Prot. IZSLER interno:

FAO Reference Centre - Annual report

(Thematic areas[1])

Title of FAO Reference Centre	**FAO Reference Centre for FMD and SVD**
Name of the Institution and contact details	**Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia - Italy**
Director of Institute	**Dr. Piero Frazzi**
Head of the FAO Reference Centre	**Santina Grazioli** **Giulia Pezzoni**
Name, title and function of reporting officer (if different from above)	
Reporting period	**2021**
Date of reporting	**12/07/2022**

[1] Applies to: Veterinary Epidemiology, VPH, Laboratory Biosafety and biocontainment, Veterinary drugs and vaccine control, Wildlife health, Human- animal -environment interface

Please report activities as specified in the paragraph "areas of collaboration" in the designation letter – *Where applicable, please always give details on beneficiary countries, project names, activities carried out, outcomes and recommendations*

INTRODUCTION: Also in 2021, the laboratory's activities were affected by the COVID-19 emergency, mainly in the logistical challenges to arrange shipments and in the organization of training in presence, affecting international activities as receiving samples from endemic countries both for diagnostics and reaserch purposes.
Travel restrictions caused by the pandemic have determined a consolidation of activities carried out remotely, including meetings, congress and training. Despite the difficulties, the laboratory was able to respond to all requests for the supply of diagnostic kit and it was observed an increase in the demand for diagnostic kits confirming the resumption of activities in foot-and-mouth disease control.

1. Test(s) carried out for FAO member countries

Foot-and-Mouth Disease

SEROLOGICAL TESTS (NSP-ELISA, SP-ELISAs for different serotypes, Virus Neutralization tests (VNT) with a variety of serotypes and strains. Sample from Azerbaijan
Samples were received to estimate effectiveness of the vaccines currently used in the country and potential cross-protection vs circulating viruses. The antibodies evaluated during the trial included only those against FMDV strains homologous to vaccine strains used in Azerbaijan. The study is part of the studies performed in the Transcaucasus region including Armenia, Georgia and Azerbaijan.

Country	**VNT** (end-point titration)				**SP-ELISA** (end-point titration)			**NSP-3ABC**
	O	A/Iran05	A/G-VII	Asia 1	O	A	Asia 1	
AZERBAIJAN	204	375	375	204	208	208	208	208

Swine Vesicular Disease

Following the EU's recognition of the eradication of the SVD in Italy (No. 470 of March 20, 2019), since March 2021, the National Surveillance Plan for SVDV was suspended. New guidance from the Central Authority has provied that a serological control must be performed only in farms that export products in compliance with the APHIS' programs, in addition the virological tests on environmental feces samples had been suspended. Consequently, diagnostic activity has decreased significantly.

July 1, the Laboratory, as WOAH Reference Lab for SVDV, receved 35 sera from Cyprus to be tested by 5B7 competive ELISA and VNT

Country	**Year**	**5B7 competitive ELISA**	**VNT**	**IgG Isotyping ELISA**	**IgM Isotyping ELISA**	**Realtime RT-PCR**
Italy	2021	27,355 (at NRL) + 20,000 (other regional labs)	56	56	56	231
Cyprus	2021	35 sera	35	//	//	//

2. Involvement in other non-FAO technical assistance projects or activity

Continuous remote assistance and advice are regularly provided to various Member countries for elaboration and interpretation of results recorded with the diagnostic kits supplied for FMD diagnosis and serology.

3. Participation in international, regional scientific collaborative studies or projects (please mention partners and/or organisation, country, objectives and activities)

Title: Small-scale trial for the evaluation of vaccine quality and immune responses in vaccinated animals in the Transcaucasus countries.
Countries involved: Azerbaijan.
Organisation: EUFMD with the collaboration of IZSLER.
Objectives: The results of the field study should provide information on:

- the expected proportion of animals that develop a specific level of antibodies following the administration of a single dose of the vaccine,
- the effect of a booster dose,
- the duration (and level) of specific antibody titers over time.

