

OIE Reference Laboratory Reports Activities

Activities in 2019

This report has been submitted : 2020-01-13 14:01:55

Name of disease (or topic) for which you are a designated OIE Reference Laboratory:	Contagious bovine pleuropneumonia
Address of laboratory:	Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale" Via Campo Boario 64100 Teramo ITALY
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Name (including Title) of Head of Laboratory (Responsible Official):	Dr Nicola D'Alterio, Acting General Director
Name (including Title and Position) of OIE Reference Expert:	Massimo Scacchia, DVM, Official Veterinarian, Head of Cooperation, Development and Research Unit
Which of the following defines your laboratory? Check all that apply:	Governmental

ToR 1: To use, promote and disseminate diagnostic methods validated according to OIE Standards

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 OIE Manual (Yes/No)	Total number of test performed last year	
		Nationally	Internationally
Indirect diagnostic tests		Nationally	Internationally
CFT	Yes	0	46
Direct diagnostic tests		Nationally	Internationally
Isolation	Yes	0	6
PCR	Yes	0	6
RT-PCR	Yes	0	6
PCR-RFLP	Yes	0	6
Immunohistochemistry	Yes	0	6
NGS	Yes	0	6

ToR 2: To develop reference material in accordance with OIE requirements, and implement and promote the application of OIE Standards. To store and distribute to national laboratories biological reference products and any other reagents used in the diagnosis and control of the designated pathogens or disease.

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

Yes

NOTE: Currently, there are 22 laboratories that produce Standard Reference Reagents officially recognised by the OIE for 19 diseases/pathogens. Please click the following link to the list of OIE-approved International Standard Sera:

<http://www.oie.int/en/our-scientific-expertise/veterinary-products/reference-reagents/>. If the reagent is not listed on this page, it is NOT considered OIE-approved. The next two questions allow you to indicate non-OIE-approved diagnostic reagents.

Disease	Test	Available from
Contagious bovine pleuropneumonia	Both irradiated and non-irradiated sera are available. Please contact the Reference Laboratory (Dr Pini) for advice on their suitability for different types of serological test (complement fixation test, indirect or competitive enzyme-linked immunosorbent assay)	Dr Massimo Scacchia CESME, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', Via Campo Boario, 64100 Teramo, Italy Tel: (39-0861) 33.24.05 Fax: (39-0861) 33.22.51 m.scacchia@izs.it

Type of reagent available	Related diagnostic test	Produced/ Supply imported	Amount supplied nationally (ml, mg)	Amount supplied internationally (ml, mg)	Name of recipient OIE Member Countries
Positive serum	For different types of serological test (complement fixation test, indirect or competitive enzyme-linked immunosorbent assay)	Produced	<input checked="" type="radio"/> <10mL <input type="radio"/> 10-100mL <input type="radio"/> 100-500mL <input type="radio"/> >500mL	<input checked="" type="radio"/> <10mL <input type="radio"/> 10-100mL <input type="radio"/> 100-500mL <input type="radio"/> >500mL	

3. Did your laboratory supply standard reference reagents (non OIE-approved) and/or other diagnostic reagents to OIE Member Countries?

Yes

Type of reagent available	Related diagnostic test	Produced/ provide	Amount supplied nationally (ml, mg)	Amount supplied internationally (ml, mg)	No. of recipient OIE Member Countries	Region of recipients
Antigen	CFT	Produced	0	40 ml	2	<input checked="" type="checkbox"/> Africa <input type="checkbox"/> Americas <input type="checkbox"/> Asia and Pacific <input type="checkbox"/> Europe <input type="checkbox"/> Middle East

4. Did your laboratory produce vaccines?

No

5. Did your laboratory supply vaccines to OIE Member Countries?

No

ToR 3: To develop, standardise and validate, according to OIE Standards, new procedures for diagnosis and control of the designated pathogens or diseases

6. Did your laboratory develop new diagnostic methods validated according to OIE Standards for the designated pathogen or disease?

