Workshop on *Laboratory capacity to diagnose equine diseases* in Asia and Pacific

Introduction to the WOAH „six diseases“ and the HHP concept

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World Organisation for Animal Health
Founded as OIE

Organisation mondiale de la santé animale
Fondée en tant qu'OIE

Organización Mundial de Sanidad Animal
Fundada como OIE

Regional Workshop, Tokyo, 17 – 18 September 2024

The HHP framework in brief: Rationale

- A certain number of sport horses are under close veterinary supervision, governed by FEI[1] and IFHA[2] rules
- They present a low health risk
- They are identified and traceable
- Their welfare is essential to their capacity to perform
- They enter countries on a temporary basis for competition purpose only, **not for breeding**

[1]FEI = Federation Equestre Internationale [2]IFHA = International Federation of Horse racing

They can be defined as a

SUB-POPULATION

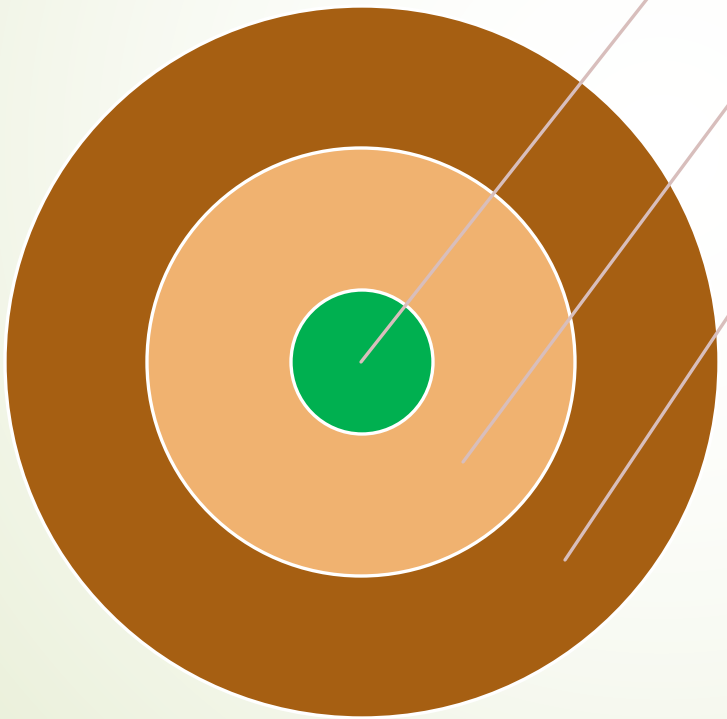
of the global equine population

They are included in the OIE Code chapter 4.17 as

HHP horse

(High health, high performance horse)

Sub-population



"High health, high performance" (HHP) sub-population

Sport horses

Other horses

Perceived challenges to travel internationally

- Application of excessive, inconsistent sanitary regulations
- Differing approaches to quarantine, laboratory testing, certification requirements
- Lack of knowledge/skills/interest/low priority for Veterinary Services
- Few countries with national regulations for **temporary** importation

Collaboration with the industry

Key partners:

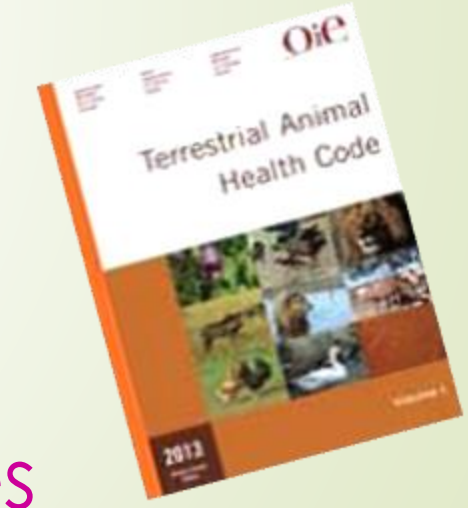
International Horse Sports Confederation (IHSC)

(formed by FEI and IFHA in 2013)

- MoU between IHSC and WOAH
- Engagement in Public-Private-Partnership to address movement constraints at **global** level

HHP framework for the facilitation of International Competition Horse Movements

Based on



OIE Standards and approaches

OIE standards and principles

- The OIE Terrestrial Animal Health Code lists 11 equine diseases and 6 multiple species diseases
- Members have reporting obligations!
  - Zoning, Disease Free Zones, and Compartmentalisation are defined in the Code
  - Animal identification and traceability described in the Code
  - Quality of Veterinary Services and the PVS Framework

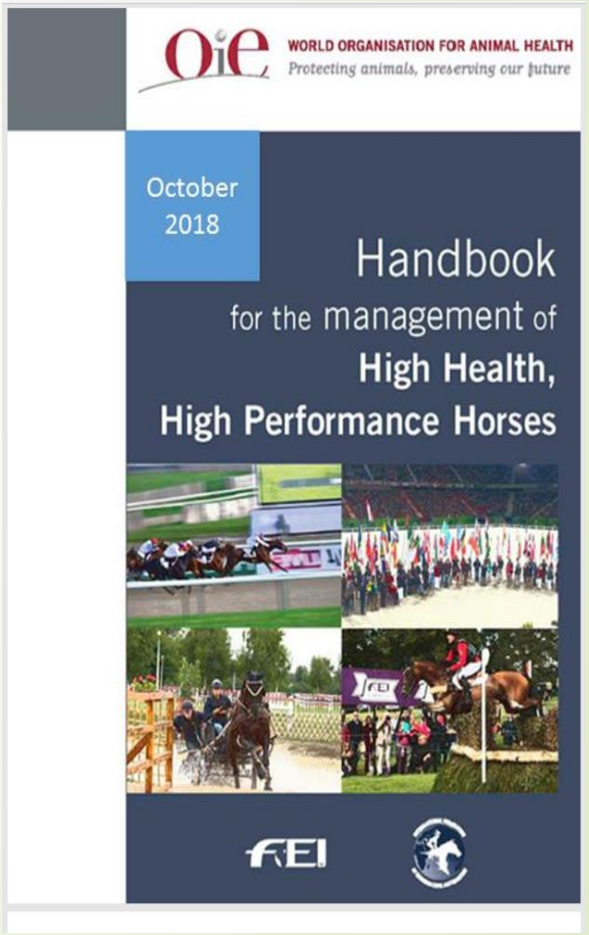
HHP sub-population complies with these principles

Based on 4 pillars:

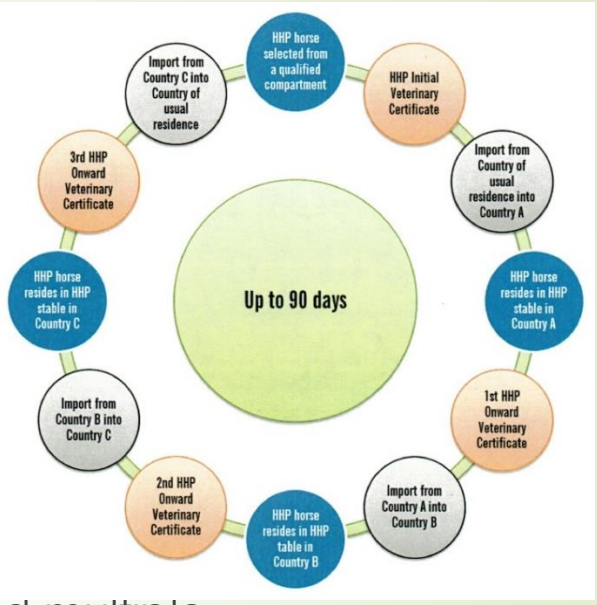
1. High Health status: vaccinations, tests, quarantine, HHP health certification
2. Performance
3. Identification and traceability
4. Biosecurity

Progress in implementation of HHP since its inception

- Update of the "HHP Handbook" in December 2018 – containing all elements of the step-wise approach to establish HHP framework at
  - Stable
  - Horse
  - Travel
  - Event



Recap of stepwise approach



1. **Stable**: qualification as compartment
2. **Horse(s)**: qualification as HHP horse
3. **Travel and venue(s)**
   - HHP certificate(s)
   - Travel up to 90 days with multiple destinations and multiple certificates
4. **Return to home base**

Single use strategy:
Compartment not maintained

Multiple use strategy:
Compartment was maintained

1. **Qualification of stable (compartment)**

- Health status of the country
  - Good reporting record of equine diseases to OIE
- Establishment
  - Must be registered with national Federation
  - Qualification period of 90 days under RV supervision
  - Special regulations for introduction of new horses (during qualification and maintenance of compartment)
  - Approval by official veterinarian
  - Once qualified – register in HHP database

2. Horse qualification as HHP

- Under regular supervision during stable qualification by RV
- Depending on country health status, required tests and vaccinations
- Once qualified, registered as HHP horse on HHP database

3. Travel and venues

- Issue of the initial HHP Health Certificate
- Observe biosecurity during lay-overs, travel and venue(s)
  - Must be registered on HHP database
  - Must have HHP biosecurity standards
  - EDFZ if not EU approved third country
- Issue of multiple HHP Health Certificates after each event

4. Return to home base

- Return after maximum 90 days continuous travel
- If new cycle of travel is desired and compartment was maintained:
  - 14 days residence in compartment if:
    - Country has known health status, no glanders for 3 yrs, no VEE for 2 years
  - 21 days residence in compartment if:
    - Country not known to be free of VEE for 2 yrs
  - 30 days residence in compartment if:
    - Country not know to be free of glanders for 3 yrs

- HHP framework has since its inception been further developed continuously
- Is in line with WOAH standards and largely fulfils the requirements of the new EU Animal health law for the "conditions of entry into the Union"
- The database, a necessity for HHP to work, has made major progress
- Regions are encouraged to trial the HHP framework for intra-regional movement

The six diseases

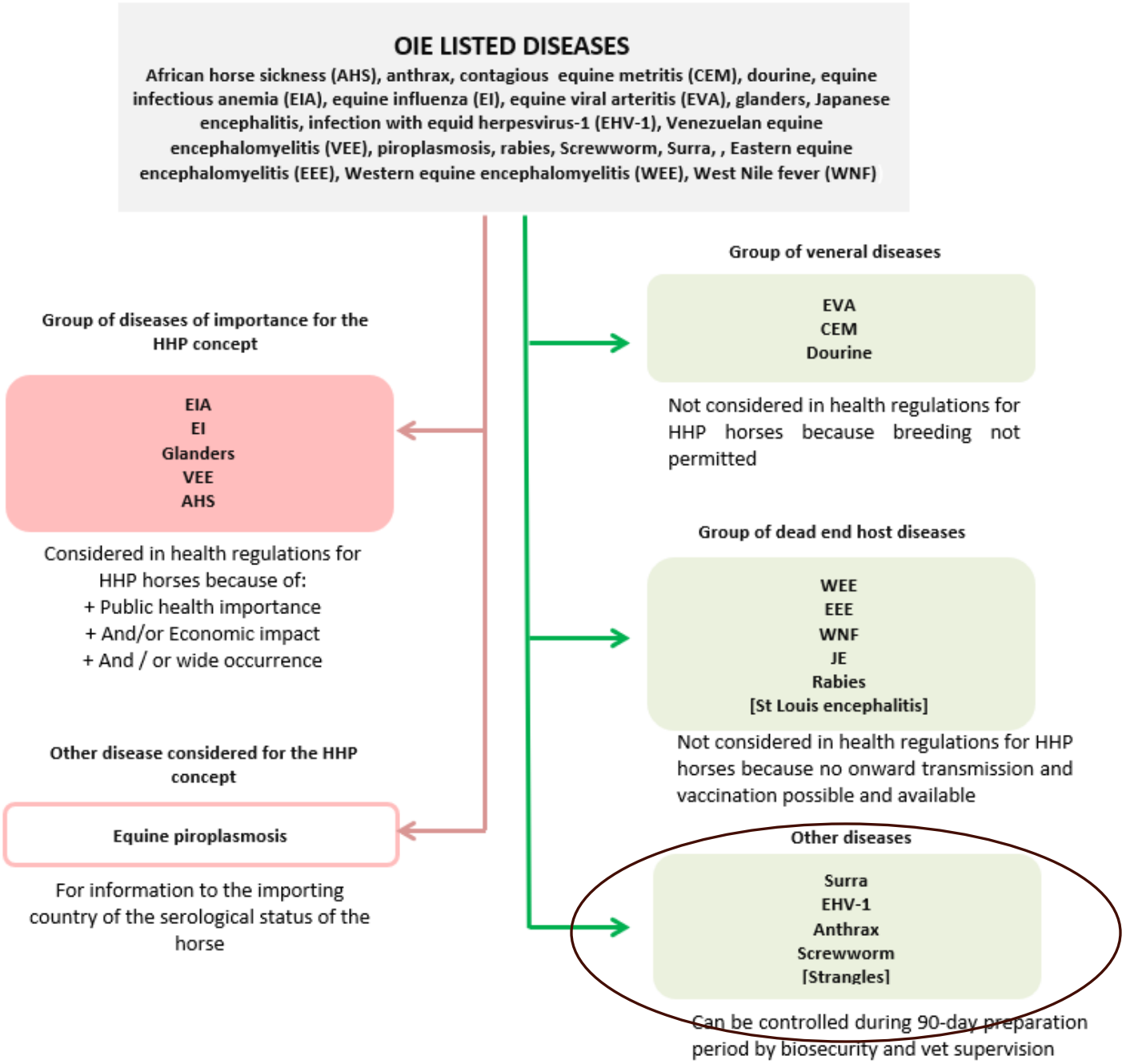
- A HHP health certificate was developed, can be found as annex to the HHP handbook
- This health certificate was commented on by WOAH members in 2015 and passed through SCAD in September 2015.
- It is based on the risk assessment that WOAH carried out globally and in which it identified six diseases that need to be regulated in a health certificate because they cannot be detected or mitigated by health management and biosecurity

Dominguez M., Munstermann S., Murray G. & Timoney P. (2015)
High-health, high-performance' horses: risk mitigation strategies for OIE-listed diseases.
Rev. Sci. Tech. Off. Int. Epiz., **34** (3), 837 - 848

Risk assessment based categorisation of WOAH listed diseases of importance

Only the „6 diseases“ are specifically regulated in the proposed Certificate

If no HHP preparation period, measures for this group of diseases need to be included



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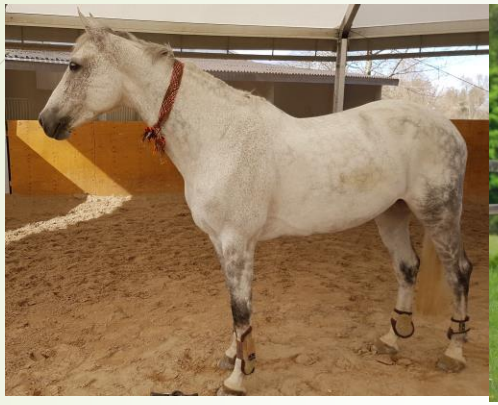






The WOAH project on *Facilitation of international horse movement* in Asia and Pacific

Proposed harmonised health certificate for competition horses

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Background

- HHP concept was developed to facilitate movement of competition horses worldwide (TAHC chapter 4.17)
  - To avoid the need for pre-export and post-arrival quarantines
- A HHP health certificate was developed, can be found as annex to the HHP handbook
- This health certificate was commented on by WOAH members in 2015 and passed through SCAD in September 2015.
- However, as the HHP concept, particularly the registration of HHP horses and premises, have not (yet) been fully adopted by the equestrian sports organisations and the EU, where most competition horses from this region are based, the certificate cannot be used as is
- Rather than to use the harmonised certificate worldwide, we now propose to develop "regional" certificates through initiatives in Asia and Latin America

Background *cont*.

- The harmonised Certificate is based on the HHP principles
- Was intended initially for several HHP movements within 90 days in the Region, but based on countries' feedback, we changed to a "single use" certificate
- Major modifications to the HHP Certificate in the HHP Handbook:
  - Replaced the „HHP horse" by „registered horse" , using the definition as given in the WOAH glossary,
  - Every competition horse will have an affiliation with a national registering body
  - Establishment must be under veterinary supervision to verify the claim of "free from listed diseases"
  - VEE was replaced by Surra, as VEE does not exist in the Region

Background *cont.*

- The general prinicples can be applied:
  - Competition horses have a high health status
  - They are under veterinary supervision
  - Biosecurity measures are applied in their establishments
  - They are registered with the national or international Federation /racing association
  - They have a passport and are individually identified
  - They enter the country temporarily, not a permanent import
  - While competing, they are generally not breeding animals
- We can put these general conditions as preamble in the health certificate
- We regulate only the six diseases that are important for competition horses because they cannot be detected or mitigated by health management and biosecurity
- We leave room for bilateral additional conditions to be added

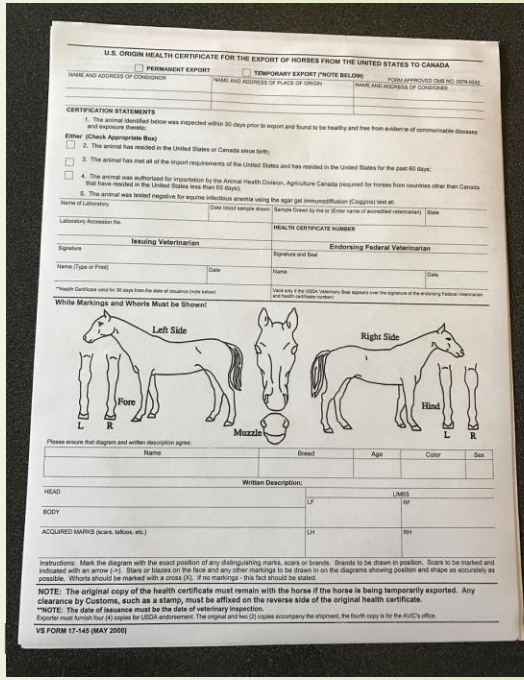
Objective of the proposed harmonised certificate

- To be used for movement within the region
- To incentivise to organise intra-regional competitions with facilitated movement for participating horses
- For competition horses resident in the region

- If agreed, it could be tested at a regional event
- If works well, could be upscaled

Current health certificates

- We requested all countries participating in the WOAH project to send us their health certificates
  - They are usually for general permanent import of equines, not specific for competition horses or temporary import, except:
    - Into Thailand for Asian Championships
    - Into China for Asian Games 2023
    - Into Indonesia for Asian Games 2018
- There is a lot of divergence, but some common features
  - Country of origin should be free from AHS for 2 years
  - Establishment/holding should be free from most WOAH listed diseases
  - A definite residence period in the establishment free from specified (mostly 6 months)
  - Horses had no contact with premises that notified a disease or horses of a l status for a definite period (mostly 90 days)
  - Horses should be treated against endo- and ectoparasites before departure
  - Horses should be vaccinated against EI



U.S. ORIGIN HEALTH CERTIFICATE FOR THE EXPORT OF HORSES FROM THE UNITED STATES TO CANADA

PERMANENT EXPORT TEMPORARY EXPORT (*NOTE BELOW)

NAME AND ADDRESS OF CONSIGNOR | NAME AND ADDRESS OF PLACE OF ORIGIN | NAME AND ADDRESS OF CONSIGNEE

CERTIFICATION STATEMENTS

1. The animal identified below was inspected within 30 days prior to export and found to be healthy and free from evidence of communicable diseases and exposure thereto;

Either (Check Appropriate Box)

2. The animal has resided in the United States or Canada since birth;

3. The animal has met all of the import requirements of the United States and has resided in the United States for the past 60 days;

4. The animal was authorized for importation by the Animal Health Division, Agriculture Canada (required for horses from countries other than Canada that have resided in the United States less than 60 days);

5. The animal was tested negative for equine infectious anemia using the agar gel immunodiffusion (Coggins) test at:

Name of Laboratory | Date blood sample drawn | Sample Drawn by me or (Enter name of accredited veterinarian) | State

Laboratory Accession No. | HEALTH CERTIFICATE NUMBER

Issuing Veterinarian | Endorsing Federal Veterinarian

Signature | Signature and Seal

Name (Type or Print) | Date | Name | Date

**Health Certificate valid for 30 days from the date of issuance (note below)

Valid only if the USDA Veterinary Seal appears over the signature of the endorsing Federal Veterinarian and health certificate number)

White Markings and Whorls Must be Shown!

Please ensure that diagram and written description agree.

Name	Breed	Age	Color	Sex

Written Description:

HEAD		LMBS
BODY	LF	RF
ACQUIRED MARKS (scars, tattoos, etc.)	LH	RH

Instructions: Mark the diagram with the exact position of any distinguishing marks, scars or brands. Brands to be drawn in position. Scars to be marked and indicated with an arrow (→). Stars or blazes on the face and any other markings to be drawn in on the diagrams showing position and shape as accurately as possible. Whorls should be marked with a cross (X). If no markings - this fact should be stated.

NOTE: The original copy of the health certificate must remain with the horse if the horse is being temporarily exported. Any clearance by Customs, such as a stamp, must be affixed on the reverse side of the original health certificate.

**NOTE: The date of issuance must be the date of veterinary inspection.

Exporter must furnish four (4) copies for USDA endorsement. The original and two (2) copies accompany the shipment, the fourth copy is for the AVIC's office.

VS FORM 17-145 (MAY 2000)

Certificates received and studied

General requirements for import into	Specific requirements
India	Australia into New Caledonia
Sri Lanka	France into New Caledonia
Taiwan	New Zealand into New Caledonia
Thailand	India into Bhutan
	Argentina into Philippines
	Australia into Philippines
For specific sport events	Indonesia into Philippines
Into Thailand for Asian Championships	USA into Philippines
Into Indonesia for Asian Games 2018	Korea into Malaysia
Into Korea for Asan Games 2014	UK into Australia

Proposal

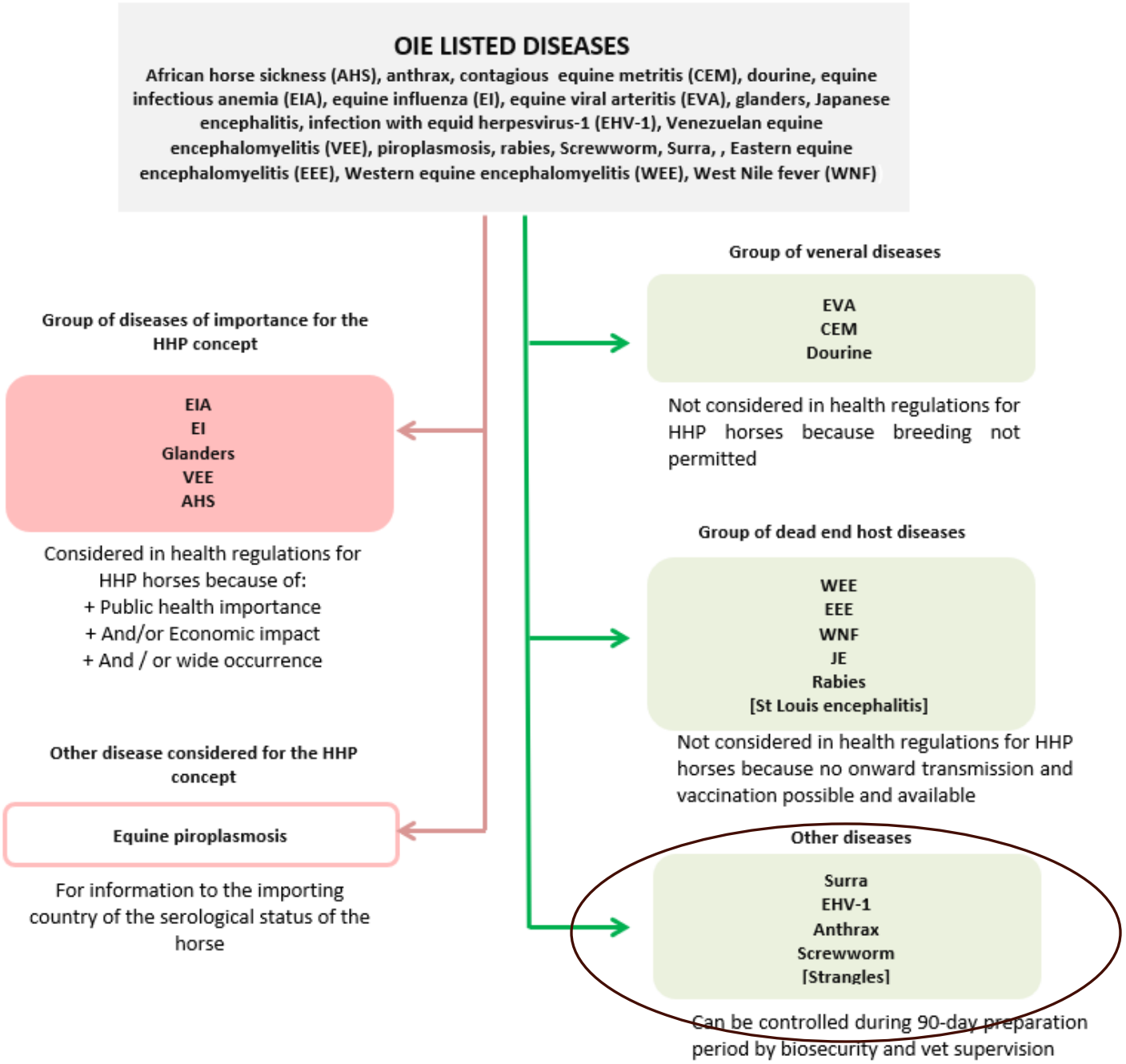
- A certificate that covers one single journey
  - Horse shall not remain in the country of the event, but return to country of permanent or temporary residence
  - Venue of competition has a high biosecurity standard (particularly vector protection and vector control)
  - In Part I it is stipulated that during the 90 day period before travel the horse was not used for breeding
  - „registered horse“ - registered with a national Federation or Racing Association
  - „registered premise“ - registered with a national Federation and under veterinary supervision

Risk assessment based categorisation of WOAH listed diseases of importance

Only the „6 diseases“ are specifically regulated in the proposed Certificate

For Asia – replace VEE with surra

If no HHP preparation period, measures for this group of diseases need to be included



How are the „6 diseases“ regulated

- **AHS**
  - „Country should be free from AHS for 2 years“ - AHS is amongst the diseases for official country freedom since 2015 – how many countries in the region have the WOAH status of country freedom? (12 out of 32)

    Proposal to include a condition for countries not officially free!
- **VEE**
  - VEE has never occured in the region, we therefore removed it - but put **Surra** instead
- **Glanders**
  - Country free for 3 years plus single CFT test
  - Establishment free for 12 mths, horse permanent resident for 30 days and double CFT test
  - New ELISA test currently under study by WOAH BSC, can eventually complement the CFT and shorten the residence period

Control measures

- **Piroplasmosis**
  - In line with the newly approved TAHC chapter 12.7.6 (temporary importation) NO test is required, but daily checks for ticks 30 days before departure, during transport and while at the venue (V.9)
- **EIA**
  - Test within 90 days before departure (Coggins)
- **EI**
  - Comes from a country free of EI and has been resident for 14 days OR
  - Is vaccinated against EI
- **Surra**
  - A TAHC chapter has been developed and been commented on by members, and is currently under study by specialist commissions – has a provision for temporary import (8.7.9) – one serological test 14 days prior to departure

Consultative process

Date	Activity	Round of revision	comment
February 24	Regional Workshop Thailand	Discussion of draft certificate	Comments by working groups
March 24	Comments were integrated: Revised certificate + Explanatory notes	1st	Country comments received by April 24
April 24	Comments from NZ, AUS, NC, Singapore, Malaysia, Thailand, Nepal, Bhutan were integrated: Revised certificate + Updated Explanatory Note	2nd	Country comments received by May 24
June 24	Three online meetings with participating countries	3rd	Final certificate with accompanying Explanatory Notes available

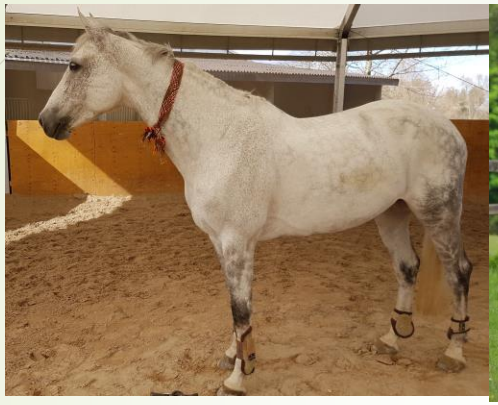
Outcome

- 13 / 16 countries participated actively in the consultation
- Some countries with a substantial horse sport industry did not participate
- 4 countries accepted the final version and would like to use it
- 9 cannot use it in ist current form for different reasons

?

- **Proposed way forward:**
  - Organise a test event and use this opportunity to address each countries specific issues

Thank you for your attention



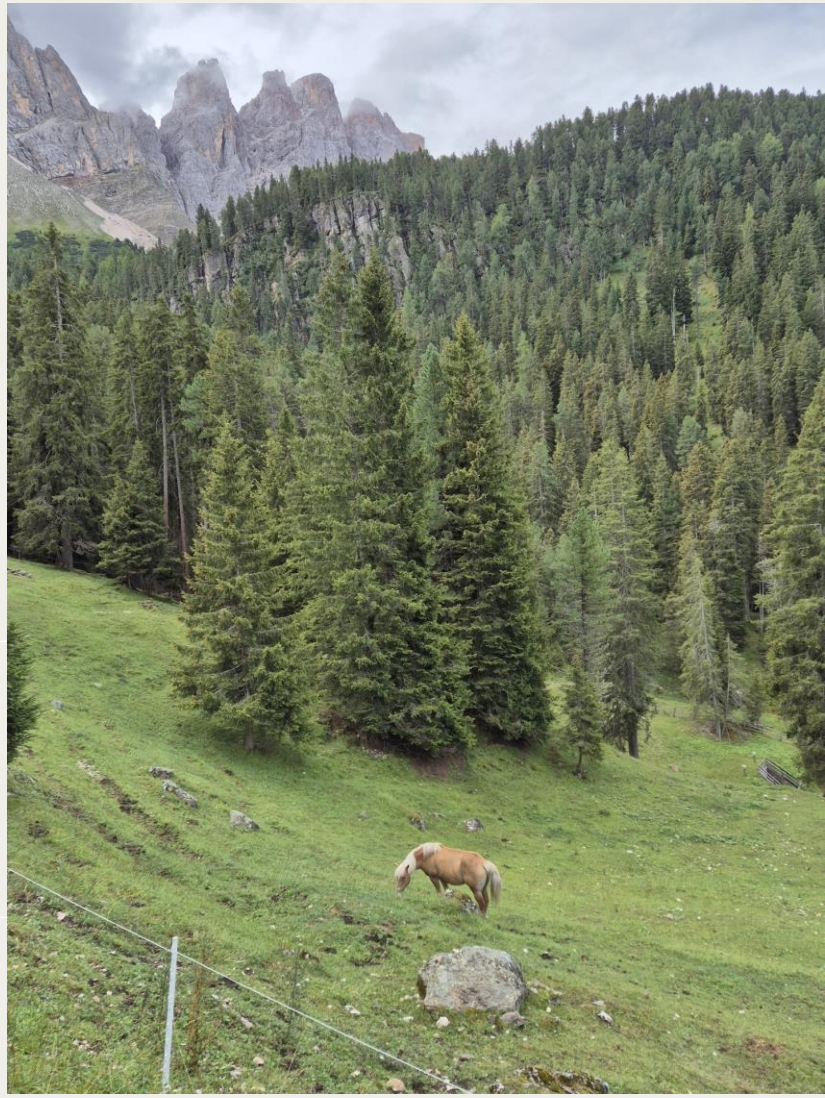





World Organisation for Animal Health | Organisation mondiale de la santé animale | Organización Mundial de Sanidad Animal

WOAH Regional Workshop on “Laboratory capacity to diagnose equine diseases” in Asia and the Pacific

17-18 September 2024- Tokyo, JAPAN

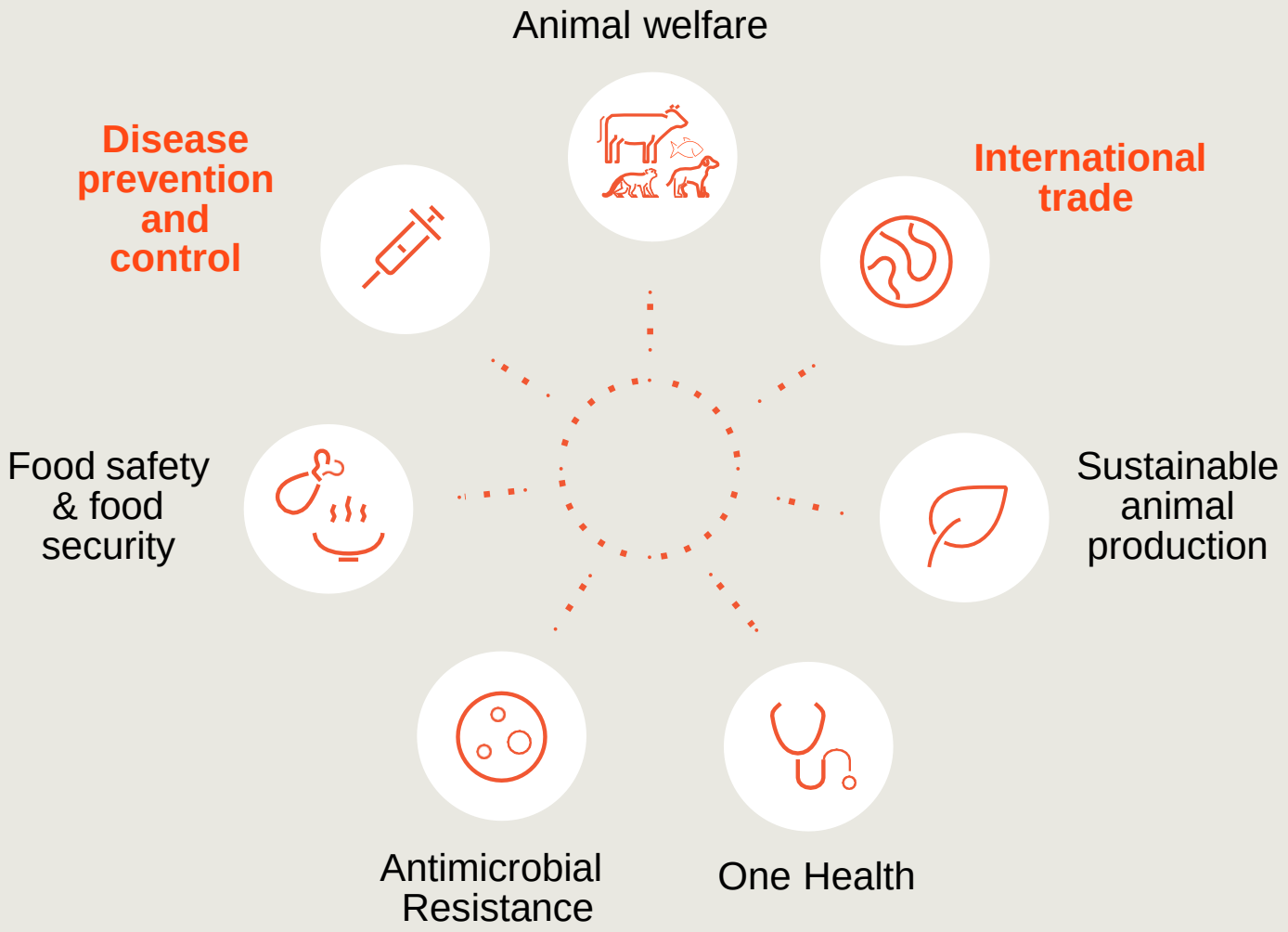


Terrestrial Animal Health Code: standards for “six diseases” and disease distribution (WAHIS)

