/No)	Total number of test performed last year	
Indirect diagnostic tests		Nationally	Internationally
ELISA	No	18	1183
Direct diagnostic tests		Nationally	Internationally
Cell Culture	Yes	19	

			37
Real-time RT-PCR(rRT-PCR)	No	415	1105
Immunohistochemistry (IHC)	No	18	14
Molecular characterization (RT-PCR and sequencing analysis)	No	12	46
Immunofluorescence (IF)	No	12	0

TOR2: REFERENCE MATERIAL

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

No

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

Yes

Type of reagent available	Related diagnostic test	Produced/ provide	Amount supplied nationally (ml, mg)	Amount supplied internationally (ml, mg)	No. of recipient WOAH Member Countries	Country of recipients
SSN-1 cell	Virus isolation	produced	0	45 ml	2	MOROCCO, TUNISIA,
Live betanodavirus reassortant RG/SJ	Virus isolation, RT-PCR, rRT-PCR	stored	0	2 ml	1	TUNISIA,
Live betanodavirus RGNNV	Virus isolation, RT-PCR, rRT-PCR	stored	0	3 ml	1	MOROCCO,
Inactivated betanodavirus RGNNV	RT-PCR, rRT-PCR	stored	1.5 ml	2 ml	3	CAMBODIA, ISRAEL, ITALY,
Inactivated betanodavirus SJNNV	RT-PCR, rRT-PCR	stored	0	1.5 ml	1	CAMBODIA,
Inactivated betanodavirus RG/SJ	RT-PCR, rRT-PCR	stored	0	0,5 ml	1	CAMBODIA,
Characterized sea bass serum anti SJNNV	IHC	stored	0	2 ml	1	AUSTRALIA,

4. Did your laboratory produce vaccines?

No

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

No

TOR4: DIAGNOSTIC TESTING FACILITIES

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

Yes

Name of WOAH 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
CAMBODIA	2024-03-01	Real time PCR	11	0
CYPRUS	2024-02-01	Real time PCR	37	0
FRANCE	2024-01-01	Real time PCR	55	0
GREECE	2024-03-01	Real time PCR-genotyping, cell culture, titration, ELISA, IHC	1269	20
ISRAEL	2024-02-01	Real time PCR, genotyping, cell culture	41	2
MALTA	2024-06-01	Real time PCR, cell culture	31	0
MAURITIUS	2024-10-01	Real time PCR	13	0
NORWAY	2024-11-01	Real time PCR, genotyping	8	6
PORTUGAL	2024-10-01	Real time PCR-genotyping, cell culture, IHC	30	29
SAUDI ARABIA	2024-05-01	Real time PCR, IHC	0	5
SPAIN	2024-01-01	Real time PCR-genotyping, cell culture, titration, IHC	740	26
THE NETHERLANDS	2024-01-01	Real time PCR	11	0
TUNISIA	2024-10-01	Real time PCR-genotyping, cell culture, titration	34	16
TURKEY	2024-03-01	Real time PCR	1	0

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

Yes

Name of the WOAH Member Country receiving a technical	Purpose	

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consultancy		How the advice was provided
CYPRUS	Diagnosis and control of VER	Diagnostic support and remote assistance
FRANCE	Diagnosis and control of VER	Diagnostic support and remote assistance
ISRAEL	Diagnosis and control of VER	Diagnostic support and training activities
MALTA	Diagnosis (exclusion of) of VER	Diagnostic support and consultancy during tuna die off
NORWAY	Diagnosis and control of VER	Virus characterization
SPAIN	Diagnosis and control of VER	Diagnostic support and remote assistance
TUNISIA	Cell culture and ELISA set up	Training and reagents provision
GREECE	Diagnosis and characterization of VER	Diagnostic support and consultancy during grouper die off
PORTUGAL	Diagnosis and characterization of VER	Diagnostic support and consultancy during grouper die off. Manuscript preparation
MOROCCO	Diagnosis and control of VER	Diagnostic support and training
CAMBODIA	Diagnosis and control of VER	Diagnostic support and training
MAURITIUS	Diagnosis (exclusion of) of VER	Diagnostic support and consultancy during red drum die off