Activities: Testing on sera for the trials conducted in Georgia and Armenia has been completed in 2019-2020, while shipment of sera, collected in the Azerbaijan trials, due to COVID-19 emergency was postponed to 2021. Testing on sera collected in Azerbaijan completes the small-scale trials in the Transcaucasus countries.

Title: Development of field diagnostic methods: antigenic detection (multiplex LFDs) for Foot and Mouth Disease Virus
Objectives and **activities:** To generate multiplex Lateral Flow Devices (LFDs) able to identify the presence of FMDV in field samples and to directly identify the serotype of detected viruses. This is the simplest system for field diagnosis applied to vesicular lesions. In addition, it also allows a safer and easier shipment of samples to reference laboratories for further investigations. In 2021 the validation study of the LFDs prototypes were designed, involving colleagues from endemic countries (Turkey Kenya) and the WRL – Pirbright.
Partner: University of Turin, Italy

Title: Eastern Africa Foot and Mouth Disease Virus (FMDV) Reference Antigen Panel (AgResults Project).
Organisations : GALVmed
Objectives: The AgResults Foot and Mouth Disease Vaccine Challenge Project supports the development and uptake of high-quality FMD vaccines, tailored to meet the needs of Eastern Africa
Activities: Antigen profiling on 67 FMDV isolates circulating in Eastern African countries, including the following lineages O/EA-2, O/EA-3, O/EA-4, A/AFRICA/G-I, A/AFRICA/G-IV, SAT1/I, SAT2/IV, using panels of 95 characterized Monoclonal Antibodies.
A selection of sixteen FMDV isolates can be used to evaluate serological responses of FMDV vaccines in the context of their suitability for use in Eastern African countries. The panel is tailored to cover the genetic diversity within the FMDV lineages that circulate in Eastern African countries
Partner: The Pirbright Institute, UK

Title: Harmonisation and calibration of VNT methods used for post-vaccination monitoring in different FMD Reference Laboratories". (EuFMD-FAR) – 2020

Organisations: The Pirbright Institute-UK

Objectives:

1. Comparison of VNT titres generated by different laboratories,
2. Analysis of available *in vivo* vaccine potency test data to establish serological cut-offs for the evaluation of vaccine performance,
3. Preparation of reference sera calibrated to "protective" responses,
4. Comparison of SP-ELISA and VNT results for post-vaccination.

Activities:

- Carry out an inter-laboratory study with the goal to harmonise the virus neutralisation test amongst the laboratories (objective 1);
- Through the review of available data on correlations between serology and protection, as well as the generation of additional correlations from sera not previously available, a pragmatic serological cut-off(s) to define a "protective" response will be established (objective 2);
- Reference sera correlating with 50% protection will be established for some widely used serotype O and A vaccines (objective 3);
- Using the outcomes from objective 2, compare SP-ELISAs with VNTs to assess whether SP-ELISAs are suitable for post-vaccination studies (objective 4).

Partner: The Pirbright Institute-UK; IZSLER-Italy, Sciensano-Belgio, ANSES-Francia, Wageningen Bioveterinary Research-Olanda

Title: Research Agreement with The Pirbright Institute (IZSLER document ID N. 275/2021 I.P. 773/2021)

Objectives: The research includes the following schedules of project

1. Continued development of antigen-detection assays for FMDV and other vesicular diseases,
2. Development and validation of new ELISAs for serological testing of FMDV,
3. Development of assays for FMD vaccine quality using VP4 as a marker for intact antigen,
4. Validation of a novel universal serology assay for diagnosis of FMD,
5. Informing vaccine quality by developing peptide-based immunoassays for serotype-specific detection of FMDV-specific antibodies,
6. Use of bio-layer interferometry (BLI) to define binding parameters of monoclonal antibodies for use in diagnostic assays.

4. **Activities**: research agreement signed in June 2021 of five-year duration. Research activities will take place over this time frame.**Organisation/participation in international/regional scientific meetings**

IZSLER experts participated in the following scientific meetings with active contributions:

Title of the events: Meetings of the Special Committee on Biorisk Management (SC-BRM)" of EuFMD. Discussion and upgrade on the following topics: reviewing of the Minimum Standards for FMD contingency laboratories presented at the 44th GS 21-24 April 2021; tranining in biorisk management; list of disinfectant methods.
Date and location: 29th January, 9th February, 18th May, 30th September, 15th November. Virtual events.
IZSLER Role: The IZLSER Biorisk Officer (BRO) is a Member of the Committee

Title of the event: "Preparedness to use emergency vaccination for FAST diseases in European countries." Organised by EuFMD.