Mauro Meske
Project Liason Officer WOAH-IHSC
Disease Status Department

Intoduction- WOAH: Who we are

WOAH's main working areas



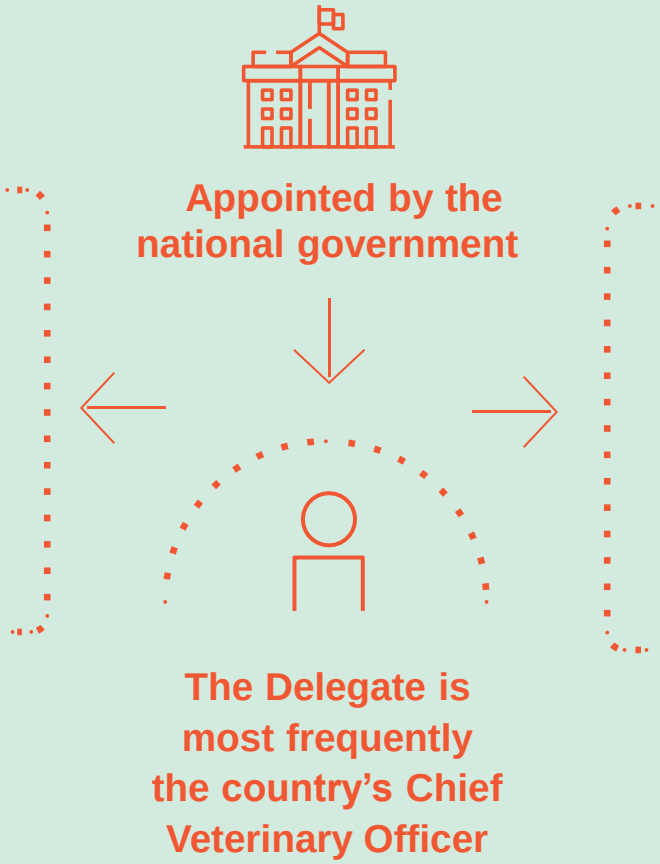
Introduction: WOAH's governance structure

The WOAH Delegate

National Representative to WOAH

Responsible for negotiating **international standards** on animal health

Convince his/her government to invest in **animal disease monitoring and prevention**



Appointed by the national government

The Delegate is most frequently the country's Chief Veterinary Officer

Is a **national representative** with **international status**

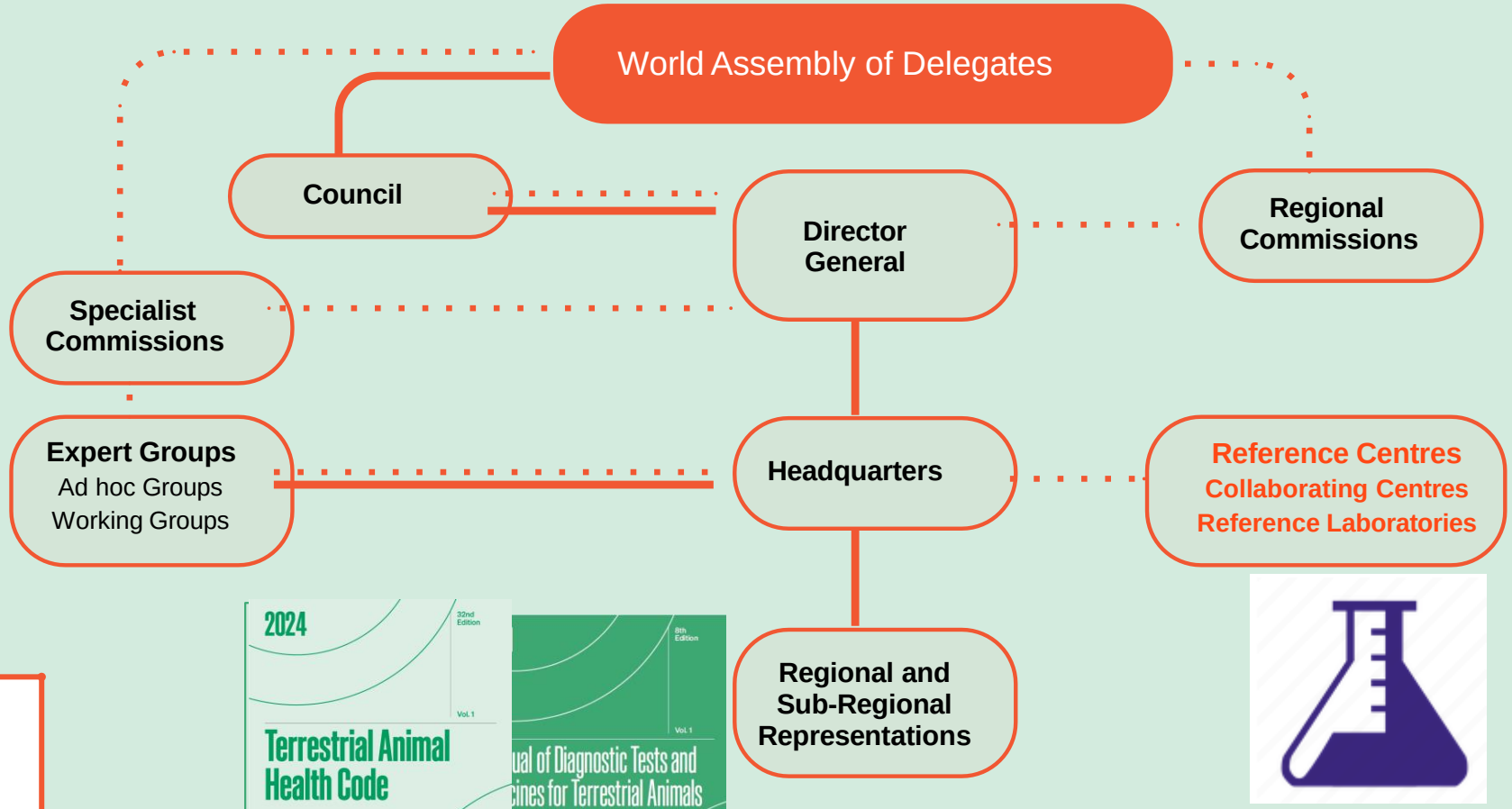
Notifies WOAH of the **national animal disease situation**

Introduction: WOAH's governance structure

World Assembly of Delegates

- Composed of all national Delegates
- Convenes at least once a year in May
- Adopts the standards published in the Codes and Manuals

1 Member = 1 Vote



WOAH international standards

WOAH establishes standards for the improvement of animal health and welfare and veterinary public health worldwide, including the prevention of disease spread through international trade of animals and animal products.

World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) recognises **WOAH** as the **reference international standard-setting organisation for animal health** and zoonoses.

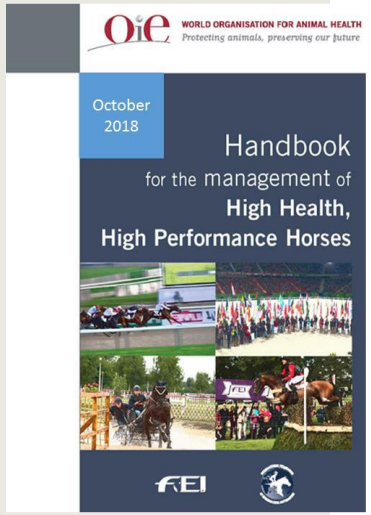
Include import sanitary measures, according to the commodity, their origin, the risk reduction measures applicable to each commodity

Veterinary Authorities should use these standards to set up measures to prevent the spread of significant transboundary diseases via international trade in animals and animal products while avoiding unjustified sanitary trade barriers

Members shall ensure that their sanitary measures are based on existing international standards or based on risk analyses conducted according to these standards.

RATIONALE FOR THE SELECTED 6 DISEASES

Risk assessment based categorisation of WOAH listed diseases of importance



OIE LISTED DISEASES

African horse sickness (AHS), anthrax, contagious equine metritis (CEM), dourine, equine infectious anemia (EIA), equine influenza (EI), equine viral arteritis (EVA), glanders, Japanese encephalitis, infection with equid herpesvirus-1 (EHV-1), Venezuelan equine encephalomyelitis (VEE), piroplasmosis, rabies, Screwworm, Surra, , Eastern equine encephalomyelitis (EEE), Western equine encephalomyelitis (WEE), West Nile fever (WNF)

Group of diseases of importance for the HHP concept

EIA
EI
Glanders
VEE
AHS

Considered in health regulations for HHP horses because of:
+ Public health importance
+ And/or Economic impact
+ And / or wide occurrence

Other disease considered for the HHP concept

Equine piroplasmosis

For information to the importing country of the serological status of the horse

Group of veneral diseases

EVA
CEM
Dourine

Not considered in health regulations for HHP horses because breeding not permitted

Group of dead end host diseases

WEE
EEE
WNF
JE
Rabies
[St Louis encephalitis]

Not considered in health regulations for HHP horses because no onward transmission and vaccination possible and available

Other diseases

Surra
EHV-1
Anthrax
Screwworm
[Strangles]

Can be controlled during 90-day preparation period by biosecurity and vet supervision

Only the 6 diseases are specifically regulated in the proposed HHP Certificate

VEE has never occured in the Asia and the Pacific Region. Therefore, it was removed from the Health Certificate for the Region and replaced it by **Surra**

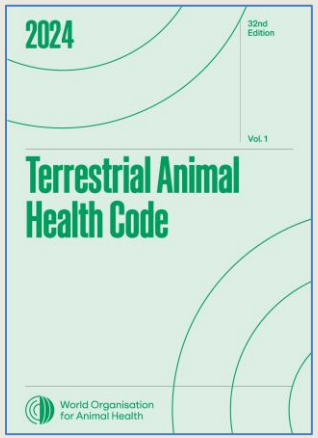
Revision of WOAH standards for the main diseases affecting horses

Equine Influenza *
Equine piroplasmosis *
Surra*
Contagious Equine Metritis *

Already reviewed and adopted in 2023- 2024

Under review (2024-2025):

- Dourine for potential adoption 2025 *
- African horse sickness -for potential adoption 2025
- Western, Eastern and Venezuelan equine encephalomyelitis & Japanese Encephalitis (2024-2025)



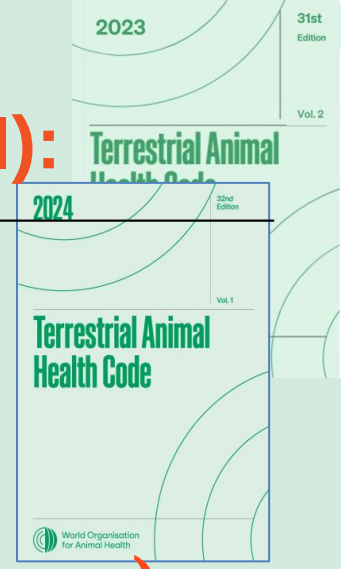
Glanders (last updated 2018)

* Provisions for temporary importation of horses

All Chapters: specific provisions for surveillance & country-zone freedom

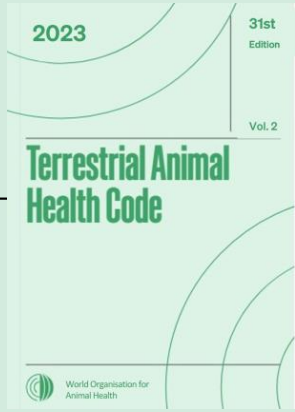
Plans to update the Code Chapter 5.12 with a Model passport for the international movement of competition horses- Digital passports (2025-2026)

Provisions in the newly drafted Chapters (EP, Surra, EI):



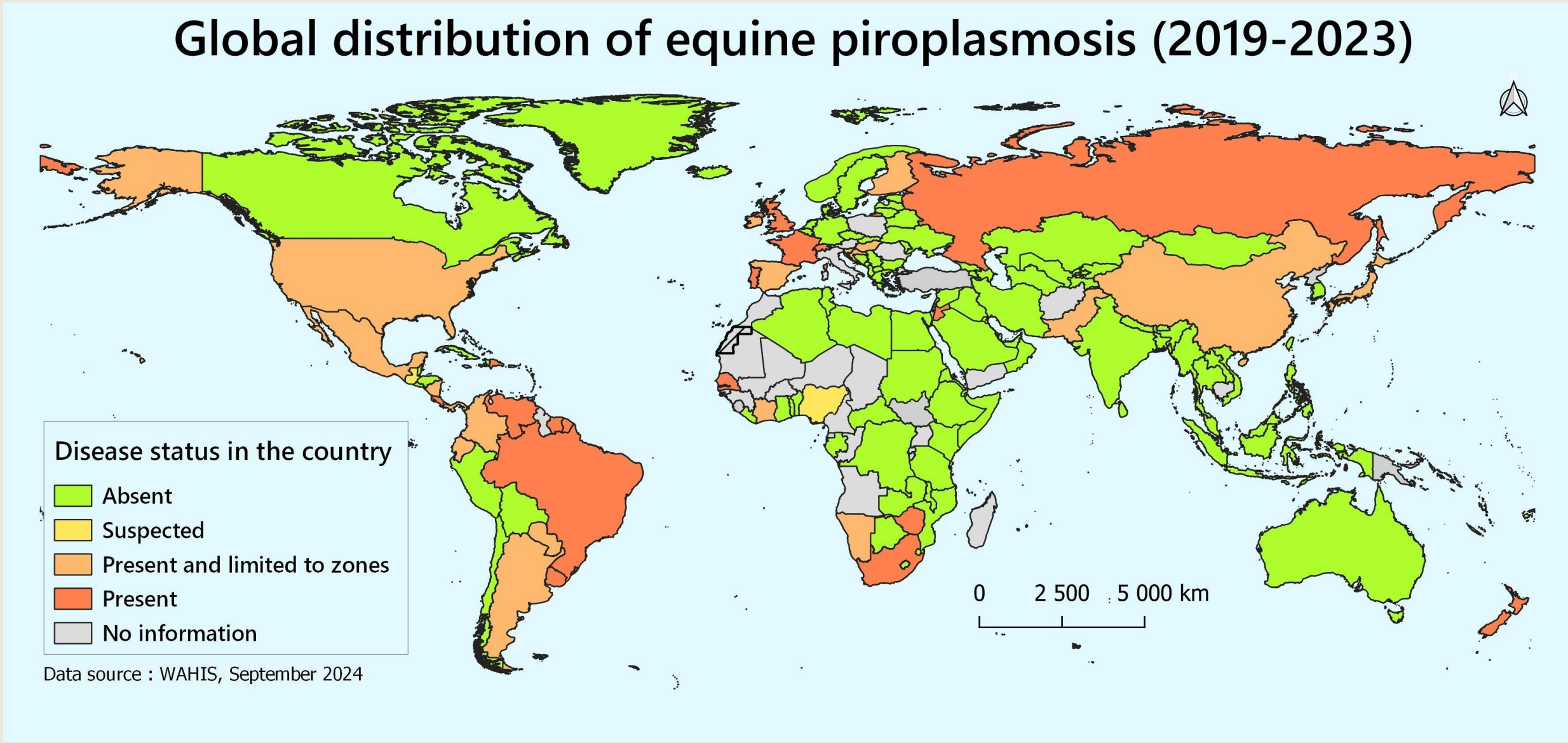
- **Case definitions** for each disease,
- List of **safe commodities**,
- **Free country, Zone (EI, EP) or compartment free (surra)** (with requisites for qualification, maintenance and recovery of freedom)
- Guidance on **Surveillance** (animal surveillance, vector surveillance-EP-)
- **Import provisions** for different commodities (live animals, animal products)
- Inclusion of provisions for **temporary importation** of competition horses

Chapter 12.7. Inf. with *Theileria Equi* and *Babesia Caballi* (Equine Piroplasmosis)

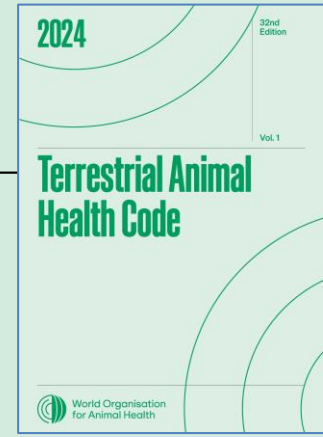


- **Recommendations for importation** of equids
- Recommendations for **temporary importation** of horses **ONLY for competition purposes and HHP horses**, lab test are not required but horses should be :
  - Accompanied by an **international veterinary certificate**, a passport and individual identification, belonging to **high health horse subpopulation** as defined in Chapter 4.17;
  - **Not subjected to any practice that may represent a risk of transmission** of infection with *T. equi and Babesia caballi*
  - **maintained free from ticks during the 30 days prior to shipment**, during **transport** and protected from ticks **during their stay** (and examined daily for the presence of ticks)
- Recommendations to protect equids from ticks
- **General principles of surveillance** (including clinical, serological, agent, and vector surveillance, and surveillance in high-risk areas)

DISEASE DISTRIBUTION - Data from the World Animal Health Information System (WAHIS)



Chapter 8.23. Surra (Inf. with *Trypanosoma Evansi*) 2024



Case definition

Country or zone free: Notifiable for at least the past 2 years + measures

to prevent the introduction of the infection (i.e., import of equids) and either:

1. Country historically free according to Art 1.4.6 (notifiability and early warning system in place for **10 years**; no cases for the last **25 years**; no infection in wildlife); or
2. Surveillance programme in place for the past two years (including active surveillance)

Compartments free

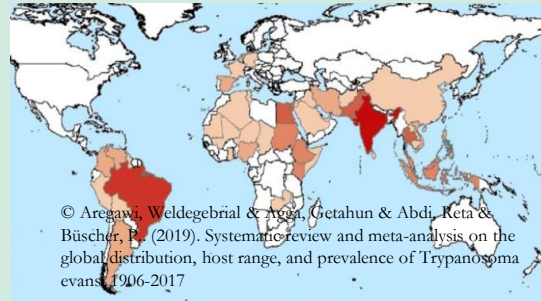
Recommendations for importation of susceptible animals from infected countries: no clinical signs the day of shipment +

kept since birth or for 6 months in a free country/ zone/ compartment) did not transit to through an infected zone or Subjected to lab test with negative results (2 antibody tests interval 30 days)

Recommendations for temporary importation of horses: (HHP+ vector protection+ 1 test)

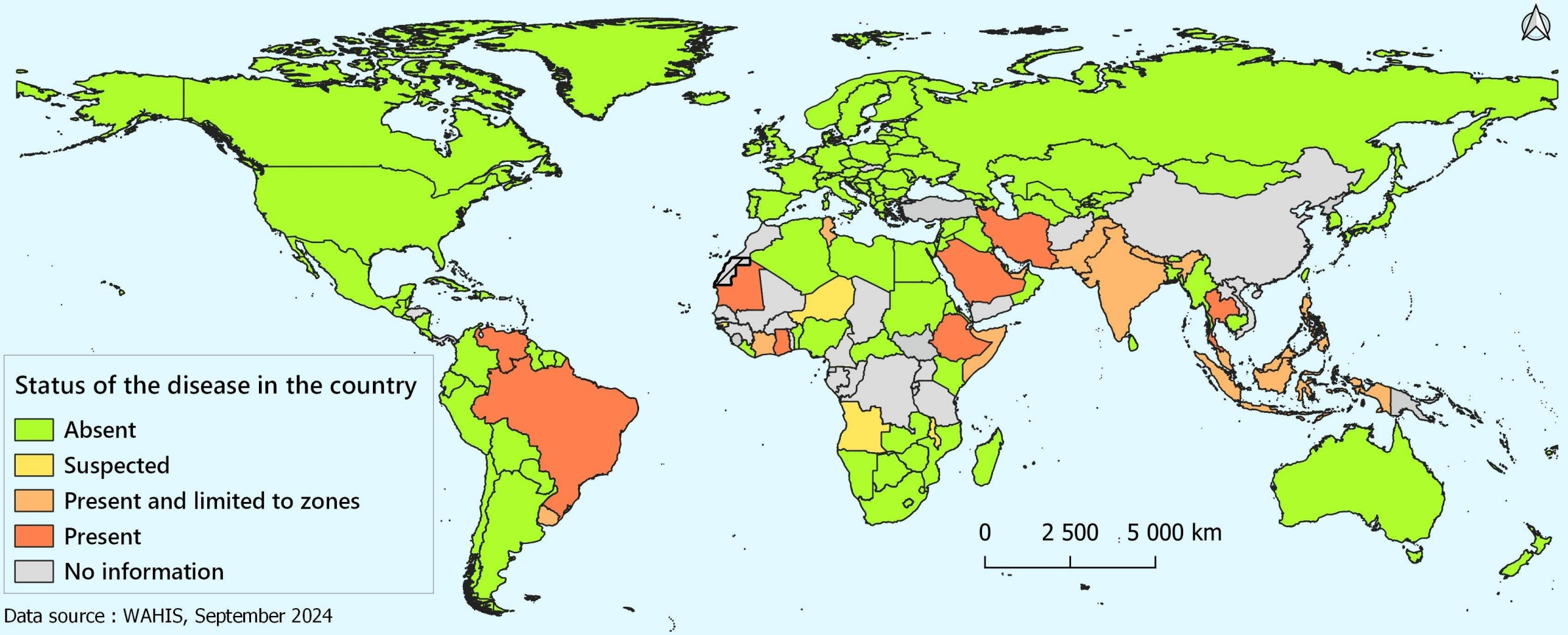
General conditions & Surveillance methods: Clinical and Pathological;

Serological; Agent identification; Sentinel animals; vector surveillance



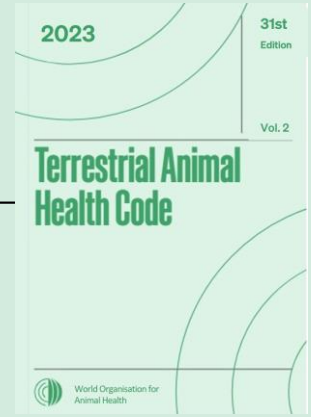
DISEASE DISTRIBUTION - Data from the World Animal Health Information System (WAHIS)

Global distribution of Surra (Trypanosoma evansi) (2019-2023)



Data source : WAHIS, September 2024

Chapter 12.6. Inf. with Equine Influenza Virus:



- **Recommendations for Temporary importation of horses**

  ONLY for horses and for competition purposes, not exceeding 90 days, lab test are not required but horses should be accompanied by:

  - **A passport** in accordance with Chapter 5.12. and individual identification or belonging to HHP horse subpopulation as defined in Chapter 4.17; AND
  - **An international veterinary certificate** attesting that horses either come from an **EI free country**/zone **OR** showed **no clinical signs of EI** in any premises horses had been resident for the 14 days prior to shipment **AND were vaccinated**
  - During their stay ensure that horses are kept separated from domestic and captive wild equids of a different EI health status through **appropriate biosecurity**

- **List of safe commodities**

- **Determination of the EI status:** Risk assessment+ notifiability+ EI awareness programmes+ surveillance in place to demonstrate the presence of infection in the absence of clinical signs

Chapter 12.6. Inf. with Equine Influenza Virus:


vaccines

Article

Evaluation of Current Equine Influenza Vaccination Protocols Prior to Shipment, Guided by OIE Standards

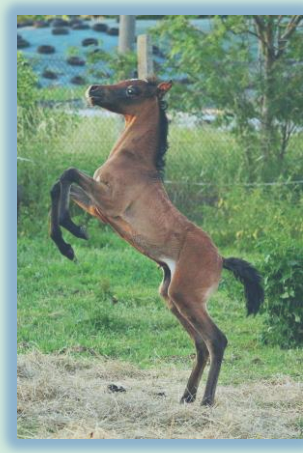
Country, zone or compartment free from Equine Influenza:

- infection with EIV is **notifiable** in the whole country;
- Evidence through an effective surveillance programme, implemented in accordance with principles in Chapter 1.4., that **no case of EI in the past two years;**

i. If **vaccination is practiced**: evidence through surveillance (serological + agent identification) of **absence of EIV circulation** in the population of domestic, captive wild, feral, and wild equids during the past 12 months;

ii. If **vaccination is not practised**: **serological surveillance** is sufficient;

- Apply appropriate **movement controls** to minimise the risk of introduction of EIV

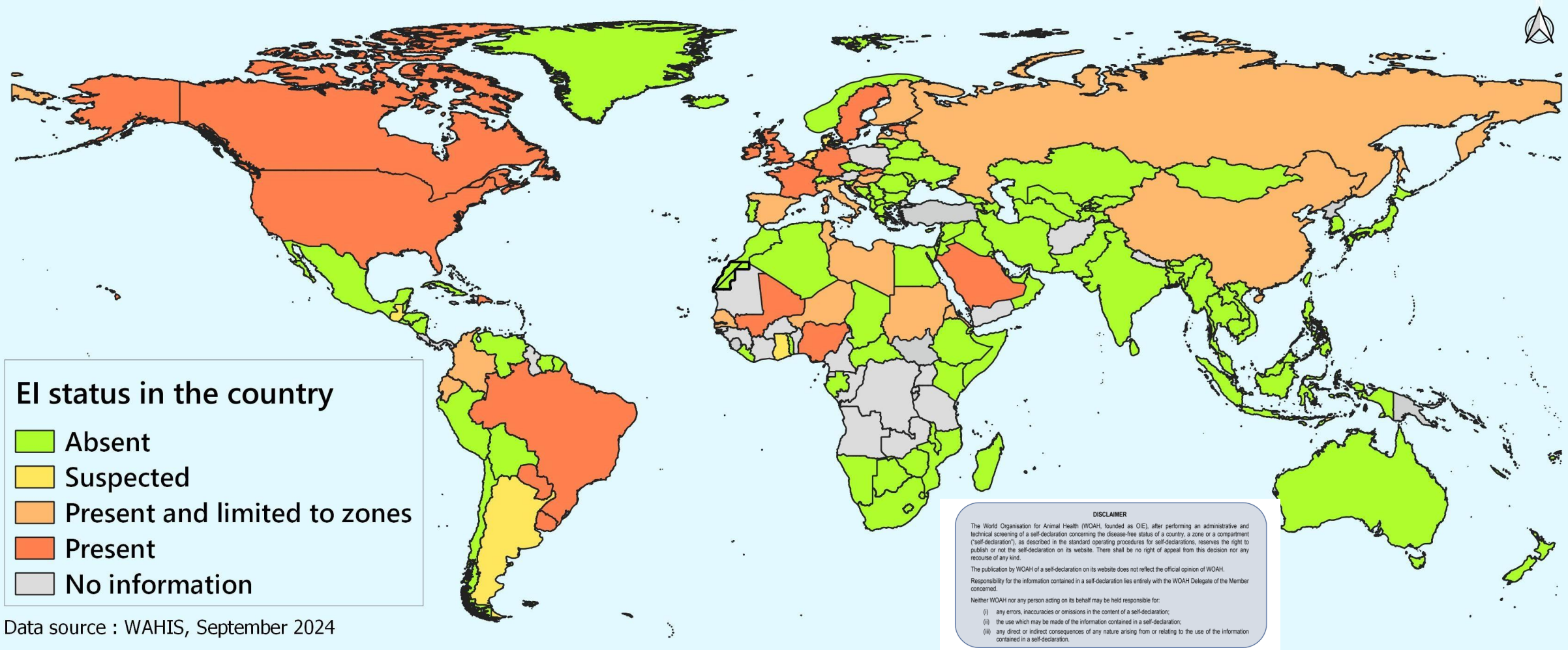


Recovery of Free Status

Vaccination protocols prior to shipment

DISEASE DISTRIBUTION - Data from the World Animal Health Information System (WAHIS)

Global distribution of equine influenza (2019-2023)



Data source : WAHIS, September 2024

DISCLAIMER

The World Organisation for Animal Health (WOAH, founded as OIE), after performing an administrative and technical screening of a self-declaration concerning the disease-free status of a country, a zone or a compartment ("self-declaration"), as described in the standard operating procedures for self-declarations, reserves the right to publish or not the self-declaration on its website. There shall be no right of appeal from this decision nor any recourse of any kind.

The publication by WOAH of a self-declaration on its website does not reflect the official opinion of WOAH.

Responsibility for the information contained in a self-declaration lies entirely with the WOAH Delegate of the Member concerned.

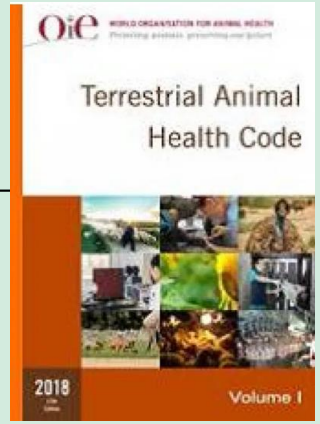
Neither WOAH nor any person acting on its behalf may be held responsible for:

(i) any errors, inaccuracies or omissions in the content of a self-declaration;
(ii) the use which may be made of the information contained in a self-declaration;
(iii) any direct or indirect consequences of any nature arising from or relating to the use of the information contained in a self-declaration.

Self-declaration of freedom from infection with Equine Influenza viruses (EI) in horses by Japan

Self-Declaration sent to the World Organisation for Animal Health (WOAH, founded as OIE) on 31 January 2024 by Dr OKITA Masatsugu, the Delegate of Japan to WOAH, Director of Animal Health Division, Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan. This self-declaration, initially established on 1 July 2009, is reiterated to reflect updates in the Terrestrial Code.

Chapter 12.10. Inf. with *Burkholderia mallei* (glanders):



Case definition

Country, zone free from glanders:

- Glanders is **notifiable** in the whole country for the past 3 years;
- Evidence through an effective surveillance programme, that **no case of glanders in the past 12 months;**
- Apply appropriate import of equids and germplasm to minimise the risk of introduction of *B. mallei*

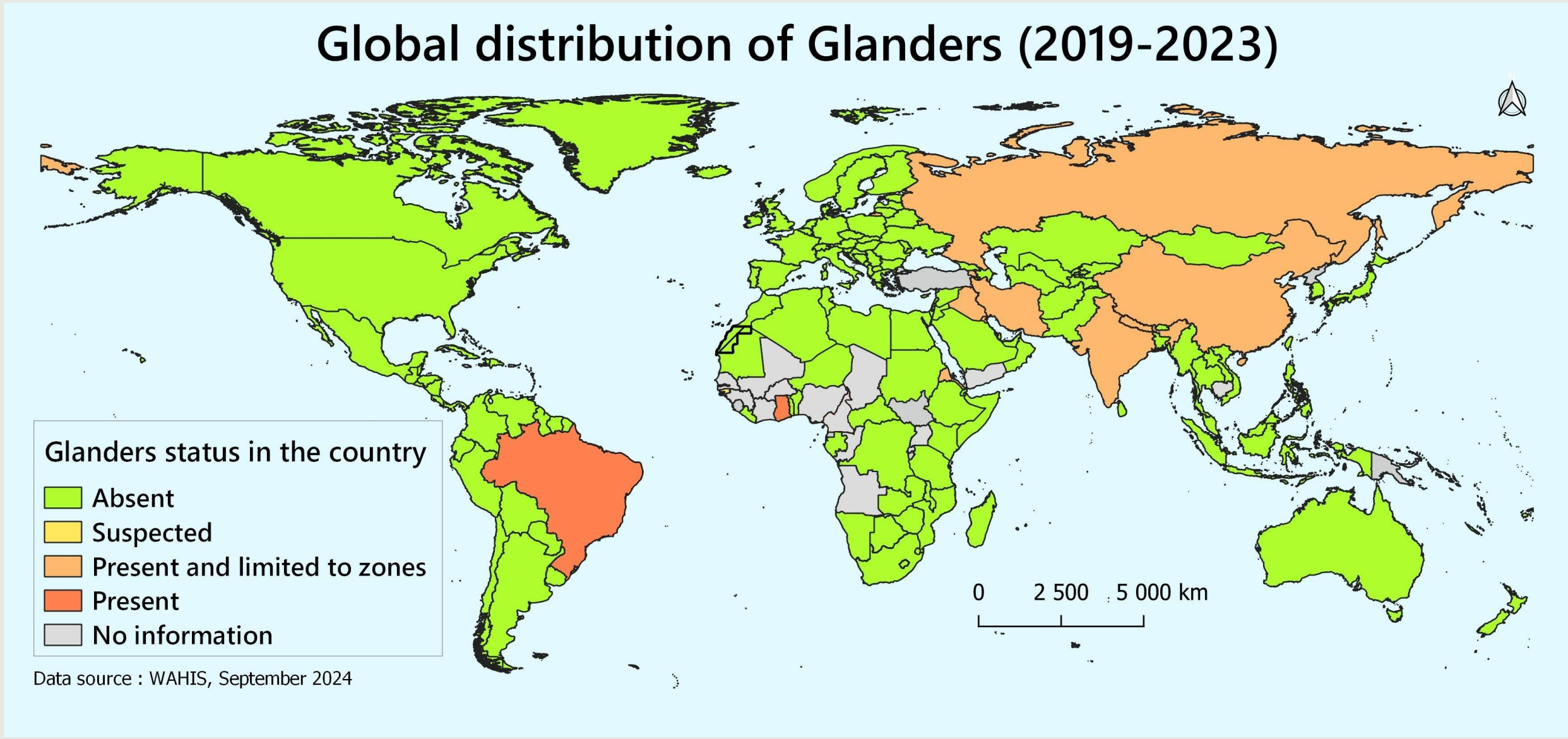
Recovery of Free Status

Recommendations for importation of equids from free countries/zones (kept 6 months in free countries-zones or from not free zones kept for 12 months in an establishment with no cases+ isolated for 30 days and tests (CFT-ELISA?)

