# **WOAH Reference Laboratory Reports Activities 2023**

## **Activities in 2023**

This report has been submitted: 30 mai 2024 15:14

## **Laboratory Information**

Name of disease (or topic) for which you are a designated WOAH Reference Laboratory:	Avian mycoplasmosis (Mycoplasma gallisepticum, Mycoplasma synoviae)	
Address of laboratory:	Via Bovolino 1c, 37060 - Buttapietra (VR), Italy	
Tel.:	+39045500285	
E-mail address:	scatania@izsvenezie.it	
Website:	https://www.izsvenezie.com/reference-laboratories/avian-mycoplasmosis/	
Name (including Title) of Head of Laboratory (Responsible Official):	Dr. Salvatore Catania	
Name (including Title and Position) of WOAH Reference Expert:	Dr. Salvatore Catania	
Which of the following defines your laboratory? Check all that apply:  Governmental Research agency		

### **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

res			
Diagnostic Test	Indicated in WOAH Manual (Yes/No)	Total number of test performed last year	
Indirect diagnostic tests		Nationally	Internationally
Indirect ELISA for Mycoplasma gallisepticum		18718	0
Indirect ELISA for Mycoplasma synoviae		4652	0
Direct diagnostic tests		Nationally	Internationally
Real-time PCR for Mycoplasma gallisepticum		1614	16
Real-time PCR for Mycoplasma synoviae		1408	11
Avian mycoplasma culturing		230	12
16s-rDNA PCR + Denaturing Gradient Gel Electrophoresis (DGGE)		108	15
mgc2 gene sequencing		35	4
vlhA gene sequencing		104	12
Multi Locus Sequence Typing for Mycoplasma gallisepticum		19	8
Multi Locus Sequence Typing for Mycoplasma synoviae		52	3

### 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?

No

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?

Yes

7. Did your laboratory validate diagnostic methods according to WOAH Standards for the designated pathogen or disease?

Yes

NAME OF THE NEW TEST OR DIAGNOSTIC METHOD  DEVELOPED	DESCRIPTION AND REFERENCES (PUBLICATION, WEBSITE, ETC.)
Multiple Locus Variable-Number Tandem Repeat Analysis (MLVA) for M. synoviae	A MLVA protocol based on six tandem repeats loci (6 loci-MsMLVA) analysis for the differentiation of M. synoviae strains. This MLVA protocol showed a good discriminatory power while being more rapid and cheaper than the other available molecular methods. Presented at: - 24th IOM Biennial Congress. 16-20 July 2023. Title: Mycoplasma synoviae genotyping: discrimination power of Multiple Locus variable-number Tandem repeat analysis for live vaccine and wild strains. Stefani Elisabetta, Bottinelli Marco, Matucci Andrea, Gastaldelli Michele, Righetti Verdiana, Nai Giorgia, Conci Veronica, Tondo Annalucia, Kreizinger Zsuzsa, Gyuranecz Miklós, Catania Salvatore 22nd WVPA Congress. 4-8 September 2023. Title: Multiple Locus variable-number Tandem repeat analysis for differentiation between Mycoplasma synoviae live vaccine strain and wild strains collected on field: preliminary results. Stefani Elisabetta, Matucci Andrea, Gastaldelli Michele, Righetti Verdiana, Nai Giorgia, Tondo Annalucia, Gyuranecz Miklós, Catania Salvatore, Bottinelli Marco. Available at: https://www.izsvenezie.it/servizi/servizi-specifici/genotipizzazione-micoplasmi/

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?

Nο

#### **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
SPAIN	2023-03-08	- Mycoplasma culturing - 16S- rDNA PCR + Denaturient Gradient Gel Electrophoresis (DGGE) - Real-time PCR for Mycoplasma gallisepticum - Real time PCR for Mycoplasma synoviae - mgc2 gene sequencing - vlhA gene sequencing	16	0
EGYPT	2023-06-22	- Real-time PCR for Mycoplasma gallisepticum - Multi Locus Sequence Typing for Mycoplasma gallisepticum	6	0
IRELAND	2023-10-31	- Real-time PCR for Mycoplasma synoviae - Multi Locus Sequence	0	3

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	Typing for Mycoplasma synoviae	

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

Voc

NAME OF THE WOAH MEMBER COUNTRY RECEIVING A TECHNICAL CONSULTANCY	PURPOSE	HOW THE ADVICE WAS PROVIDED
SPAIN	Consultancy on how to interprete mgc2 gene sequencing results.	Remotely (e-mail)
HONG KONG	- Consult on the validation of diagnostic tests, specifically real-time PCR for Mycoplasma gallisepticum and Mycoplasma synoviae Request for the provision of positive controls (Mycoplasma strains).	Remotely (e-mail, Zoom meeting)
SRI LANKA	<ul> <li>Provision of the developed SOP (Standard Operating Procedure) and related documents for diagnosing Mycoplasma synoviae and isolating the bacteria from clinical submissions.</li> <li>Possibility of initiating a collaborative project to improve the diagnostic conditions in Sri Lanka Possibility of organizing a training period at the WOAH reference laboratory.</li> </ul>	Remotely (e-mail, Zoom meeting)
ITALY	- Consult on aspects of ELISA accreditation: 1) Use and calibration of laboratory machine. 2) Approach to internal validation of the method.	In person, during a visit to the laboratory.
ITALY	<ul> <li>Provision of reference materials for molecular biology tests for an accredited laboratory within the national territory.</li> </ul>	Remotely (e-mail)
EGYPT	Consultancy on interpretation of Mylti Locus Sequence Typing (Mycoplasma gallisepticum) results.	Remotely (e-mail)
IRELAND	Consultancy on ELISA tests for avian Mycoplasmas.	Remotely (e-mail)