TOR5: COLLABORATIVE SCIENTIFIC AND TECHNICAL STUDIES

12. Did your laboratory participate in international scientific studies in collaboration with WOAH 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
PathoGelTrap - New Blue Revolution through a pioneering pathogen-trapping technology based on bioselective hydrogel-forming proteins - H2020 EU Project (2020-	3 years	To innovate infectious disease management practices, providing industry with a technology capable of effectively removing specific pathogens directly from water.	1. Smartwaterplanet, high-tech SME specialized in the development of technology solutions for the aquaculture sector. Madrid, Spain; 2. The Higher Council for Scientific Research (CSIC - State Agency for scientific research and technological development) Madrid, Spain; 3. University College Dublin (UCD) Ireland; 4. IZSVe, Italy; 5. IFPAN Institute of Physics, Polish Academy of Sciences, Warsaw, Poland. 6. LOMARTOV environmental	IRELAND ITALY POLAND SPAIN

2023)			engineering SME, specialized in supporting R&D, industrial and technology-based projects with a multidisciplinary approach, Valencia, Spain. https://pathogeltrap.eu/project/	
AQUAE-STRENGHT	3 years	Strengthening capacity on aquatic animal health and epidemiological surveillance	Advisors: Norwegian Veterinary Institute - NORWAY • Technical University of Denmark – DENMARK • Centre for Environment, Fisheries and Aquaculture Science – UK Beneficiaries: Institut National des Sciences et Technologies de la Mer – Laboratoire D'Aquaculture – TUNISIA • Office National de Sécurité Sanitaire des Produits Alimentaires - MOROCCO • Ministry of Agriculture, Forestry and Rural Development – Israeli Veterinary Services and Animal Health – ISRAEL • Ministry of Agriculture, Forestry and Fisheries – Fisheries Administration (Maff) – CAMBODIA	CAMBODIA DENMARK ISRAEL MOROCCO NORWAY TUNISIA UNITED KINGDOM

13. In exercising your activities, have you identified any regulatory research needs* relevant for WOAH?

Yes

Research need : 1

Please type the Research need: The withdrawal of the chapter devoted to Viral Encephalopathy and Retinopathy in the new edition of the WOAH Aquatic Code brought to light some regulatory gaps and the need for additional technical documents. In particular, the lack of guidelines recommending the recognised laboratory methods has emerged.

Relevance for WOAH Disease Control, Standard Setting,

Relevance for the Code or Manual Code,

Field Diagnostics,

Animal Category Aquatic,

Disease:

Viral encephalopathy and retinopathy

Kind of disease (Zoonosis, Transboundary diseases) Transboundary diseases,

If any, please specify relevance for Codes or Manual, chapter and title

(e.g. Terrestrial Manual Chapter 2.3.5 - Minimum requirements for aseptic production in vaccine manufacture)

Answer:

Notes:

Answer:

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:
Data repository of NNV strains/sequences collected during mortality events in groupers (Epinephelus spp.) and other wild fish in the Mediterranean basin; Data collected from NNV outbreaks in European hatchery of sea bream; Data obtained from questionnaires sent in the frame of the proficiency test organized by the RL.

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:
•Through the publication of peer reviewed articles at international level and supporting other laboratories in manuscript writing; through the participation in international scientific conferences (i.e. organised by EAFP-European Association of fish Pathology, EAS- European Aquaculture Society). • As partner of EVAg (https://www.european-virus-archive.com/), which is a non-profit organization based on a global network of virology experts, through the collection, characterization, standardization, authentication and distribution of viruses and derived products. An international group of 36 laboratories, including 29 EU and 7 non-EU institutions, are actively involved in the EVAg project, representing a wide range of virological disciplines. IZSVe has made viruses of veterinary interest and derived products available to the international scientific community. In particular, the viral archive of aquatic viruses has been added to this catalogue, including all known Betanodavirus species (RGNNV, SJNNV, TPNNV, BFNNV and reassortant strains).