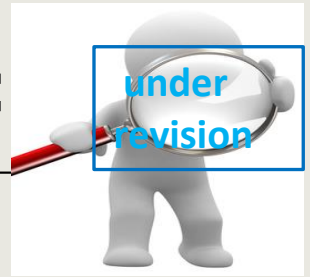
Recommendations for the importation of equine semen and embryos

General Principles of Surveillance and Surveillance Methods

DISEASE DISTRIBUTION - Data from the World Animal Health Information System (WAHIS)



Chapter 12.1. Inf. with African Horse Sickness virus (AHS)(2014):



Case definition

Country, zone free from AHS:

Infection with AHS is **notifiable** in the whole country;

No AHS cases in the past 2 years

AND either

- Historical freedom (25 years without evidence of AHS infection+ early warning system) or
  - AHS absence for the past two years demonstrated through an effective surveillance programme, or
  - Demonstrated AHS absence for 40 days + absence of Culicoides
- Plus, an AHS free country or zone which is adjacent to an infected country or zone should include a zone in which surveillance is conducted

Chapter 12.1. Inf. with African Horse Sickness (AHS):

Recommendations for importation of equids from free countries/zones

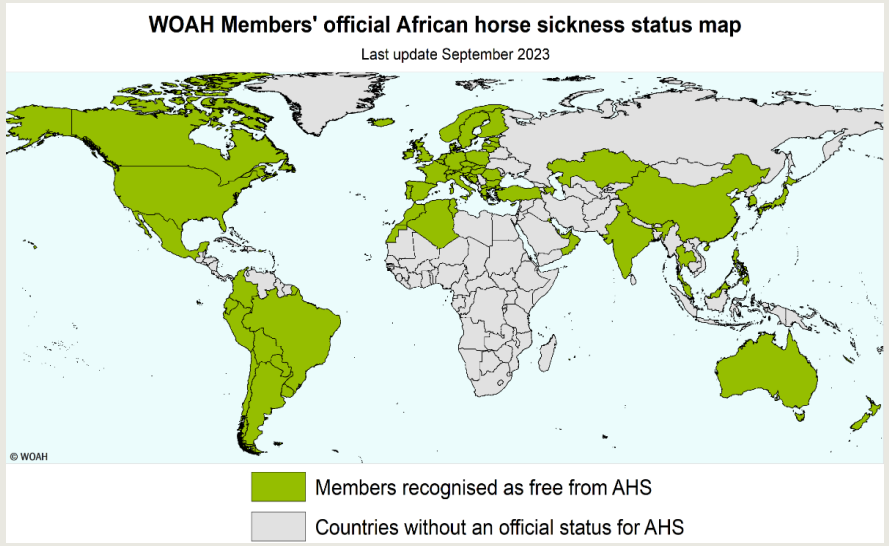
Recommendations for importation of equids from free countries/zones not free from AHS

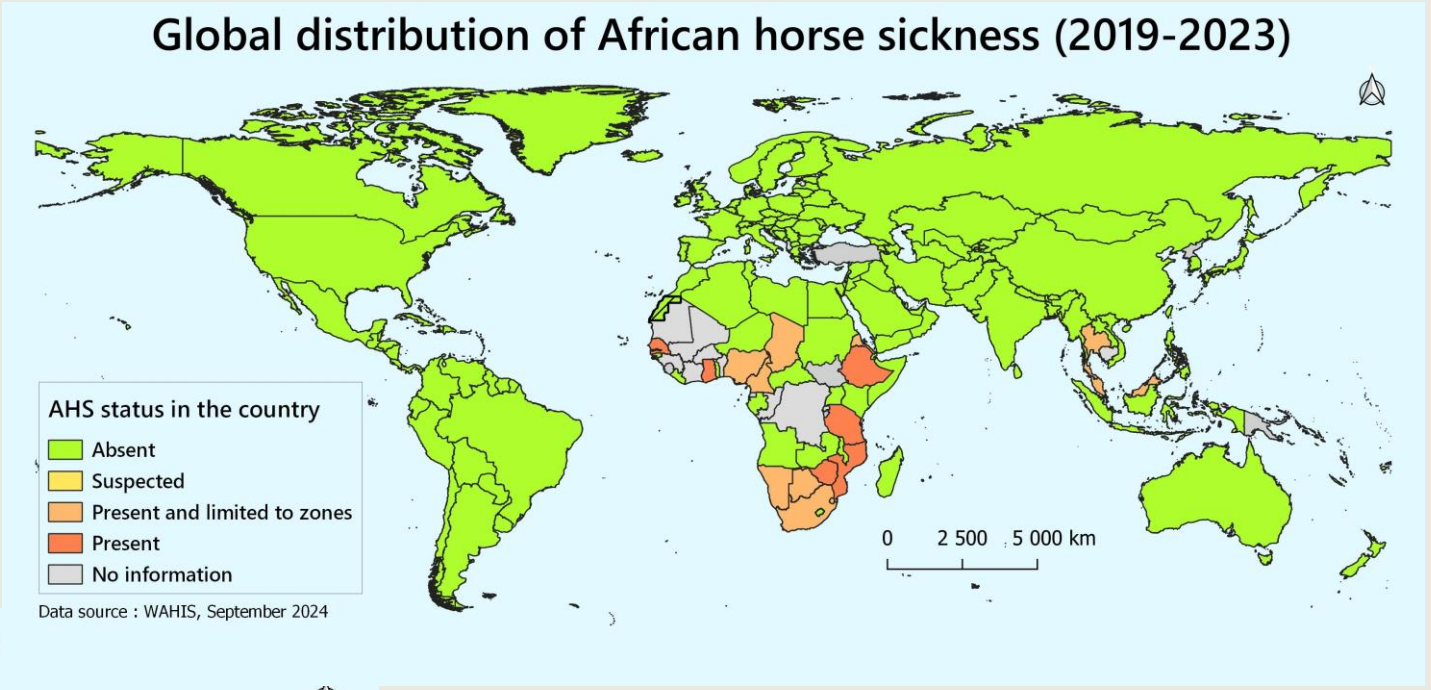
kept 6 months in free countries-zones or from not free zones kept for 12 months in an establishment with no cases+ isolated for 30 days and tests (PCR-ELISA)

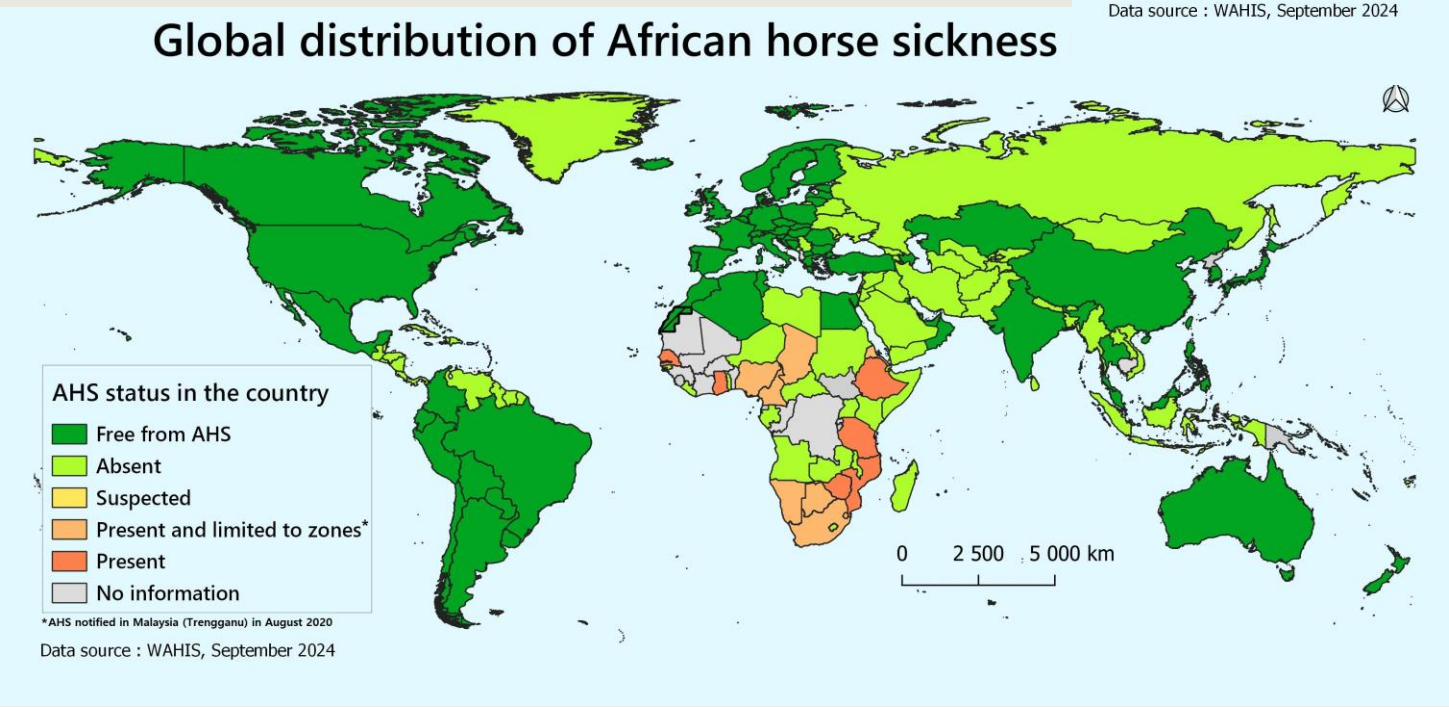
Recommendations for the importation of equine semen and embryos

Protecting animals from Culicoides attacks

DISEASE DISTRIBUTION - Data from the World Animal Health Information System (WAHIS)







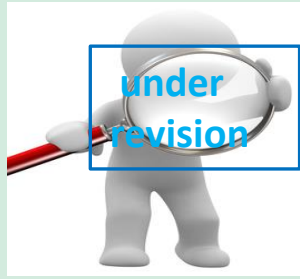
Equine Infectious Anaemia (EIA). Adopted in 2007

Recommendations for the importation of equines:

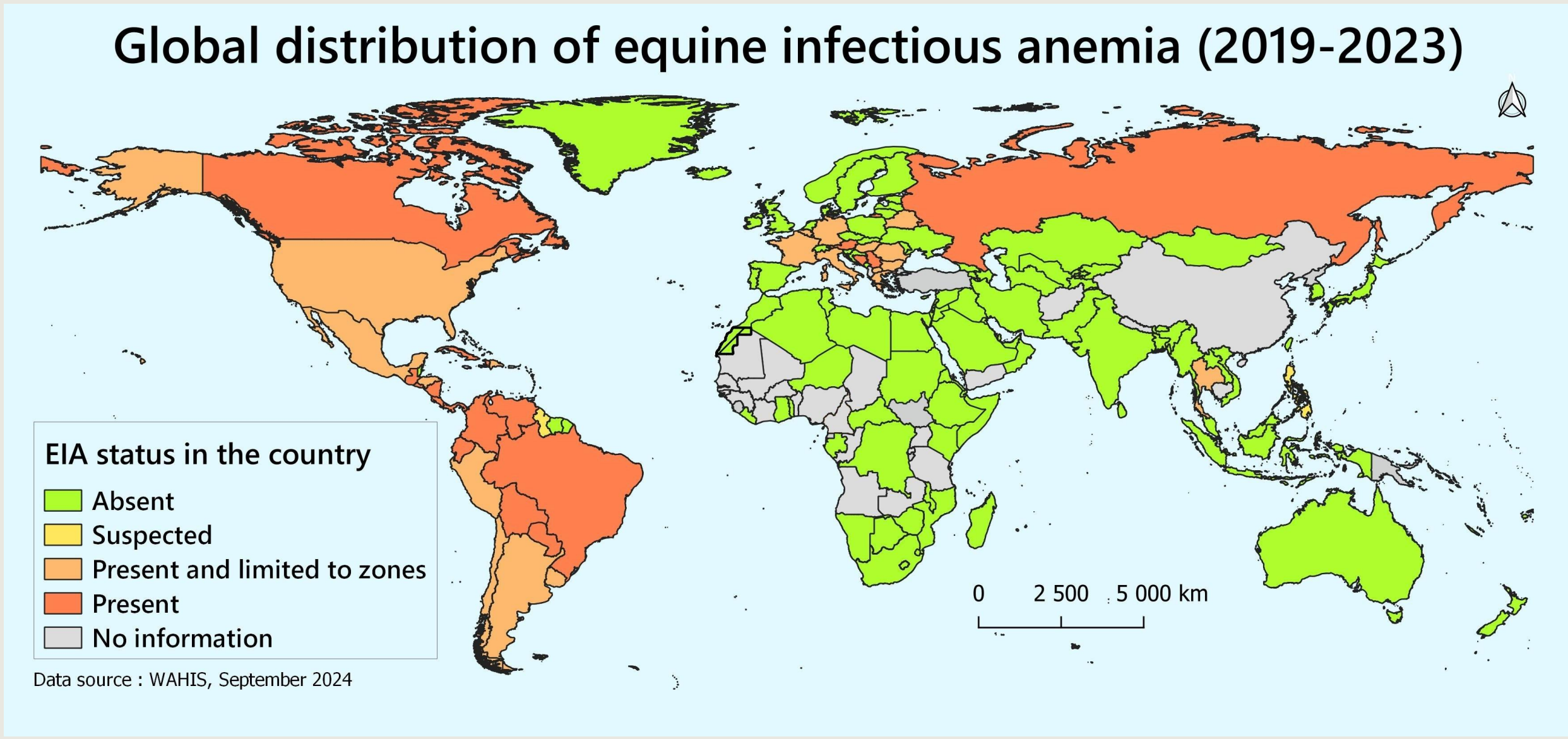
- No clinical signs 48 prior to shipment
- No case of EIA in the premises where the animals were kept 3 months prior to shipment
- Subjected to diagnostic tests: blood samples collected 30 days prior to shipment for permanent importations and 90 days for temporary importations

Other Chapters under revision:

- **Eastern, Western, and Venezuelan Equine Encephalomyelitis**
- **Japanese encephalitis**
- **Dourine**



DISEASE DISTRIBUTION - Data from the World Animal Health Information System (WAHIS)



Thank you

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F. +33 (0)1 42 67 09 87

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The role of the European Union Reference Laboratory on Equine Diseases: an example of a regional approach for specialised diagnostic capacities and vaccine development

Stéphan Zientara

Director of the Animal Health Laboratory
Maisons-Alfort, France

EU/WOAH/FAO REFERENCE Lab on FMD
EU REFERENCE Lab on EQUINE DISEASES
WOAH REFERENCE Lab on EHDV

European Commission

New official control regulation (OCR) and EURLs

DG SANTE
Unit G3

Health and Food Safety

Mission of DG SANTE/Unit G3 – Official Controls and Eradication of diseases in animals

- Coordinate the implementation of the **new legislative framework** on **official controls** in the food chain, applicable from December 2019;

- Ensure the application of EU rules for the **prevention, control and eradication of animal diseases**, including mobilisation of EU **crisis management** instruments;

- Provide a general framework for the verification of compliance with the EU rules on food & feed, and animal health.

European Commission

EURL network 2016

- **45 EURLs** according Commission Implementing Decision of 27/07/2015
  - **30 Food and Feed safety**
  - **15 Animal Health**

- Budget for 2 years (2016+2017): **31.656.500 €**
- Budget for 2 years (2019+2020): **41.350.500 €**
- Budget for 3 years (2025+2027): **42.000.000 €**

There is **continuous Government support** over a period of about 12 years to support this network

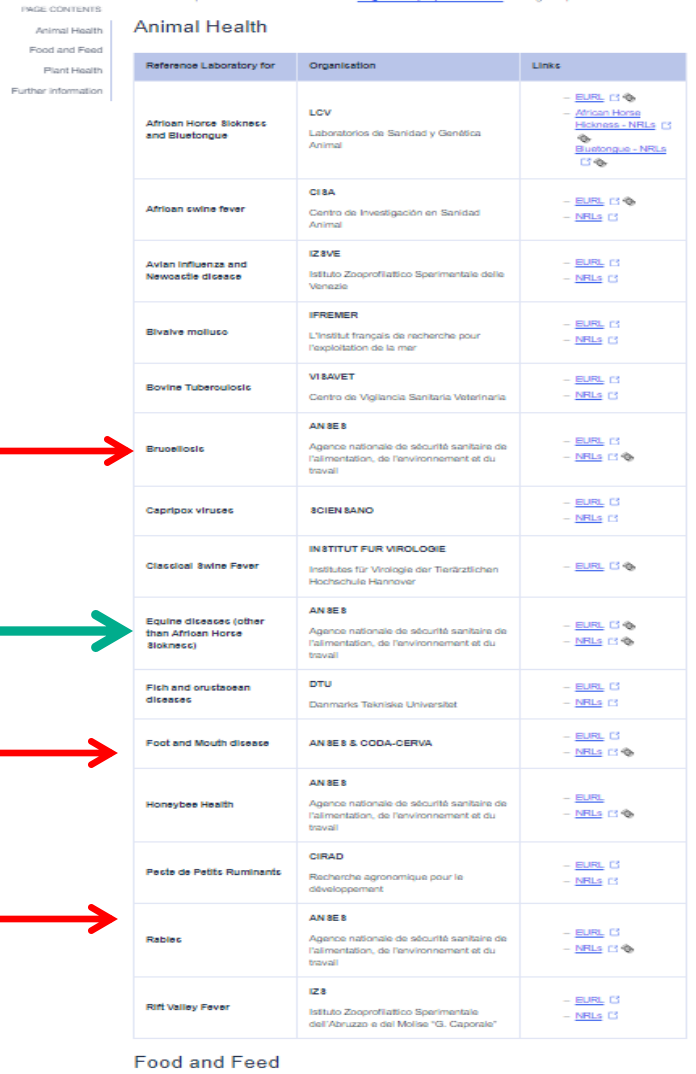
Link to all EURLs on website DG SANTE

https://food.ec.europa.eu/horizontal-topics/european-union-reference-laboratories_en

15 EU RL on animal health

ANSES : 4

EU RL on equine diseases (except AHS)



PAGE CONTENTS

Animal Health

Food and Feed

Plant Health

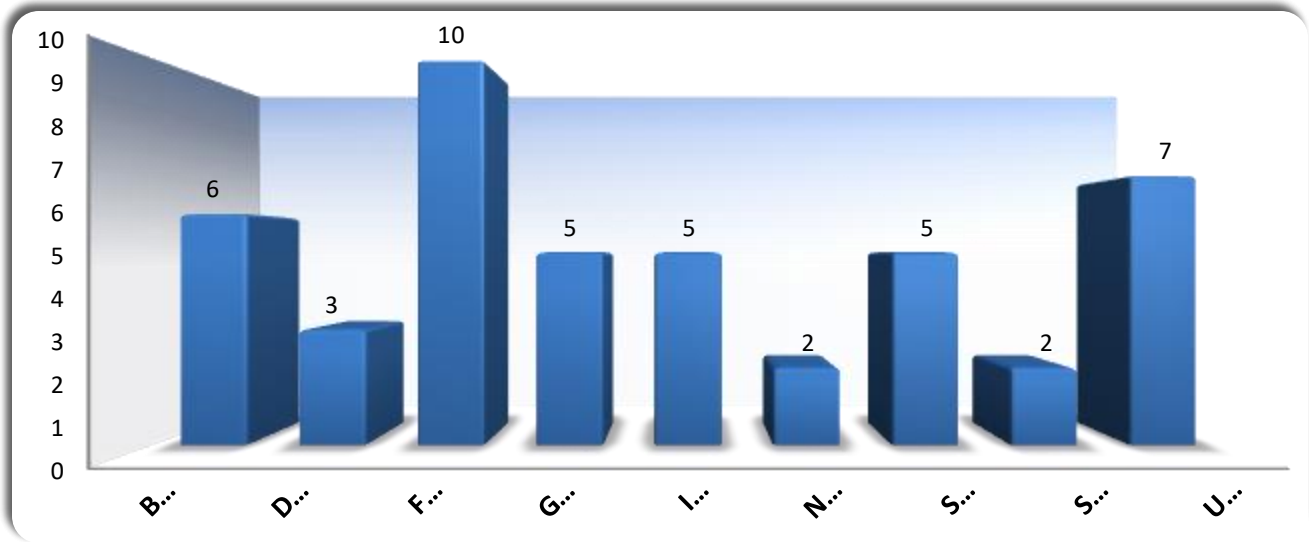
Further information

Animal Health

Reference Laboratory for	Organisation	Links
African Horse Sickness and Bluetongue	LCV Laboratorios de Sanidad y Genética Animal	– EURL – African Horse Sickness - NRLs Bluetongue - NRLs
African swine fever	CISA Centro de Investigación en Sanidad Animal	– EURL – NRLs
Avian influenza and Newcastle disease	IZSVE Istituto Zooprofilattico Sperimentale delle Venezie	– EURL – NRLs
Bivalve mollusc	IFREMER L'Institut français de recherche pour l'exploitation de la mer	– EURL – NRLs
Bovine Tuberculosis	VISAVET Centro de Vigilancia Sanitaria Veterinaria	– EURL – NRLs
Brucellosis	ANSES Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail	– EURL – NRLs
Capripox viruses	SCIENSANO	– EURL – NRLs
Classical Swine Fever	INSTITUT FUR VIROLOGIE Institutes für Virologie der Tierärztlichen Hochschule Hannover	– EURL
Equine diseases (other than African Horse Sickness)	ANSES Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail	– EURL – NRLs
Fish and crustacean diseases	DTU Danmarks Tekniske Universitet	– EURL – NRLs
Foot and Mouth disease	ANSES & CODA-CERVA	– EURL – NRLs
Honeybee Health	ANSES Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail	– EURL – NRLs
Peste de Petits Ruminants	CIRAD Recherche agronomique pour le développement	– EURL – NRLs
Rabies	ANSES Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail	– EURL – NRLs
Rift Valley Fever	IZS Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"	– EURL – NRLs

Food and Feed

Distribution EURLs per Member State



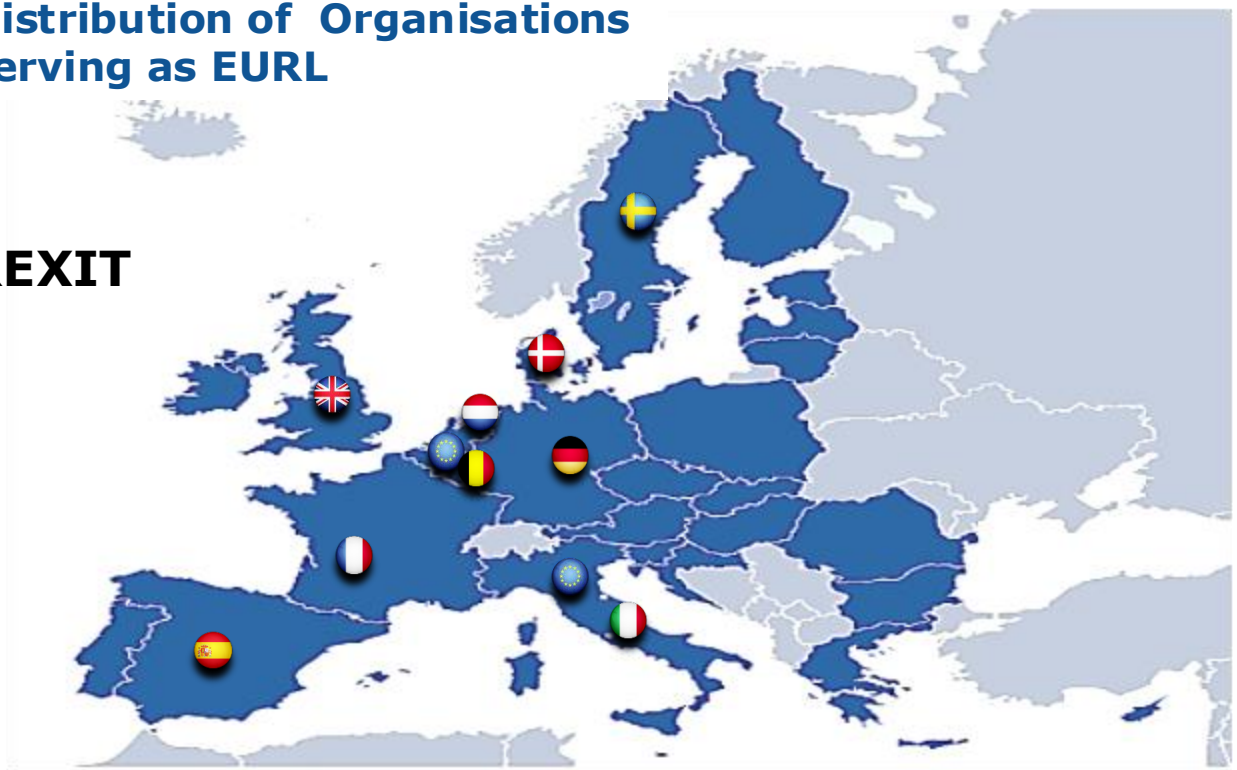
Variety of organisations serving as EURL

- Official Laboratories (as designated by Competent Authority)
- Research Institutes
- University Laboratories
- Private Laboratories

- Also need to fulfil the requirements !
- Accredited: EN ISO/IEC 17025, EN 45002, EN 45003

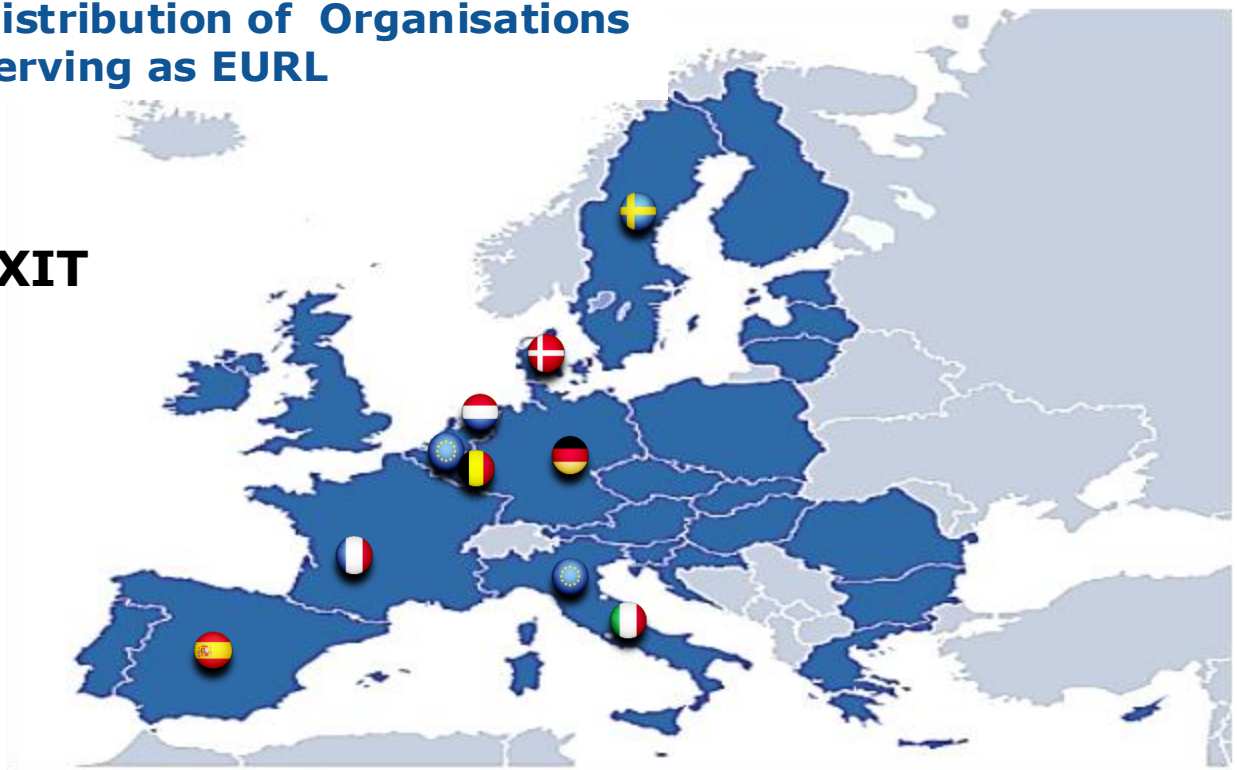
Distribution of Organisations serving as EURL

Before BREXIT



Distribution of Organisations serving as EURL

After BREXIT



Laboratorio de Referencia de la UE

- About us
- Bluetongue disease
- Horse sickness
- Diagnosis

European Reference Laboratory for African horse sickness and Bluetongue (AHS&BT)

Welcome to the website of the European Reference Laboratory for African horse sickness and Bluetongue (AHS&BT EURL)

You will find on the site information on Bluetongue and African horse sickness and pathogens that cause them, as well as activities of the EURL.

NEWS Click here
Publications, events, recent outbreaks...
related to African horse sickness and Bluetongue

Versión en español

anses
French agency for food, environmental and occupational health safety

The role of the European Union Reference Laboratory on Equine Diseases: an example of a regional approach for specialised diagnostic capacities and vaccine development

Since 1st July 2008

COMMISSION REGULATION (EC) No 180/2008

of 28 February 2008

concerning the Community reference laboratory for equine diseases other than African horse sickness and amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council

RÉPUBLIQUE FRANÇAISE
Liberté Égalité Fraternité

anses

Search on the site OK

ANSES has a network of nine reference and research laboratories located throughout France, close to industry. These laboratories operate in three main areas: animal health and welfare, food safety (chemical and microbiological) and plant health. They are internationally recognised in their respective fields of expertise: epidemiology, microbiology and antimicrobial resistance, toxins and physico-chemical contaminants. Thanks to their work in providing expertise, epidemiological monitoring, alerts and scientific and technical assistance, they play a vital role in understanding various threats and in collecting data from the network of accredited laboratories.

Find a laboratory

List of laboratories corresponding to the search

Nancy Laboratory for Hydrology
The Nancy Laboratory for Hydrology contributes to ensuring the health quality of water for human consumption, by carrying out missions on a French national scale. It provides scientific and technical support to public policymakers on behalf of the French Directorate ...

Laboratoire d'hydrologie de Nancy
40, rue Lionnois
Nancy
Phone : +33 (0) 3 83 38 87 20

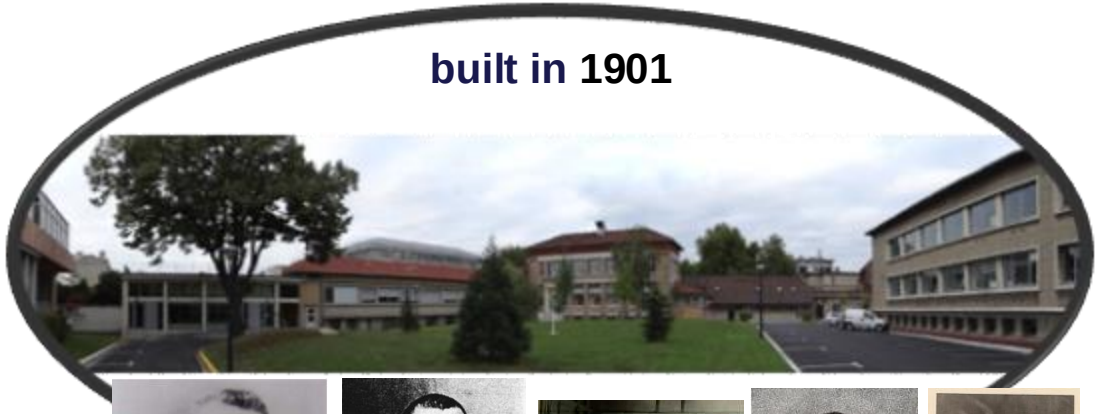
Fougères Laboratory
The Fougères Laboratory is primarily involved in improving knowledge of the benefits and risks of veterinary medicinal products and disinfectants used by the food processing industry and in evaluating the toxicity of residues and contaminants.
...

Laboratoire de Fougères
10B rue Claude Bourgelat

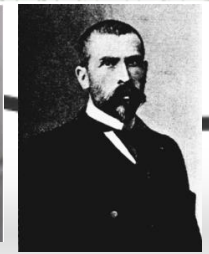
1,400 staff total with 700 staff in laboratories

Animal Health laboratory

built in 1901



Edmond Nocard
(1850-1903)



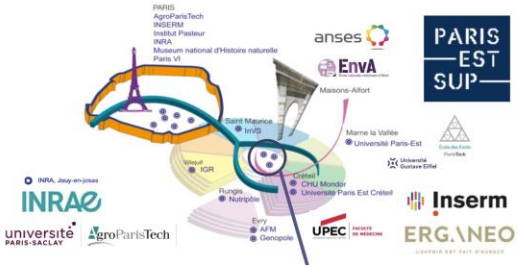
Emile Roux
(1853-1933)

Henri Carré
(1870-1938)

Henri
Vallée
(1874-1947)

Emmanuel
Leclainche
(1861-1953)

Maisons-Alfort campus



Normandy campus

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Normandie Université · NORMANDIE ÉQUINE VALLÉE · hippolia · Fonds Éperon · RÉGION NORMANDIE · Calvados LE DÉPARTEMENT

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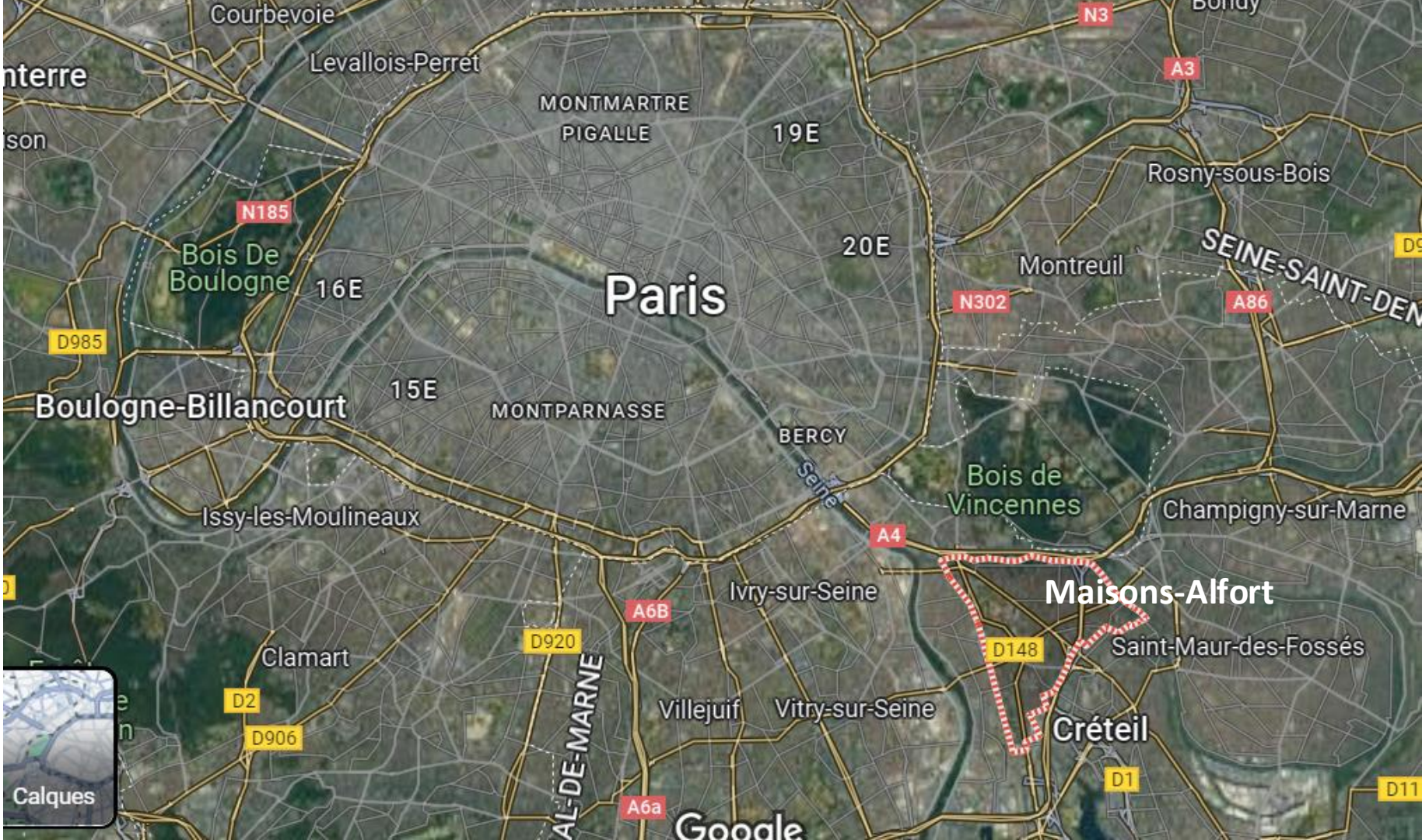
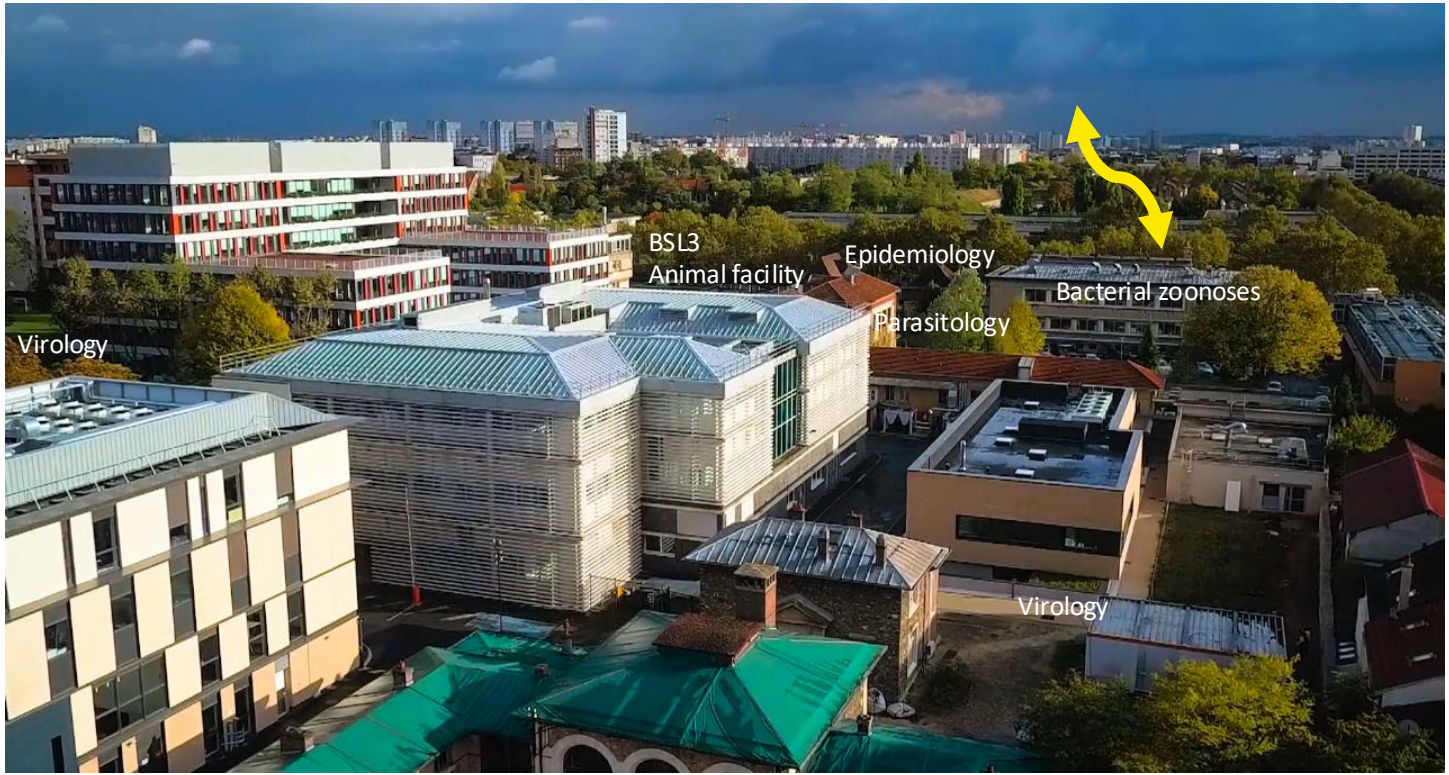
GIS CENTAURE


Courbevoie
Levallois-Perret
MONTMARTRE
PIGALLE
19E
N3
A3
Rosny-sous-Bois
N185
Bois De
Boulogne
16E
20E
Paris
Montreuil
SEINE-SAINT-DEN
N302
A86
D985
Boulogne-Billancourt
15E
MONTPARNASSE
BERCY
Seine
Bois de
Vincennes
Champigny-sur-Marne
Issy-les-Moulineaux
A4
Ivry-sur-Seine
Maisons-Alfort
A6B
D920
D148
Saint-Maur-des-Fossés
Clamart
D2
D906
Villejuif
Vitry-sur-Seine
Créteil
D1
A6a
Google
Calques