Yes

7. Did your laboratory develop new vaccines according to OIE Standards for the designated pathogen or disease?

No

Name of the new test or diagnostic method or vaccine developed	Description and References (Publication, website, etc.)
Immunoblotting method based on the use of a recombinant Mmm antigen	An Immunoblotting method based on the use of a recombinant Mmm antigen has been developed. Luciani M., Armillotta G., Manna L., Ciarelli A., Di Febo T., Krasteva I., Pini A., Sacchini F., D'Angelo A.R. "Selection of a monoclonal antibody by ELISA, immunoblotting and Quartz Crystal Microbalance technology for immunohistochemical detection of Mycoplasma mycoides subsp. mycoides". Veterinaria Italiana, In press.
NGS analyses for a deep molecular characterisation of Mmm strains	The OIE Reference Laboratory established protocols for NGS analyses for a deep molecular characterisation of Mmm strains to be applied for functional and evolutionary studies and epidemiological investigations. 1. Presentation made by Di Federico M. "Phylogenomic analysis of Mycoplasma mycoides subsp. mycoides strains circulating in Nigeria during 2014-2016". European Mycoplasma Conference", Londra, UK, 18-19 Marzo 2019. 1. Di Federico M, Orsini M, Ancora M, Marcacci M, Di Domenico M, Krasteva I, Zilli K, Musa JA, Francis MI, Sacchini F, Scacchia M, Cammà C. 2019. Draft Genome Sequences of Mycoplasma mycoides subsp. mycoides Strains APF9 and AP108, Isolated in Nigeria in 2014 to 2016. Microbiol Resour Announc. 2019 Sep 19;8(38). pii: e00783-19. doi: 10.1128/MRA.00783-19.

ToR 4: To provide diagnostic testing facilities, and, where appropriate, scientific and technical advice on disease control measures to OIE Member Countries

8. Did your laboratory carry out diagnostic testing for other OIE Member Countries?

Yes

Name of OIE Member Country seeking assistance	Date (month)	No. samples received for provision of diagnostic support	No. samples received for provision of confirmatory diagnoses
NIGERIA	March	0	6

9. Did your laboratory provide expert advice in technical consultancies on the request of an OIE Member Country?

Yes

Name of the OIE Member Country receiving a technical consultancy	Purpose	How the advice was provided
AUSTRIA	VETLAB NETWORK – ERFAN, Can Africa be in a possible stage for a scientific cooperation.	Presentation made as invited speakers at FAO/IAEA VETLAB Directors' Meeting, 19-23 August 2019, IAEA Headquarters, Vienna-Austria Member countries; BOTSWANA, ZIMBABWE, NAMIBIA, MOZAMBIQUE, ZAMBIA ZIMBABWE, TANZANIA, ETHIOPIA, TUNISIA, ALGERIA, MAROCCO, NEPAL
NAMIBIA	ERFAN-Enhancing Research for Africa Network; Presentation of Working Groups and Working Sections opening. ERFAN Budget.	2 presentations made at the First Working groups meeting in SADC Region, Windhoek, Namibia, 9-11 September 2019 Member countries; SOUTH AFRICA, BOTSWANA, ZIMBABWE, ZAMBIA, NAMIBIA, MOZAMBIQUE, ANGOLA, TANZANIA
TUNISIA	Enhancing Research for Africa Network: Un Réseau pour renforcer la recherche en Afrique: organisation et objectifs du projet. ERFAN Budget	2 presentations made at the Second ERFAN North-West Africa Workshop, Tunis, Tunisia, 5-7 November 2019 Member countries; TUNISIA, LIBYA, MOROCCO, ALGERIA, MAURITANIA, SENEGAL, EGYPT
ITALY	ERFAN-Enhancing Research in Veterinary Science for Africa	Invited speakers, event organised by the Italian Ministry of Foreign Affairs for the African Diplomatic corps: ERFAN presentation. Italian Ministry of Foreign Affairs, Rome, 4 December 2019 Member countries; Diplomatic Delegates from 23 African Countries.
CYPRUS	ERFAN PROJECT-Enhancing Research for Africa Network	Presentation made as invited speakers at the 19th JPC/REMESA – 9-10 December 2019, Larnaca, Cyprus Member countries; MOROCCO, ALGERIA, TUNISIA, LIBYA, EGYPT, LIBANO, MAURITANIA, SPAIN, FRANCE, ITALY, PORTUGAL, MALTA, CYPRUS
ETHIOPIA	OIE Reference Laboratory for CBPP, role and activities	Presentation made at the OIE Twinning project Kick off meeting with NAHDIC- Ethiopia, Teramo, Italy, 10-12 December 2019 Member country; ETHIOPIA
ETHIOPIA	OIE Twinning project with NAHDIC- Ethiopia; activities description	Presentation made at the OIE Twinning project Kick off meeting with NAHDIC- Ethiopia, Teramo, Italy, 10-12 December 2019 Member country; ETHIOPIA