Date and location 31st March . Virtual meeting
IZSLER Role: WOHA/FAO Lab expert

Title of the event: Special Committee for Surveillance and Applied Research
Date and location: 22 January 2021. Virtual event
IZSLER Role: Member of Committee

Title of the event : Virtual meeting on the Protocol for the submission of FMD samples to SAP Institute. Follow-up of the Meeting between the Veterinary Services of Turkey, Islamic Republic of Iran, Pakistan and the EuFMD held on 10 May 2021.Organised by EuFMD.
Date and location: 29 June 2021. Virtual event
IZSLER Role: WOHA/FAO Lab expert.

Title of the event: Follow-up meeting on Risk-Based Strategic Plan with Lebanon. Organised by EuFMD.
Date and location: 26 August 2021. Virtual event
IZSLER Role: WOHA/FAO Lab expert

Title of the event: 5th virtual GF-TADs Foot-and-Mouth Disease roadmap meeting for the Middle East, combined with Epi and Lab networks meeting.
Date and location: 29 June 2021. Virtual event
IZSLER Role: WOHA/FAO Lab expert

Participation of an IZSLER veterinary epidemiologist in webinar on EuFMDiS, which is a multi-country Foot-and-Mouth Disease outbreak simulation model adapted fromthe Australian Animal Disease Spread Model (AADIS).

- 26th February: "How to improve the data quality of the EuFMDiS model", focused on the upgrade of EUFMDis.
- 31st March 2021: "Preparedness to use emergency vaccination for FAST diseases in European countries."

5. Publication and dissemination of information relevant to the work of FAO including list of scientific publications, internet publishing activities, presentations at international, regional conferences

a) Articles published in peer-reviewed journals

1. Paton, David; Di Nardo, Antonello; Knowles, Nick; Wadsworth, Jemma; Pituco, Maristela; Cosivi, Ottorino; Rivera, Alejandro; Bakkali-Kassimi, Labib; **Brocchi, Emiliana**; de Clercq, Kris; Carrillo, Consuelo; Maree, Francois; Singh, Raj; Vosloo, Wilna; Park, Min; Sumption, Keith; Ludi, Anna; King, Donald.
   The history of foot-and-mouth disease virus serotype C: the first known extinct serotype?
   Virus Evolution, 2021, 7 (1): veab009. doi: http://orcid.org/0000-0002-9097-2262
2. **Foglia EA**, Lembo T, Kazwala R, Ekwem D, Shirima G, **Grazioli S, Brocchi E, Pezzoni G**
   Combining Multiple Assays Improves Detection and Serotyping of Foot-and-Mouth Disease Virus. A Practical Example with Field Samples from East Africa.
   Viruses. 2021 Aug 10;13(8):1583. doi: 10.3390/v13081583
3. Richard Bradhurst, Graeme Garner, Mark Hovari, Maria de la Puente, Koen Mintiens, Shankar Yadav, Tiziano Federici, Ian Kopacka, Simon Stockreiter, Ivanka Kuzmanova,

Samuil Paunov, Vladimir Cacinovic, Martina Rubin, Jusztina Szilagyi, Zsofia Szepesine, Kokany, **Annalisa Santi**, Marco Sordilli, Laura Sighinas, Mihaela Spiridon, Marko Potocnik, Keith Sumption.
Development of a transboundary model of livestock disease in Europe.
Transboundary and Emerging Diseases, 2021, 1–20. https://doi.org/10.1111/tbed.14201

4. Pezzoni G, Bregoli A, Chiapponi C, Grazioli S, Di Nardo A, Brocchi E. Retrospective Characterization of the 2006-2007 Swine Vesicular Disease Epidemic in Northern Italy by Whole Genome Sequence Analysis. Viruses. 2021 Jun 22;13(7):1186. doi: 10.3390/v13071186. PMID: 34206208.