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:

3

1. Toffan A., Buratin A., Pascoli F., Toson M., Ellero F., Abbadi M. (2024) Participating in VER-IPT: is it worth the effort? Bulletin of the European Association of Fish Pathologists, May 2024. https://doi.org/10.48045/001c.118456;

2. Zrnčić S., Katharios P., Padrós F., Fioravanti M., Gustinelli A., Le Breton A., Toffan A. (2024) New Challenges and Achievements in Mediterranean Fish Health Management. Bulletin of the European Association of Fish Pathologists, May. https://doi.org/10.48045/001c.118459.

3. Leandro MG, Zornu J, Le Breton A, Chérif N, Basurco B, Furones D, Muniesa A, Toffan A, Dalla Pozza M, Franzago E, Zrnčić S, Varvarigos P, Saleh H, Cagirgan H, Dverdal Jansen M, Brun E, Tavornpanich S. Quantification of biosecurity measures in Mediterranean European seabass and gilthead sea bream farms, Aquaculture, Volume 596. 2025. https://doi.org/10.1016/j.aquaculture.2024.741898.

b) International conferences:

c) National conferences:

1
Berto P., Pascoli F., Biasini L., Marsella A., Buratin A., Toffan A. (2024) Intra host distribution of viral genome in European sea bass post vaccination against betanodavirus. In: XXVIII Convegno nazionale S.I.P.I. . Cesenatico (FC), Italy, 27-28 June 2024, pag. 39 (P2)

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

8
Projects at National level (2):
1. RC 13/22 VERTICAL – "Study on vertical transmission of immunity and VER virus infection in sea bream" (the RL participates as principal investigator). Funded by the Italian Ministry of Health
2. Study to identify genetic markers associated to Viral Encephalo-Retinopathy (VER) resistance in experimentally infected sea bass (Dicentrarchus labrax). Research agreement with the University of Padua, Dept. of Comparative Biomedicine and Nutrition –BCA

Working groups (3):
1. Participation in the national network of the IIZZSS (Istituti Zooprofilattici) for the study of stranded aquatic animals. Through technical and scientific assistance to the Ministry of Health, the aim of the network is to develop surveillance strategies and disease control in aquatic populations that are vulnerable to stranding
2. Co-chair of the Strategic Working group FISH (Standing Committee on Agricultural Research SCAR –FISH)
3. Fish health Expert for the Scientific Advisory board of the EU PAHW- European Partnership on Animal Health & Welfare (PAHW)

Links from IZSVe's web site (3):
National reference laboratory for fish, crustacean and mollusc pathology / WOAH reference laboratory for viral encephalopathy and retinopathy of marine fish
http://www.izsvenezie.com/reference-laboratories/fish-crustacean-and-mollusc-pathology/

IZSVE's contribution to Pathogeltrap:
https://www.izsvenezie.it/proteine-intelligenti-catturano-virus-batteri-pesci/
https://pathogeltrap.eu/

IZSVE's contribution to AQUAE STRENGTH:
https://www.izsvenezie.it/progetto-aquae-strength/

TOR7: SCIENTIFIC AND TECHNICAL TRAINING

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

Yes

a) Technical visit : 0

b) Seminars : 0

c) Hands-on training courses: 6

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
C	MOROCCO	2
C	ISRAEL	4
D	TUNISIA	1

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 EN ISO/IEC 17025:2018	Certificate of Accreditation	17025.pdf

19. Is your quality management system accredited?

Yes

Test for which your laboratory is accredited	Accreditation body
Virus Isolation in Cell Cultures	ACCREDIA – Italian Accreditation System

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

Yes

The IZSVe implements biorisk management actions to prevent diseases among personnel and to protect the community from harm caused by potentially infectious pathogens. The IZSVe Biosafety Committee was established in 2023 and is responsible for the following tasks: - Evaluation of the safety risks for workers and for the environment connected to the activities to be performed under BSL2/BSL3 conditions that involve the use of microorganisms, animals and Genetically Modified Microorganisms MOGM; - Evaluation of the emergency procedures - Evaluation of all the management and operative procedures to be applied inside the BSL-3 laboratory and animal facilities including potential biosecurity issues. All Standard Operative Procedures (SOPs) and handling of pathogens are written and performed accordingly to the WHO Laboratory Biosafety Manual (4th Ed.). However, the RL in the specific handles risk group 1 agents and biosafety level 1 (BSL-1) are therefore applied to the laboratory setting in which personnel work with low-risk microbes that pose little to no threat of infection in healthy adults. Safety protocols for biosafety level 1 labs are anyway applied, although no special contaminant equipment is used and there is no need to be isolated from surrounding facilities.