ToR 5: To carry out and/or coordinate scientific and technical studies in collaboration with other laboratories, centres or organisations

10. Did your laboratory participate in international scientific studies in collaboration with OIE Member Countries other than the own?

Yes

Title of the study	Duration	Purpose of the study	Partners (Institutions)	OIE Member Countries involved other than your country
Phylogenetic study of Mmm strains in SADC Region. PhD Research study.	3 Years	Characterize Mmm strains using last generation molecular techniques and link them to a defined geographical region. PhD Research study.	National Veterinary Laboratory, Gaborone Veterinary School -Teramo University, Italy	BOTSWANA
Pathogenic studies of Mmm. PhD Research study	3 Years	Study of interaction between Mmm and host cells using OMIC approach	NVL Gaborone, CVRI-Lusaka, Veterinary School -Teramo University, Italy	ZAMBIA
Phylogenetic study of Mmm strains in Nigeria.	3 Years	Characterize Mmm strains using last generation molecular techniques and link them to a defined geographical region. PhD Research study.	Faculties of Veterinary Medicine of Zaria, Ilorin and Abuja, Nigeria	NIGERIA
Pathogenic studies of CCPP. PhD Research study	3 Years	Study of interaction between Mccp and host cells using Immunohistochemistry and Confocal microscopy	National Veterinary Laboratory, Gaborone Veterinary School -Teramo University, Italy. Pendik Institute, Turkey	BOTSWANA

ToR 6: To collect, process, analyse, publish and disseminate epizootiological data relevant to the designated pathogens or diseases

11. Did your Laboratory collect epizootiological data relevant to international disease control?

Yes

If the answer is yes, please provide details of the data collected:

"Phylogenomic analysis of Mycoplasma mycoides subsp. mycoides strains circulating in Nigeria during 2014-2016".

12. Did your laboratory disseminate epizootiological data that had been processed and analysed?

Yes

If the answer is yes, please provide details of the data collected:

1. Di Federico M, Orsini M, Ancora M, Marcacci M, Di Domenico M, Krasteva I, Zilli K, Musa JA, Francis MI, Sacchini F, Scacchia M, Cammà C. 2019. Draft Genome Sequences of *Mycoplasma mycoides* subsp. *mycoides* Strains APF9 and AP108, Isolated in Nigeria in 2014 to 2016. *Microbiol Resour Announc.* 2019 Sep 19;8(38). pii: e00783-19. doi: 10.1128/MRA.00783-19.