b) International conference:

1. A multiplex lateral flow device for on-field identification and serotyping of Foot-and-Mouth disease virus. **Efrem Alessandro Foglia, Santina Grazioli, Giulia Pezzoni**, Laura Anfossi, Sergio Rosati, **Emiliana Brocchi**. DISCONTOOLS "Filling the knowledge gaps in animal disease control" – 20 October 2021, Bruxelles.
2. Molecular evolution of swine vesicular disease virus in italy from 1992 to the eradication. Arianna Bregoli, Dennis Benedetti, Mattia Calzolari, Chiara Chiapponi, Santina Grazioli , Efrem Alessandro Foglia, Giulia Pezzoni, Emiliana Brocchi. DISCONTOOLS "Filling the knowledge gaps in animal disease control" – 20 October 2021, Bruxelles
3. Preliminary experiments for the development of a new serotyping assay for Foot-and-Mouth disease virus. **Efrem Alessandro Foglia, Giulia Pezzoni, Santina Grazioli, Emiliana Brocchi**. EAVLD 2021 Virtual Meeting – 17 November 2021
4. Exploring Foot-and-mouth disease virus antibody interactions using bio-layer interferometry. Abrew shaw, Alison Burman, amin Asfor, Anna Ludi, **Emiliana Brocchi**, **Santina Grazioli**, Donald King. Scientific Meeting of the Global Foot-and-Mouth Disease Research Alliance 1-3 November 2021-Buenos Aires, Argentina - Virtual event
5. A universal test for the quality of conventional FMD vaccine: VP4 to distinguish between intact and dissociated antigen. Stephen Berryman, amin Asfor, Amina Yasmin, **Santina Grazioli. Emiliana Brocchi**, Tobias Tuthill. Scientific Meeting of the Global Foot-and-Mouth Disease Research Alliance 1-3 November 2021-Buenos Aires, Argentina - Virtual event

c) National conference:

Title of event: Workshop for IIZZSS on topics covered by the National Reference Center for Foot and Mouth and Vesicular Diseases (CERVES). Organised by CERVES
Date and location: 10 November 2021. Webinair: Virtual event.
Work presented:

a) Feedback on PTS 2020 and introduction of coming PTS 2021
b) FMD 2021: an update of global events from the WRLFMD and the WOAH/FAO FMDLaboratory Network
c) Combining Multiple Assays Improves Detection and Serotyping of Foot-and-Mouth Disease Virus. A Practical Example with Field Samples from East Africa.

d) Scientific meetings

Title of the event: Group for Vaccination Advice, Guidance and Consultation (GVA) for FAST1 diseases: Designation of the core group in the South-East European Neighbourhood (SEEN). Organised by EuFMD

Date and location: 18 May 2021. Virtual event
Work presented: Field trials to estimate the effectiveness of the vaccination program implemented in Transcaucasian countries (Georgia & Armenia)

Title of the event: Annual meeting of the FMD/SVD/VSV National Reference Laboratories within EU.
Date and location: 14 October 2021 - EURL, ANSES, France. Virtual event
Role: National/WOAH/FAO Reference Laboratory for vesicular diseases.
Work presented: Combining multiplex assays improves detection and serotyping of foot-and-mouth Disease virus. A practical example with field samples from East Africa.

Title of the event: 15th WOAH/FAO FMD Laboratory Network Meeting.
Date and location: 23-24 November 2021. Virtual event.
Role: WOAH/FAO Lab expert.
Work presented: Updates from the WOAH/FAO reference lab-IZSLER.

Title of event: Knowledge-sharing on official Recognition of foot and mouth disease-free status. General Administration of Customs of the people's Republic of China (GACC) Asian Development Bank in collaboration con Urumqi customs District of GACC e Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences. Co-organized by the World Organization for Animal Health (WOAH), General Administration of Customs of the People's Republic of China (GACC), and Asian Development Bank in collaboration with Urumqi Customs District of GACC and Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences
Date and location: 25-26 November 2021. Virtual event
Role: WOAH/FAO Lab expert.
Work presented: Elements of foot and mouth disease (FMD)-free status

Title of the event: Virtual workshop on Development of Risk-Based Strategic Plan (RBSP) in Lebanon and in the Syrian Arab Republic. Organised by EuFMD.
Date and location: 28-29 July 2021. Virtual event
Role: WOAH/FAO Lab expert.
Work presented: FMD lab diagnosis (FMD clinical aspects and sample submission).