## 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
MyMIC: Standardization of diagnostics and antimicrobial susceptibility testing and clinical interpretation in animal mycoplasmas.	2022-2025	This project aims to set up a network of laboratories working on mycoplasma diagnostics and their susceptibility to ATBs to compare the different methods used and the results of minimum inhibitory concentrations.	- Universidad de Las Palmas de Gran Canaria. (Spain) - National Veterinary Institute (Sweden) - University of Melbourne (Australia) - University of Maiduguri (Nigeria) - University of Agriculture Peshawar (Pakistan) - Anses (France) - CIRAD (France) - PIWET (Poland) - University of Giessen (Germany) - University of Bern (Switzerland) - University of Veterinary Medicine, Vienna (Austria) - Veterinary Medical Research Institute (Hungary) - Kimron Veterinary Institute (Israel) - Finnish Food Authority (Finland) - GD Animal Health (The Netherlands) - Centro Nacional	AUSTRALIA AUSTRIA BELGIUM CUBA FINLAND FRANCE GERMANY HUNGARY ISRAEL NIGERIA PAKISTAN POLAND SPAIN SWEDEN SWITZERLAND THE NETHERLANDS UNITED KINGDOM

de Sanidad Agropecuaria (CENSA
, Cuba) - University of Ghent
(Belgium)

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

Yes

#### -Research need: 1-

Please type the Research need: There is a need to develop a diagnostic test that allows for the discrimination between vaccine and wild strains in positive animal groups with a high degree of specificity.

Relevance for WOAH Disease Control,

Relevance for the Codes or Manual Code, Manual,

Field Epidemiology and Surveillance, Diagnostics, Vaccines,

Animal Category Terrestrial,

Disease

Avian mycoplasmosis (Mycoplasma gallisepticum, Mycoplasma synoviae)

Kind of disease (Zoonosis, Transboundary diseases)

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

 $(e.g.\ Terrestrial\ Manual\ Chapter\ 2.3.5-Minimum\ requirements\ for\ a septic\ production\ in\ vaccine\ manufacture)$ 

Answer:

Notes:

Answer

#### TOR6: EPIZOOLOGICAL DATA

14. Did your Laboratory collect epidemiological data relevant to international disease control?

No

15. Did your laboratory disseminate epidemiological data that had been processed and analysed?

Νo

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

a) Articles published in peer-reviewed journals:

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- Buni D, Kovács ÁB, Földi D, Bányai K, Bali K, Domán M, Wehmann E, Bradbury J, Bottinelli M, Catania S, Stefani E, Lysnyansky I, Kovács L, Grózner D, Gyuranecz M, Kreizinger Z. Development of molecular assays for the analysis of genetic relationships of Mycoplasma iowae. Vet Microbiol. 2023 Dec;287:109909. doi: 10.1016/j.vetmic.2023.109909. Epub 2023 Nov 3. PMID: 37925876.
- Ramírez AS, Poveda JB, Dijkman R, Poveda C, Suárez-Pérez A, Rosales RS, Feberwee A, Szostak MP, Ressel L, Viver T, Calabuig P, Catania S, Gobbo F, Timofte D, Spergser J. Mycoplasma bradburyae sp. nov. isolated from the trachea of sea birds. Syst Appl Microbiol. 2023 Nov;46(6):126472. doi: 10.1016/j.syapm.2023.126472. Epub 2023 Oct 7. PMID: 37839385.
- Matucci A, Stefani E, Tondo A, Righetti V, Bottinelli M, Gavazzi L, Merenda M, Catania S. Isolation and characterization of an atypical Mycoplasma gallisepticum strain showing a new mgc2 variant. Vet Microbiol. 2023 Jul;282:109768. doi: 10.1016/j.vetmic.2023.109768. Epub 2023 May 3. PMID: 37148622.
- Zacometti C, Tata A, Stella R, Leone S, Pallante I, Merenda M, Catania S, Pozzato N, Piro R. DART-HRMS allows the detection of toxic alkaloids in animal autopsy specimens and guides the selection of confirmatory methods in accidental plant poisoning. Anal Chim Acta. 2023 Jul 11;1264:341309. doi: 10.1016/j.aca.2023.341309. Epub 2023 May 3. PMID: 37230724.
- Tommasoni C., Schiavon E., Lisuzzo A., Gianesella M., Merenda M., Coin P., Patregnani T., Tola S., Catania S., Barberio. Salmonella enterica serovar Dublin infection in dairy cattle: a case study on the management of an outbreak in Italy. Large Animal Review, (2023), 99-103, 29(2)
- b) International conferences:

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24th Congress of the International Organization for Mycoplasmology:

- Investigation on the effects of two storage methods of diagnostic samples on the isolation of avian Mycoplasma strains. Conci, V., Bottinelli, M., Picchi, M., Bottazzari, M., Rossi, I., Catania, S., Tondo, A.