**13. What method of dissemination of information is most often used by your laboratory?
(Indicate in the appropriate box the number by category)**

a) Articles published in peer-reviewed journals: 3

1. Di Federico M, Orsini M, Ancora M, Marcacci M, Di Domenico M, Krasteva I, Zilli K, Musa JA, Francis MI, Sacchini F, Scacchia M, Cammà C. 2019. Draft Genome Sequences of *Mycoplasma mycoides* subsp. *mycoides* Strains APF9 and AP108, Isolated in Nigeria in 2014 to 2016. *Microbiol Resour Announc.* 2019 Sep 19;8(38). pii: e00783-19. doi: 10.1128/MRA.00783-19.
2. Lartigue C, Valverde Timana Y, Labroussaa F, Schieck E, Liljander A, Sacchini F, Posthaus H, Batailler B, Sirand-Pugnet P, Vashee S, Jores J, Blanchard A. Attenuation of a Pathogenic *Mycoplasma* Strain by Modification of the *obg* Gene by Using Synthetic Biology Approaches. *mSphere.* 2019 May 22;4(3). pii: e00030-19. doi: 10.1128/mSphere.00030-19.
3. Liljander A, Sacchini F, Stoffel MH, Schieck E, Stokar-Regenscheit N, Labroussaa F, Heller M, Salt J, Frey J, Falquet L, Goovaerts D, Jores J. Reproduction of contagious caprine pleuropneumonia reveals the ability of convalescent sera to reduce hydrogen peroxide production in vitro. *Vet Res.* 2019 Feb 8;50(1):10.

b) International conferences: 9

1. Presentation made by Di Federico M. "Phylogenomic analysis of *Mycoplasma mycoides* subsp. *mycoides* strains circulating in Nigeria during 2014-2016". European *Mycoplasma* Conference", Londra, UK, 18-19 Marzo 2019;
2. Poster made by Di Federico M. Ancora M., Luciani M., Krasteva I., Marruchella G., Sacchini F., Orsini M., Orsini G., Di Febo T., Rossi E., Cammà C., Scacchia M., Mattioli M. Pro-inflammatory response of bovine polymorphonuclear cells induced by *Mycoplasma mycoides* subsp. *mycoides*: further insights from in vitro investigations. European *Mycoplasma* Conference", Londra, UK, 18-19 Marzo 2019;
3. Poster made by Liljander A., Sacchini F., Nicholson P., Frey J., Labroussaa F., Cippa V.L., Salt J., Goovaerts D., Schieck E. and Jores J. Attenuation of *Mycoplasma capricolum* subsp. *capripneumoniae* based on individual SNPs. European *Mycoplasma* Conference", Londra, UK, 18-19 Marzo 2019;
4. Presentation made as invited speakers at FAO/IAEA VETLAB Directors' Meeting, 19-23 August 2019, IAEA Headquarters, Vienna-Austria; VETLAB NETWORK – ERFAN, Can Africa be in a possible stage for a scientific cooperation ERFAN-Enhancing Research for Africa;
5. Speaker at the First Working groups meeting in SADC Region, Windhoek, Namibia, 9-11 September 2019 – Two Presentations on: Working Groups and Working Sections opening- and: ERFAN Budget;
6. Speaker at the Enhancing Research for Africa Network, Presentation made at the Second ERFAN North-West Africa Workshop, Tunis, Tunisia, 5-7 November 2019 – Two Presentations on: Un Réseau pour renforcer la recherche en Afrique: organisation et objectifs du projet - and: ERFAN Budget;
7. Invited speakers, event organised by the Italian Ministry of Foreign Affairs for the African Diplomatic corps. Presentation on: ERFAN presentation. Italian Ministry of Foreign Affairs, Rome, 4 December 2019;
8. Presentation made as invited speakers at the 19th JPC/REMESA – 9-10 December 2019, Larnaca, Cyprus, ERFAN PROJECT-Enhancing Research for Africa Network;
9. Speaker at the OIE Twinning project, Kick off meeting with NAHDIC- Ethiopia, Teramo, Italy, 10-12 December 2019– Two Presentations on: OIE Reference Laboratory for CBPP, role and activities- and: OIE Twinning project with NAHDIC- Ethiopia; activities description.