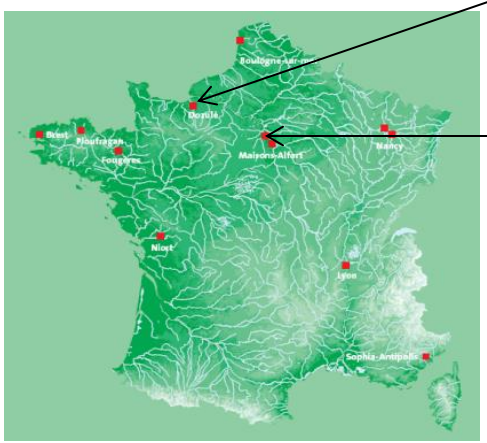

Veterinary school
Auchan Supermarché (Bourgelat)
Access Alfort
Villa Fragonard
D19
D6
École Vétérinaire de Maisons-Alfort
Rue Paul Bert
Rue Naville
Rue Ernest Renan
Rue Edmond Nocard
Office National des Forêts
Rue Bouley
Rue Chabert
Chem. Latéral
Hertz Location De Voitures - Hertz...
Le Temple du Ninja
All. des Fleurs
All. des Amaryllis
All. des Bégonias
Imp. Fiocre
Le Bar Belge
All. des Platanes
Rue André Soladier
Agence Nationale Sécurité Sanitaire...
ue de Seine
Lycée Eugène Delacroix
Gymnase Eugène Delacroix


BSL3
Animal facility
Epidemiology
Bacterial zoonoses
Parasitology
Virology
Virology


Animal health laboratory, Normandy campus

I. Organisation of the EU RL

EU RL in the **Animal Health Laboratory** on 2 sites



campus of Dozulé (Normandy region)

Campus of Maisons-Alfort (near Paris)

The EU RL has a mandate with 10 diseases: glanders, CEM, dourine, EAV, EIAV, WNV, exotic encephalitides (EEEV, WEEV, VEEV and JEV)

I. Organisation of the EU RL

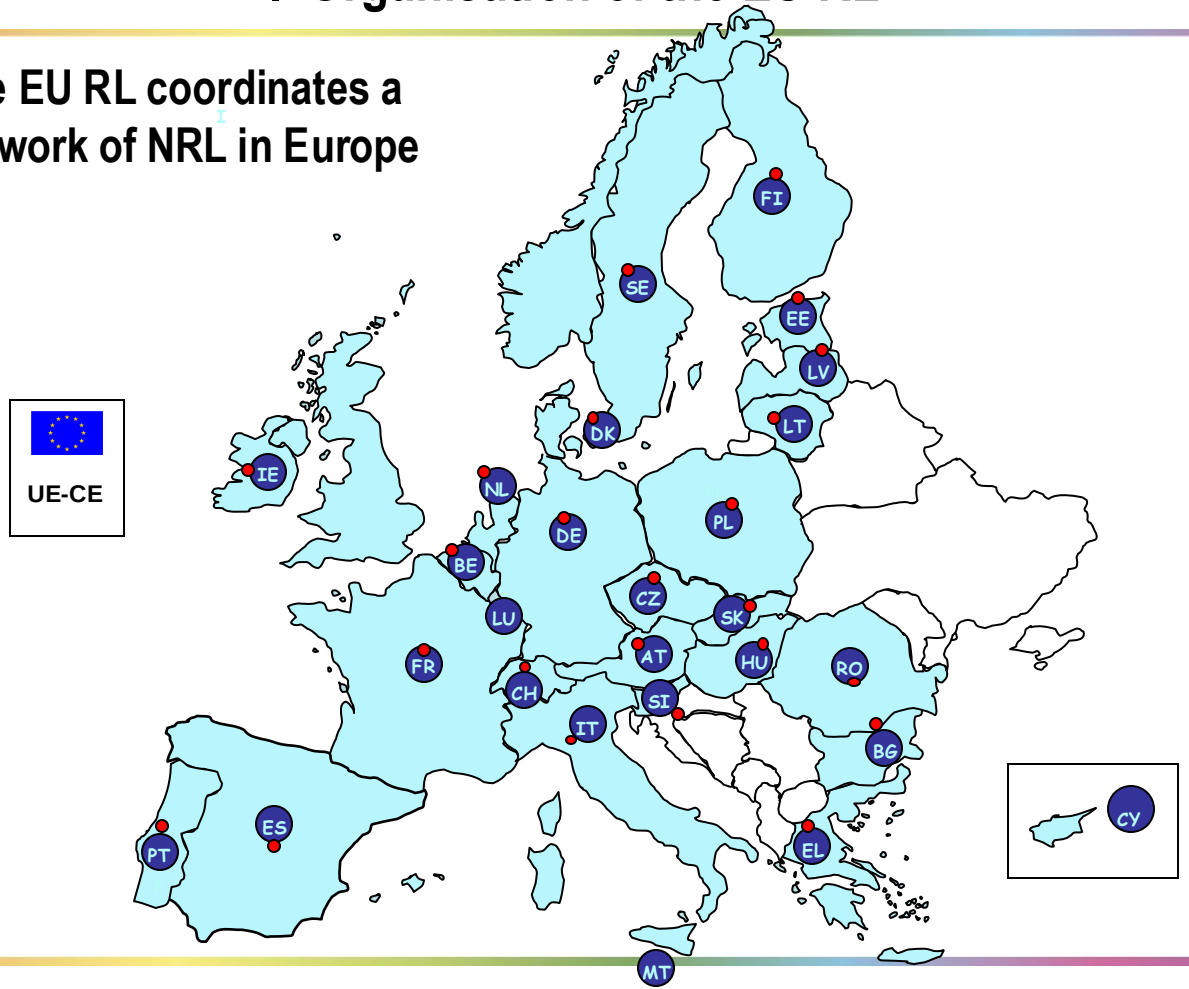
Diseases	Heads
WN, EEE, EEV,EEW	G. Gonzalez
Glanders	K. Laroucau
EIAV, EAV, EHV	JC Valle-Casuso/D. Gaudaire
CEM	S. Petry
Dourine	L. Hebert

all of my colleagues have assisted in organising WOAH webinars on equine diseases
https://rr-asia.woah.org/en/projects/horse-movement/international-horse-movement-in-asia-and-the-pacific/

Director => S.ZIENTARA
Deputy Director => G.GONZALEZ

I. Organisation of the EU RL

The EU RL coordinates a network of NRL in Europe



Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001

- Art. 94.2.a Providing national reference laboratories with details and guidance on the methods of laboratory analysis, testing or diagnosis, including reference methods.

- Art. 94.2.b Providing reference materials to national reference laboratories

- Art. 94.2.c Coordinating the application by the national reference laboratories and, if necessary, by other official laboratories of the methods referred to in point (a), in particular, by organising regular inter-laboratory comparative testing or proficiency tests and by ensuring appropriate follow-up of such comparative testing or proficiency tests in accordance, where available, with internationally accepted protocols, and informing the Commission and the Member States of the results and follow-up to the inter-laboratory comparative testing or proficiency tests.

- Art. 94.2.l Where relevant for their area of competence, cooperate among themselves and with the Commission, as appropriate, to develop methods of analysis, testing or diagnosis of high standards.

Missions of the EU RL: communicate with NRL

flyers, papers, social networks,

…

13th Newsletter
October 2016

EAVLD Newsletter

EUROPEAN UNION REFERENCE LABORATORY FOR EQUINE DISEASES

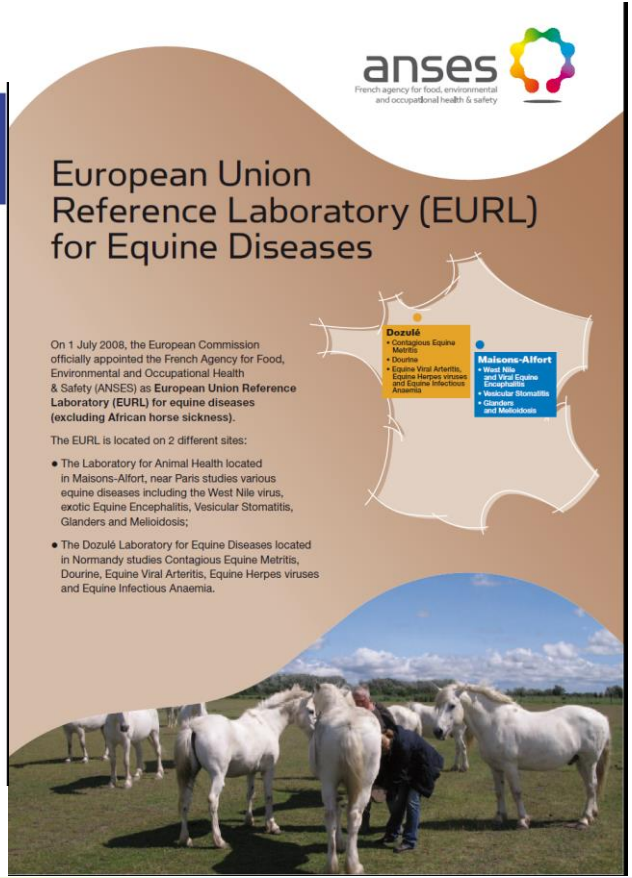
On 1 July 2008, the European Commission officially appointed the French Agency for Food, Environmental and Occupational Health &safety (ANSES) as European Union Reference Laboratory (EU RL) for equine diseases (excluding African horse sickness).

The Laboratory for Animal Health located in Maisons-Alfort near Paris studies various equine diseases including the West Nile virus (WNV), exotic Equine Encephalitis (i.e. Eastern, Western , Venezuelan and Japanese encephalitis), Vesicular Stomatitis (VS), Glanders and Melioidosis.

The Dozulé Laboratory for Equine Diseases located in Normandy studies Contagious Equine Metritis (CEM), Dourine, Equine Viral Arteritis (EVA), Equine herpes viruses (EHV) and Equine infectious Anemia (EIA).

All these diseases belong to the **OIE list in forced in 2015 of notifiable terrestrial and aquatic animal diseases** (chapter for multispecies disease or chapter for equine diseases). This EURL is therefore specific as compared with others EURL because it's responsible of many diseases and these diseases are due to virological, bacteriological or parasitological pathogens.

According to the diseases managed by the EURL, there is or not a disease-free status in Europe.



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French agency for food, environmental and occupational health & safety

European Union Reference Laboratory (EURL) for Equine Diseases

On 1 July 2008, the European Commission officially appointed the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) as **European Union Reference Laboratory (EURL) for equine diseases (excluding African horse sickness).**

The EURL is located on 2 different sites:

- The Laboratory for Animal Health located in Maisons-Alfort, near Paris studies various equine diseases including the West Nile virus, exotic Equine Encephalitis, Vesicular Stomatitis, Glanders and Melioidosis;
- The Dozulé Laboratory for Equine Diseases located in Normandy studies Contagious Equine Metritis, Dourine, Equine Viral Arteritis, Equine Herpes viruses and Equine Infectious Anaemia.

Dozulé
- Contagious Equine Metritis
- Dourine
- Equine Viral Arteritis, Equine Herpes viruses and Equine Infectious Anaemia

Maisons-Alfort
- West Nile and Viral Equine Encephalitis
- Vesicular Stomatitis
- Glanders and Melioidosis

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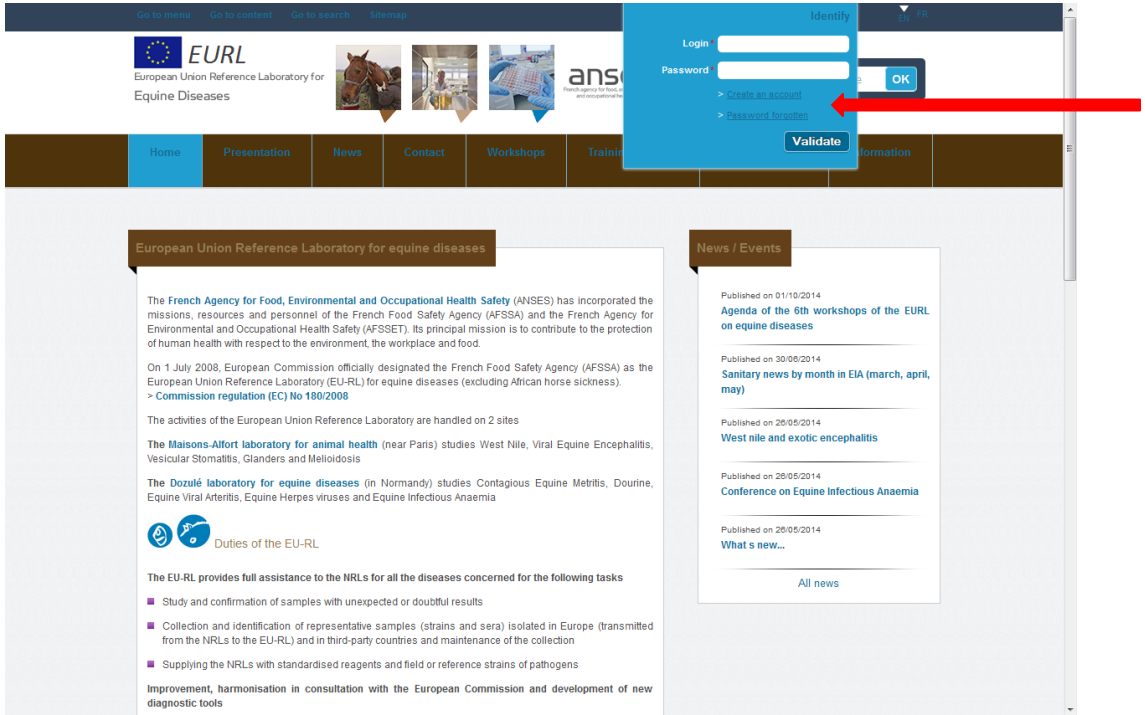
Missions of the EU RL: communicate with NRL

EU RL website :https://eurl-equinediseases.anses.fr

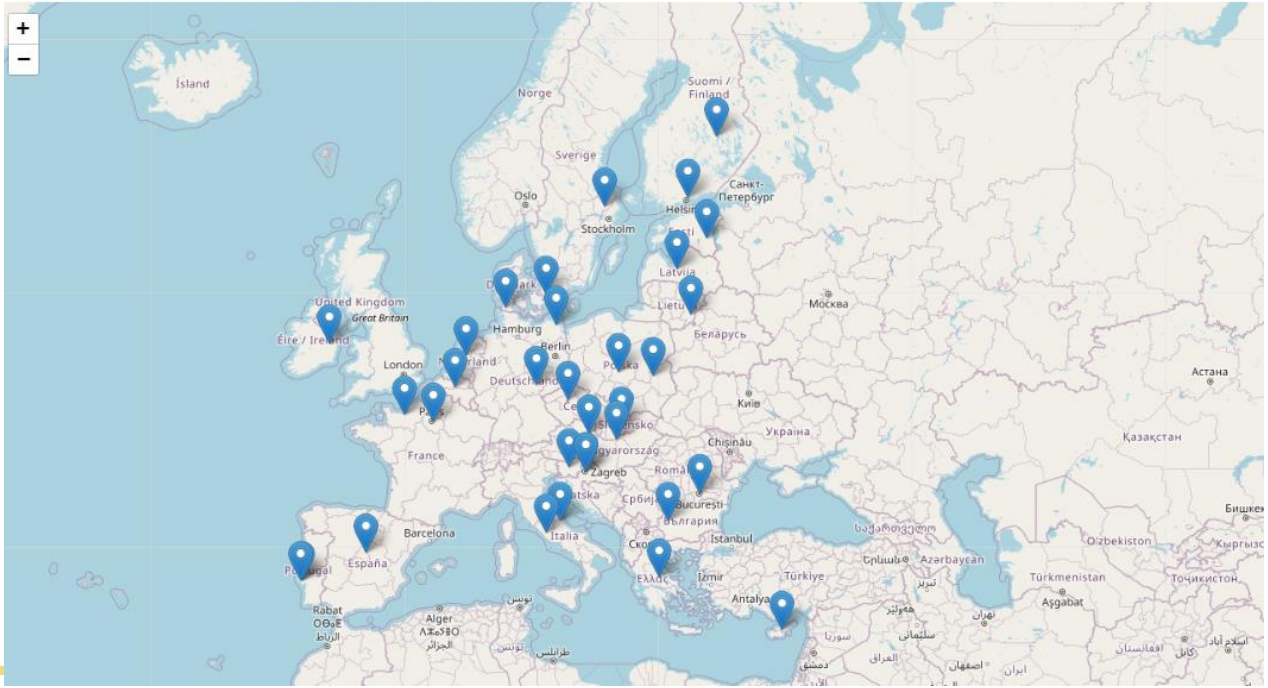


LABORATORY NETWORK NATIONAL REFERENCE LABORATORIES

lational Reference Laboratories

The EURL for equine diseases is supported by a network of collaborating laboratories in each Member State.

To display information on the laboratory, display on map



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EURL DUTIES

The activities of the European Union Reference Laboratory are handled on 2 sites

The Laboratory for animal health, Maisons-Alfort site (near Paris) studies West Nile, Viral Equine Encephalitis, Vesicular Stomatitis, Glanders and Melioidosis
The Laboratory for animal health, Dozulé site (in Normandy) studies Contagious Equine Metritis, Dourine, Equine Viral Arteritis, Equine Herpes viruses and Equine Infectious Anaemia
Duties of the EU-RL

The EU-RL provides full assistance to the NRLs for all the diseases concerned for the following tasks

Study and confirmation of samples with unexpected or doubtful results
Collection and identification of representative samples (strains and sera) isolated in Europe (transmitted from the NRLs to the EU-RL) and in third-party countries and maintenance of the collection
Supplying the NRLs with standardised reagents and field or reference strains of pathogens

Improvement, harmonisation in consultation with the European Commission and development of new diagnostic tools

Coordinate in consultation with the Commission the methods employed by Member States for equine diagnosis diseases
Set up of new diagnosis methods as Enzyme Link Immunosorbent Assay (ELISA) kits or molecular diagnosis for equine diseases

Research on phenotypic and molecular diversity and epidemiological surveillance

Genotyping of European strains isolated in the fields - Development of new vaccine in horses Information
Organisation of Workshops, proficiency tests or training sessions each year for the NRLs

Information

Organisation of Workshops, proficiency tests or training sessions each year for the NRLs

NRL CONTACTS

To display information on the laboratories that work on a given disease, please click on the corresponding image.

- Equine viral arteritis (EVA)
- Equine infectious anaemia (EIA)
- Contagious equine metritis (CEM)
- Dourine
- Glanders, melioidosis
- West Nile virus (WNV)
- Show all

Country	Institution	Adress	Adress 2	Postal code	Town	Diseases
AUSTRIA	AGES Austrian Agency for Health and Food Safety, Institute for Veterinary Disease Control Moedling		Robert Koch-Gasse 17	2340	Moedling	WNV, EVA, EIA, CEM Dourine, Glanders, VSV
BELGIUM	Sciensano		Groeselenberg 99	1180	Brussels	WNV, EVA, EIA, CEM Dourine, Glanders, VSV
BULGARIA	NRL Equine Viral diseases	Exotic and Emerging Diseases	190 Lomsko Shose blvd	1231	Sofia	WNV, EVA, EIA
CROATIA	ARTERIab	Dpt of Microbiology and Infectious Diseases with clinic, faculty of veterinary medecine	Heinzelova 55	10000	Zagreb	WNV, EVA, EIA, CEM Dourine
CROATIA	Croatian Veterinary Institute		Savska cesta 143	10000	Zagreb	Glanders
CZECH REPUBLIC	State Veterinary Institute in Prague, NRL for equine diseases		Sidlistni 136/24	16503	Prague 6 - Lysolaje	WNV, EVA, EIA, CEM Dourine, Glanders, VSV
CYPRUS	Virology Laboratory virology service			1417	Nicosia	WNV, EIA
DENMARK	Technical University of Denmark, National Veterinary Institute, Section for Diagnostic and Contingency plans		Bulowsvej 27	1870	Frederiksberg C	EVA, EIA, CEM, Dourine, Glanders
DENMARK			Lindholm	DK-4771	Kalvehave	WNV and VSV
ESTONIA	Estonian National Centre for Laboratory Research and Risk Assessment (LABRIS)		Kreutzwaldi 30	51006	Tartu	WNV, EVA, EIA, CEM Dourine, Glanders
FRANCE	ANSES	Laboratory for animal health,Maisons-Alfort site	14 rue Pierre et Marie Curie	94704	Maisons-Alfort	WNV, Glanders, VSV
FRANCE	ANSES	Dozulé Laboratory for animal health, Dozulé site		14430	Goustranville	EVA, EIA, CEM, Dourine
FINLAND	Finnish Food Authority		Mustialankatu 3	FI-00790	Helsinki	WNV, EVA, EIA, Dourine, Glanders and

REFERENCE MATERIALS

Search a document

Title : Title

Risk Pathogene : Risk

SEARCH

Listing of molecular reagents available in the EURL for equine diseases

Reference materials, Published on 09/02/2018

DOWNLOAD

Listing of serological reagents available in the EURL for equine diseases

Reference materials, Published on 09/02/2018

DOWNLOAD

Listing of reagants available in the EURL for equine diseases

Diseases	Serum ref	Product name	Packaging	Expected titre	Titre range	Contact	Comments
EVA	S00653	EAV positive serum	Lyophilized (1ml)	128	64-256	delphine.gaudaire@anses.fr	
	S00653	EAV positive serum	Lyophilized (1ml)	24	12-48		
	S00653	EAV positive serum	Lyophilized (1ml)	192	96-384		
	S00653	EAV positive serum	Lyophilized (1ml)	8	4-16		
	Serum ref	**Product name**	**Packaging**	**Expected titre**	**Titre range**	**Contact**	**Comments**
EIA	S00652	EIAV positive serum	Lyophilized (1ml)	/	/	delphine.gaudaire@anses.fr	
	Serum ref	**Product name**	**Packaging**	**WNV competition : % S/N*100 (IDVet kit)**	**WNV IgM capture % S/P*100 (IDVet kit)**	**Contact**	**Comments**
JEV		JEV positive serum (8 days post inoculation)	Lyophilized (0,5ml)	76	0	cecile.beck@anses.fr	Sera available for ELISA and VNT
		JEV positive serum (20 days post inoculation)	Lyophilized (0,5ml)	33	0		
		JEV positive serum (35 days post inoculation)	Lyophilized (0,5ml)	26	0		
		JEV positive serum (58 days post inoculation)	Lyophilized (0,5ml)	27	0		
WNV		WNV lineage 1 positive serum (8 days post inoculation)	Lyophilized (0,5ml)	55	110		
		WNV lineage 1 positive serum (20 days post inoculation)	Lyophilized (0,5ml)	19	85		
		WNV lineage 1 positive serum (35 days post inoculation)	Lyophilized (0,5ml)	10	27		
		WNV lineage 1 positive serum (58 days post inoculation)	Lyophilized (0,5ml)	7	4		
		WNV lineage 2 positive serum (8 days post inoculation)	Lyophilized (0,5ml)	35	143		
		WNV lineage 2 positive serum (20 days post inoculation)	Lyophilized (0,5ml)	16	135		
		WNV lineage 2 positive serum (35 days post inoculation)	Lyophilized (0,5ml)	8	102		
		WNV lineage 2 positive serum (58 days post inoculation)	Lyophilized (0,5ml)	6	28		
USUV		USUV positive serum (8 days post inoculation)	Lyophilized (0,5ml)	93	-1		
		USUV positive serum (20 days post inoculation)	Lyophilized (0,5ml)	30	0		
		USUV positive serum (35 days post inoculation)	Lyophilized (0,5ml)	33	0		
		USUV positive serum (58 days post inoculation)	Lyophilized (0,5ml)	6	18		
TBEV		TBEV positive serum (8 days post inoculation)	Lyophilized (0,5ml)	79	0		
		TBEV positive serum (20 days post inoculation)	Lyophilized (0,5ml)	35	1		
		TBEV positive serum (35 days post inoculation)	Lyophilized (0,5ml)	21	0		
		TBEV positive serum (58 days post inoculation)	Lyophilized (0,5ml)	16	0		
	Serum ref	**Product name**	**Packaging**			**Contact**	**Comments**
CEM	S00650 / S00651	Polyclonal antibodies anti-*Taylorella equigenitalis* (produced on rabbits)	1 ml (S00650) 0,2 ml (S00651)			sandrine.petry@anses.fr	for slide agglutination test
	Serum ref	**Product name**	**Packaging**			**Contact**	**Comments**
Dourine	S00654	Dourine high titer positive serum (OVI strain)	1 ml	2+ 1/80	40(2+)-160(2+)	laurent.hebert@anses.fr	
	S00655	Dourine low titer positive serum (OVI strain)	830 µl	2+ 1/5	5(1+)-5(4+)		
	S00656	Dourine antigens (OVI strain)	1 ml	n/a	n/a		
	Serum ref	**Product name**	**Packaging**			**Contact**	**Comments**
All diseases	S00653	Equine negative serum for 8 equine diseases	Lyophilized (1ml)	Negative	/	delphine.gaudaire@anses.fr	

SOP

Search a document

Title : Title

Risk Pathogene : Risk

SEARCH

SOP CEM culture method _Rev3

SOP, Published on 19/10/2023

DOWNLOAD

TECHNICAL

PCR Glanders SOP

SOP, Published on 05/07/2021

KEYWORDS

SOP, GLANDERS, PCR

DOWNLOAD

Serodiagnosis Surra CATT/T evansi

SOP, Published on 30/04/2021

KEYWORDS

SURRA, CATT, SERODIAGNOSIS

DOWNLOAD

Detection of WNV RNA genome by WNV rtRTPCR (adapted Linke et al 2007)

SOP, Published on 28/04/2021

KEYWORDS

WNV STARTHOR

DOWNLOAD

Detection and titration of WNV specific antibodies using VNT

SOP, Published on 28/04/2021

KEYWORDS

WNV PRNT MNT

DOWNLOAD

DETECTION OF *BURKHOLDERIA MALLEI* AND *BURKHOLDERIA PSEUDOMALLEI* BY REAL-TIME PCR

Written by: Thomas DESHAYES
Karine LAROUCAU

Approved by: Karine LAROUCAU

This protocol is an OIE-based method used at the EU-RL, all OIE-CFT based methods validated and used successfully in the proficiency tests can be used for this assay.

1. TOPIC AND SCOPE

This document describes the PCR methods for the detection of *Burkholderia* sp, *B. mallei* and *B. pseudomallei* according to the world organisation for animal health (OIE) international standard: OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals – Chapter 3.5.11, glanders and melioidosis (https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.05.11_GLANDERS.pdf).

Several systems are described in this document:

- Detection of the *B. pseudomallei* complex (including *B. mallei*, *B. pseudomallei* and *B. thailandensis* species)
- Specific detection of *B. mallei* (fliP)
- Specific detection of *B. pseudomallei* (orf11)

For detection, the PCR systems implemented are based on the use of specific oligonucleotide primer pairs and probes labelled in the 5' with the FAM fluorochrome. The detection of the exogenous gene added at the sample extraction stage is carried out using the associated PCR kit (Diagenode, DICD-YD-L100) and includes primers and a probe labelled in 5' with the fluorochrome VIC. This internal control system makes it possible to check the correct operation of the DNA extraction and the possible presence of PCR inhibitors. It constitutes a control of the DNA extraction steps and of the PCR method.

2. MATERIAL TO BE EXAMINED

2.1. SERUM

The PCR diagnosis of glanders is performed on DNA extracted from tissues or swabs.
Upon reception, the samples should be kept refrigerated (5 ± 3°C) or frozen (≤ -16°C).

2.2. TRANSPORT OF SAMPLES

Samples must be stored at ≤ -16°C.

EQUINE ARTERITIS AND GLANDERS/MELIOÏDOSIS WORKSHOPS

Please save the dates!

Two workshops are organized this year on **Equine arteritis virus and Glanders/Melioïdosis.**

The workshops will be held at the International Center of Deauville – Congress Center, 1 av. Lucien Barrière – 14800 DEAUVILLE (Normandy, France) as follows:

- Monday September 30 , 2024: Equine Arteritis
- Friday October 4, 2024: Glanders – Melioïdosis

This year the 2 workshops are organized in conjunction with the International Equine Infectious Diseases Conference (IEIDC 2024)

The hybrid mode in-person / virtual attendance will be offered.

II. Missions of the EU RL: organise PT and workshop

II. Missions of the EU RL: organise training sessions

- According to the results to the PT, training sessions are organized each year by the EU RL

Non-EU countries can participate in ANSES in PTs and training but they have to pay the costs

Biosecurity training

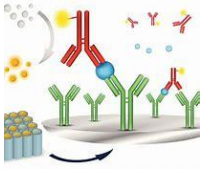
Molecular diagnosis

Virus isolation

1. Equine Viral Arteritis

Equine viral arteritis

- EURL develops :
  - New serological diagnostic tools to overcome the challenge to implement VNT in labs and to facilitate result interpretation
  - Innovative diagnostic tools for early detection of EAV infections or in particular, when viral load is very low, and thus the number of viral nucleotides targeted is limited
  - New molecular tools to better characterize EAV to investigate its biological properties of isolated strain and its impact on the health status of the horse population
  - New *in-vitro* models to identify new therapeutic treatments



J Clin Microbiol. 2015 Jan; 53(1): 287–291. PMCID: PMC4290937
Published online 2014 Dec 18. Prepublished online 2014 Oct 22. doi: 10.1128/JCM.01935-14 PMID: 25339390

Combination of an Unbiased Amplification Method and a Resequencing Microarray for Detecting and Genotyping Equine Arteritis Virus

Aymeric Haas,[a] Delphine Gaudaire,[a] Jean-Claude Manuguerra,[b] Albertine Leon,[c] Antoine Gessain,[d,e] Claire Laupier,[a] Nicolas Berthet,[d,e,*] and Stephan Zientara[f]

Article | Open access | Published: 22 June 2020

Replication of Equine arteritis virus is efficiently suppressed by purine and pyrimidine biosynthesis inhibitors

Jose-Carlos Valle-Casuso, Delphine Gaudaire, Leslie Martin-Latrie, Anthony Madeline, Patrick Dallemagne, Stéphane Pronost, Hélène Munier-Lehmann, Stephan Zientara, Pierre-Olivier Vidalain & Aymeric Haas

Scientific Reports 10, Article number: 10100 (2020) Cite this article

Interlaboratory proficiency testing

✓ IL-PT1 : serological diagnosis of EVA using Virus Neutralization Test ☞ **EVA VNT**

2009
23 labs
- 12 sera :
  - 4- & 8+

2013
21 labs
- 24 sera :
  - 9- & 15+

2016
23 labs
- 24 sera :
  - 13- & 11+

2020
23 labs
- 22 sera :
  - 9- & 13+

✓ IL-PT2 : virological diagnosis of EVA using two methods ☞ **EAV VI**

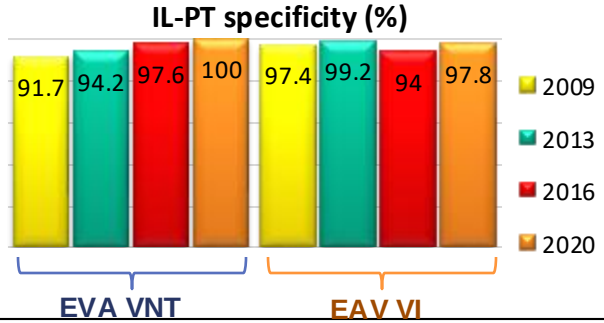
1. *Detection of EAV genome using RT-PCR method ⇒ EAV PCR*
2. *Detection of EAV infectious particles using Virus Isolation on cell culture ⇒ EAV VI*

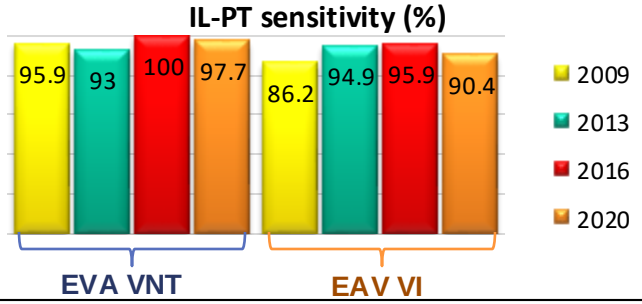
2009
19 labs
- EAV PCR:
  - 10 semen
    - 3- & 7+
- EAV VI :
  - 3 semen
    - 1- & 2+

2013
19 labs
- EAV PCR:
  - 15 semen
    - 11- & 4+
- EAV VI :
  - 5 semen
    - 3- & 2+

2016
24 labs
- EAV PCR:
  - 16 semen
    - 7- & 9+
- EAV VI :
  - 4 semen
    - 3- & 1+

2020
24 labs
- EAV PCR:
  - 15 semen
    - 3- & 12+
- EAV VI :
  - 3 semen
    - 1- & 2+





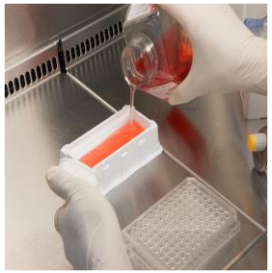
Training session

Theorical training

- Introduction regarding EVA:
  - Disease
  - Diagnostic
  - Epidemiology

On-the-job training

- Virus Neutralization test (VNT) plating and result reading
- Virus isolation on cell culture
- PCR diagnosis



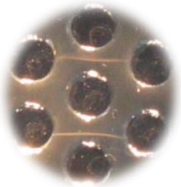
Year	2011	2014	2019	2021	2024
Nb of participants	5	3	2	3	2

2021 EVA training session

2. EQUINE INFECTIOUS ANEMIA

Interlaboratory proficiency testing

✓ EIA AGID IL-PT : serological diagnosis of EIA using the Agar Immuno-Diffusion Test



2010
24 participants
22 sera (13- & 9+)

2014
28 participants
24 sera (7- & 17+)

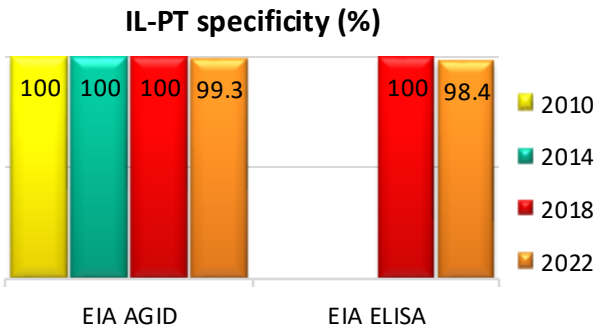
2018
32 participants
24 sera (12- & 12+)

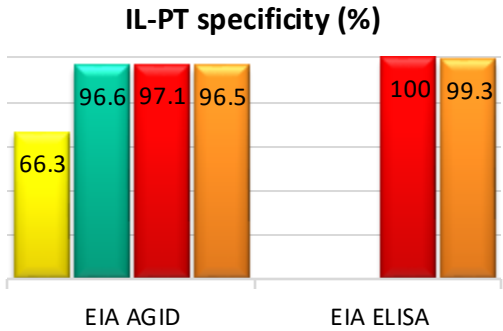
2022
24 participants
24 sera (6- & 18+)

✓ EIA ELISA IL-PT : serological diagnosis of EIA using ELISA

2018 IL-PT
32 participants
24 sera (12- & 12+)

2022 IL-PT
24 participants
24 sera (6- & 18+)





Training session

Theorical training

- Introduction regarding EIA:
  - Disease
  - Diagnostic
  - Epidemiology

Year of EIA training session	Nb of participants
2011	5
2015	4
2018	4
2023	4

On-the-job training

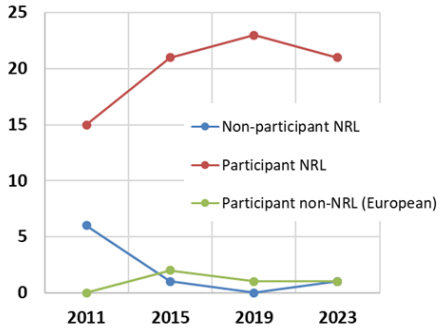
- Agar Gel ImmunoDiffusion Test : plating and result reading
- ELISA

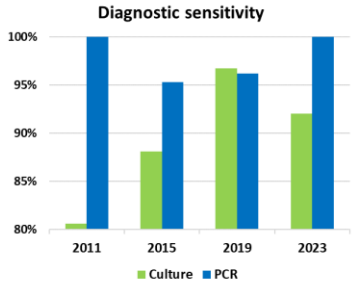
2023 EVA training session

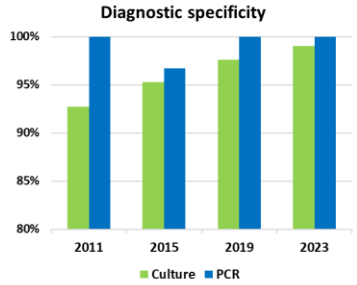
3. CONTAGIOUS EQUINE METRITIS

EURL CEM: management of a network of 22 NRLs

- **Proficiency test** organized every 4 years



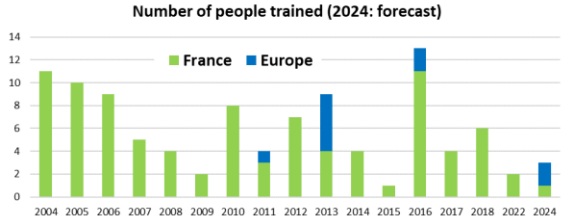




- **PCR**: >95%
- **Culture**: Sensitivity greatly depends on the selectivity & productivity of media / Specificity is progressing over time

- **Workshop** organized the same year than the PT: epidemiological review, review of changes to the CEM chapter of the WOAH Terrestrial Manual, results of the PT, latest scientific and technical advances…

- **Training** organized for the worst-performing NRLs in PT



EURL CEM highlights

- **Build up and maintain a *Taylorella* collection:** 700 strains including 10% *T. asinigenitalis*

- **MLST genotyping strains** (Duquesne et al., 2021) from NLRs and curation of the international MLST database "*Taylorella spp. | PubMLST*"

- Methodological advances to genotype *Taylorella* spp. isolates
  1. MLST: **replacement of Sanger sequencing of PCR products by whole genome sequencing**
  2. core genome MLST in development

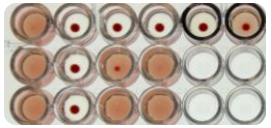
- **Observation of subspecies lineages into the *T. asinigenitalis* species** (Kozak et al., 2023)

- Current R&D on **improving bacteriological diagnosis** (Breuil et al., 2022; Duquesne et al., 2021)

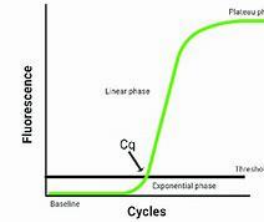
4. GLANDERS

European Laboratory Reference activities

- **Diagnosis Glanders & Melioidosis** (1st line and confirmatory)



- **Coordinating laboratory networks**
  - Interlaboratory **proficiency test: every 3 years**
  - Training sessions (*on request and for lowest-performing NRLs*)



- **Lyophilised positive glanders serum / Molecular markers**

- **International collaborations**
  - Brazil, India, Kuwait, Nepal Mongolia,….