6. Any major change in staff or institution, including governmental institution(s), during the reporting period

In December 2021 the BRO, Dr. Cesare Berneri, retired and Dr. Nadia Vicari was appointed as a replacement.
The selections to fill the vacancies at the FAO/WOAH were held in November 2021.
Dr. G. Macabiani (Biologist) and Dr. T. Trogu (Veterinarian) were employed in January 2022.

7. Update on accredited tests (list of tests)

FMD *Diagnostic tests – Indirect tests*	Accreditation status
Competitive ELISA – Structural Proteins (serotypes O, A, C, Asia 1, SAT1, SAT2)	ISO 17025
Virus Neutralization Test (all seven serotypes)	ISO 17025
NSP Ab ELISA (3ABC trapping ELISA)	ISO 17025

FMD *Diagnostic tests – Direct tests*

Virus Isolation (IB-RS2, BHK21, LFBK)	in process of accreditation
Real Time RT-PCR-3D gene	ISO 17025
Real Time RT-PCR-5UTR region	ISO 17025
Ag detection and serotyping ELISA (MAbs based)	ISO 17025
Real Time topotypes-specific	lab internal protocol
VP1 sequencing	lab internal protocol
Complete genome sequencing	lab internal protocol

SVD *Diagnostic tests – Indirect tests*

Competitive ELISA (WOHA prescribed test for screening)	ISO 17025
IgG-specific ELISA	lab internal protocol
IgM-specific ELISA	lab internal protocol
Virus Neutralization Test	ISO 17025

SVD *Diagnostic tests – Direct tests*

Virus Isolation (cell culture)	in process of accreditation
Conventional RT-PCR (3D-fragment)	ISO 17025
Realtime RT-PCR (3D-fragment)	ISO 17025
Sandwich ELISA (mAbs-based) based)	ISO 17025
Sequencing (3D region, IRES, VP1)	lab internal protocol
Complete genome sequencing	lab internal protocol

8. Other activities indicated in areas of collaboration

INTERNATIONAL COOPERATION AGREEMENT

Signature of cooperation agreement between IZSLER and National Food and Veterinary Risk Assessment Institute (NMVRI) of Lithuania. With the present agreement IZSLER, upon request of NMVRI, commits to:

a. Analyze samples submitted by NMVRI, in order to confirm or rule out the presence of FMD virus;
b. Perform antigenic and genomic characterization of virus isolates;
c. Provide consultancy and collaboration in planning a serological survey in case of need to recover the FMD-free status after an outbreak

Signature by IZSLER of the Global Foot-and-Mouth Research Alliance (GFRA) MoU as partner.

TRAINING AND TECHNICAL VISITS ORGANIZED

The Lab has given the availability to organize training for a PhD Scientist from Pathology Section at Animal Health Department Food Safety and Veterinary Institute, Tirana, Albania supported by IAEA. We are waiting for the funding agency's feedback.

PROFICIENCY TESTS

IZSLER **participated** in the following international Proficiency Tests:

1. FMD/SVD Proficiency Test 2021, organized by the FMD-EURL, ANSES, France. It aimed to evaluate testing laboratory capability for early detect FMD/SVD outbreaks using virological and serological methods. Panels 1-live viruses (six samples) for FMDV/SVDV detection, typing and sequencing (tests applied: VI, Ag detection and serotyping ELISA, rtRT-PCR, VP1 sequencing with phylogenetic analysis); Panel 3 (5 samples) for FMDV serological tests (tests

applied: NSP-Ab ELISA, SP-Ab ELISA, VNT); Panel 4 (four samples) for SVD serological tests (tests applied: 5B7 competitive ELISA, SVDV isotype-specific ELISA for IgG and IgM, VNT).
Feedback: All reported results are consistent with expected results

2. FMDV One-Step triplex Real-Time RT-PCR organized by the FMD-EURL, ANSES
   The FMD-EURL is developing a new one-step triplex real-time RT-PCR for FMDV detection. As part of its validation, the EURL organized an inter-laboratory method validation test (IMVT) to evaluate the performance of the method developed. The objective of this method validation test was to assess the reproducibility of the triplex rtRT-PCR assay
   Participants labs: 10 laboratories.