c) National conferences: 2

1. Di Federico M., Orsini M., Ancora M. Krasteva I., Zilli K, Musa J.A., Francis M.I, Sacchini F., Scacchia M., D'Alterio N., Cammà C. Analisi filogenomica di ceppi di *Mycoplasma mycoides* subsp. *mycoides* isolati in Nigeria tra il 2014 e il 2017. Congresso Nazionale S.I.Di.L.V. (XIX), Matera, 23-25 Ottobre 2019, Poster;
2. Di Federico M., Keokilwe L., Ancora M., Krasteva I., Di Febo T., Sacchini F., Befacchia G., Orsini G., Luciani M., Scacchia M., Cammà C. Pleuropolmonite contagiosa bovina: studio di espressione genica applicato ad un modello sperimentale ex vivo di espanti polmonari bovino. Congresso Nazionale S.I.Di.L.V. (XIX), Matera, 23-25 Ottobre 2019, Poster.

d) Other:

(Provide website address or link to appropriate information) 1

ERFAN Website: <http://evet4africa.org/wp/>**ToR 7: To provide scientific and technical training for personnel from OIE Member Countries****To recommend the prescribed and alternative tests or vaccines as OIE Standards**

14. Did your laboratory provide scientific and technical training to laboratory personnel from other OIE Member Countries?

Yes

a) Technical visits: 1

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	MOZAMBIQUE	20
b	SOUTH AFRICA, BOTSWANA, ZIMBABWE, ZAMBIA, NAMIBIA, MOZAMBIQUE, ANGOLA, TANZANIA	60
b	TUNISIA, LIBYA, MOROCCO, ALGERIA, MAURITANIA , SENEGAL, EGYPT	53

ToR 8: To maintain a system of quality assurance, biosafety and biosecurity relevant for the pathogen and the disease concerned

15. Does your laboratory have a Quality Management System?

Yes

Quality management system adopted	Certificate scan (PDF, JPG, PNG format)
ISO 17025	Accredia certificate.pdf

16. Is your quality management system accredited?

Yes

Test for which your laboratory is accredited	Accreditation body
CFT	ILAC-ACCREDIA
Immunoblotting	ILAC-ACCREDIA
PRC-RFLP	ILAC-ACCREDIA

17. Does your laboratory maintain a “biorisk management system” for the pathogen and the disease concerned?

Yes

(See *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 1.1.4*)

ToR 9: To organise and participate in scientific meetings on behalf of the OIE

18. Did your laboratory organise scientific meetings on behalf of the OIE?

Yes

National/ International	Title of event	Co-organiser	Date (mm/yy)	Location	No. Participants
International	ERFAN-Enhancing Research for Africa Network First Working groups meeting in SADC Region	Central Veterinary Laboratory, Windhoek Faculty of Veterinary Science, UNAM, OIE Sub-Regional Office- Gaborone	9/19	Windhoek, Namibia	60
International	Enhancing Research for Africa Network, Second North-West Africa Workshop	OIE Sub-Regional Office- Tunis	11/19	Tunis, Tunisia	53
International	OIE Twinning project; Kick off meeting with NAHDIC- Ethiopia,		12/19	Teramo, Italy	25

19. Did your laboratory participate in scientific meetings on behalf of the OIE?

Yes

Title of event	Date (mm/yy)	Location	Role (speaker, presenting poster, short communications)	Title of the work presented
FAO/IAEA VETLAB Directors' Meeting	8/19	IAEA Headquarters, Vienna-Austria	Invited speaker	VETLAB-ERFAN, Can Africa be in a possible stage for a scientific cooperation
Event organised by the Italian Ministry of Foreign Affairs for the African Diplomatic corps	12/19	Italian Ministry of Foreign Affairs, Rome-Italy	Invited speaker	ERFAN Presentation
19th JPC/REMESA	12/19	Larnaca- Cyprus	Invited speaker	ERFAN OIE PROJECT- Enhancing Research for Africa Network

ToR 10: To establish and maintain a network with other OIE Reference Laboratories designated for the same pathogen or disease and organise regular inter-laboratory proficiency testing to ensure comparability of results

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

Yes

21. Was your laboratory involved in maintaining a network with OIE Reference Laboratories designated for the same pathogen or disease by organising or participating in proficiency tests?

