

WOAH Reference Laboratory Reports Activities 2024

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LABORATORY INFORMATION

*Name of disease (or topic) for which you are a designated WOAH Reference Laboratory:	Marek's disease	
*Address of laboratory:	Ash Road, Pirbright, Woking, Surrey, GU24 0NF UNITED KINGDOM	
*Tel:	+441487231493	
*E-mail address:	yongxiu.yao@pirbright.ac.uk	
Website:	https://www.pirbright.ac.uk/diagnostics-surveillance/mareks-disease-virus- referencelaboratory	
*Name (including Title) of Head of Laboratory (Responsible Official):	Dr. Yongxiu Yao	
*Name (including Title and Position) of WOAH Reference Expert:	Dr. Yongxiu Yao, Head of Viral Oncogenesis group	
*Which of the following defines your laboratory? Check all that apply:	Academic institution	

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) Ves

Diagnostic Test	Indicated in WOAH Manual (Yes/No)	Total number of test	performed last year
Indirect diagnostic tests		Nationally	Internationally
None		0	0

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Direct diagnostic tests		Nationally	Internationally
Real-time PCR for virulent MDV- 1, MDV-2, HVT vaccine, CVI988 vaccine, and Prevexxion vaccine	Yes	2367	326

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?

Not applicable

5. Did your laboratory supply vaccines to WOAH Members?

Not applicable

TOR3: NEW PROCEDURES

6. Did your laboratory develop new diagnostic methods for the designated pathogen or disease?

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Name of the new test or diagnostic method developed	Description and References (Publication, website, etc.)
Real-time PCR for specific detection of the new Marek's disease vaccine 'Prevexxion- RN®'	Validation of this test as an ISO/IEC 17025-accredited test is in progress; the report (when completed) could be requested from: Marek's Disease Virus Reference Laboratory, The Pirbright Institute, Ash Road, Woking, Surrey, GU24 0NF, UK (https://www.pirbright.ac.uk/diagnostics-surveillance/mareks-disease-virus-reference-

7. Did your laboratory validate diagnostic methods according to WOAH Standards for the designated pathogen or disease? No

8. Did your laboratory develop new vaccines for the designated pathogen or disease?

No 9. Did your laboratory validate vaccines according to WOAH Standards for the designated pathogen or disease?

No

TOR4: DIAGNOSTIC TESTING FACILITIES

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

Yes

Name of WOAH Member Country seeking assistance	Date	Which diagnostic test used	No. samples received for provision of diagnostic support	No. samples received for provision of confirmatory diagnoses
THE NETHERLANDS	2024-07-29	Real-time PCR for Prevexxion vaccine	60	0

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GERMANY	2024-08-12	Real-time PCR for CVI988 vaccine, HVT vaccine, MDV-1 field strains, MDV- 2 field strains	20	20
IRELAND	2024-08-19	Real-time PCR for HVT vaccine	120	0
DENMARK	2024-09-09	Real-time PCR for CVI988 vaccine, HVT vaccine, MDV-1 field strains, MDV- 2 field strains	8	8
DENMARK	2024-10-21	Real-time PCR for HVT vaccine	10	0
DENMARK	2024-12-04	Real-time PCR for HVT vaccine	12	0
DENMARK	2024-12-11	Real-time PCR for HVT vaccine	8	0
UKRAINE	2024-12-11	Real-time PCR for HVT vaccine	4	4

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

No

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
The consequences of transmissible vaccines on disease ecology and pathogen evolution: Marek's disease virus as a case study	5 years	To use a Marek's disease virus vaccine-challenge model to quantify the consequences of transmissible vaccine use	Pennsylvania State University	UNITED STATES OF AMERICA
Examining the molecular diversity of MDV field isolates	5 years	Epidemiological investigation, pathogenicity analysis, and evaluation of vaccine protection against the MDV isolates from MD- vaccinated poultry farms	Henan academy of Agricultural Science	CHINA (PEOPLE'S REP. OF)
Exploring the potential of MDV-2 as a vaccine vector	4 years	To study if MDV-2 infection is a good candidate for vectored	MSD Animal Health	THE NETHERLANDS

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vaccines against MD and	
other avian viral diseases	

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

No

TOR6: EPIZOOLOGICAL DATA

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

Yes

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

We have an ongoing project to sequence the meq gene (closely linked to virulence of Marek's disease virus) of MDV isolated from samples submitted from UK backyard chicken flocks and commercial chicken flocks, to investigate the phylogeny, pathology, and distribution of these viruses.

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

No

16. What method of dissemination of information is most often used by your laboratory? (Indicate in the appropriate box the number by category and list the details in the box)

a) Articles published in peer-reviewed journals:

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Ortigas-Vasquez A, Pandey U, Renner DW, Bowen CD, Baigent SJ, Dunn J, Cheng H, Yao Y, Read AF, Nair V, Kennedy DA, Szpara ML. Comparative analysis of multiple consensus genomes of the same strain of Marek's disease virus reveals intrastrain variation. Virus Evol. 2024 Jun 21;10(1):veae047. doi: 10.1093/ve/veae047. eCollection 2024.