First glanders cases detected in Nepal underscore the need for surveillance and border controls

Molecular characterization of Burkholderia mallei strains isolated from horses in Brazil (2014–2017)

A genetic variant of Burkholderia mallei detected in Kuwait: Consequences for the PCR diagnosis of glanders

Molecular epidemiology of Burkholderia mallei isolates from India (2015–2016): New SNP markers for strain tracing

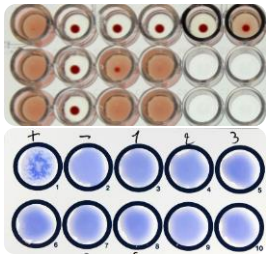
Provide a framework to ensure the reliability of official controls

5. DOURINE

European Laboratory Reference activities

- **Diagnosis** (1st line and confirmatory)

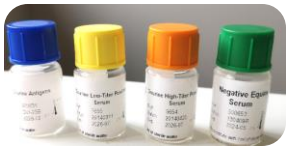
Diag test	# of tests 2023
Dourine Complement fixation test	158
CATT/T.evansi	122



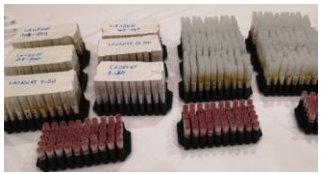
- **Coordinating laboratory networks**
  - Interlaboratory **proficiency test: every 3 years**
  - Training sessions (*on request and for lowest-performing NRLs*)
  - Bilateral comparison *(on request)*:

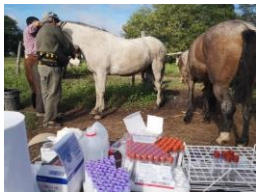
- **Reagent supply for dourine CFT**

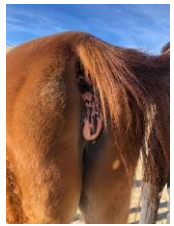
	2023	
Reagent	**# of vial**	**# of countries**
High Titer serum	31	7
Low titer serum	17	6
Negative control	16	5
Antigen	155	14



- **International epidemiological surveys**
  - Tunisia, Egypt, Argentina, Saoudi Arabia, Sri Lanka…







Provide a framework to ensure the reliability of official controls

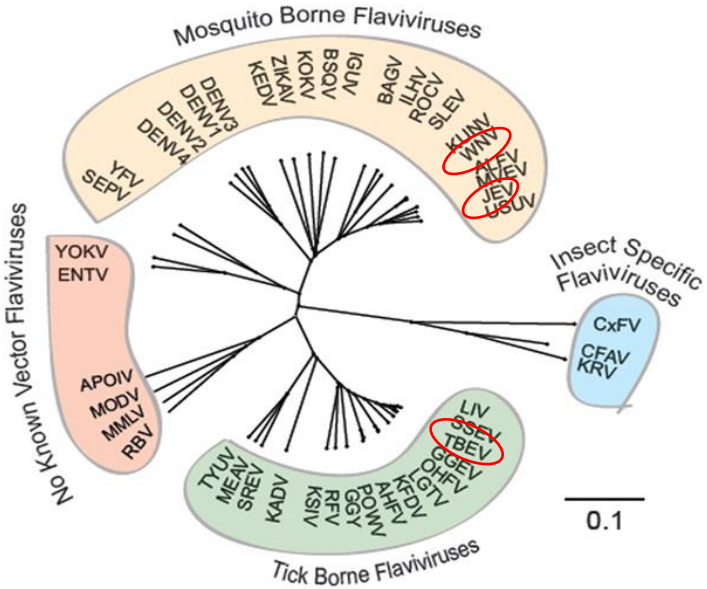
5. VIRAL ENCEPHALITIS

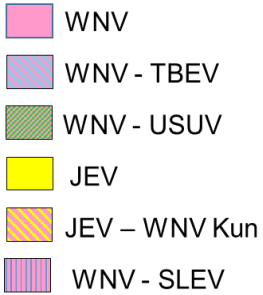
Arthropod-borne equine encephalitis viruses – Orthoflavivirus and Alphavirus

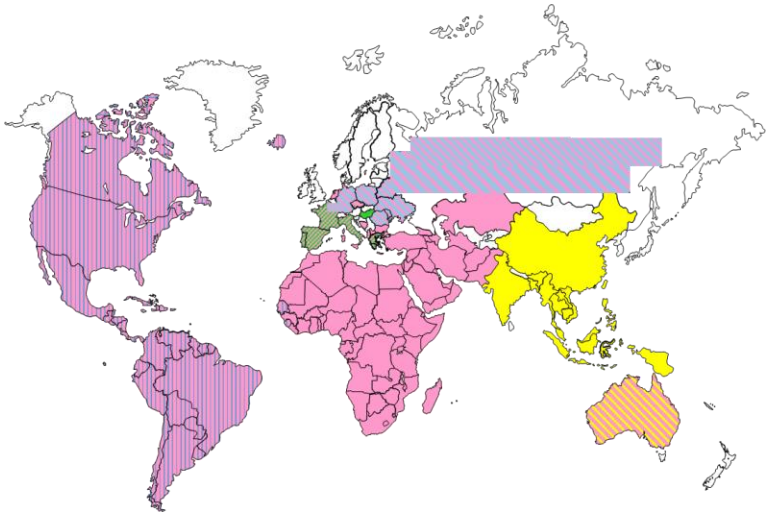
European Union Reference Laboratory for Equine Diseases

WEST NILE AND JAPANESE ENCEPHALITIS ORTHOFLAVIVIRUS

Flaviviridae family
Orthoflavivirus genus







Reemerging public and veterinary health burdens

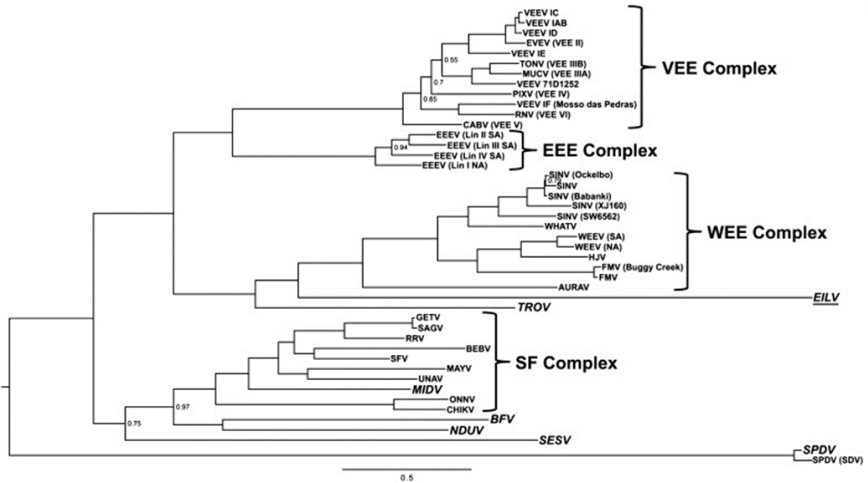
Villordo et al., *Trends microbiol.* 2016

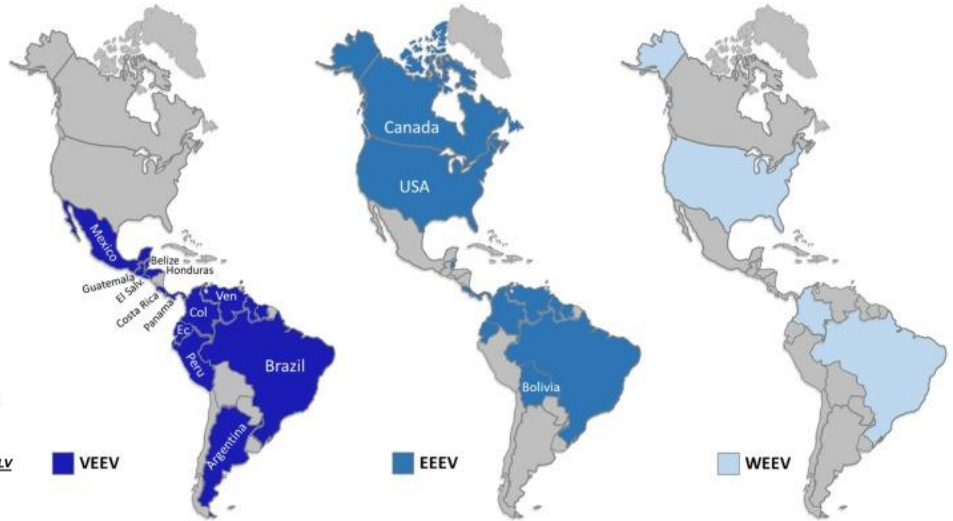
Arthropod-borne equine encephalitis viruses – Orthoflavivirus and Alphavirus

European Union Reference Laboratory for Equine Diseases

ALPHAVIRUSES: EASTERN / WESTERN AND VENEZUELA EQUINE ENCEPHALITIS VIRUS

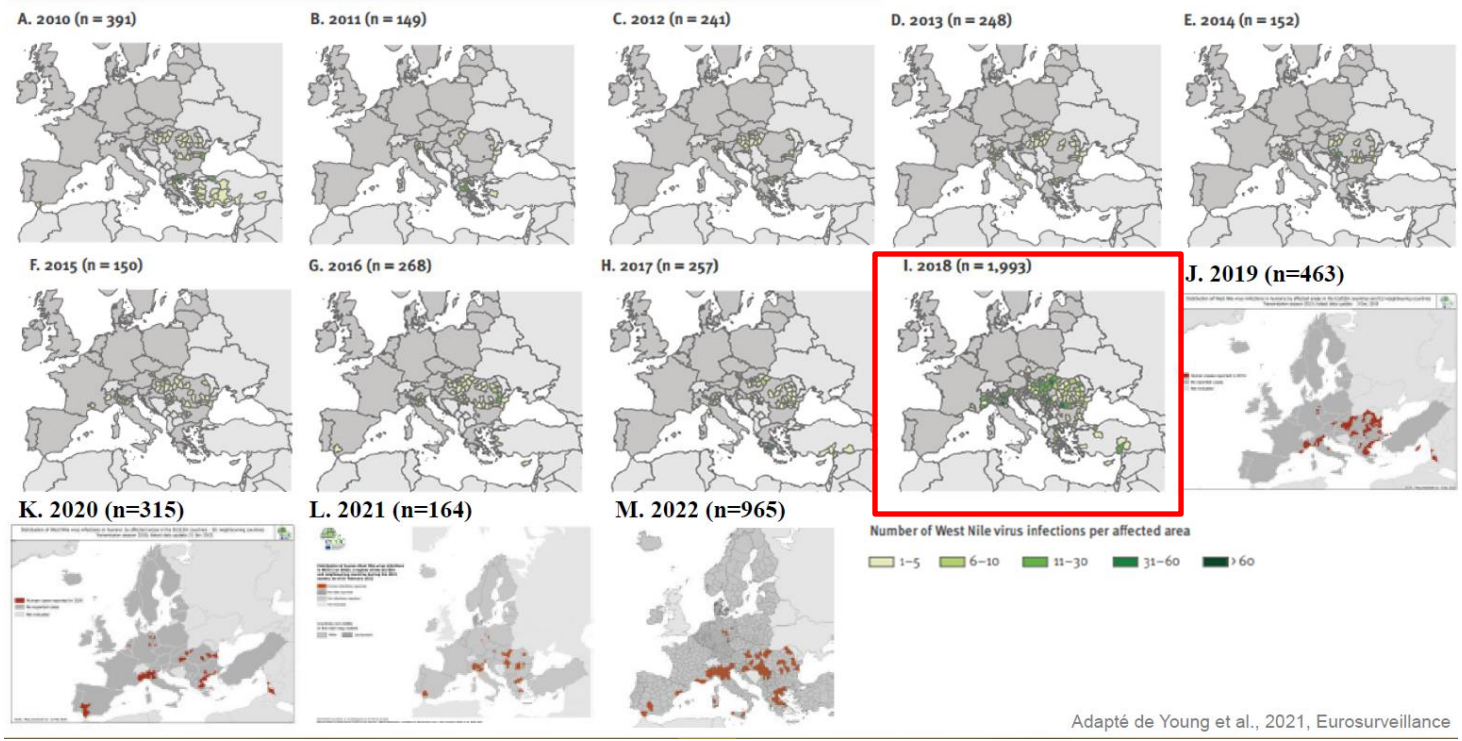
Togaviridae Family
Alphavirus genus





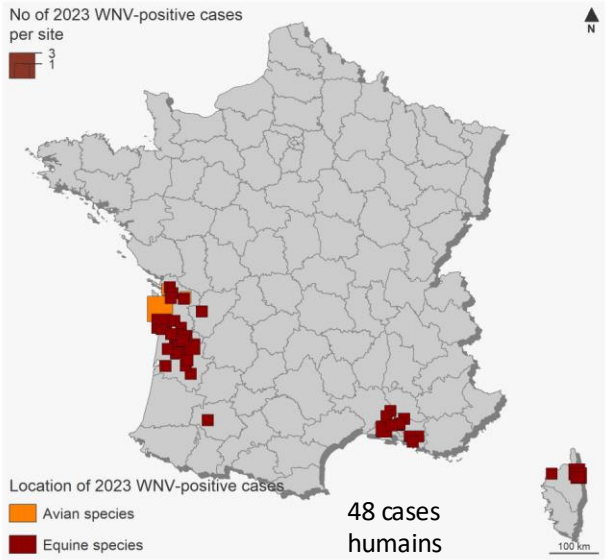
Equine arboviroses at risk of introduction into Europe

Guzmán-Terán C, et al. 2020

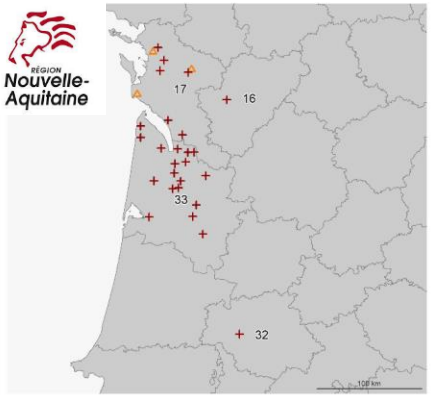


Adapté de Young et al., 2021, Eurosurveillance

2023: exceptional circulation of WNV in France



48 cases humains



Transmission 2023:

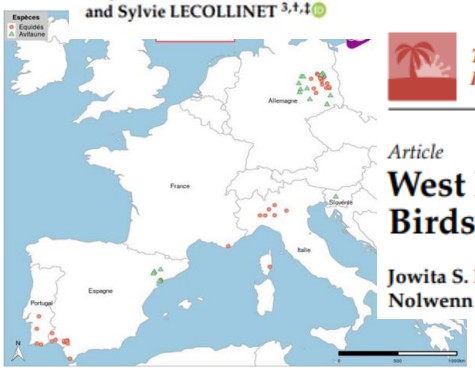
49 equine cases with 32 in Nouvelle-Aquitaine

7 avian cases in Nouvelle-Aquitaine

Epidemiosurveillance of WNV in Europe

European Union Reference Laboratory for Equine Diseases

➢ Expansion of WNV geographical distribution to northern and southwestern Europe



(source : Commission Européenne ADIS le 20/12/2021)

(source : Commission Européenne ADIS on 19/12/2022 and ECDC on 26/12/2022)

(source: Commission Européenne ADIS le 6/11/2023 et ECDC le 25/10/023)

viruses — MDPI

Article

Detection of West Nile Virus Lineage 2 in Eastern Romania and First Identification of Sindbis Virus RNA in Mosquitoes Analyzed using High-Throughput Microfluidic Real-Time P

Luciana Alexandra CRIVEI [1,*], Sara MOUTAILLER [2], Gaëlle GONZALEZ [3], Steeve LOWENSKI [3], Ioana Cristina CRIVEI [1], Daniela POREA [1], Dragoș Constantin ANITA [1], Ioana Alexandra RATOI [1], Stéphan ZIENTARA [3], Luanda Elena OSLOBANU [1], Alexandru TOMAZATOS [4], Gheorghe SAVUTA [1,†] and Sylvie LECOLLINET [3,†,‡]

Tropical Medicine and Infectious Disease

Article

West Nile Virus Lineage 2 in Free-Living *Corvus cornix* Birds in Poland

Jowita S. Niczyporuk [1,*], Wojciech Kozdrun [1], Agnieszka Czujkowska [2], Yannick Blanchard [3], Mariteragi Helle [4], Nolwenn M. Dheilly [4] and Gaelle Gonzalez [4]

pathogens — MDPI

Article

Contrasted Epidemiological Patterns of West Nile Virus Lineages 1 and 2 Infections in France from 2015 to 2019

Cécile Beck [1,*], Isabelle Leparc Goffart [2,3], Florian Franke [4], Gaelle Gonzalez [1], Marine Dumarest [1], Steeve Lowenski [1], Yannick Blanchard [5], Pierrick Lucas [5], Xavier de Lamballerie [3], Gilda Grard [2,3], Guillaume André Durand [2,3], Stéphan Zientara [1], Jackie Tapprest [6], Grégory L'Ambert [7], Benoit Durand [8], Stéphanie Desvaux [9,†] and Sylvie Lecollinet [1,†]

Example of research activities: Orthoflavivirus – Hosts interaction and Pathogenicity

- Identification of molecular determinants of WNV L1 virulence in mammalian and avian hosts

International Journal of *Molecular Sciences*

MDPI

Très virulente

Review

Molecular Determinants of and Pathogenesis in Verteb

Forte

Lise Fiacre [1,2,3], Nonito Pagès [2,3], Emmanuel Albi
Sylvie Lecollinet [1,*,†] and Gaëlle Gonzalez [1,†]

→ fo

viruses

MDPI

Article

Evaluation of NS4A, NS4B, NS5 and 3′UTR Genetic Determinants of WNV Lineage 1 Virulence in Birds and Mammals

[illegible] [1,2,3,†], Steeve Lowenski [1,†], Céline Bahuon [1], Marine Dumarest [1], Bénédicte Lambrecht [4], Maha Dridi [4], [illegible] [1], Stéphan Zientara [1], Miguel-Ángel Jiménez-Clavero [5,6], [illegible] and Sylvie Lecollinet [1,*,‡]

Front Microbiol. 2024 Jan 17:14:1324069. doi: 10.3389/fmicb.2023.1324069. eCollection 2023.

Different viral genes modulate virulence in model mammal hosts and *Culex pipiens* vector competence in Mediterranean basin lineage 1 West Nile virus strains

Lise Fiacre [1 2], Antoine Nougairède [3], Camille Migné [1], Maëlle Bayet [2], Maxime Cochin [3], Marine Dumarest [1], Teheipuaura Helle [1], Antoni Exbrayat [4], Nonito Pagès [2], Damien Vitour [1], Jennifer P Richardson [1], Anna-Bella Failloux [5], Marie Vazeille [5], Emmanuel Albina [4], Sylvie Lecollinet [# 2], Gaëlle Gonzalez [# 1]

- Identific transmission, its virulence in vertebrate hosts

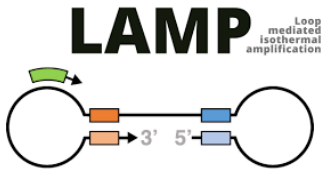
European Union Reference Laboratory for Equine Diseases

EURL Arthropod-borne equine encephalitis viruses: management of 25 NRLs

- **First line and confirmatory diagnosis** of WNV and exotic equine encephalitis viruses (JEV, V/E/WEEV)

- **Coordinating a network of 25 NRLs**
  - Interlaboratory **proficiency test: every 3 years**
  - **Training sessions** (on request and for lowest-performing NRLs)

- Optimization and development of **high-throughput detection tools**



- **International collaborations**
- **Reagent supply for NRLs (sera, RNA, etc)**

European Union Reference Laboratory for Equine Diseases

Activities 2024

European Union Reference Laboratory for Equine Diseases

Stephan Zientara
Directeur

Gaëlle Gonzalez
Adjointe au Directeur

José-Carlos Vallé-Casuso
Anémie infectieuse équine
Artérite virale équine

Laurent Hébert
Dourine/Surra

Sandrine Petry
Métrite contagieuse équine

Karine Larouceau
Morve/ Melioïdose

Gaëlle Gonzalez
WNV et encéphalites équines exotiques

❑ REFERENCE ACTIVITIES JANUARY-JUNE 2024

- Training on EIAV
  (12 to 14 March 2024 – Dozulé (Normandy)

  WAGENINGEN UNIVERSITY & RESEARCH NRL The Netherlands: 3 people

- Organisation of **2 PTs**: **Glanders/Melioidosis** and **Equine viral arteritis**
  - ➢ Samples panels sent in July

- **Diagnosis**
  - ➢ WEEV in Argentina: collection of horse samples of infected horses – characterization of the infection
  - ➢ Dévelopment of an VEEV ELISA

❑ OLYMPIC GAMES of PARIS 2024: equestrian OG

European Union Reference Laboratory for Equine Diseases

PARIS 2024

Versailles castle

350 horses

- **Operational committee** ANSES : participation of Stéphan Zientara and José-Carlos Vallé Casuso
- **Investigation of equine diseases:** WNV/VEEV – CEM – EIA – EVA - necropsy

❑ Organisation of wokshops EAV and Glanders/Melioidosis: 30 September and 4 October 2024

Deauville

EAV: 30 September 2024

Glanders- melioïdosis: 4 October 2024

NORMANDY

IEIDCXII 2024

International Equine Infectious Diseases Conference

Deauville, France

30th September – 4th October 2024

Local organisation committee

✓ Conferences:
S. Zientara, K. Laroucau

✓ Conferences Practitionners day:
S. Zientara, JC Vallé-Casuso, G. Gonzalez

✓ Session « Meet the experts » : implication EURL + NRLs participants to workshops

anses

Thank you for your attention

20 mn + 5 mn

The role of the EU Reference Lab on Equine Diseases: an example of a regional approach for specialised diagnostic capacities and vaccine development (25 min): one of the goals of this workshop is to encourage the region to set up a regional network of Reference Labs, labs with specific capacity and to assist each other in the region to build capacity. It would therefore be very important that you explain the modalities how your network operates, e.g. how it was set up, the schedule of meetings, the schedule for PTs, the services the network offers, how it supports its partners, how it is funded etc. if you could make it a convincing “blue print” for the Asia labs to take as shining example, that would be wonderful!

RÉPUBLIQUE FRANÇAISE
Liberté
Égalité
Fraternité

anses

Few comments on the ppt on ANSES as EURL:

71 slides for 25 min is definitely too much, even if some only show a picture!

What about the budget after 2017? I think it would be more interesting to show budget availability over a longer time period rather than just 2 years in the past
On slide 21 you could mention that all of your colleagues have assisted in organising WOAH webinars on equine diseases that can be found on our website: https://rr-asia.woah.org/en/projects/horse-movement/international-horse-movement-in-asia-and-the-pacific/

Slide 24: the articles quoted here are Articles of which agreement/regulation/document? Not clear!
Slides 37 – 65 on the different diseases: please shorten, particularly on those diseases that are covered in the webinar (glanders, EIA, dourine/surra). It would be a pity if you have to rush through rather than to have few slides and have time to fully explain them
Please add something on how non-EU countries can participate in ANSES activities, particularly in PTs and training!

African Horse Sickness

Global importance for trade; standards and novel diagnostic tests (WOAH manual); existing vaccines, vaccine development

Stéphan Zientara

ANSES-Animal Health Laboratory
European Union reference laboratory on equine diseases
UMR ANSES/INRAE/ENVA
WOAH reference laboratory on EHDV, FMDV
EU reference laboratory on FMDV

Maisons-Alfort, France

World Organisation for Animal Health
Founded as OIE

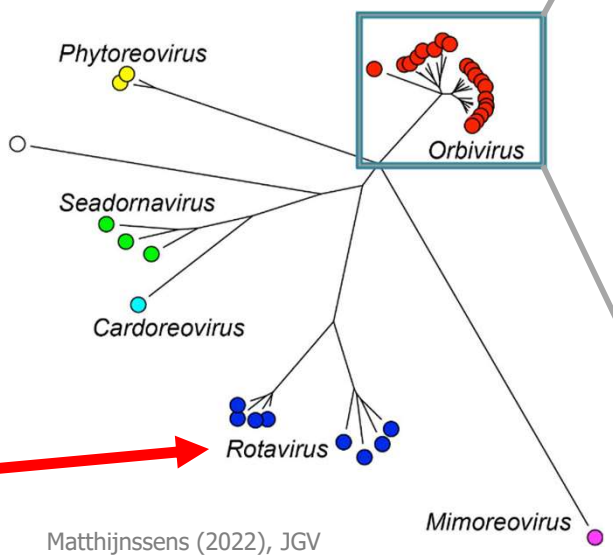
European Commission

European Union Reference Laboratory for Equine Diseases

anses

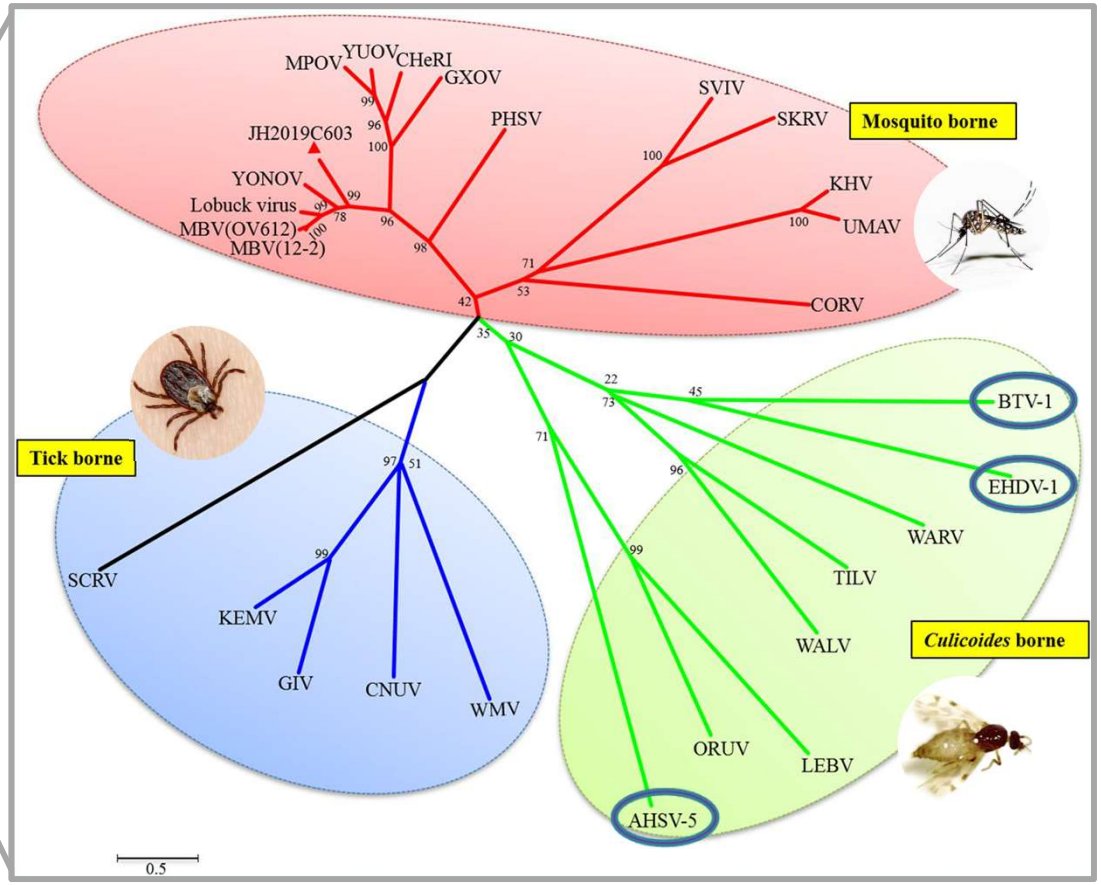
Orbiviruses

Family *Sedoreoviridae*



Matthijnssens (2022), JGV

Genus *Orbivirus*: 22 species

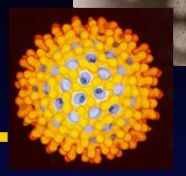


Yang (2021), Virus genes

World Organisation for Animal Health
Founded as OIE

- 3 important diseases
  - **BTV:** Bluetongue virus
  - **EHDV:** Epizootic hemorragic disease virus
  - **AHSV:** African horse sickness virus

AHSV/BTV/EHDV



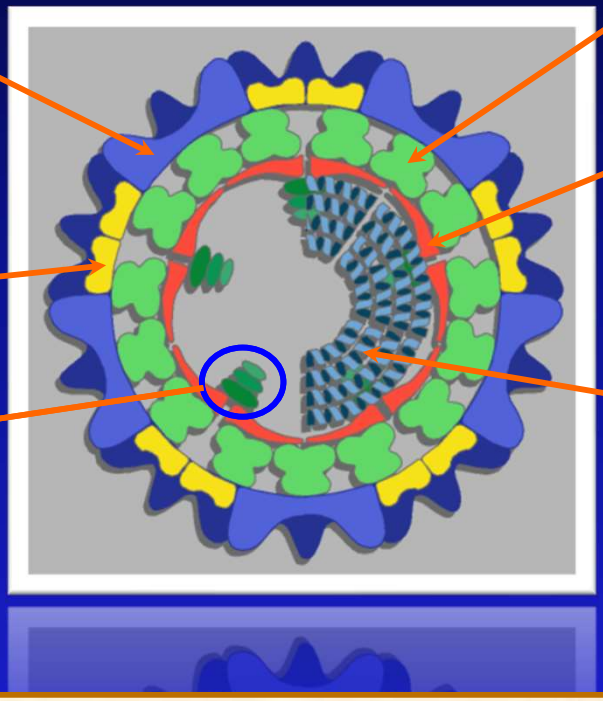
- Family *Sedoreoviridae* ; Genus *Orbivirus*

VP2 :
- binding to the receptor, virus entry
- neutralisation, type specificity

VP7 :
- conserved among serotypes

VP3 :

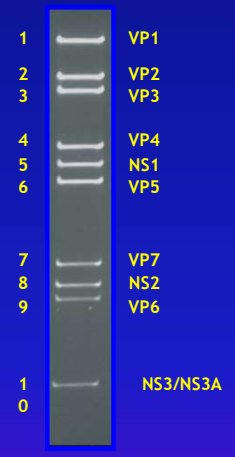
VP5 : - pénétration



transcription complex :
- **VP1** : RNA polymerase
- **VP4** : capping enzyme
- **VP6** : RNA helicase

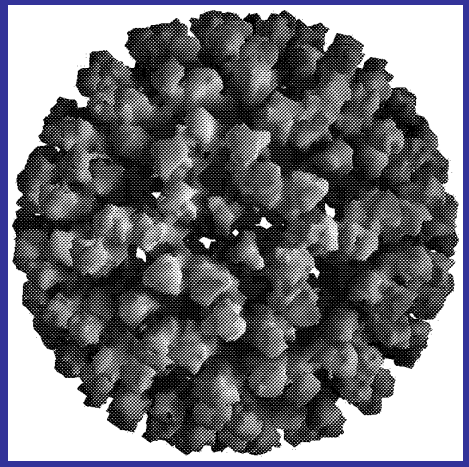
virus genome:

10 segments dsARN



5 non structural proteins (NSP):
- NS1 : pathogenesis and morphogenesis of BTV (tubules)
- NS2 : RNA encapsidation
- NS3/3A : virion release
- NS4 : determinant factor of virulence
- NS5 : discovery 2015

Orbivirus



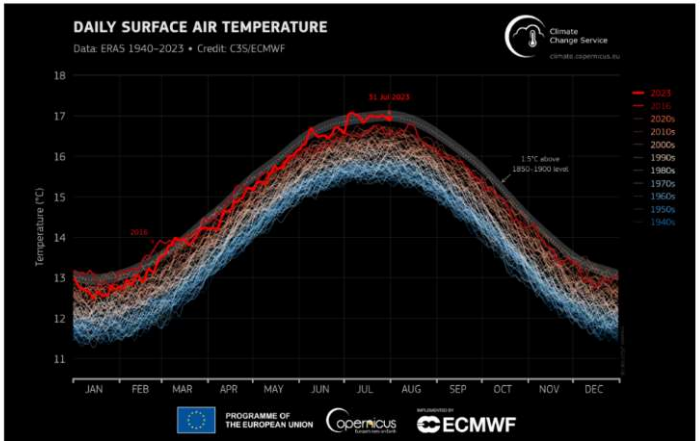
reassortment

36 serotypes BTV
9 serotypes AHSV
7 serotypes EHDV

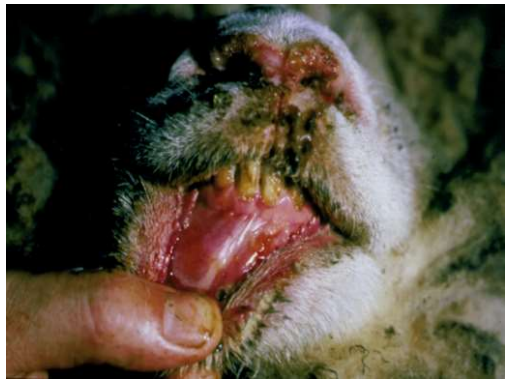
Vectors: *Culicoides*
***(imicola, bolitinos**, obsoletus,…)*

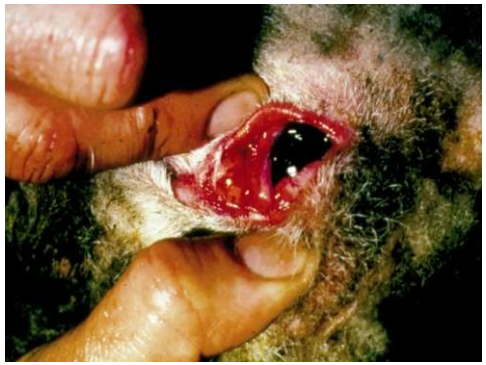
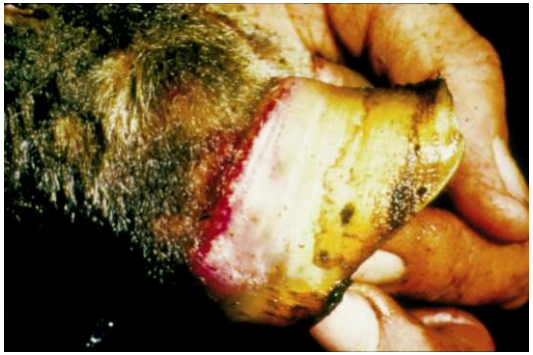
July 2023 sees multiple global temperature records broken

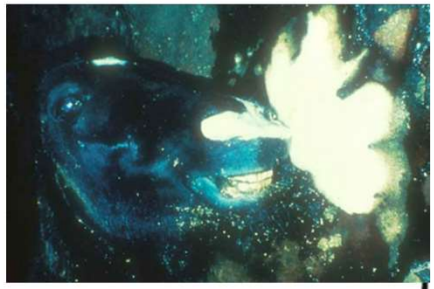
Following the hottest June on record and a series of extreme weather events, including heatwaves in Europe, North America and Asia, and wildfires in Canada and Greece, ERA5 data from the Copernicus Climate Change Service (C3S) show that July 2023 was the hottest month on record globally and broke several records within the month.*



Viral Hemorrhagic Fevers



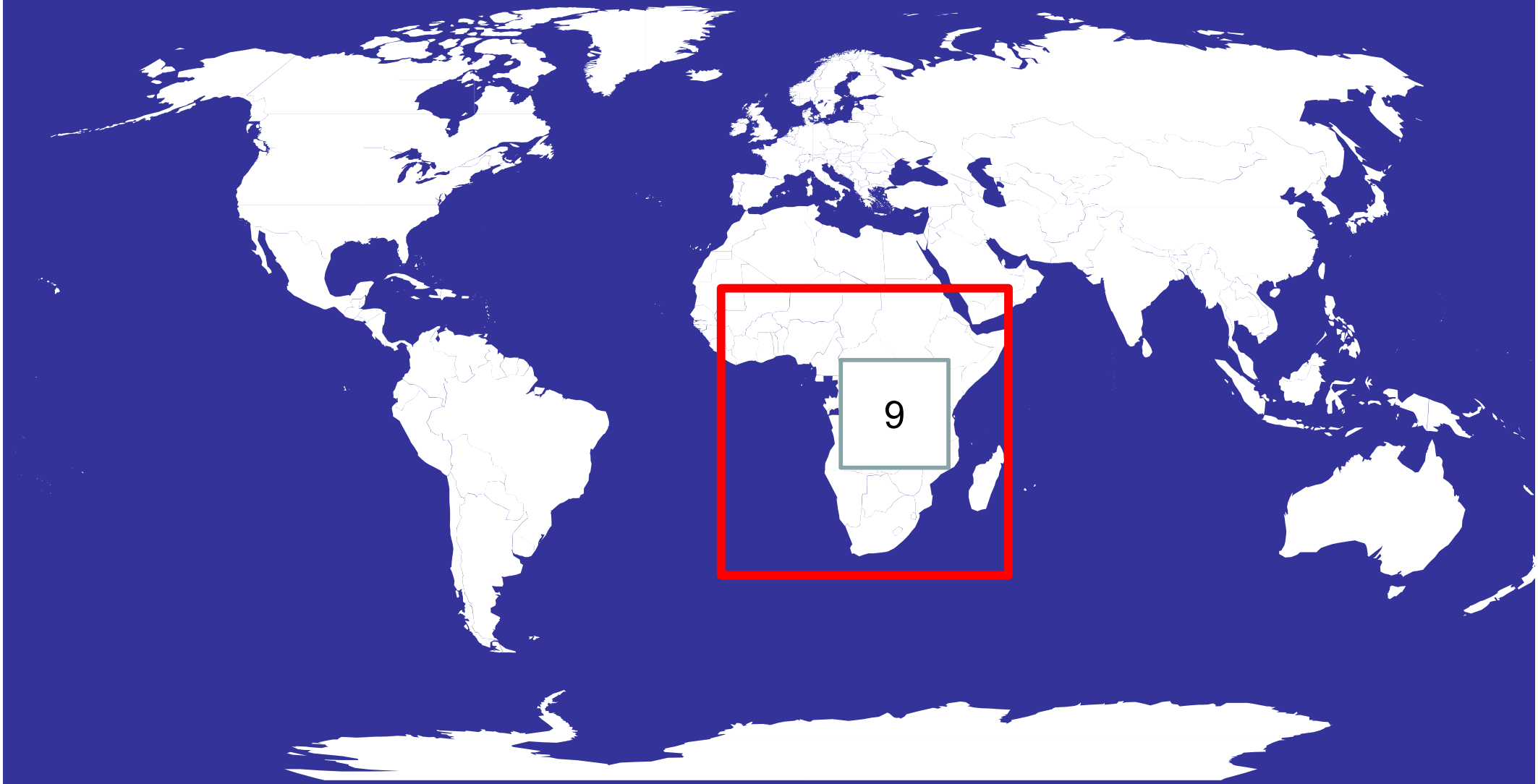
BTV

AHSV

AHS

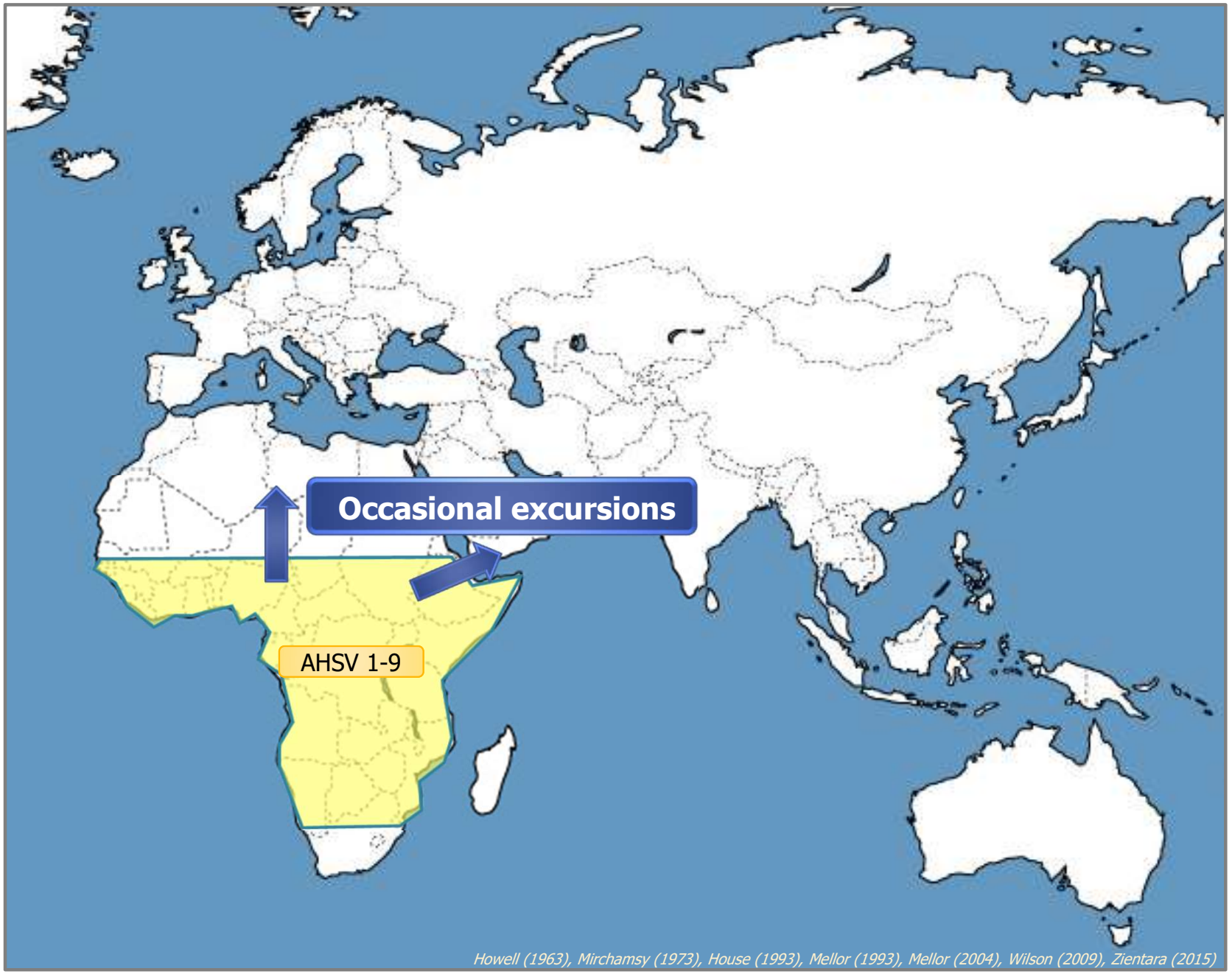
Courtesy Alan Guthrie, SA

Distribution of AHS



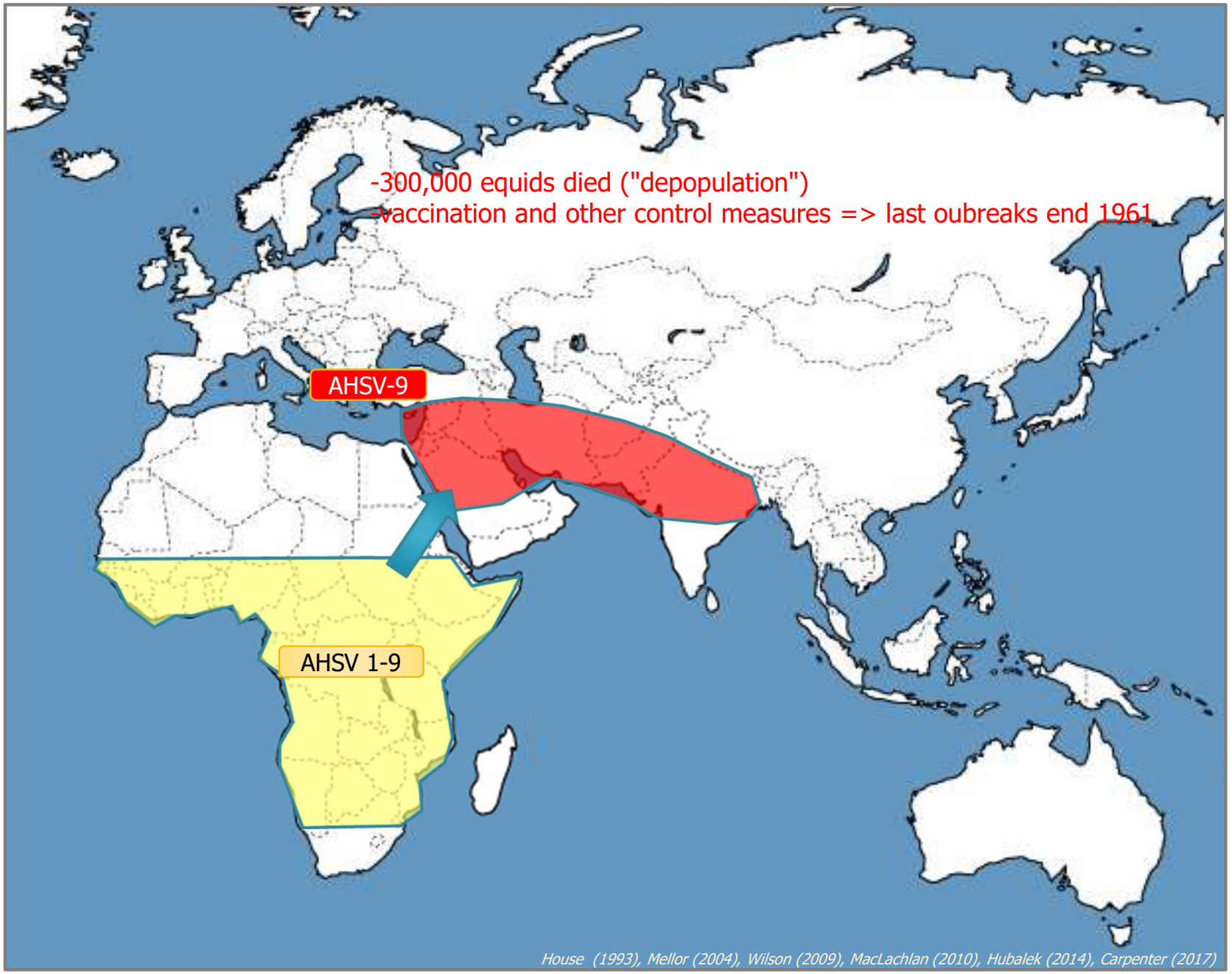
Why is AHS important for trade?

AHS distribution in the mid-20th century



AHS distribution: mid to end 20th

1959-61



AHS distribution: mid to end 20th

1965-66

- 77 equidae died or were clinically affected and 600 contacts were slaughtered.
- More than 600,000 in the buffer area were progressively vaccinated,
- .

Cadiz Province, Oct. 1966

Morocco, 1965

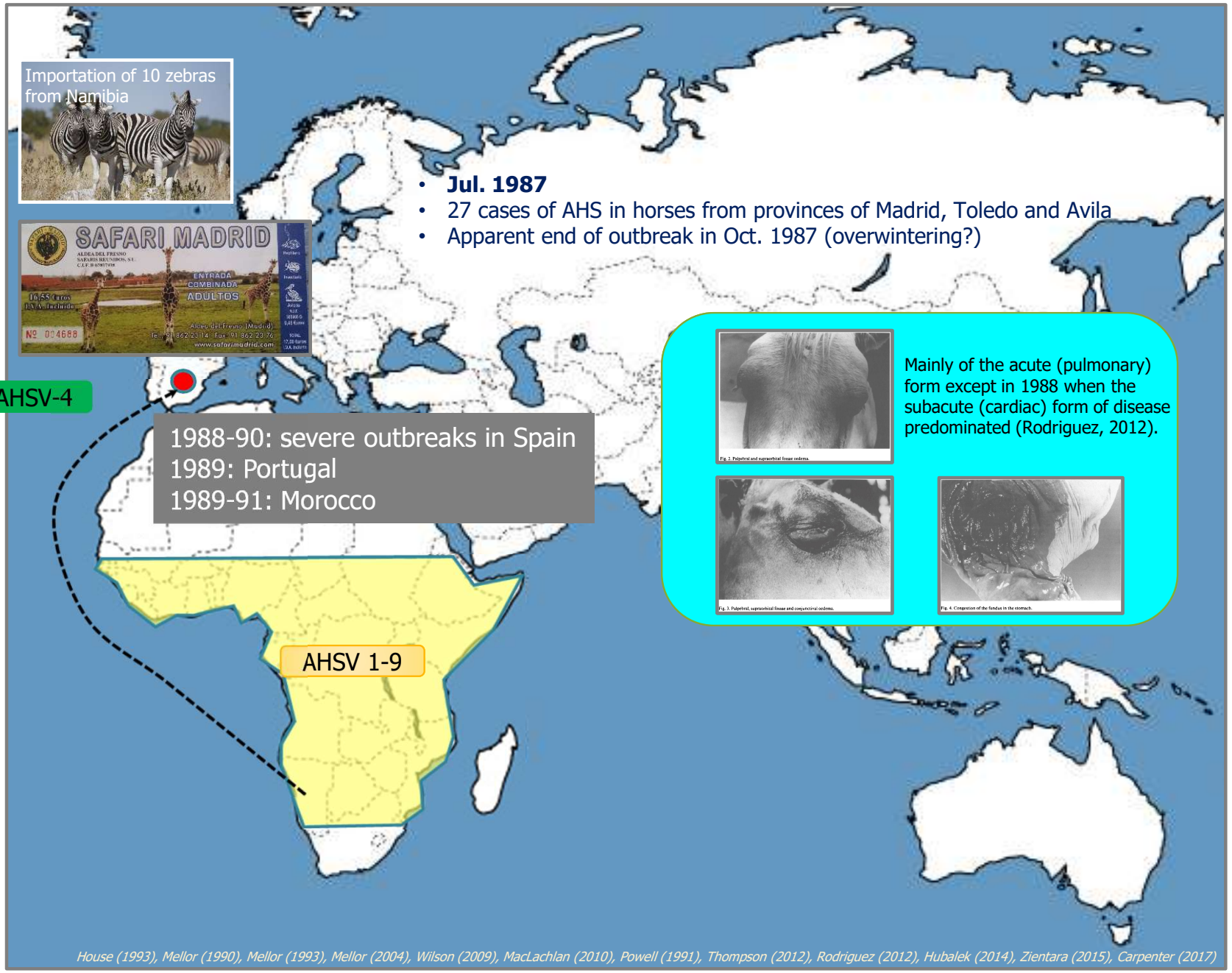
AHSV-9

AHSV 1-9

House (1993), Mellor (1993), Mellor (2004), Wilson (2009), MacLachlan (2010), Hubalek (2014), Zientara (2015), Carpenter (2017)

AHS distribution: mid to end 20th

1987-91

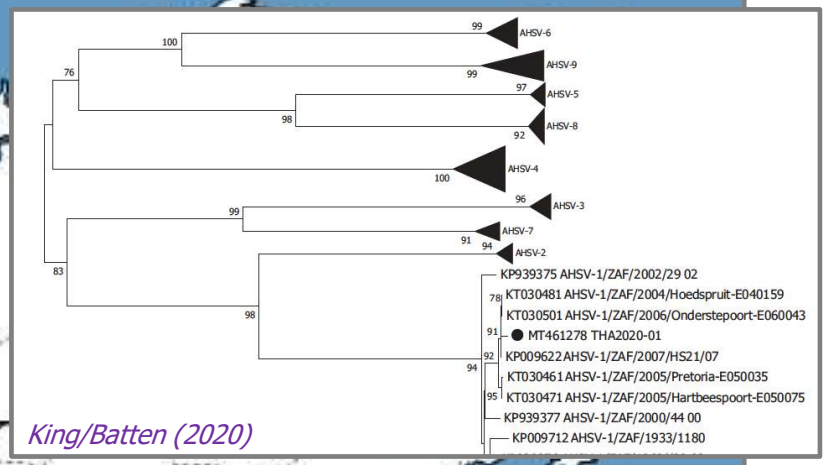


House (1993), Mellor (1990), Mellor (1993), Mellor (2004), Wilson (2009), MacLachlan (2010), Powell (1991), Thompson (2012), Rodriguez (2012), Hubalek (2014), Zientara (2015), Carpenter (2017)

AHS distribution: end 20th - 21st century

2020

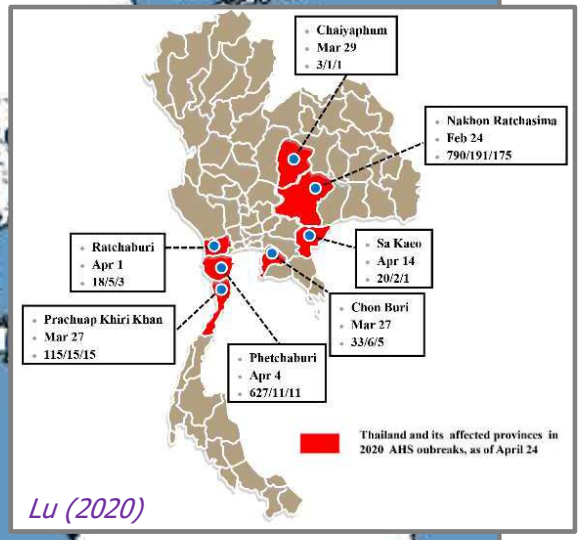
- **Feb. 24** Nakhon Ratchasima province, 175/191 deaths.
- **Mar.** blood samples from 4 horses showing AHS clinical signs sent to the Pirbright Institute. AHSV confirmed by rtRT-PCR and virus isolation. Sequencing and serotyping => **type 1**.
- **Apr. 24**: 6 other outbreaks in different provinces, 211/231 deaths.
- Control measures including mass **vaccination** campaign (**LAV**).
- **Aug. 6**: AHSV (+) horses in Malaysia (WOAH)



King/Batten (2020)

AHSV-1

AHSV 1-9



Lu (2020)

Lu (2020), King (2020)

John Grewar, SA

Diagnosis

Laboratory diagnosis

Serology ← Serum

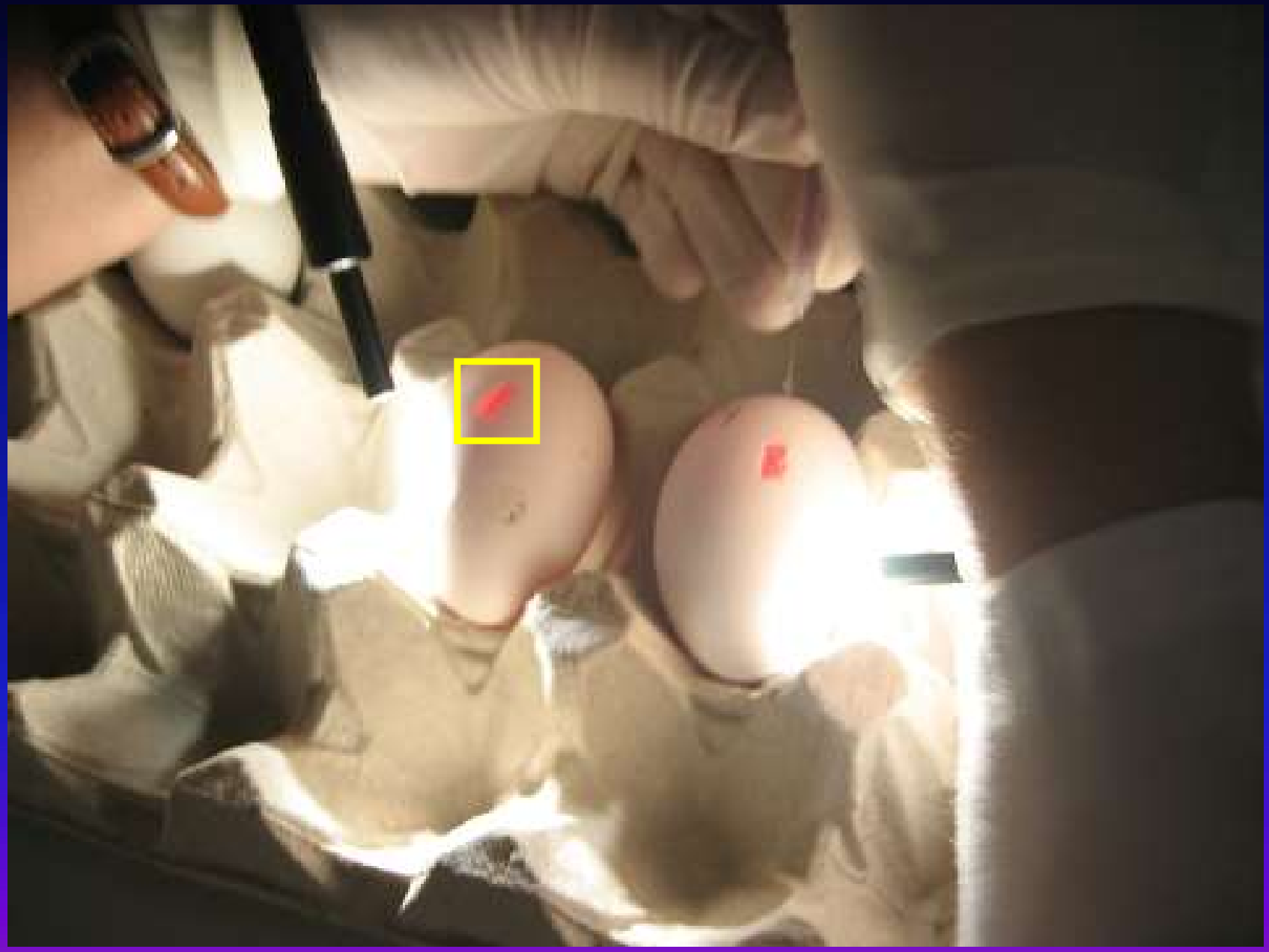
- AGID: group specific Ab
- ELISA : group specific Ab
- IFI
- Seroneutralisation: type specific Ab

Virology ← spleen, blood (EDTA)

- Virus isolation
  - embryonated chicken eggs (IV)
  - BHK 21, Vero, KC cells
  - brains of newborn mice
- Virus neutralisation: serotype

RT-PCR
rt-RT-PCR

INOCULATION OF AHSV TO ECE



embryos negative infected (haemorrhages)

Arch Virol Suppl. 1998:14:317-27. doi: 10.1007/978-3-7091-6823-3_28.

Use of reverse transcriptase-polymerase chain reaction (RT-PCR) and dot-blot hybridisation for the detection and identification of African horse sickness virus nucleic acids

S Zientara [1], C Sailleau, S Moulay, C Crucière, M el-Harrak, W W Laegreid, C Hamblin

viruses

MDPI

Article

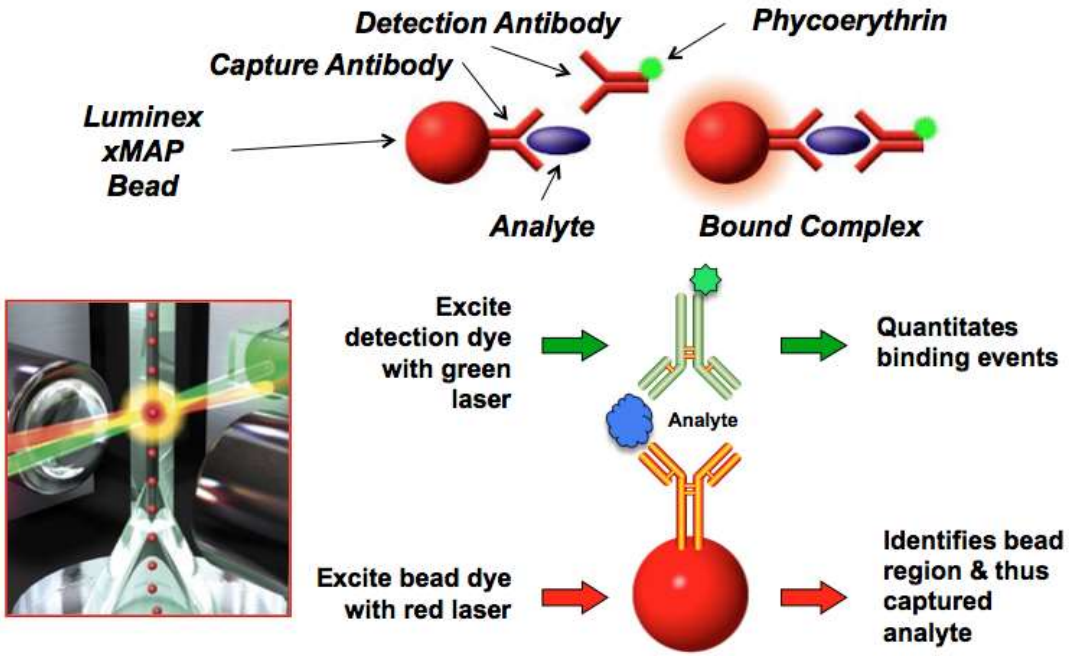
Development and Validation of Three Triplex Real-Time RT-PCR Assays for Typing African Horse Sickness Virus: Utility for Disease Control and Other Laboratory Applications

Rubén Villalba [1], Cristina Tena-Tomás [2], María José Ruano [1], Marta Valero-Lorenzo [1], Ana López-Herranz [1], Cristina Cano-Gómez [1] and Montserrat Agüero [1,*]

› Transbound Emerg Dis. 2016 Aug;63(4):353-9. doi: 10.1111/tbed.12503. Epub 2016 Apr 19.

Development of a Luminex–Based DIVA Assay for Serological Detection of African Horse Sickness Virus in Horses

A Sánchez-Matamoros [1 2 3], E Nieto-Pelegrín [1 2], C Beck [4], B Rivera-Arroyo [1 2], S Lecollinet [4], C Sailleau [4], S Zientara [4], J M Sánchez-Vizcaíno [1 2]



B. DIAGNOSTIC TECHNIQUES

Table 1. *Test methods available for the diagnosis of African horse sickness and their purpose*

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification[1]						
Real-time RT-PCR	+	+++	+	+++	++	–
Agarose gel-based RT-PCR	–	+	+	++	+	–
Virus isolation	–	++	–	+++	–	–
Detection of immune response						
ELISA (serogroup specific based on VP7)	+++	++	++	++	+++	++
CFT	+	+	+	+	+	+
VN	+	+	–	+	+	+++

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose; n/a = not applicable.

Although not all of the tests listed as category +++ or ++ have undergone formal validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

RT-PCR = reverse-transcription polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay; VN = virus neutralisation; CFT = complement fixation test.

vaccination

Vaccination

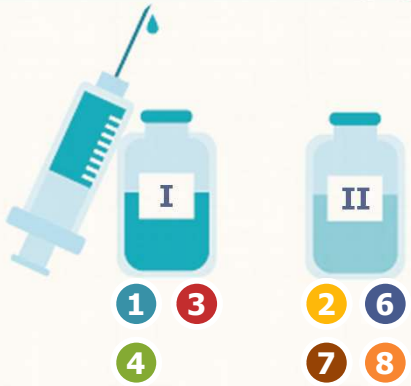
Emergency vaccination is an important tool for protecting equine populations and to fight against the spread of the disease

L.A.V. – Live-attenuated vaccines (or modified-live vaccines, MLV)

Live-attenuated vaccines

Polyvalent LAVs

Thailand (2020)



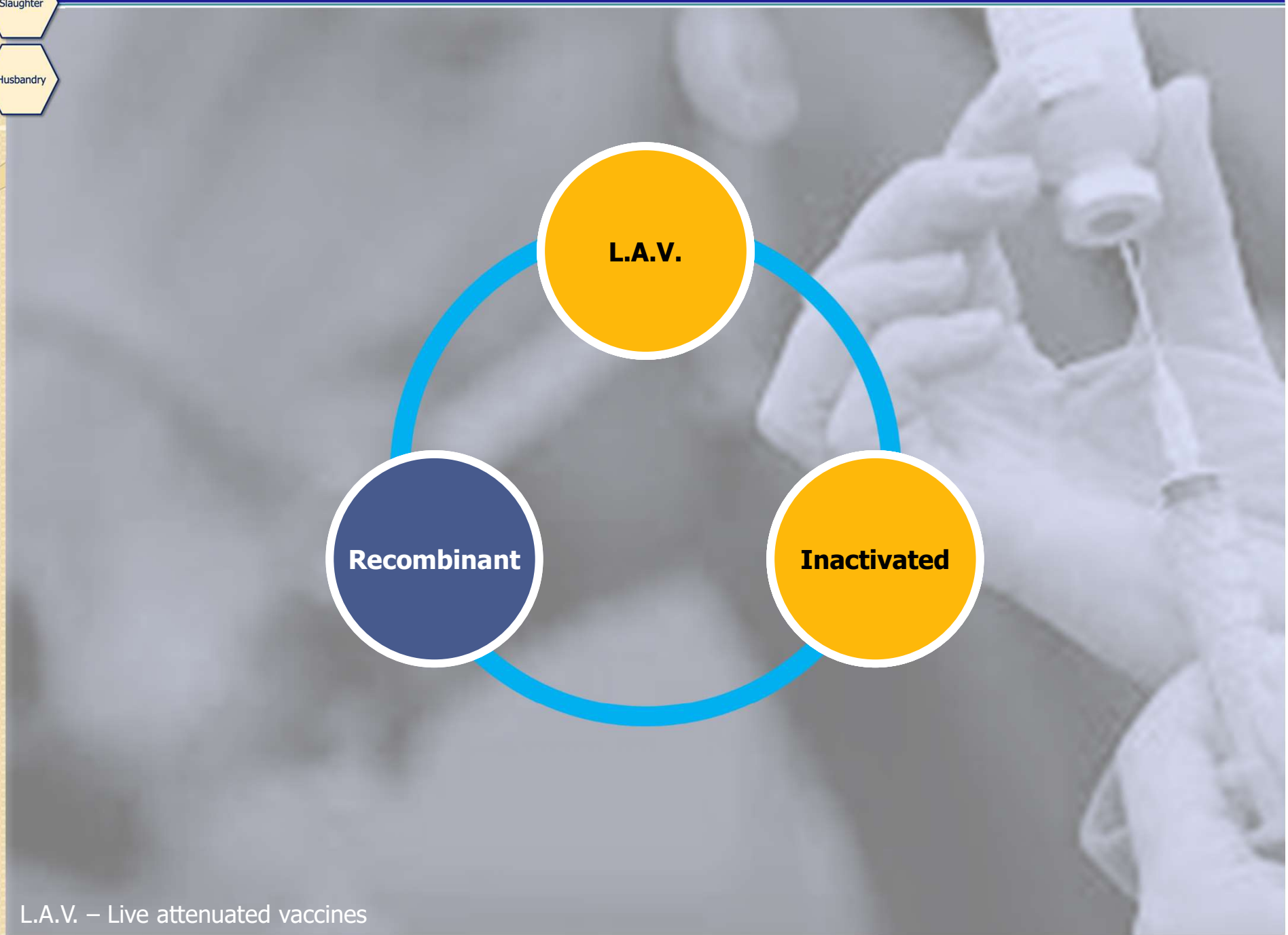
But…

Need for alternatives vaccines

- Possible reversion to virulence
- Few standard formulations
- Variable protection
  - Viraemia
  - Not DIVA
  - No license in Europe
- Transmission by vectors
- Reassortments with field strains

Powell (1991), MacLachlan (2007), Von Teichman (2010), Verwoerd (2012), Zientara (2015), Robin (2016), Carpenter (2017), Dennis (2019)

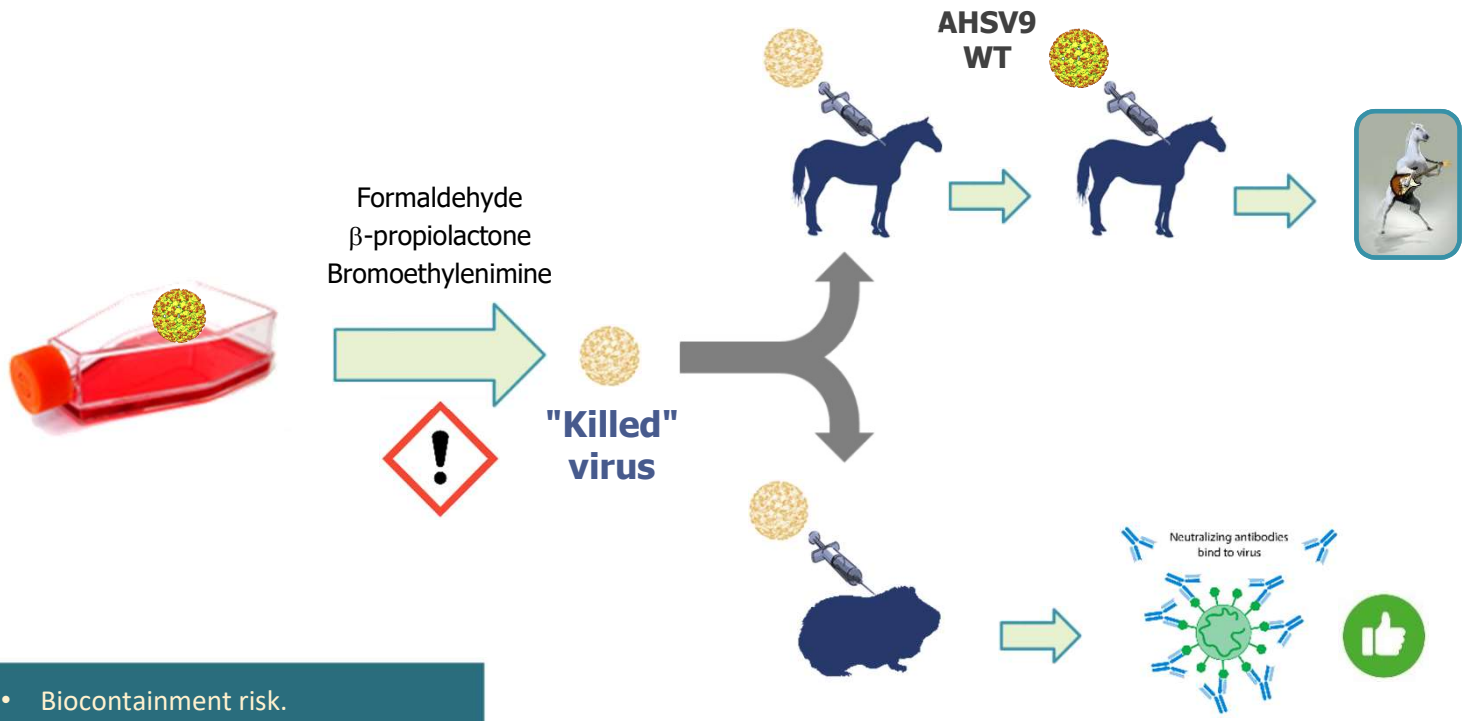
Vaccination



L.A.V. – Live attenuated vaccines

Inactivated vaccines

Mirchamsy (1968), Weyer (2013), Lelli/Pini (2013), Van Rijn (2020)



- Biocontainment risk.
- Several inoculations.
- Expensive.
- Risk of uncomplete inactivation.
- Not DIVA.