3. FMD Proficiency Testing scheme 2021, organized by the WRLFMD.
   This study aimed to assess laboratory diagnostic performance and facilitate harmonization of testing between national and international FMDV reference laboratories.
   Two panels focused on outbreak scenario (Panel 1) and post-surveillance serology (Panel 2) were distributed.
   - Panel 1: N.8 samples containing live viruses for FMDV detection, typing and sequencing (tests applied: VI, Ag detection and serotyping ELISA, rtRT-PCR, VP1 sequencing with phylogenetic analysis);
   - Panel 2: N. 4 sera for FMDV serological tests (tests applied: NSP-Ab ELISA, SP-Ab ELISA, VNT).

   Participating Labs: No-EU laboratories and WOHA/FAO Reference Laboratories. The Laboratories have been scored according to expectations defined by the PCP status of their country or their international reference laboratory status.
   Organizing Lab: The Pirbright Institute, UK.
   Feedback: For the results submitted, the lab performance has been scored as category 4 consistent with WOAH/FAO Reference Laboratory requiremets.

IZSLER **organized** the following PTs addressed to maintain and to practice preparedness of regional laboratories to support the NRL in case of FMDV emergency

1. National Proficiency Test for FMD serology.
   Samples: Panel of 20 blind sera.
   Tests: ELISA for NSP Ab (by 3ABC trapping IZSLER kits)
   Participants: N. 10 Italian regional Laboratories.

2. National Proficiency Test for FMD molecular testing.
   Samples: Panel of 8 blind samples and positive control
   Tests: Real-time RT-PCR (3D) using two protocols provided to the participants
   Participants: N. 10 Italian regional Laboratories.

3. Annual inter-laboratory test to monitor the harmonisation and performance of the 5B7-competitive ELISA for SVDV Ab detection carried out in 10 Italian regional laboratories for the testing to detect the Ab against SVD.The panel was composed by 9 sera (5 positive sera and 4 negative sera).

DISTRIBUTION OF DIAGNOSTICS

Calibrated reagents for the Competitive ELISA for SVDV antibody (WOAH prescribed test for screening) were supplied to Canada and Polonia, in addition to the Italian network of regional laboratories for conduction of serological testing (for approximately 20,000 sera

testing in Italian regional laboratories and approximately 28,000 tests at the national reference lab).
During 2021, IZSLER produced and distributed 7 different typologies of ELISA kits for FMD diagnostic. Summary table showing details of kits typology and global distribution are provided below.

Type of reagent		Quantity	Recipient of the reagent (Laboratories / Countries)
FMDV Ag detection ELISA type O, A, C, Asia1, SAT1-2		145	Distributed in 37 countries
	NSP Ab ELISA KIT (3ABC)	35	
SP-Antibody ELISA Kit	FMDV O	87	
	FMDV A	81	
	FMDV Asia 1	55	
	FMDV SAT 1	8	
	FMDV SAT 2	16	

In 2021, the lab distributed the minimum number of kits since 2014 and a decrease of about 20% compared with 2020 in the countries that requested kits was observed.
Viceversa the request for FMDV antigen detection ELISA kit was increased by about 30% compared to 2020.

IZSLER, in collaboration with EUFMD, provided to 11 Balkans countries the reagents which were used for the proficiency test provided by the EURL,. The reagents included a ready-to-use master mix to perform the pan-FMDV real-time RT-PCR, based on 3D genomic portion, and IZSLER ELISA kits and positive controls.

9. Comments or remarks on any general or technical matter/finding/trend

Nothing to report

Reporting officer

Dr. Santina Grazioli & Dr. Giulia Pezzoni

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