Teng M, Luo J, Zhang Y, Reddy VRAP, Samuel P, Yao Y, Nair V. Viral miRNA delivered by exosomes from Marek's disease virus-transformed lymphoma cell line exerts regulatory function in internalized primary chicken embryo fibroblast cells. Tumour Virus Res. 2024 Dec; 18:200286. doi: 10.1016/j.tvr.2024.200286. Epub 2024 Jun 22.

Zhu ZJ, Teng M, Liu Y, Chen FJ, Yao Y, Li EZ, Luo J. Immune escape of avian oncogenic Marek's disease herpesvirus and antagonistic host immune responses. NPJ Vaccines. 2024 Jun 15;9(1):109. doi: 10.1038/s41541-024-00905-0.

Xu H, Xu X, He H, Shao H, Yao Y, Qin A, Qian K. Regulation of Wnt/β-catenin signaling by Marek's disease virus in vitro and in vivo. Front Microbiol. 2024 Apr 3;15:1388862. doi: 10.3389/fmicb.2024.1388862. eCollection 2024.

b) International conferences:

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Professor Venugopal Nair has delivered the following talks:

1. Invited talk on "Innovations in control of avian viral diseases" at the International Symposium on Animal Virus, Vaccines and Immunity AVVI 2024 at Siksha O Anusandhan, Bhubaneswar, Odisha, India 9-11 February 2024.



2. Invited talk on "Challenges in the control of infectious animal diseases including zoonosis" at Anhui Agricultural University, Hefei, China 16 November 2024.

3. Invited talk on "Animal Health Research in the 21st Century: Challenges & Opportunities" at the International Symposium on Important Animal Diseases and Zoonoses, Yangzhou, China 19 November 2024.

4. Invited talk at 7th UK-China CERAD Symposium on "Recent advances in avian disease research", Binzhou, China, 20-21 November, Title: Animal Health Research in the 21st Century: Challenges & Opportunities.

Dr. Yongxiu Yao has given the following talks:

1. Talk on "Marek's Disease Virus Reference Laboratory at the Pirbright Institute" at 14th International Symposium on Marek's Disease and Avian Herpesviruses, St. Louis, MO, USA 12-14 July 2024.

2. Talk on "Identification of host factors critical for the proliferation of MDV transformed cell line using CRISPR-Cas9 screen" at 14th International Symposium on Marek's Disease and Avian Herpesviruses, St. Louis, MO, USA 12-14 July 2024.

3. Invited talk at 7th UK-China CERAD Symposium on "Recent advances in avian disease research", Binzhou, China, 20-21 November, Title: CRISPR/Cas9 gene editing: Powerful tool to study virus-host interactions.

4. Invited talk at the Yazhouwan National Laboratory, Sanya, China, 12th November 2024. Title: CRISPR/Cas9 gene editing: Powerful tool to study virus-host interactions & vaccine development.

5. Invited talk at the International Veterinary Vaccinology Network Conference 2024, Ho Chi Minh City, Vietnam 9-10 November 2024. Title: Genome editing of avian herpesviruses for recombinant vaccine development.

Dr. Yaoyao Zhang has given the following presentations:

1. Talk on "Genome editing of Herpesvirus of turkeys for recombinant vaccine development" at 14th International Symposium on Marek's Disease and Avian Herpesviruses, St. Louis, MO, USA 12-14 July 2024.

2. Poster presentation on "An efficient approach to develop recombinant HVT vector vaccines" at the International Veterinary Vaccinology Network Conference 2024, Ho Chi Minh City, Vietnam 9-10 November 2024.

3. Talk on "Gene editing in Marek's disease virus-transformed cell lines using CRISPR/Cas9 system" at the International Symposium on Important Animal Diseases and Zoonoses, Yangzhou, China 19 November 2024.

4. Invited talk at 7th UK-China CERAD Symposium on "Recent advances in avian disease research", Binzhou, China, 20-21 November, Title: Efficient approaches to develop recombinant HVT vectored vaccines.

5. Invited talk at Huazhong Agricultural University, China, 20 December 2024. Title: Application of CRISPR/Cas9 system in MDVtransformed cell lines and recombinant HVT vectored vaccine development.

The following people have also presented at 14th International Symposium on Marek's Disease and Avian Herpesviruses, St. Louis, MO, USA 12-14 July 2024:

1. Dr. Soumendu Chakravarti talked on "Effects of co-infections on Marek's disease in UK poultry farms and development of novel rapid diagnostic strategies".

2. Miss Sophie Cutts talked on "Characterising the activity of the Marek's disease virus virion host shutoff protein".

3. Poster presentation by Dr. Liping Liu on "Generation of recombinant HVT vaccines expressing glycoprotein B (gB) of MDV-1".

4. Poster presentation by Dr. Vishwanatha R. A. P. Reddy on "Optimising in ovo Herpesvirus of turkey (HVT) - vectored vaccines: Defining the role of the HVT vNr-13 protein in late-stage embryonic tissues".

c) National conferences:

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Dr. Susan Baigent has given the following presentation:

1. As a guest lecturer, Susan gave an online lecture to Animal Biotechnology MSc course students, University of Nottingham on "Development and commercialisation of molecular tests for Marek's disease", February 2024

2. Invited talk on "Marek's disease: diagnostics and research" at UK Animal and Plant Health Agency Avian Science Day, Alderley Edge, Cheshire, UK, 13 November 2024.



Dr. Soumendu Chakravarti has given a talk on "Effects of co-infections on Marek's disease in UK poultry farms and development of novel rapid diagnostic strategies" at British Society of Animal Sciences (BSAS)-2024, Belfast, Northern Ireland, 10 April 2024. Miss Sophie Cutts has given a talk on "Characterising the activity of the Marek's disease virus virion host shutoff protein" at Microbiology Society Annual Conference, Edinburgh, UK 8-11 April 2024.

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

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	Certificate issued by UK accreditation service	Pirbright UKAS Certificate.pdf

19. Is your quality management system accredited?

Yes

Test for which your laboratory is accredited	Accreditation body
Real-time PCR testing of chicken organs, feathers, and poultry dust	
to detect Marek's disease virus (vMDV), MDV-2, and vaccine strains	United Kingdom Accreditation Service (UKAS) 17025
CVI988 and HVT	

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

Yes

MDV (including MDV serotypes 1, 2 and HVT) causes diseases in poultry species such as chickens, turkey, and quails. The viruses are exclusive pathogens of avian species and hence are unlikely to infect and cause any harm to humans. Also, no known risks to the environment have been associated with these viruses. However, when samples are prepared for qPCR and culture of the virus, all work is strictly performed with the appropriate PPE (nitrile gloves and lab coat) and in a containment level 2 environment solely for sample protection and for sterility of cultures in line with group practices for culture of avian herpesviruses.

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?

TOR10: NETWORK WITH WOAH REFERENCE LABORATORIES

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

Not applicable (only WOAH Reference Laboratory designated for the disease

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

Not applicable (only WOAH Reference Laboratory designated for the disease

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

Not applicable (Only WOAH Reference Laboratory designated for the disease) We are the only WOAH Reference Laboratory designated for the disease.

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

Not applicable (only WOAH Reference Laboratory designated for the disease

TOR11: OTHER INTERLABORATORY PROFICIENCY TESTING

27. Did your laboratory organise or participate in inter-laboratory proficiency tests with laboratories other than WOAH Reference Laboratories for the same pathogen during the past 2 years?

No

We have recently identified another laboratory (CESAC Centre de Sanitat Avícola de Catalunya i Aragó, Spain) which is using the vMDV real-time PCR and CVI988 real-time PCR which we developed (although not accredited for these tests). After completing the due diligences, CESAC could be considered to partake in interlaboratory comparison testing.

Currently, as part of the ongoing discussion on this topic with our accreditation body, we test samples for a previous collaborator of known provenance (EQA).

TOR12: EXPERT CONSULTANTS

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

No

29. Additional comments regarding your report:

Yes

Unlike high consequential animal diseases with epidemic/pandemic potential, Marek's disease is endemic in most member countries. As it is not a notifiable disease in these countries, testing the samples for diagnosis may be seen as less of a priority and hence large numbers of samples are not submitted to the MDVRL from many countries.

However, MDVRL activities increased significantly during 2023 and 2024, compared with previous years. In addition, we worked on developing a real-time PCR specific for Prevexxion vaccine which is now undergoing validation as an ISO/IEC 17025-accredited test. We continue to provide technical advice to queries, diagnostic support and confirmation diagnoses from other member countries, and supply standard reference reagents upon request. Other activities include collecting epidemiological data relevant to international disease control, participation in international scientific studies in collaboration with WOAH members, and dissemination of information by publishing in peer-reviewed journals and presenting at international meetings. Most of the activities are carried out using the funding from the Pirbright Institute MDVRL or from charges for the tests, as we do not receive any funding support from the WOAH or local



government.

MDVRL is open to receive requests to deliver training to other members but has not yet been successful on this. We are currently having discussions with Zoetis Egypt to provide a 2.5-day visit to MDVRL for hands-on training in DNA preparation, real-time PCR and virus isolation for 3 people in 2025.

Working with Pirbright's training team, we prepared a Marek's disease e-learning course, which can be accessed via this link: https://www.pirbright.ac.uk/engage-with-us/training-pirbright/science-courses/mareks-disease-md-elearning

Furthermore, although there has been no successful in-person training conducted, we have been actively providing technical advice and responding to queries from other WOAH member countries on MD testing, disease control, and vaccination by emails, video calls, phone calls, and during our visits to other countries.

As MDVRL at Pirbright is the only accredited MDV reference laboratory, there is no other MDV reference laboratory to network with for inter-laboratory proficiency testing. We have recently identified another laboratory (CESAC Centre de Sanitat Avícola de Catalunya i Aragó, Spain) which is using the vMDV real-time PCR and CVI988 real-time PCR which we developed (although not accredited for these tests). After completing the due diligences, CESAC could be considered to partake in interlaboratory comparison testing.

Currently, as part of the ongoing discussion on this topic with our accreditation body, we test samples for a previous collaborator of known provenance (EQA).