- "***Equipest***®" (Rhône Mérieux) used during the 1987-1991 AHSV-4 epidemic.
- No inactivated vaccine commercially available today.

RESEARCH ARTICLE Open Access

Immune response of horses to inactivated African horse sickness vaccines

Marina Rodríguez[1*], Sunitha Joseph[1], Martin Pfeffer[2], Rekha Raghavan[1] and Ulrich Wernery[1]

Mellor (2004), Dennis (2019)

Vaccination

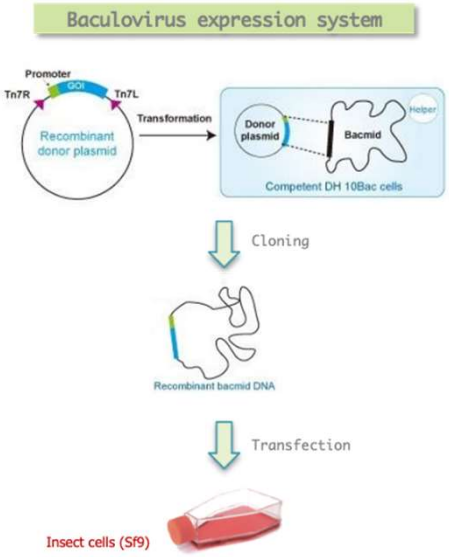
- Subunit
- Virus-like particles
- DNA
- Virus-vectored
- Reverse genetics

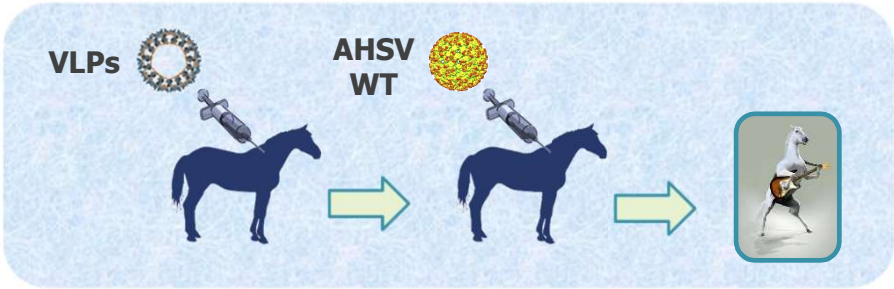
L.A.V. – Live attenuated vaccines

VLPs vaccines

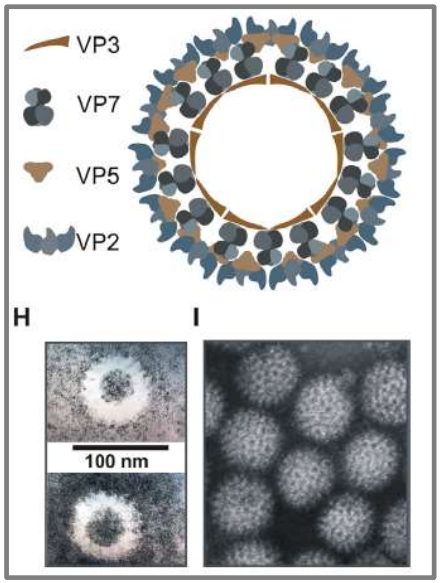
Du Plessis (1998), Scanlen/van Dijk (2002), Kanai (2013), Maree (2016)

P. Roy





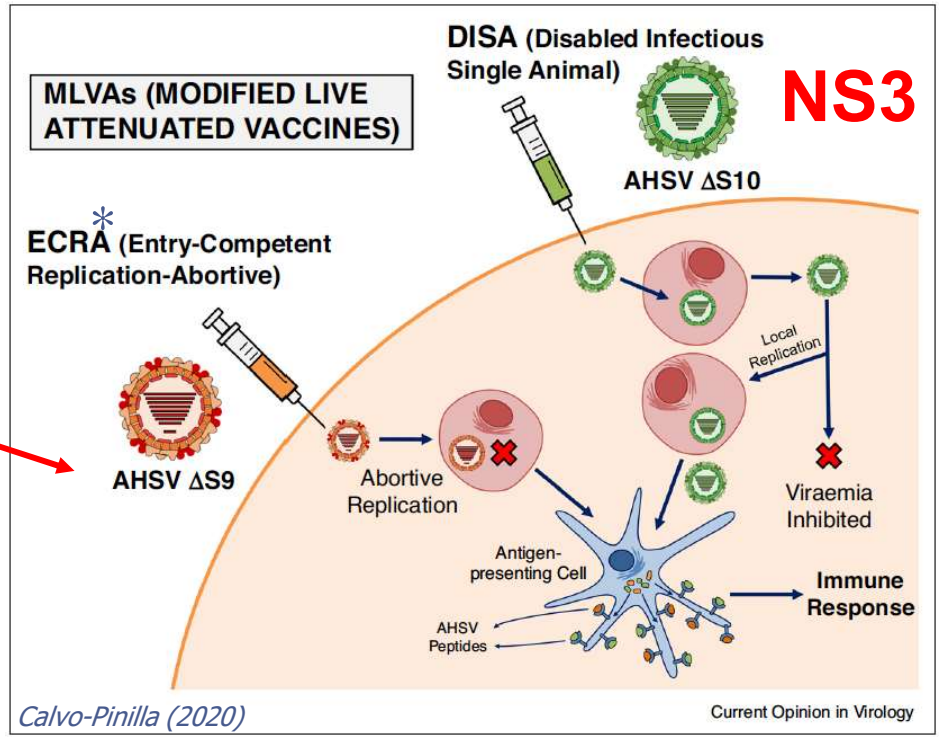
Virus-like particles



- Self-adjuvanting properties
- Highly immunogenic
- DIVA compliant
- No risk of reversion to virulence nor reassortment
- Baculo growth under sterile conditions
- High cost media

AHSV vaccines based on reverse genetics

VP6



Calvo-Pinilla (2020)

› Vaccine. 2021 May 27;39(23):3161-3168. doi: 10.1016/j.vaccine.2021.04.034. Epub 2021 May 3.

Entry-competent-replication-abortive African horse sickness virus strains elicit robust immunity in ponies against all serotypes

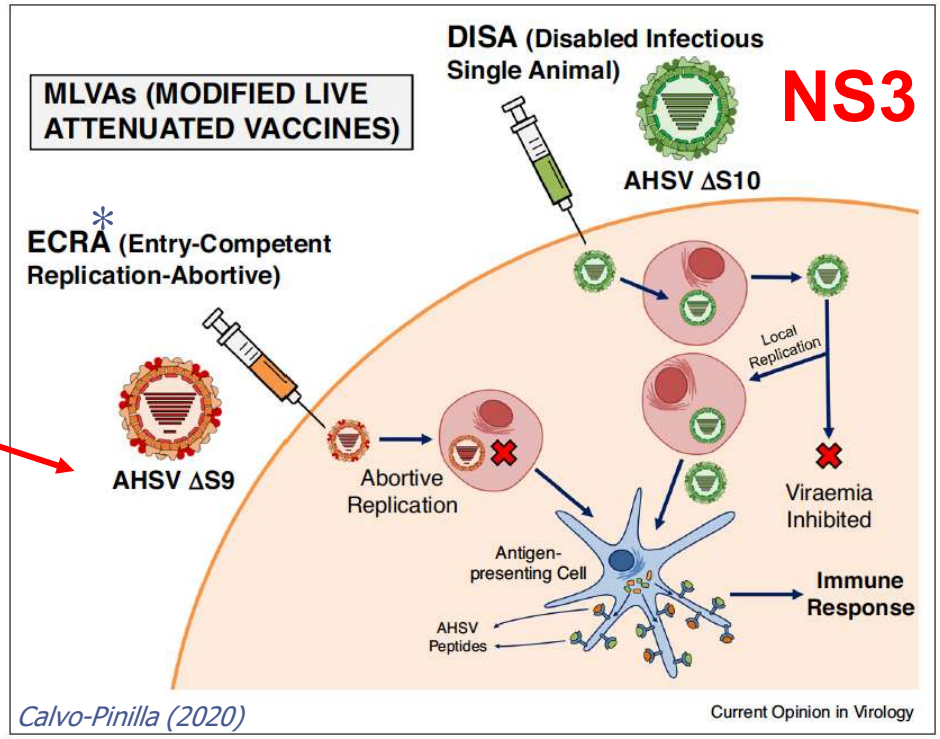
Edward Sullivan [1], Sylvie Lecollinet [2], Adeline Kerviel [1], Erika Hue [3], Stéphane Pronost [3], Cécile Beck [2], Marine Dumarest [2], Stephan Zientara [2], Polly Roy [4]

Dennis (2019), Roy (2020), Calvo-Pinilla (2020)

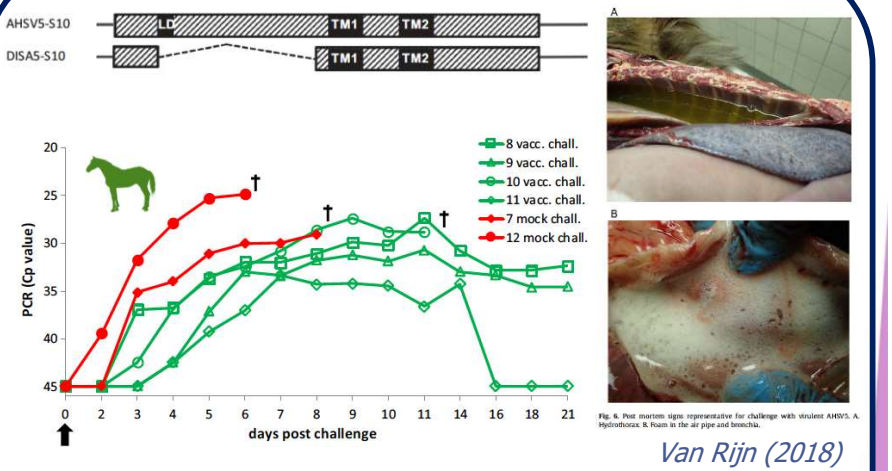
AHSV vaccines based on reverse genetics

ECRA: Lulla (2016, 2017)
DISA: Van de Water (2015), Feenstra (2015), Van Rijn (2018-1/2)

VP6



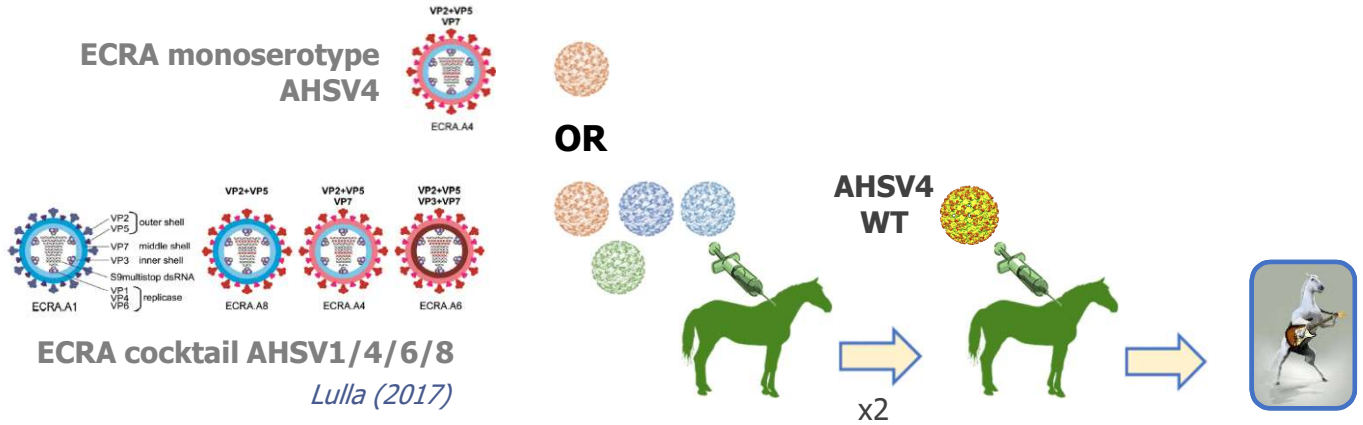
DISA



Van Rijn (2018)

DIVA companion test (Van Rijn, 2018-2)

ECRA



*previously called Disabled Infectious Single Cycle (DISC) vaccines

Dennis (2019), Roy (2020), Calvo-Pinilla (2020)

Vaccine 36 (2018) 1925–1933

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

African horse sickness virus (AHSV) with a deletion of 77 amino acids in NS3/NS3a protein is not virulent and a safe promising AHS Disabled Infectious Single Animal (DISA) vaccine platform

Piet A. van Rijn [a,b,*], Mieke A. Maris-Veldhuis [a], Christiaan A. Potgieter [b,c], René G.P. van Gennip [a]

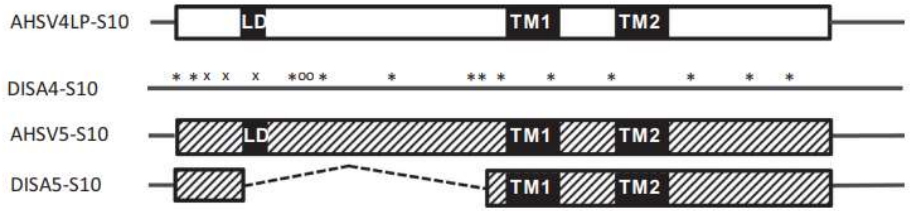


Fig. 1. Schematic presentations of mutated genome segment 10. S10 of AHSV4 and AHSV5 are shown by open and striped boxes, respectively. Domains Late Domain (LD), and trans membrane regions TM1 and TM2 are indicated by filled boxes. AUG->GCC mutations, and introduction of STOP codons and silent mutations in S10 of DISA4 are indicated by *, x, and o, respectively. Untranslated RNA sequences are indicated by lines and putatively translated NS3-ORFs are represented by boxes. The in-frame deletion of 77 amino acid codons in S10 of DISA5 is shown.

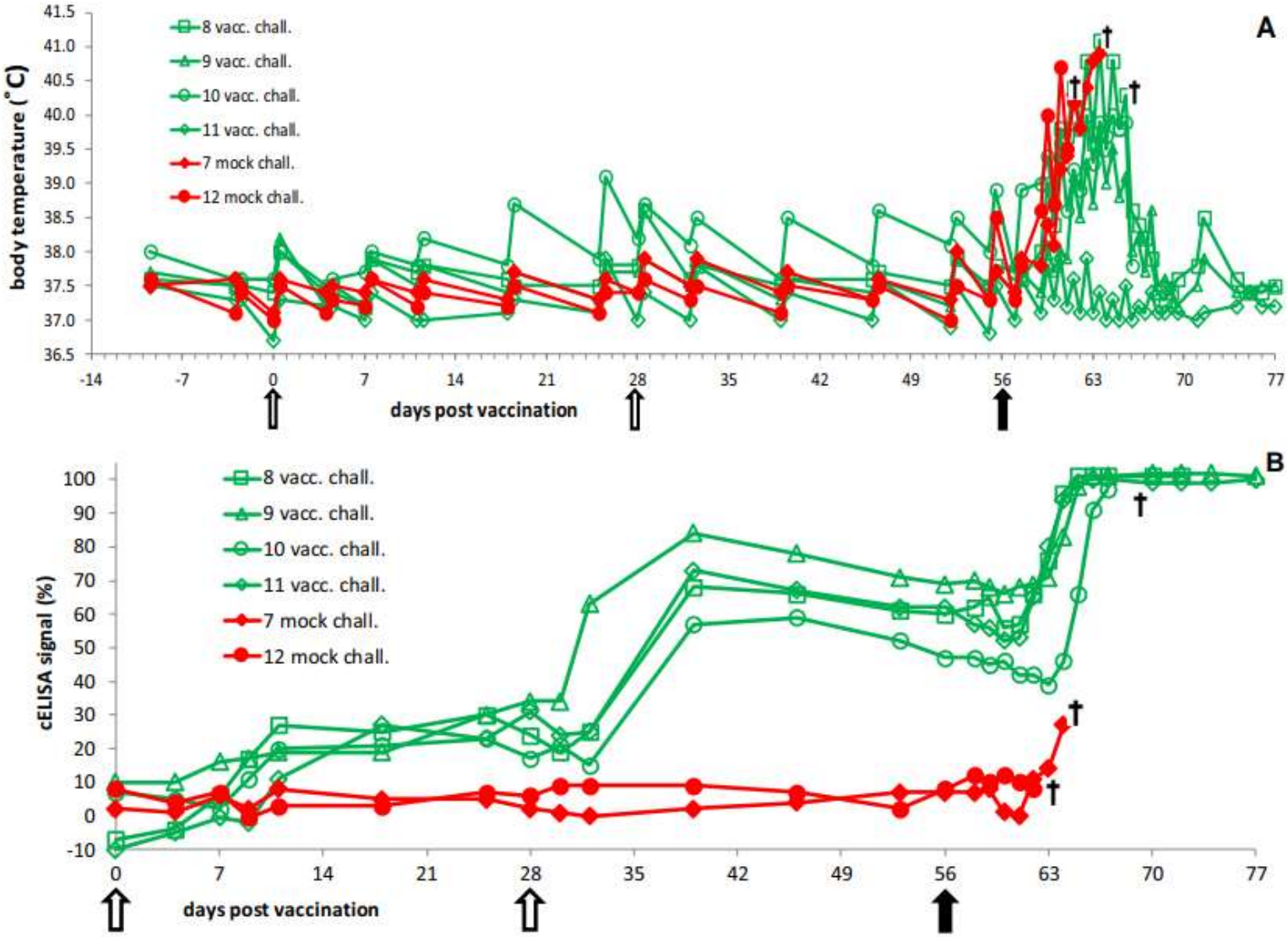


Fig. 4. Time course of body temperature and VP7 seroconversion. Four ponies were intramuscularly vaccinated twice with a standard dose of DISA5 (open symbols) on day 0 and 28 (open arrows). Two ponies served as challenge controls and were not vaccinated (filled symbols). All ponies were challenged on day 56 with virulent AHSV5 (filled arrow). A cross indicates death or euthanizing for ethical reasons in the time course of the experiment. A: The body temperature was frequently measured twice daily in the morning and the afternoon on indicated days and every day after challenge. B: VP7 seroconversion was determined by cELISA and expressed as blocking percentage (100-value).

- Call Horizon Europe -SPIDVAC (coordination FLI-Anses) – AHSV-FMDV

Safe Priority Infectious Diseases VACcines

Improved control of priority animal diseases:
Novel vaccines and companion diagnostic tests for African horse sickness, peste des petits ruminants and foot-and-mouth disease

List of participating organisations

#	Participating Organisation Legal Name	Country	Action
1	FRIEDRICH LOEFFLER INSTITUT - BUNDESFORSCHUNGSINSTITUT FUER TIERGESUNDHEIT	Germany	
2	AGENCE NATIONALE DE LA SECURITE SANITAIRE DE L ALIMENTATION DE L ENVIRONNEMENT ET DU TRAVAIL	France	
3	CENTRE DE COOPERATION INTERNATIONALE EN RECHERCHE AGRONOMIQUE POUR LEDEVELOPPEMENT - C.I.R.A.D. EPIC	France	
4	ASOCIACION CENTRO DE INVESTIGACION COOPERATIVA EN BIOCIENCIAS	Spain	
5	INNOVATIVE DIAGNOSTICS	France	
6	BOEHRINGER INGELHEIM INTERNATIONALGMBH	Germany	
7	THE UNIVERSITY OF NOTTINGHAM	United Kingdom	
8	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	Spain	
9	STICHTING WAGENINGEN RESEARCH	Netherlands	
10	UNIVERSITY OF PRETORIA	South Africa	
11	UNIVERSITY OF SURREY	United Kingdom	
12	INSTITUT SENEGALAIS DE RECHERCHES AGRICOLES	Senegal	
13	MINISTERIO DE AGRICULTURA, PESCA Y ALIMENTACION	Spain	

Multimeric protein scaffold particle vaccines (MPSP)

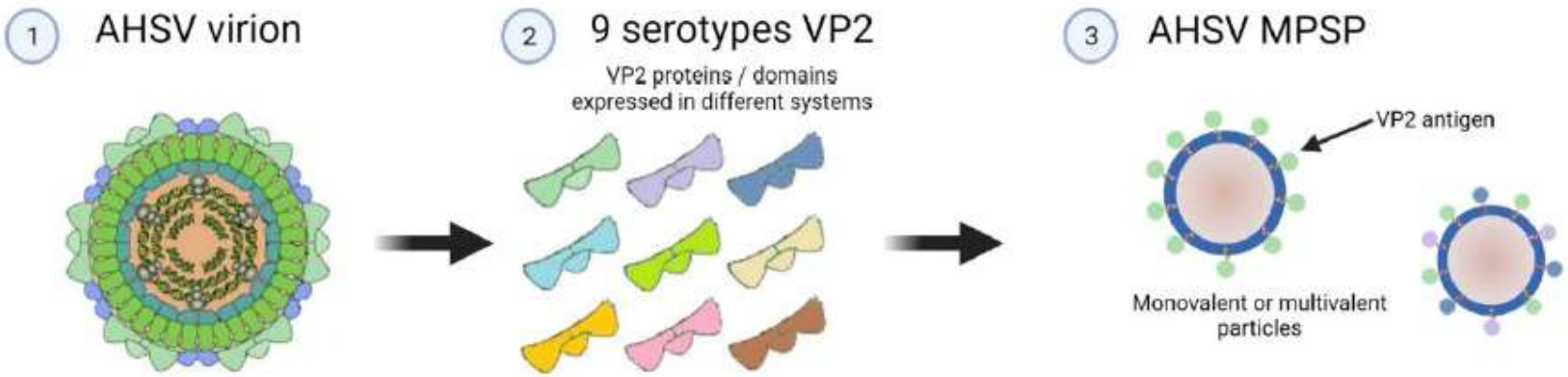


Figure 1: Flow chart of AHS MPSP vaccine design and construction.

Antigen production in different biological systems

Expression of the immunogenic proteins and domains of interest for attachment to MPSPs will be done in different systems using plants, insect cells, mammalian cells and bacteria (WP1). Together with the industrial partner BI (P06), this allows the selection of the best expression system for the prototype vaccine to be used for the pre-industrial scale-up and testing.

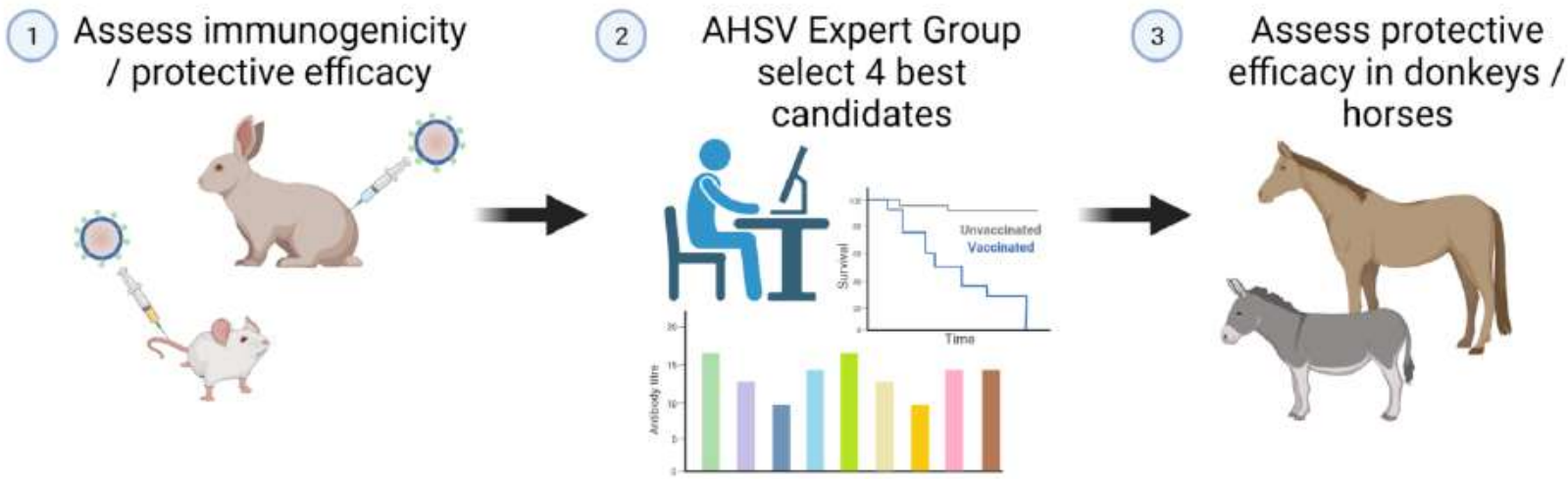


Figure 2: Flow chart of AHS MPSP vaccine evaluation.

European Union Reference Laboratory for Equine Diseases

Thank you for your attention

Contexte

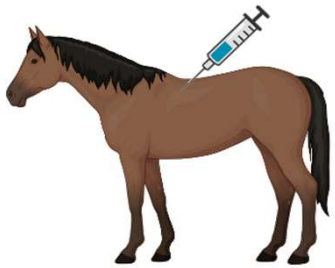
Projet européen SPIDVAC :

⟶ Développer des vaccins innovants contre la peste équine et évaluer leurs efficacité *in vitro* et *in vivo*

Production de protéines recombinantes pour vaccin

⇒ Protection totale chez les souris

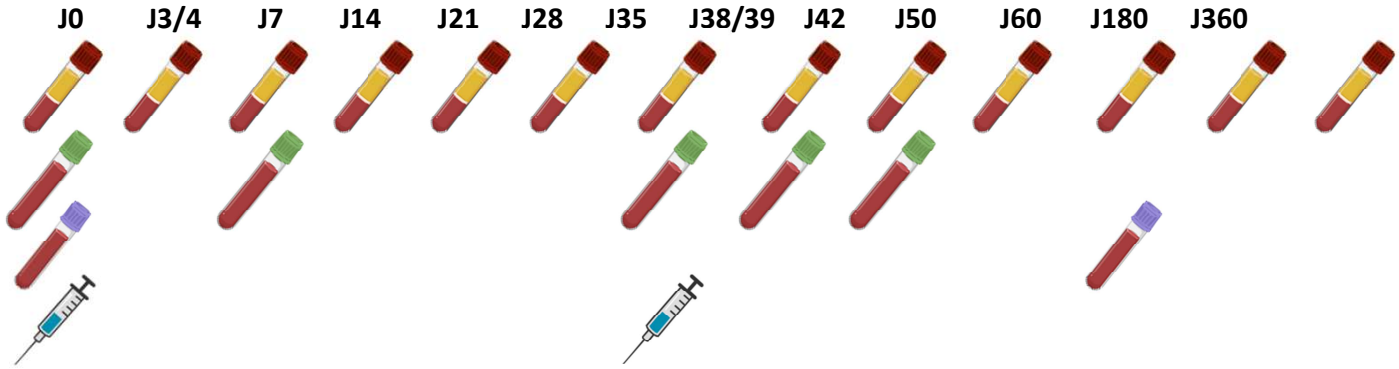
Vaccination sur cheval



Protocole expérimental

Objectifs : Cinétique des Ac neutralisants ; étude de la réponse immunitaire innée ; collecte de matériel biologique de référence et développement d'outils de diagnostic.

Vaccination de 3 groupes de 5 chevaux

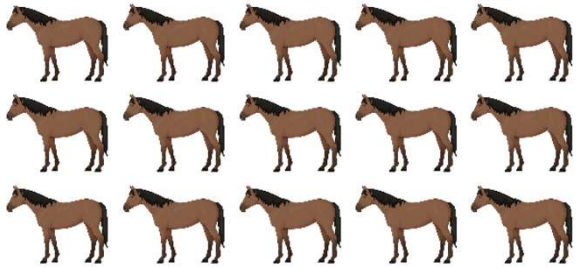


Tubes Héparine, sang complet (IDVet)
Juste groupe B 50 µg

Groupe A 25 µg

Groupe B 50 µg

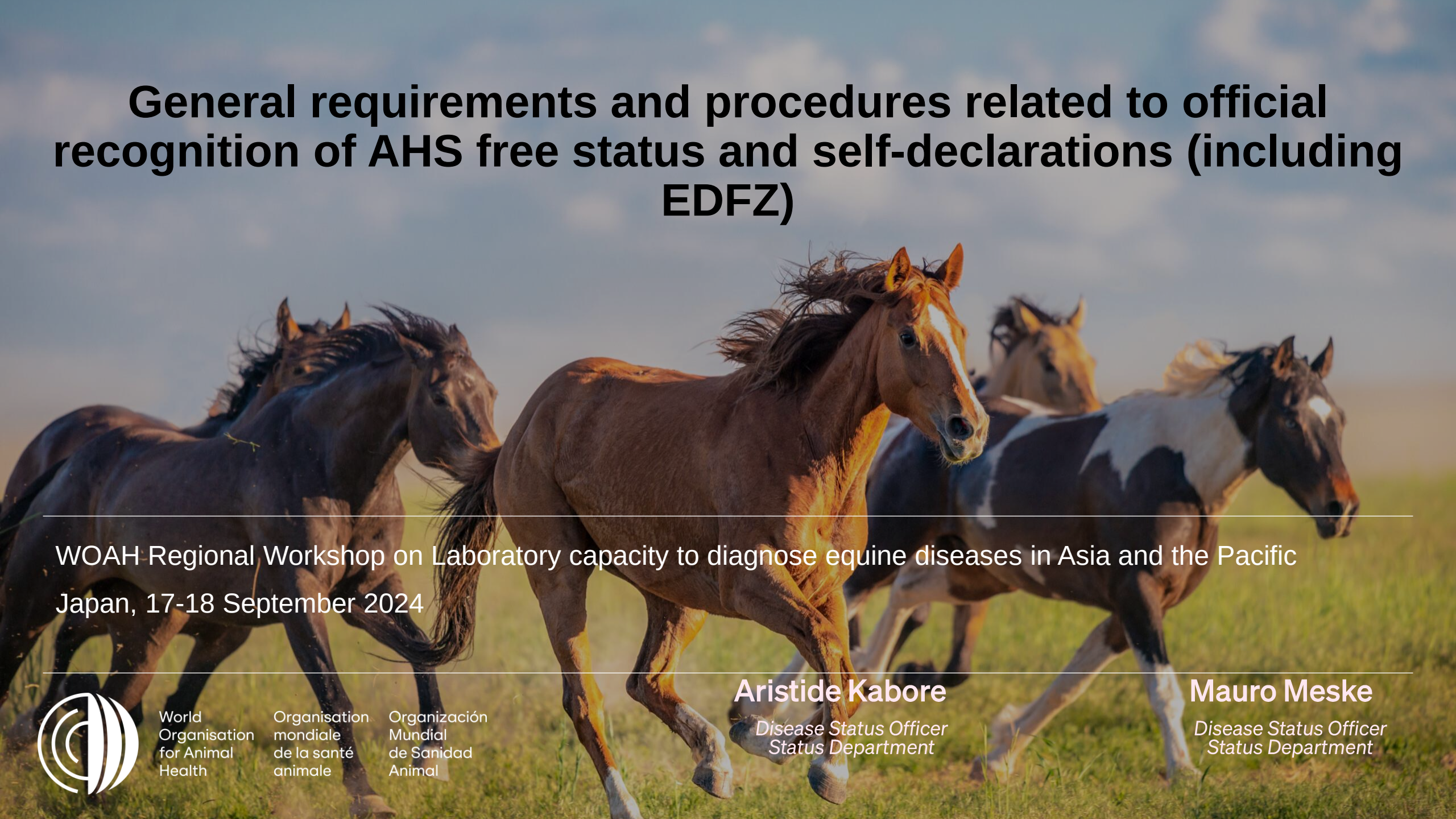
Groupe C 100 µg



1. Production de l'Ag + conjugaison aux nanoparticules par le FLI
2. Envoi de l'Ag couplé à l'ANSES
3. Adjuvant (Saponine – Hydroxyde d'aluminium) fourni par BI à l'ANSES pour formulation
4. Vaccination en **intra-musculaire** par l'ANSES
5. Prélèvements sanguins par les professionnels sur place

Adapted from Steinbach F and Bréard E

General requirements and procedures related to official recognition of AHS free status and self-declarations (including EDFZ)



WOAH Regional Workshop on Laboratory capacity to diagnose equine diseases in Asia and the Pacific

Japan, 17-18 September 2024

World Organisation for Animal Health
Organisation mondiale de la santé animale
Organización Mundial de Sanidad Animal

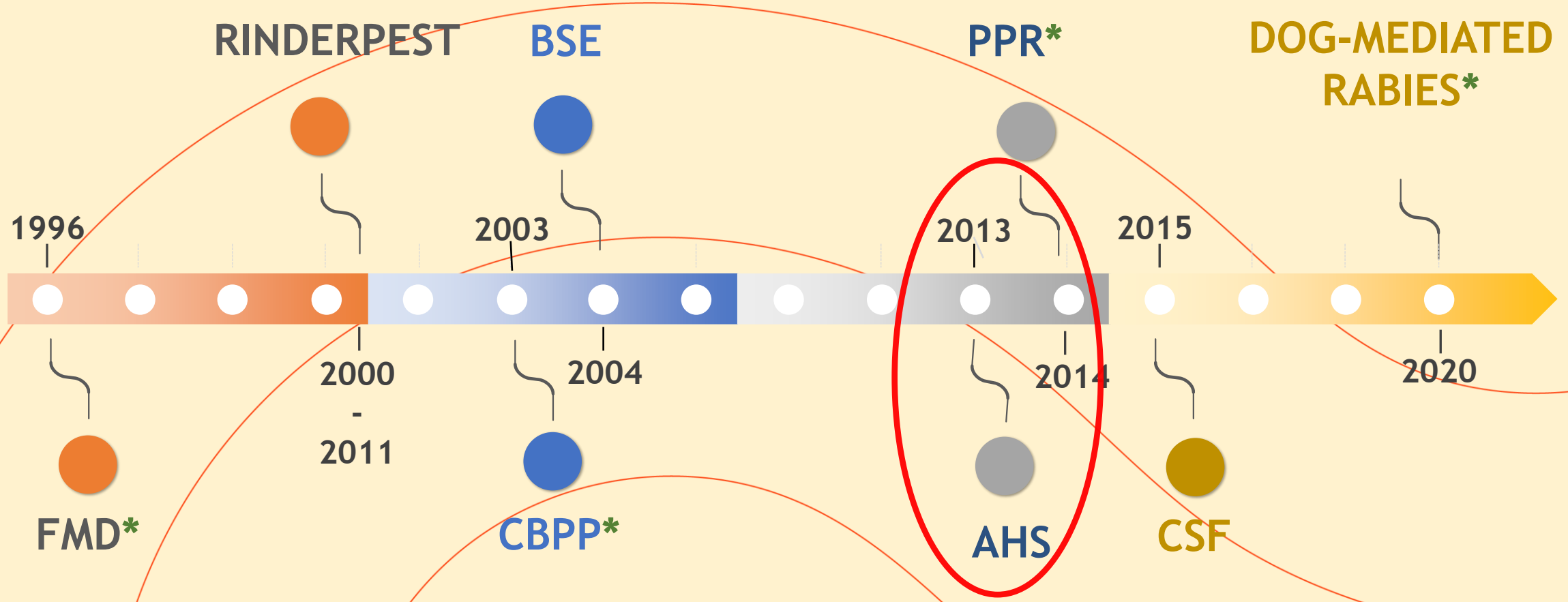
Aristide Kabore
Disease Status Officer
Status Department

Mauro Meske
Disease Status Officer
Status Department

Outline

- Historical information of official recognition
- Procedures for official recognition of animal health status
- Self-declarations of freedom from equine diseases
- Publication of a self-declaration for the establishment of an Equine Disease-Free Zone (EDFZ)

WOAH official recognition of animal health status



* WOAH endorsement of official control programme

WOAH official recognition of animal health status

The main objectives:

- Protect animal and human health in international trade;
- Facilitate trade;
- Guarantees to trading partners;

  Long term perspective documented and updated evidence on the disease-free situation;

  Veterinary Services meet baseline requirements concerning disease control and trade activities.