TOR9: SCIENTIFIC MEETINGS

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

No

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

Yes

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Title of event	Date	location	Role (speaker, presenting poster, short communications)	Title of the work presented
4th cycle training of Aquatic Animal Health Focal Points in Africa (Regional Training Workshop for WOAH National Focal Points for Aquatic Animals in Africa (French))	2024-07-07	Tunisi	Invited speaker	Projet de jumelage IZSVe-IRVT Amélioration de la capacité de diagnostic de l'IRVT en relation à l'encéphalopathie et rétinopathie virale des poissons de mer; Le Projet Aquae Strength: Renforcement des capacités en matière de santé et de surveillance épidémiologique des animaux aquatiques; https://rr-africa.woah.org/en/trainings/tunis-hosts-the-4th-cycle-training-of-aquatic-animal-health-focal-points-in-africa/

TOR10: NETWORK WITH WOAH REFERENCE LABORATORIES

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

Not applicable (only WOAH Reference Laboratory designated for the disease

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

Not applicable (only WOAH Reference Laboratory designated for the disease

25. Did you organise or participate in inter-laboratory proficiency tests with WOAH Reference Laboratories designated for the same pathogen during the past 2 years?

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

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

Not applicable (only WOAH 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 WOAH Reference Laboratories for the same pathogen during the past 2 years?

Yes

Purpose for inter-laboratory test comparisons1	Role of your reference laboratory (organizer/participant)	No. participating laboratories	Name of the test	WOAH Member Countries
Fifth Proficiency Test (VER-IPT) 2024 Every two years the NRL organises and				AUSTRALIA, CAMBODIA, CANADA, CROATIA, DENMARK, FRANCE,

conducts the Viral Nervous Necrosis Interlaboratory Proficiency Test (VER-IPT) for molecular detection of Betanodavirus.	Organiser. The NRL organises and conducts the VER-IPT on Behalf of the WOAH.	27	5th VER-IPT	ISRAEL, ITALY, MOROCCO, PORTUGAL, SINGAPORE, SPAIN, THE NETHERLANDS, TUNISIA, TURKEY, UNITED KINGDOM,
Molecular detection of the following viral agents: • Koi herpesvirus (KHV) • Megalocytivirus, represented in the panel by red sea bream iridovirus (MCV/RSIV) • Nervous necrosis virus (NNV) and • Spring viraemia of carp virus (SVCV)	Participant for WOAH member countries involved: check CSIRO website https://acdp.csiro.au/info/pt.aspx	42	Asia-Pacific Aquatic PT Program Fin fish diseases PCR– Organized by CSIRO	

TOR12: EXPERT CONSULTANTS

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

Yes

Kind of consultancy	Location	Subject (facultative)
Webinar "Aquaculture: Modern Systems, Obstacles, and Challenges, Improper Management" WOAH FAO - STOR (Scientific and Technical Office of REMESA)	Online 18/03/2024	Invited speaker : "Aquaculture: Modern Systems, Obstacles, and Challenges, Improper Management"
Mission in the frame of the WOAH AQUAE STRENGHT project	Cambodia 12/01/2024	Training in loco sampling and diagnostic methods of fish diseases
Mission in the frame of the WOAH AQUAE STRENGHT project	Morocco 19-24/05/2024	Training in loco sampling and diagnostic methods of fish diseases
Mission in the frame of the WOAH AQUAE STRENGHT project	Morocco 29/09-04/10/2024	Training in loco sampling and diagnostic methods of fish diseases

29. Additional comments regarding your report: