



Prot. IZSLER interno:



FAO Reference Centre - Annual report (Thematic areas¹)

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	2020
Date of reporting	13/05/2021

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: The impact of the COVID-19 pandemic on surveillance activities of the OIE/FAO FMD Laboratories due to the repurposing of laboratories and staff for SARS-CoV-2 testing, local shortages in laboratory reagents and PPE, logistical challenges to arrange shipments to international Reference Laboratories has affected the smooth running of international activities as receiving samples for diagnostic confirmation on a suspect case, training and meetings.

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.

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 the Transcaucasus region (Armenia, Georgia, Azerbaijan).

Sera from Georgia and Armenia were received respectively in 2019 and 2020. Due to the COVID-19 emergency and difficulties with transport logistics, we did not receive sera from Azerbaijan. We are in contact with the colleagues to organize soon the shipment of samples.

Country	Year	Purpose testing	NSP ELISA	SP ELISA (end-point titration)			VNT (end-point titration)		
				O	A	Asia 1	O	A	Asia 1
Armenia	2020	Field vaccination trial	120	197	117	117	118	117 (A/Iran05) 118 (A/G-VII)	114

Virological tests (Virus Isolation in BHK-21, IBRS-2 and LKBK; Ag detection and serotyping ELISA, VP1 sequencing)

Samples from years 2012-2018. Serological and virological surveillance in Northern Tanzania is being performed to inform predictive models of foot-and-mouth disease spread

Year testing	Country	samples	VI	Ag-ELISA	Pan-FMDV RealTime PCR	Topotypes-specific RealTime PCRs	VP1 sequence
2020	Tanzania	Epith	68	20	31	155(35x5)	53

Swine Vesicular Disease

Extensive testing is carried out every year for the SVD Italian surveillance plan. The request for SVD testing from other countries is occasional and limited to confirmatory testing, in particular, to clarify sporadic serological reactivities (singleton reactors).

Country	Year	5B7 competitive ELISA	VNT	IgG Isotyping ELISA	IgM Isotyping ELISA	Realtime RT-PCR
Italy	2020	60,279 (ref lab) + 250,000 (other regional labs)	239	239	239	950

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

In 2020, IZSLER received the request from the Tunisian National laboratory (Laboratoire de Virologie Institut de la Recherche Vétérinaire de Tunisie) for the evaluation of a project for a new BSL3 laboratory. The IZSLER Biorisk Officer (BRO) provided feedback and advice to the Tunisian colleagues.

The BRO, as a member of Special Committee on Biorisk Management (SC-BRM)” of EUFMD, participated in the reviewing of the Minimum Standards for FMD contingency laboratories presented at the 44th GS 21-24 April 2021.

In 2020 IZSLER, in collaboration with EUFMD, provided reagents to Balkans countries with the addition of Ukraine and Belarus. The reagents included a ready-to-use mastermix to perform the pan-FMDV real-time RT-PCR, based on 3D genomic portion, and IZSLER ELISA kits. All the reagents were used for the proficiency test annually provided by the EURL. This collaboration is in the framework of a project namely “Diagnostic Bank proposal for preparedness in South-East Europe” to improve the preparedness of those countries to exotic disease incursions.

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: Georgia, Armenia, 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 has been almost completed for the trials conducted in Georgia and Armenia, while shipment of sera, collected in the Azerbaijan trials, has been postponed due to the COVID-19 emergency.

IZSLER has established a collaboration with the University of Glasgow for the study entitled:

Title: Serological and virological surveillance in Northern Tanzania to inform predictive models of foot-and-mouth disease spread.

Objectives and activities:

- To time outbreaks of specific serotypes and inform epidemiological models of disease spread.
- To determine FMD virus serotype diversity in endemic settings by measuring the level of antibodies reactive against the serotypes O, A, SAT2 and SAT1. This would also enable the exploitation and validation of IZSLER kits for use in East Africa.
- Exploitation of real-time RT-PCR specific for FMDV topotypes circulating in the East Africa region (virus pool n. 4)

Partner: University of Glasgow, UK

Country: Tanzania

IZSLER has established a collaboration with the University of Turin for the study entitled

Title: Development of field diagnostic methods antigenic (multiplex LFDs) detection of 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 type the serotype of detected viruses. This is the simplest system for field diagnosis applied to vesicular lesions. In addition, it also allows the direct and easier shipment of samples (trapped in the LFD) to reference laboratories for further investigations.

Partner: University of Turin, Italy

IZSLER has the following research collaborations ongoing:

Title: Research agreement for the development of new and improved diagnostic ELISAs and reagents

Objectives and activities:

- Continued development of antigen-detection assays for FMDV and other vesicular diseases
- Development of assays for FMD vaccine quality using VP4 as a marker for intact antigen.
- Validation of a novel universal serology assay for diagnosis of FMD
- Informing vaccine quality by developing a peptide-based immunoassays for serotype-specific detection of FMDV-specific antibodies
- Use of bio-layer interferometry (BLI) to define binding parameters of monoclonal antibodies for use in diagnostic assays
- Cross-reactivity of SP Ab-ELISAs
- Development of diagnostic tools for SAT3

Partners: The Pirbright Institute, UK

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 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

4. Organisation/participation in international/regional scientific meetings

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

Title of event: Online meeting: Activités de suivi de la réunion REPIVET-RELABSA pour l'amélioration de la surveillance et la détection précoce des FAST en Afrique du Nord

Date and location: 10 - 12 février 2020. Virtual event

Title of event: Annual meeting of the FMD/SVD/VSV National Reference Laboratories within EU.

Date and location: 26-27 November - EURL, ANSES, France. Virtual event

Role: National/OIE/FAO Reference Laboratory for vesicular diseases.

Title of event: 14th OIE/FAO FMD Laboratory Network Meeting.

Date and location: 1-2 December 2020-virtual event.

Role: OIE/FAO Lab expert.

Work presented: Updates from the OIE/FAO reference lab-IZSLER.

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. Nthiwa D, Bett B, Odongo D, Kenya E, Wainaina M, Grazioli S, Foglia E, Brocchi E, Alonso S. Seroprevalence of foot-and-mouth disease virus in cattle herds raised in Maasai Mara ecosystem in Kenya. *Prev Vet Med.* 2020 Mar;176:104929. doi: 10.1016/j.prevetmed.2020.104929.
2. Asfor A, Howe N, Grazioli S, Berryman S, Parekh K., Wilsden G, Ludi A, King DP, Parida S, Brocchi E, Tuthill TJ. Detection of antibodies against a conserved capsid epitope as the basis of a novel universal serological test for foot-and-mouth disease. *Journal of Clinical Microbiology (JCM).* *J Clin Microbiol.* 2020 May 26;58(6):e01527-19. doi: 10.1128/JCM.01527-19.
3. Clare F. J. Browning, Antonello Di Nardo, Lissie Henry, Tim Pollard, Lynne Hendry, Aurore Romey, Anthony Relmy, Phaedra Eble, Emiliana Brocchi, Santina Grazioli, Donald P. King, Anna B. Ludi. An inter-laboratory exercise to compare two ELISA kits used for foot-and-mouth disease virus non-structural protein serology. *J Vet Diagn Invest.* 2020 Nov;32(6):933-937. doi: 10.1177/1040638720962070.
4. Grazioli S, Ferris NP, Dho G, Pezzoni G, Morris AS, Mioulet V, Brocchi E. Development and validation of a simplified serotyping ELISA based on monoclonal antibodies for the diagnosis of foot-and-mouth disease virus serotypes O, A, C and Asia 1. *Transbound Emerg Dis.* 2020;67:3005–3015.
5. Michael Eschbaumer, Andrea Vögtlin, David J Paton, Charles K Nfon, Emiliana Brocchi, Labib Bakkali Kassimi, David J Lefebvre, Kris De Clercq, Donald P King, Stéphan Zientara, Christian Griot, Martin Beer. Non-discriminatory exclusion testing as a tool for the early detection of foot-and-mouth disease incursions. *Front Vet Sci.* 2020 Nov 19;7:552670. doi: 10.3389/fvets.2020.552670.
6. 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*, in press

b) International conference:

1. E.A. Foglia, D. Ekwem, G. Pezzoni, M. Calzolari, S. Grazioli, J. Buza, G. Shirima, R. Reeve, E. Brocchi, T. Lembo. The role of local and regional livestock movements in Foot-and-

- Mouth Disease spread in East Africa. EuFMD OPEN SESSION Special Edition; 8-17 December 2020, Virtual Event
2. S. Grazioli, L. Anfossi, C. Nogarol, C. Cavalera, E.A. Foglia, G. Pezzoni, S. Rosati, E. Brocchi. A multiplex lateral flow device for on-field identification and serotyping of Foot-and-Mouth Disease virus. EuFMD OPEN SESSION Special Edition; 8-17 December 2020, Virtual Event
 3. G. Pezzoni, E.A. Foglia, S. Grazioli, D. Ekwem, R. Kazwala, T. Lembo, G. Shirima, E. Brocchi. Diagnostic performance of Foot-and-Mouth Disease virus detection and serotyping assays with field samples from East Africa. EuFMD OPEN SESSION Special Edition; 8-17 December 2020, Virtual Event
 4. E.A. Foglia, T. Chaligava, S. Kharatyan, G. Pezzoni, S. Grazioli, C. Pöttsch, F. Rosso, E. Brocchi. Field trial to estimate effectiveness of vaccination program against Foot-and-Mouth disease in Transcaucasian countries – Georgia and Armenia. EuFMD OPEN SESSION Special Edition; 8-17 December 2020, Virtual Event

c) National conference:

Due to COVID emergency, no national meeting in which the reference centre could participate was organized.

d) Scientific meetings

1. Activités de suivi de la réunion REPIVET-RELABSA (10 - 12 février 2020) pour l'amélioration de la surveillance et la détection précoce des FAST en Afrique du Nord – April 2020. Online edition
2. Annual meeting of National Reference Laboratories in EU for FMD, organized by EURL, ANSES-Maison-Alfort, France, 26-27 November 2020. Online edition.
3. 15^h Annual meeting of the Network of OIE/FAO Reference Laboratories for FMD, 3-5 December. Online edition. Report on activities conducted by the FMD Reference Laboratory during 2020.

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

In December 2020 Dr. Emiliana Brocchi retired. The activities of the FAO Reference Centre are managed and supervised by Dr. Santina Grazioli and Dr. Giulia Pezzoni who have worked in Dr. Brocchi's staff for a long time.

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 (OIE 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

In addition to the topics reported above, IZSLER has been active in some other areas of collaboration, namely hosting of training, organization of/participation to proficiency tests, distribution of diagnostics.

TRAINING AND TECHNICAL VISITS ORGANIZED

The impact of the COVID 19 emergency and restrictions imposed by the the National Central Authority to counter the spread of the virus did not allow to host trainers and/or visitors.

PROFICIENCY TESTS

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

1. FMD/SVD Proficiency Test 2020, organized by the FMD-EURL. It aimed to evaluate testing laboratory capability to early detect FMDV/SVDV outbreaks using virological and serological methods. Panels 1-live viruses for FMDV/SVDV detection, typing and sequencing (tests applied: VI, Ag detection and serotyping ELISA, rtRT-PCR, VP1 sequencing with phylogenetic analysis); Panel 3 for FMDV serological tests (tests applied: NSP-Ab ELISA, SP-Ab ELISA, VNT); Panel 4 for SVD serological tests (tests applied: competitive ELISA, IgG/IgM ELISA, VNT).
Participating Labs: 26 FMD National Reference Laboratories of EU member countries (which include 4 OIE Ref Labs), 5 EU candidate countries, 6 strategic countries supported by EUFMD.
Organizing Lab: EURL, ANSES, France.
2. FMD Proficiency Testing scheme 2019/2020, 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 OIE/FAO Reference Laboratories. The Laboratories has 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.

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 Ab to serotype Asia 1 (by IZSLER kits)
 Participants: N. 10 Italian regional Laboratories.
2. National Proficiency Test for FMD molecular testing.
 Samples: Panel of 3 blind samples.
 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 national surveillance plan.

DISTRIBUTION OF DIAGNOSTICS

Assembled reagents for 5B7-competitive ELISA (OIE prescribed test for SVD Ab detection) were produced and nationally provided for testing of about 450,000 sera in Italian regional labs to accomplish the serosurveillance plan for SVD.

Assembled reagents for 5B7-competitive ELISA were also provided to NRLs in Canada and Poland.

During 2020, IZSLER produced and distributed 7 different typologies of ELISA kits for FMD diagnostic. Summary tables showing details of kits typology and global distribution are provided below.

Type of kit	FMDV antigen Detection and serotyping ELISA type O, A, C, Asia 1, SAT1-2	NSP Ab ELISA KIT (DIVA test)	Competitive ELISA Kits for serotype-specific Ab				
			FMDV O	FMDV A	FMDV Asia 1	FMDV SAT2	FMDV SAT1
Total N. kits	130	56	1152	221	724	18	5

	Country or organization	FMDV antigen detection ELISA type O, A, C, Asia1,	NSP Ab ELISA KIT	SP Antibody ELISA Kit				
			3ABC	FMDV O	FMDV A	FMDV Asia1	FMDV SAT2	FMDV SAT1
	Training FAO	18	21					
Asia	Nepal	6						
	Myanmar	8						
	South Korea					2	1	
	China		5	420	180	2		
	Japan	1						
	Malaysia	2						
	Taiwan	1	3	1	2	1	1	1
	Bangladesh			2	2	2		
	Singapore			1	1	1		
	Mongolia	2		11	11			
Central Asia & WestEur asia	Kazakhstan			692		692		
	Azerbaijan			1	1	1		
	Armenia		20	3	3	3		
	Turkey	5						
	Jordan	1						
	Belarus	1		1	1	1		
	Israel	6	2	1	1	1	1	
Middle East	UAE	3						
	Oman	2		1	1	1	1	
	Saudi Arabia	4						
Africa	Egypt	1	2				1	
	Uganda	2						
	Algeria						1	
	Morocco			1	1		1	1
	Botswana	5		3	3	3	3	
	Kenya	5						
	Ethiopia	3		1	1		1	1
	Romania	1	1	1	1	1		
	Serbia	1		2	2	2	1	
	New Zeland					1	1	
	Polonia	3	1	1	1	1	1	
	Ireland	1					1	1
	Bulgaria	1		1	1	1		
	Latvia	1						
	Estonia	3		1	1	1	1	
	Ukraine	1						
	Macedonia	1		1	1	1		
	Cyprus	6						
	Montenegro	1		1	1	1		
	Moldova	1		1	1	1		
	Swiss	1						
	Sveden	1		1	1	1	1	1
	Greece	1		1	1	1		
	Malta	2						
	Austria		1	1	1	1	1	
Albania	1		1	1	1			
	Total N. kits	103	56	1152	221	724	18	5

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

Nothing to report

Reporting officer

Dr. Santina Grazioli & Dr. Giulia Pezzoni

Documento prodotto in originale informatico firmato digitalmente ai sensi del "Codice dell'Amministrazione Digitale" D.Lgs. 82/2005 e s.m.i.