- Mycoplasma synoviae genotyping: discrimination power of multiple locus variable-number tandem repeat analysis for live vaccine and wild strains. Stefani, E., Bottinelli, M. Matucci, A.; Gastaldelli, M.; Righetti, V.; Nai, G.; Conci, V.; Tondo, A.; Kreizinger, Z.; Gyuranecz, M.; Catania, S.

#### 22nd WVPA Congress:

- IN VITRO SUSCEPTIBILITY OF MYCOPLASMA IOWAE ISOLATES TO ANTIMICROBIAL AGENTS. D. Buni, L. Udvari, D. Földi, N. Belecz, Y. Cécile, S. Catania, J. Bradbury, I. Lysnyansky, L. Kovács, M. Gyuranecz, Z. Kreizinger.
- MULTIPLE LOCUS VARIABLE-NUMBER TANDEM REPEAT ANALYSIS FOR DIFFERENTIATION BETWEEN MYCOPLASMA SYNOVIAE LIVE VACCINE STRAIN AND WILD STRAINS COLLECTED ON FIELD: PRELIMINARY RESULTS. E. Stefani, A. Matucci, M. Gastaldelli, V. Righetti, G. Nai, A. Tondo, M. Gyuranecz, S. Catania, M. Bottinelli.
- DEVELOPMENT OF MOLECULAR ASSAYS FOR THE ANALYSIS OF GENETIC RELATIONSHIPS OF MYCOPLASMA IOWAE. D. Buni, A.B. Kovács, D. Földi, K. Bányai, K. Bali, J. Bradbury, M. Bottinelli, S. Catania, I. Lysnyansky, L. Kovács, D. Grózner, M. Gyuranecz, Z. Kreizinger.
- c) National conferences:

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10th Edition of the Scientific Event of the National Veterinary Association of Portugal. 14-16 April 2023.

- "Mycoplasmas in the poultry sector: where to start for a better management of these pathogens".
- "Mycoplasma synoviae infection in the poultry sector: a special focus on this intriguing microorganism".

#### ERFAN COURSES & IZS Sicilia, Palermo 26 January 2023.

- "Industrial poultry production and risk of AMR emergence: what we have learned from the mischievous mycoplasmas"
- d) Other (Provide website address or link to appropriate information):

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#### Website:

- https://www.izsvenezie.com/reference-laboratories/avian-mycoplasmosis/
- https://www.izsvenezie.it/istituto/centri-di-referenza/micoplasmosi-aviarie/
- https://www.izsvenezie.it/servizi/servizi-specifici/genotipizzazione-micoplasmi/

#### TOR7: SCIENTIFIC AND TECHNICAL TRAINING

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

No

## TOR8: QUALITY ASSURANCE

18. Does your laboratory have a Quality Management System?

Yes

Quality management system adopted	Certificate scan (PDF, JPG, PNG format)	
ISO/IEC 17025:2017	Accreditation Certificate available at:  https://www.izsvenezie.it/documenti/servizi/qualita- accreditamento/certificato-ISO-17025.pdf	Certificato di accreditamento_ACCREDIA_2024.pdf

19. Is your quality management system accredited?

Yes

Test for which your laboratory is accredited	Accreditation body
<ul> <li>Real-time PCR for Mycoplasma gallisepticum - Real-time PCR for Mycoplasma synoviae - Indirect ELISA for Mycoplasma gallisepticum - Indirect ELISA for Mycoplasma synoviae - Indirect ELISA for Mycoplasma meleagridis</li> </ul>	ACCREDIA

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

Yes

USe of MSC Class II biosafety cabinets.

#### **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?

No

### TOR10: NETWORK WITH WOAH REFERENCE LABORATORIES

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

No

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

NIO

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

No

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?

No

#### 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?

Purpose for inter- laboratory test comparisons1	Role of your reference laboratory (organizer/participant)	No. participating laboratories	Name of the Test	WOAH Member Countries
Quality Insurance -Ring trial	Participant	1	Mycoplasma culturing	UNITED KINGDOM,
Quality Insurance -Ring trial	Participant	1	16S-rDNA PCR + Denaturing Gradient Gel Electrophoresis (DGGE)	UNITED KINGDOM,
Quality Insurance -Ring trial	Participant	1	Indirect ELISA for Mycoplasma gallisepticum	UNITED KINGDOM,
Quality Insurance -Ring trial	Participant	1	Indirect ELISA for Mycoplasma meleagridis	UNITED KINGDOM,

### **TOR12: EXPERT CONSULTANTS**

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

No

29. Additional comments regarding your report:

No