No

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

Yes

Title of the project or contract	Scope	Name(s) of relevant OIE Reference Laboratories
Phylogenetic and biomolecular epidemiology study on Mmm strains isolated in the South African region	The IZSAM has activated and financed in collaboration with the Faculty of Veterinary Medicine of Teramo a PhD for a veterinarian at the National Veterinary Laboratory of Gaborone, Botswana, aimed at the phylogenetic and biomolecular epidemiology study to be carried out on Mmm strains isolated in the South African region. More in detail, the activities, now in its second year, involve the collection of Mmm isolates from some African countries and their phylogenetic study by NGS sequencing in addition to bioinformatics analysis to assess possible antibiotic resistance factors. In the context of the PhD, research activities continued on bovine lung explants to investigate some aspects related to the pathogenesis of CBPP, in full respect and implementation of the 3Rs. In order to assess the host-pathogen interaction in the early stages of infection, a gene expression study (based on RT-qPCR assays) applied to an in vitro model of neutrophil cultures and an ex vivo model of bovine lung tissue explants was conducted. From the analysis of the data derived from the two experimental models it was possible to understand the changes induced by Mmm in terms of expression of selected target genes (n=7) involved in the inflammatory process.	National Veterinary Laboratory of Gaborone, Botswana (OIE RefLab CBPP)
Studies on pathogenesis of CBPP	In the context of the PhD, research activities continued on bovine lung explants to investigate some aspects related to the pathogenesis of CBPP, in full respect and implementation of the 3Rs. In order to assess the host-pathogen interaction in the early stages of infection, a gene expression study (based on RT-qPCR assays) applied to an in vitro model of neutrophil cultures and an ex vivo model of bovine lung tissue explants was conducted. From the analysis of the data derived from the two experimental models it was possible to understand the changes induced by Mmm in terms of expression of selected target genes (n=7) involved in the inflammatory process.	National Veterinary Laboratory of Gaborone, Botswana (OIE RefLab CBPP)
ERFAN CBPP and Contagious agalactiae Working group	Networking in SADC Region and in North West Africa	National Veterinary Laboratory of Gaborone, Botswana (OIE RefLab CBPP), IZS Palermo, Italy OIE (OIE RefLab Contagious agalactiae)
Studies on pathogenesis of CCPP	In addition to the CBPP model for bovine animals, the use of respiratory tissue explants such as trachea, bronchi and lungs from goat is being developed to investigate pathogenic mechanisms caused by Mycoplasma capricolum subsp. capripneumoniae, the agent of CCPP,	OIE Reference Laboratory for CCPP at the Veterinary Control Institute, Pendik, Turkey.

ToR 11: To organise inter-laboratory proficiency testing with laboratories other than OIE Reference Laboratories for the same pathogens and diseases to ensure

equivalence of results

23. Did your laboratory organise or participate in inter-laboratory proficiency tests with laboratories other than OIE Reference Laboratories for the same disease?

No

Note: See Interlaboratory test comparisons in: *Laboratory Proficiency Testing at: <http://www.oie.int/en/our-scientific-expertise/reference-laboratories/proficiency-testing> see point 1.3*

ToR 12: To place expert consultants at the disposal of the OIE

24. Did your laboratory place expert consultants at the disposal of the OIE?

Yes

Kind of consultancy	Location	Subject (facultative)
CBPP OIE ad hoc Group (19-20 November 2019)	OIE Headquarter	Evaluation of CBPP status of OIE Members

25. Additional comments regarding your report:

OIE Twinning Project on CBPP:

- A Twinning project between the OIE Reference Laboratory at IZSAM, as Parental Laboratory and the National Animal Health Diagnostic Centre of Sebeta, Ethiopia (NAHDIC), as Candidate Laboratory, started. The Kick off meeting was organised in Teramo in December 2019. Seven NAHDIC colleagues guests attended the meeting;
- The IZSAM OIE Reference Laboratory continued to support the former candidate laboratory, Pendik Veterinary Control Institute, Turkey, during the submission of the application to become OIE Reference Laboratory for Mycoplasmosis. Finally, the Turkish Laboratory became OIE Reference Laboratory in May 2019.

The CBPP OIE Reference Laboratory also carries out the following activities:

- Funding of PhD studentship on:
 1. "Use of molecular epidemiology related to *Mycoplasma mycoides* subsp. *mycoides* strains isolated in Sub-Saharan countries to support CBPP control strategies in Africa". PhD thesis in Infectious Animal Diseases, Faculty of Veterinary Medicine, University of Teramo, AY 2018-2021; The PhD student is a Veterinarian from Botswana National Veterinary Laboratory. The objectives of his doctoral project are to characterize Mmm strains using last generation molecular techniques and link them to a defined geographical region; to retrieve all the relevant information related to the epidemiology of CBPP in the different regions considered; to generate a comprehensive Mmm genome database containing all the relevant epidemiological information related to the region where the strain originated; to combine genetic and epidemiological information to draw a CBPP distribution map; to develop a molecular epidemiology approach able to identify the most relevant risk factors for single areas predicting the most likely source of infection;
 2. "Contagious Bovine Pleuropneumonia: molecular basis of host-pathogen interactions in the early stage of the inflammatory response". PhD thesis in Cellular and Molecular Biotechnology XXXII cycle, University of Teramo in collaboration with the National Reference Centre for Genomic Sequences of Pathogenic Microorganisms: Bioinformatics Database and Analysis. AY 2016-2019. The PhD student is a Bio-technologist. As part of a doctoral project RT-qPCR assays have been developed to analyse the gene expression of a "panel" of targets involved in the inflammatory process of bovine immune cells. Also the transcription of inflammatory targets has been

monitored, using an in vitro model, based on bovine neutrophils cell culture, experimentally infected with Mmm as useful alternative tool for investigating CBPP pathogenesis;

- Research and networking projects

Presented to OIE the Enhancing Research for Africa Network (ERFAN) project, designed by the expert at the OIE Reference Laboratory for CBPP in Teramo, Italy. The General Director of OIE approved ERFAN project in November 2018. ERFAN was funded in April 2019. ERFAN is an official OIE project. ERFAN main task is to enhance research and development in Africa through OIE Reference Laboratories and Collaborating Centres, and Poles of Excellence created in North-West Africa and SADC regions, as result of previous OIE Twinning Projects. ERFAN objectives are to enhance research collaborations and to foster animal health, welfare and food security in the framework of a globalised world; to push forward the activities of Veterinary Institutions, as laboratories/faculties, by improving technology, innovation and knowledge based on capacities; to control and protect public health.

The 29 partners include 24 African Veterinarian Institutions from 15 African countries as Algeria, Angola, Botswana, Egypt, Libya, Mauritania, Morocco, Mozambique, Namibia, Senegal, South Africa, Tanzania, Tunisia, Zambia and Zimbabwe. The Botswana National Veterinary Laboratory is OIE Reference Laboratory for CBPP. The Veterinary School at Pretoria University is Collaborating Centre for Training in Veterinary Science. Also Onderstepoort Veterinary Research Institute with its OIE Reference Laboratories is an ERFAN partner.

The Italian partners are the: Italian Ministry of Health-General Director of Animal Health and Veterinary Drug-Rome; Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, with the OIE Reference Laboratories for Brucellosis, BT, CBPP and WND and the Collaborating Centres for Animal Welfare, Food Security, Epidemiology and Training in Veterinary Science. The expert at the OIE Reference Laboratory for CBPP in Teramo is a component of the ERFAN Secretariat; Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici; Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Val D'Aosta, Torino with the OIE Reference Laboratory for TSE; Istituto Zooprofilattico Sperimentale della Puglia and Basilicata, Foggia; Istituto Zooprofilattico Sperimentale della Sicilia, Palermo with the OIE Reference Laboratory for Contagious Agalactiae (CA).

The ERFAN is organised in seven Working Groups: i) Animal Welfare, ii) CBPP and CA, iii) Food Hygiene, iv) One Health: Anthrax-Brucellosis and Bovine Tuberculosis, v) Training in Veterinary science, vi) Vectors Born Diseases, in particular RVF, and vii) TSE.

The ERFAN activities carried out during the four years of the project will be:

Annual organization of General, Regional and Working Group meetings, in NWA and SADC Regions.

Implementation of activities related to the seven working groups.

Provide support to African national veterinary laboratories in order to become poles of excellence of the OIE for: i) production of reference materials as Positive and Negative sera and diagnostic antigens; ii) organisation and management of Proficiency tests; iii) implementation of new and innovative diagnostic tests; iv) improvement of research activities; v) production of didactic material (as scientific reports, video materials, photos, etc.) for disseminating research outputs and project results; vi) organization of training programs; vii) creation of web-based community.

In 2019 two meetings have been organised with 113 participants.

- NGS characterization of Mmm strains

IZSAM provided technical support and expertise to African partners for characterization of Mmm strains by NGS; A hundred and two Mmm strains are available at the CBPP OIE Reference Laboratory in Teramo, collected over more than 26 years (1992-2018) and more than 8 countries (both African and European). This collection also includes some vaccination strains and isolates collected before the 1970. A genomic database of Mmm strains available at IZSAM obtained by NGS sequencing was created. Using this approach, for the first time a phylogenetic analysis of Nigerian Mmm strains (n=34) circulating in the some areas of the Country was conducted. This may represent a molecular tool to support CBPP epidemiological investigations and disease surveillance in the country.

- Studies on pathogenesis of CBPP and CCP

Contagious bovine Pleuropneumonia

The IZSAM has activated and financed in collaboration with the Faculty of Veterinary Medicine of Teramo a PhD for a veterinarian at the National Veterinary Laboratory of Gaborone, Botswana, aimed at the phylogenetic and biomolecular epidemiology study to be carried out on Mmm strains isolated in the South African region. More in detail, the activities, now in its second year, involve the collection of Mmm isolates from some African countries and their phylogenetic study by NGS sequencing in addition to bioinformatics analysis to assess possible antibiotic resistance factors.

In the context of the PhD, research activities continued on bovine lung explants to investigate some aspects related to the pathogenesis of CBPP, in full respect and implementation of the 3Rs.

In order to assess the host-pathogen interaction in the early stages of infection, a gene expression study (based

on RT-qPCR assays) applied to an in vitro model of neutrophil cultures and an ex vivo model of bovine lung tissue explants was conducted. From the analysis of the data derived from the two experimental models it was possible to understand the changes induced by Mmm in terms of expression of selected target genes (n=7) involved in the inflammatory process.

Contagious caprine Pleuropneumonia

In addition to the CBPP model for bovine animals, the use of respiratory tissue explants such as trachea, bronchi and lungs from goat is being developed to investigate pathogenic mechanisms caused by *Mycoplasma capricolum* subsp. *capripneumoniae*, the agent of CCPP, which is exotic in Italy but widely spread in Africa and the Middle East. This activity is carry on in collaboration with the OIE Reference Laboratory for CCPP at the Veterinary Control Institute, Pendik Turkey.