Procedure for official recognition of AHS-free status

World Organisation for Animal Health | Organisation mondiale de la santé animale | Organización Mundial de Sanidad Animal

Application_SOP

Standard Operating Procedure for official recognition of animal health status and for the endorsement of official control programmes of Members

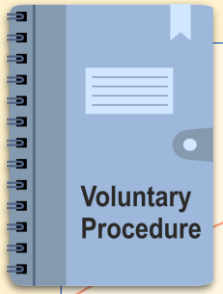
Description/ Scope:	This procedure describes the process for the preparation, assessment and approval of dossiers for the official recognition of animal health status and for the endorsement of official control programmes of Members.
Related documents:	Guidelines for the Official Status Recognition Process[1] (Annexed) Resolution No. 15 of the 2020 Adapted Procedure Resolution No. 16 of the 2020 Adapted Procedure
Related processes:	Expert Mission Deployment - Procedure (Mission_SOP) - Guidelines (Mission_Guidelines) Reconfirmation of a Status or Programme - Procedure (Reconfirmation_SOP) - Guidelines (Reconfirmation_Guidelines) Suspension, Recovery and Withdrawal - Procedure (Suspension_SOP) - Guidelines (Suspension_Guidelines) Addendum: Establishment of a protection zone
List of acronyms:	AHG: *ad hoc* Group Assembly: World Assembly of Delegates DDG: Deputy Director General, Standards and Science DG: Director General SD: Status Department GS: General Session SCAD: Scientific Commission for Animal Diseases *Terrestrial Code: Terrestrial Animal Health Code*

Step	Time Reference	Responsible person	Action	Reference Document
1.	After the GS	DG	Sends letter to Delegates confirming SCAD and AHG meeting dates and deadlines for dossier submission.	
	2 months			6.4

Countries recognised with AHS-free status in Asia and the Pacific: 11

Members of the Regional Commission for Asia and the Pacific without official recognition: 21

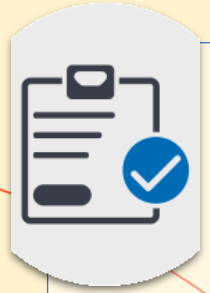
Procedure for official status



A voluntary procedure developed on Members' request to facilitate trade

Resolutions adopted by the World Assembly

SOPs and rules & guidelines

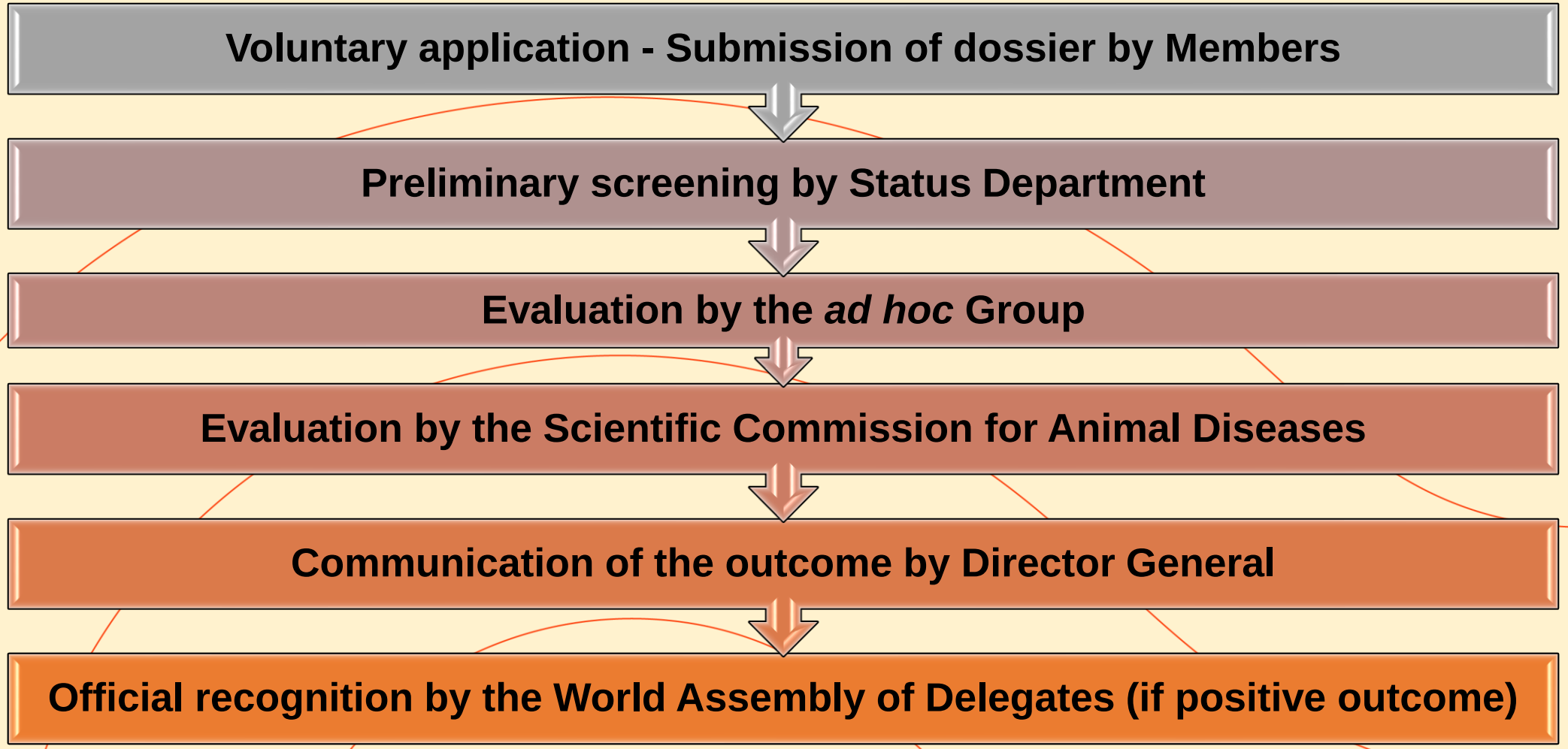


Updated when relevant

Available online in the three WOAH official languages

https://www.woah.org/en/what-we-do/animal-health-and-welfare/official-disease-status/

Procedure for official status



Procedure for official status: submission of an application

WOAH calendar meetings:

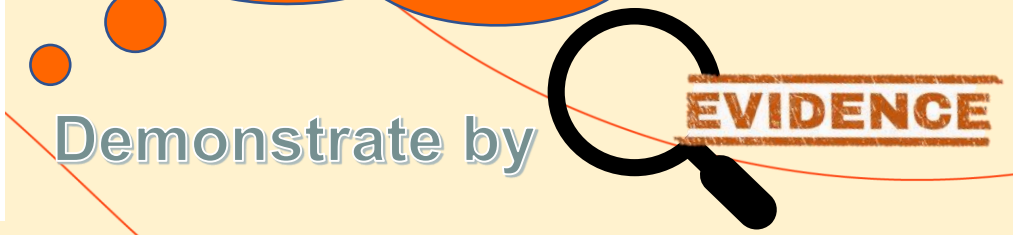
- Cycle from a General Session to the following one;
- Letter from the DG after each General Session announcing dates of the AHG;
- Deadline to submit dossier: 2 months before the meeting of the AHG.

Tentative dates of official status evaluation ad hoc Groups (AHG)		
Disease	**Meeting**	**Submission deadline**
BSE	1-4 October 2024	2 August 2024
Rabies	8-10 October 2024	9 August 2024
AHS	**9-11 October 2024**	**10 August 2024 (01)**
CBPP	29-31 October 2024	30 August 2024
FMD	5-7 November 2024	6 September 2024
PPR	12-14 November 2024	13 September 2024
CSF	19-21 November 2024	20 September 2024

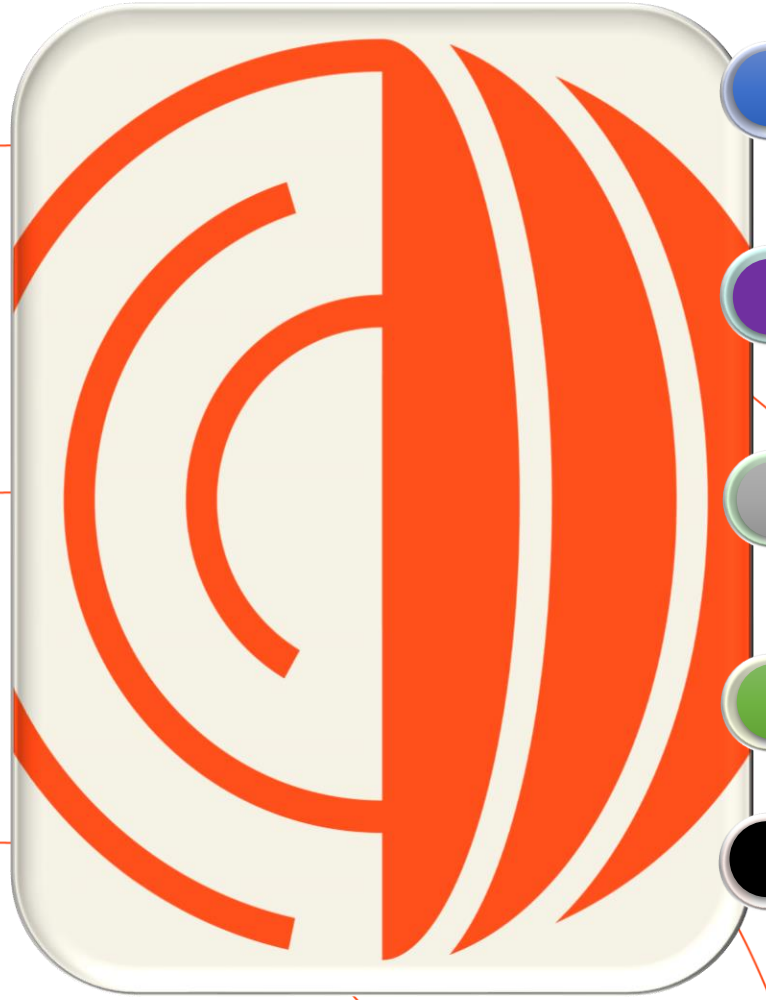
Procedure for official status: submission of an application

- Compliance with the relevant provisions of the *Terrestrial Code*.
- Complete – relevant to template **Questionnaire** in Chapter 1.7. of the *Terrestrial Code* (also in WOAH website) – **documented evidence**
- 50 pages + appendices (properly cross-referenced) + executive summary
- Digitalised map if relevant (free zone)
- Proof of payment - fee
- Contact details of technical staff

Does my dossier comply with the requirements of the Terrestrial Animal Health Code?



Procedure for official status: preliminary screening by Status Dept



- Archiving and screening
- Acknowledge receipt of the submission to the Delegate
- Request of additional information or amended dossier, if gap(s) identified
- Translation in English if needed
- Processing for the *ad hoc* Group

Procedure for official status: evaluation by the *ad hoc* group

➢ **An *ad hoc* Group:**

- Usually comprises six internationally recognised experts
- take into consideration geographical representation and gender balance, when possible.

➢ **Assessment:**

- Against *Terrestrial Code* requirements based on:
  - Information from the application (esp. evidence) and other reports (e.g., WAHIS, PVS)
  - Experts' knowledge
  - Information available in the public domain
- Possible interaction (electronic) with the applicant Member
- Detailed evaluation report and recommendations forwarded to the Scientific Commission

Procedure for official status: evaluation by the SCAD

- Scientific Commission: Members elected by the World Assembly of Delegates for a three-year term
- Assess the dossier, including the *ad hoc* Group recommendations
- Possible interaction with
  - The chairperson of the *ad hoc* Group
  - Applicant Members, including the possible visit of a delegation

    /!\ official request to WOAH by the Delegate before 31 December
- Final recommendation proposed for adoption by the World Assembly of Delegates
- May request an expert mission (field or virtual) to verify the dossier

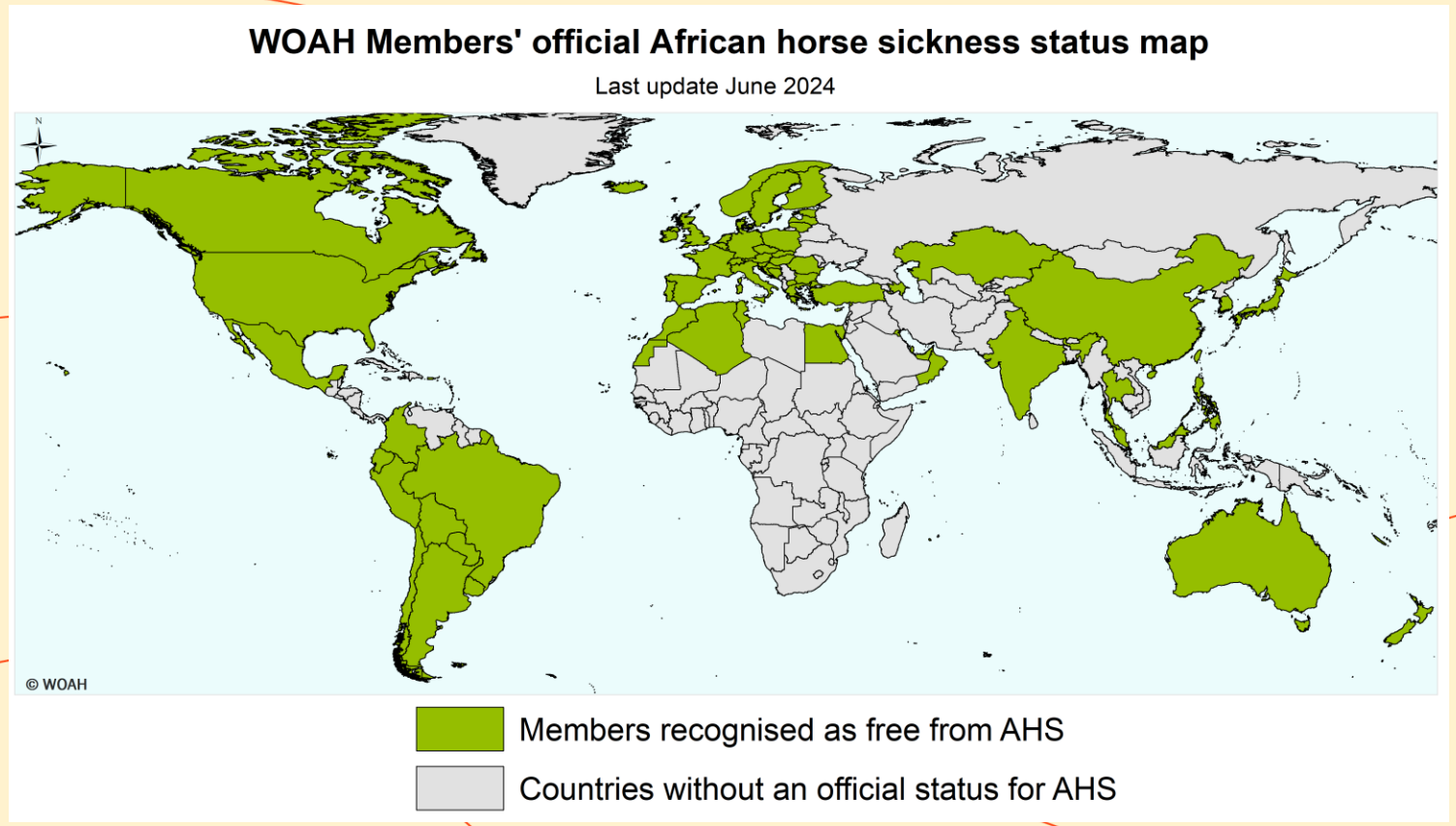
Procedure for official status: communication of the outcome

Letter from the DG to the applicant Member(s)

- Positive outcome
  - Assessment published in the Scientific Commission report
  - Letter to all Delegates
    60-day period
    - World Assembly of Delegates
- Negative outcome
  - Assessment kept confidential
    Report provided only to the applicant Member
- Pending a mission

Procedure for official status: official recognition

Through the adoption of a Resolution by the World Assembly of Delegates at the General Session

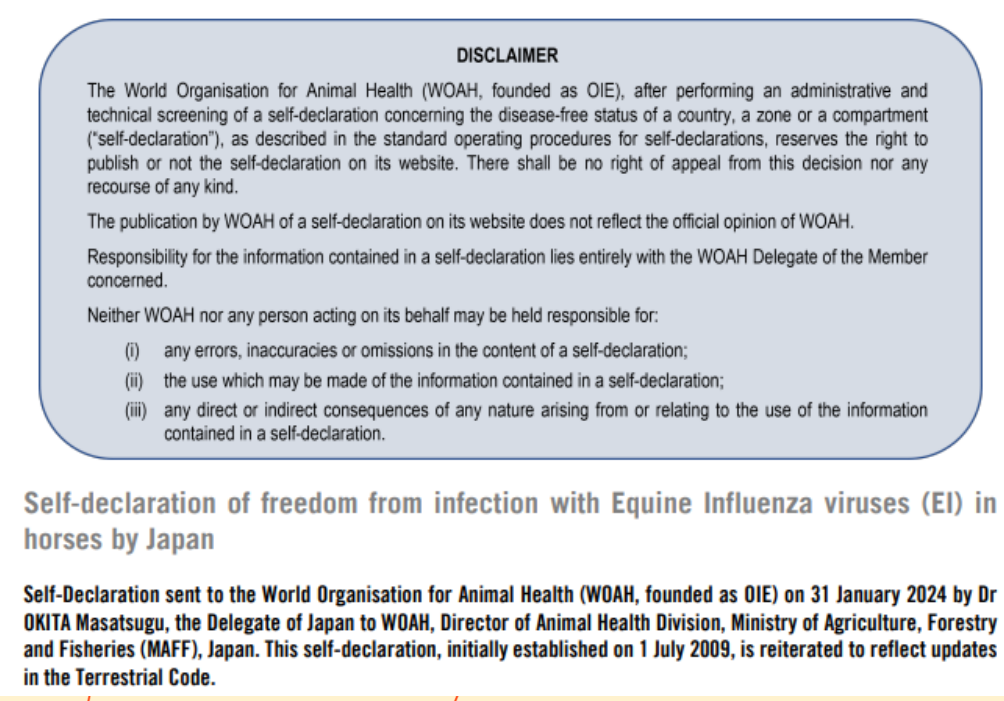


Additional procedures: self-declarations

Self-declarations of freedom from equine diseases without official status recognition

Main objectives:

- To allow Delegates to self-declare their country, a zone, or a compartment within their territory, free from any disease (except AHS)



DISCLAIMER

The World Organisation for Animal Health (WOAH, founded as OIE), after performing an administrative and technical screening of a self-declaration concerning the disease-free status of a country, a zone or a compartment ("self-declaration"), as described in the standard operating procedures for self-declarations, reserves the right to publish or not the self-declaration on its website. There shall be no right of appeal from this decision nor any recourse of any kind.

The publication by WOAH of a self-declaration on its website does not reflect the official opinion of WOAH.

Responsibility for the information contained in a self-declaration lies entirely with the WOAH Delegate of the Member concerned.

Neither WOAH nor any person acting on its behalf may be held responsible for:

(i) any errors, inaccuracies or omissions in the content of a self-declaration;

(ii) the use which may be made of the information contained in a self-declaration;

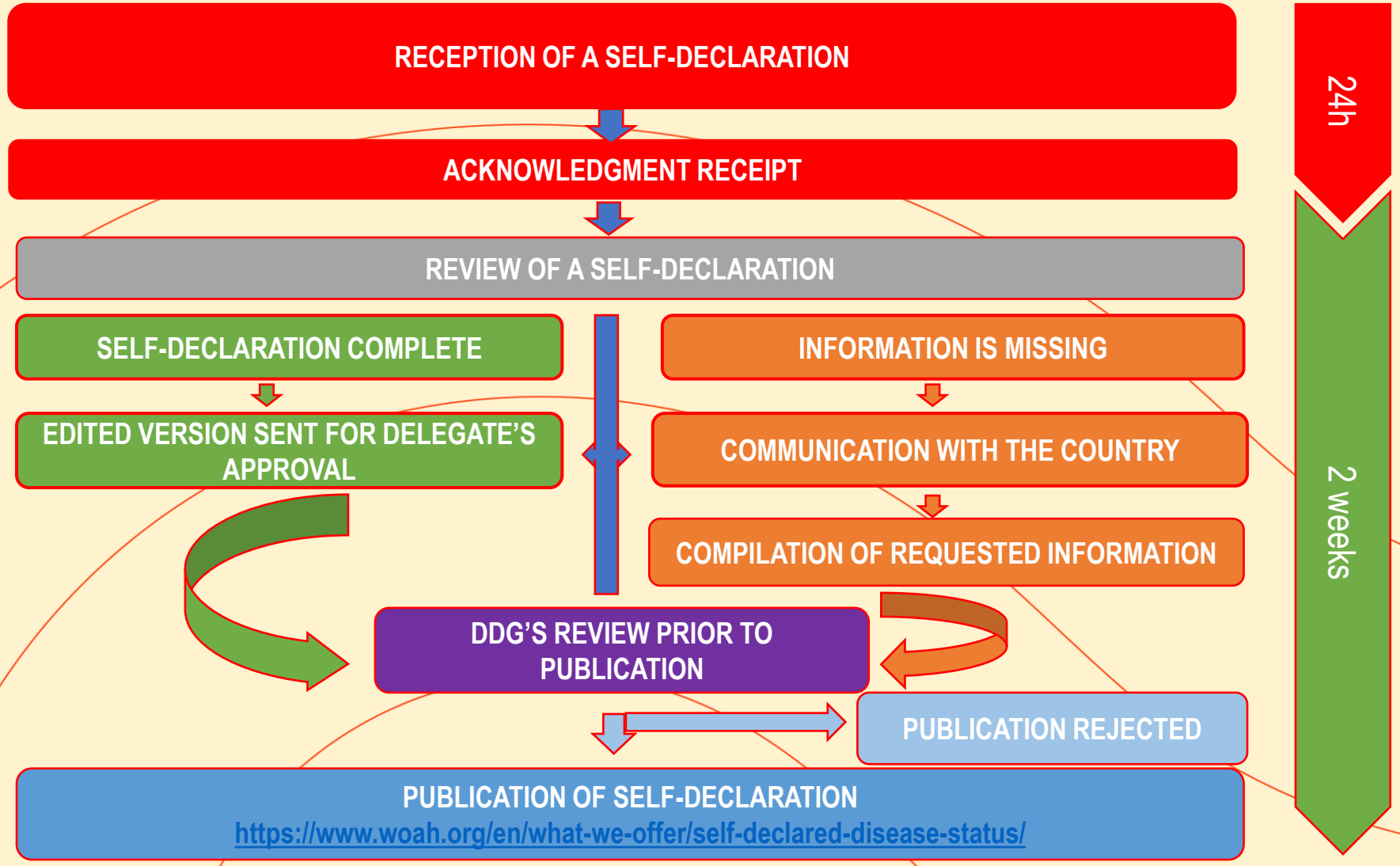
(iii) any direct or indirect consequences of any nature arising from or relating to the use of the information contained in a self-declaration.

Self-declaration of freedom from infection with Equine Influenza viruses (EI) in horses by Japan

Self-Declaration sent to the World Organisation for Animal Health (WOAH, founded as OIE) on 31 January 2024 by Dr OKITA Masatsugu, the Delegate of Japan to WOAH, Director of Animal Health Division, Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan. This self-declaration, initially established on 1 July 2009, is reiterated to reflect updates in the Terrestrial Code.

Member	Self-declared freedom from	Category	From*	To	Country/zone/ compartment	Status
Japan	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2024-06-02		Country	Active
India	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2024-05-01		Compartment	Active
Singapore	African swine fever virus (Inf. with)	Terrestrial	2024-05-01		Country	Active
Thailand	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2024-04-24		Country	Active
Malaysia	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2024-04-23		Country	Active
Malaysia	Nipah virus encephalitis	Terrestrial	2024-04-23		Country	Active
Japan	Equine influenza virus (Inf. with)	Terrestrial	2024-01-31		Country	Active
Japan	Newcastle disease virus (Inf. with)	Terrestrial	2024-01-31		Country	Active
Indonesia	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2023-10-05		Compartment	Active
Chinese Taipei	Sheep pox and goat pox	Terrestrial	2023-09-15		Country	Active
New Zealand	Brucella abortus (Inf. with)	Terrestrial	2021-04-17		Country	Active
Japan	Brucella abortus, Brucella melitensis and Brucella suis (inf. with)	Terrestrial	2021-04-01		Country	Active
Japan	Mycobacterium tuberculosis complex (Inf. with)	Terrestrial	2021-04-01		Country	Active
New Zealand	Theileria equi	Terrestrial	2020-10-08		Country	Active
New Zealand	Equine arteritis virus (Inf. with)	Terrestrial	2014-06-24		Country	Active
Sri Lanka	Haemorrhagic septicaemia (Pasteurella multocida serotypes 6:b and 6:e)	Terrestrial	2012-12-12		Country	Active
New Zealand	Echinococcus granulosus (Inf. with)	Terrestrial	2002-11-18		Country	Active
China (People's Rep. of)	EDFZ	Terrestrial	2022-01-04	2023-12-31	Zone	Not active
Japan	EDFZ	Terrestrial	2021-07-06	2021-09-05	Zone	Not active

Self-declarations

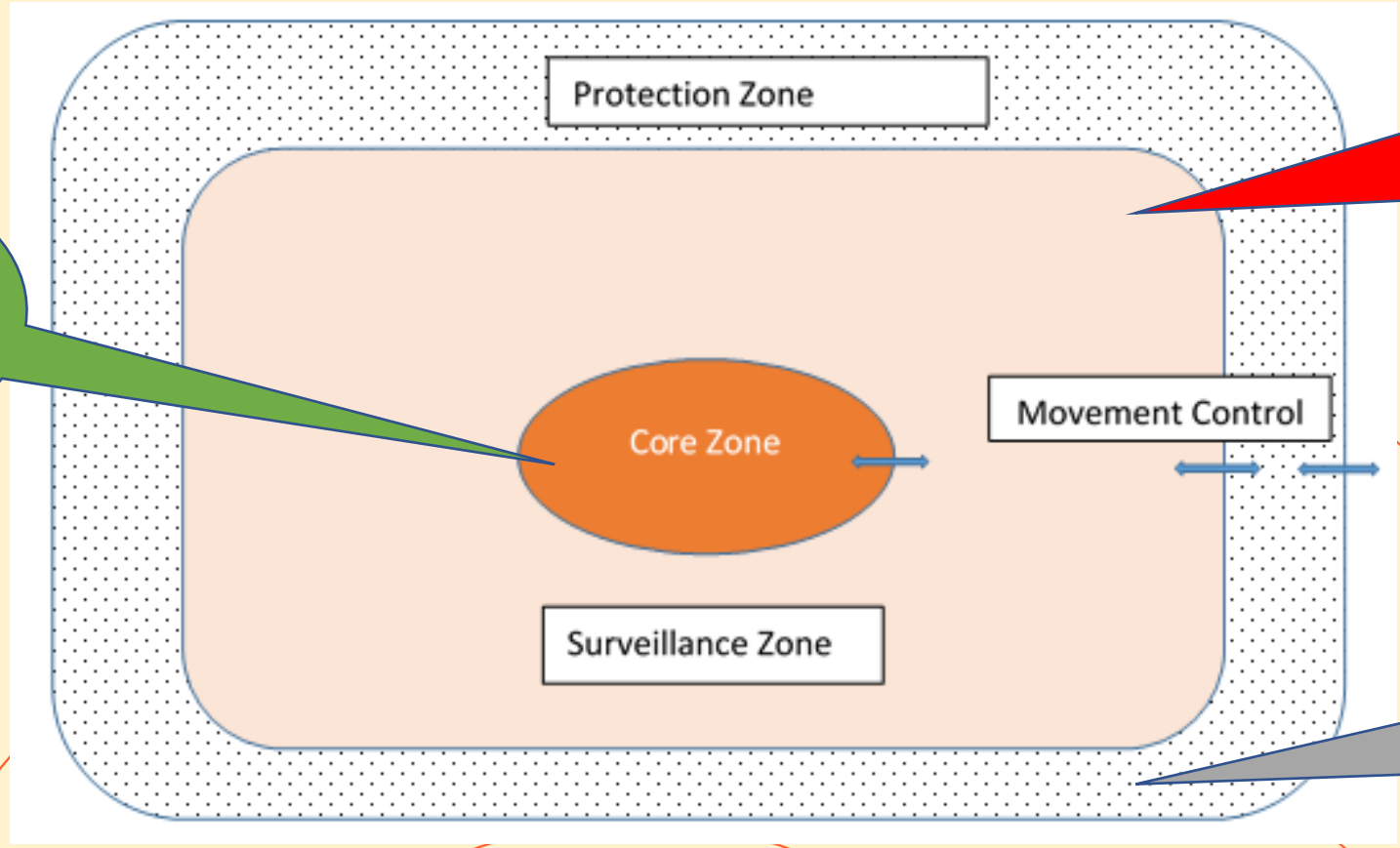


Establishment of Equine disease-free zones (EDFZ)

Main objectives:

- to establish (temporarily) a higher health status for a zone with a limited animal population within a country;
- to protect equids in the EDFZ from diseases that may occur in other parts of the territory;
- to protect equids outside of the EDFZ from diseases that potentially could be imported into the zone;
- to enable the safe entry and exit of horses.

Establishment of Equine disease-free zones (EDFZ)

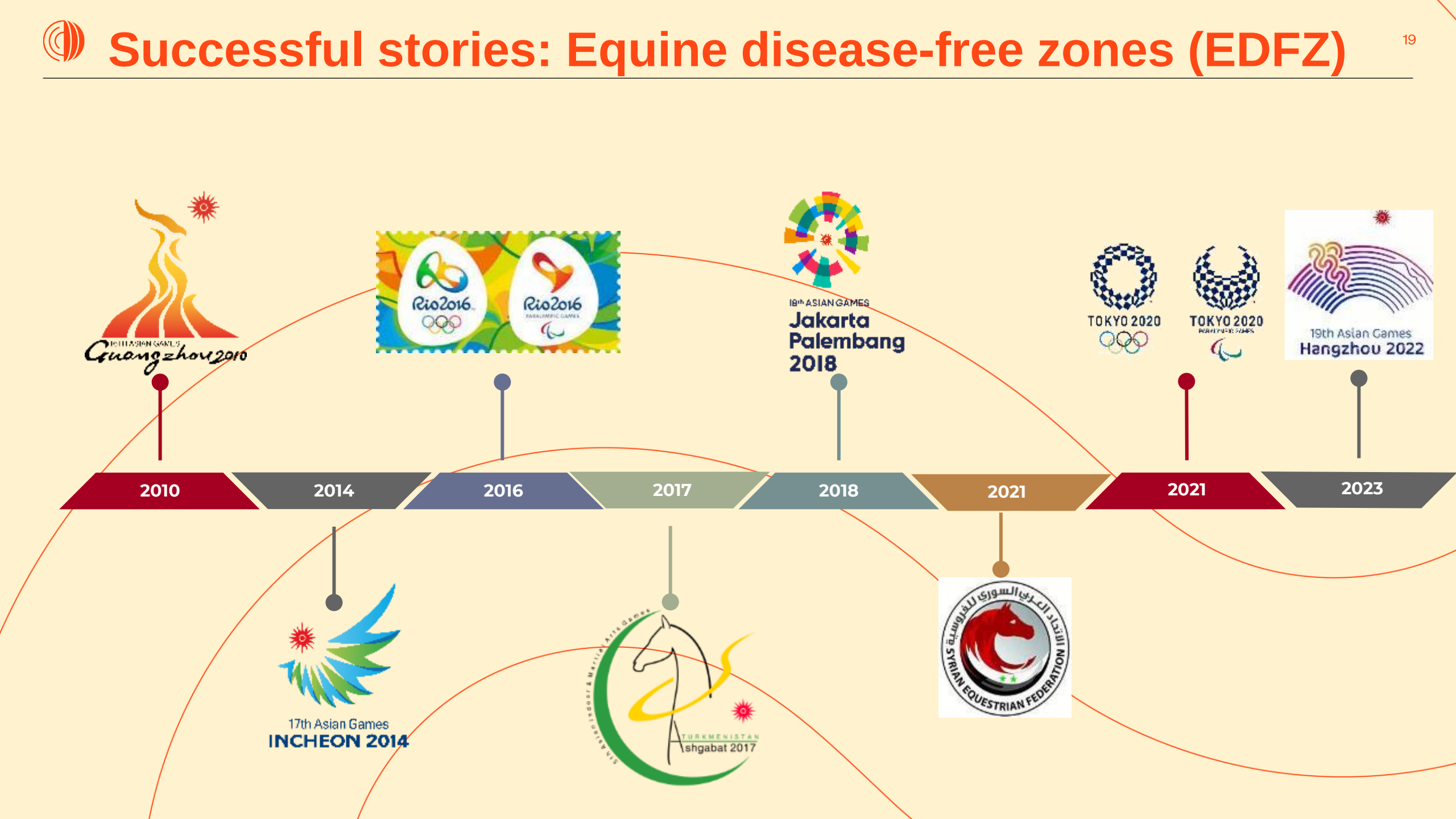


Event venue (core Zone)

control of movements, Surveillance and early detection system in place

PZ: buffer zone, optional

Successful stories: Equine disease-free zones (EDFZ)



Self-declarations from the WOAH Region RRAP

Self-declarations of freedom from equine diseases without official status recognition

Self-Declarations

Disease name

Search

Category filter
- Seleccionar to…
- Aquatic
- Terrestrial

Status filter
- Active
- Not active

Temporary
Todas

Regional filter
- Seleccionar to…
- Africa
- Americas
- Asia Pacific
- Europe
- Middle East

Member filter
Todas

Member
Search

View quick data export guide

Member	Self-declared freedom from	Category	From*	To	Country/zone/ compartment	Status	Temporary
Japan	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2024-06-02		Country	Active	No
New Zealand	Echinococcus granulosus (Inf. with)	Terrestrial	2024-05-06		Country	Active	No
India	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2024-05-01		Compartment	Active	No
Singapore	African swine fever virus (Inf. with)	Terrestrial	2024-05-01		Country	Active	No
Thailand	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2024-04-24		Country	Active	No
Malaysia	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2024-04-23		Country	Active	No
Malaysia	Nipah virus encephalitis	Terrestrial	2024-04-23		Country	Active	No
Japan	Equine influenza virus (Inf. with)	Terrestrial	2024-01-31		Country	Active	No
Japan	Newcastle disease virus (Inf. with)	Terrestrial	2024-01-31		Country	Active	No
Indonesia	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2023-10-05		Compartment	Active	No
Chinese Taipei	Sheep pox and goat pox	Terrestrial	2023-09-15		Country	Active	No
New Zealand	Brucella abortus (Inf. with)	Terrestrial	2021-04-17		Country	Active	No
Japan	Brucella abortus, Brucella melitensis and Brucella suis (inf. with)	Terrestrial	2021-04-01		Country	Active	No
Japan	Mycobacterium tuberculosis complex (Inf. with)	Terrestrial	2021-04-01		Country	Active	No
New Zealand	Theileria equi	Terrestrial	2020-10-08		Country	Active	No
New Zealand	Equine arteritis virus (Inf. with)	Terrestrial	2014-06-24		Country	Active	No
Sri Lanka	Haemorrhagic septicaemia (Pasteurella multocida serotypes 6:b and 6:e)	Terrestrial	2012-12-12		Country	Active	No

Publications of Self-declarations for the establishment of EDFZ (Temporary-inactive): China 2022 (Asian Games); Japan 2021-2020 (Olympic Games)

Self-declarations of freedom from diseases from the Region: Nipah (Malaysia); EI (Japan), EP & EVA (New Zealand)

Self-Declarations

Disease name

Search

Category filter
- Seleccionar to…
- Aquatic
- Terrestrial

Status filter
- Active
- Not active

Temporary
Todas

Regional filter
- Seleccionar to…
- Africa
- Americas
- Asia Pacific
- Europe
- Middle East

Member filter
Todas

Member
Search

View quick data export guide

Member	Self-declared freedom from	Category	From*	To	Country/zone/ compartment	Status	Temporary
Korea (Rep. of)	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2023-05-16	2023-12-05	Country	Not active	No
Japan	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2023-05-13	2023-11-24	Country	Not active	No
Japan	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2022-06-13	2023-01-23	Country	Not active	No
China (People's Rep. of)	EDFZ	Terrestrial	2022-01-04	2022-12-31	Zone	Not active	Yes
China (People's Rep. of)	EDFZ	Terrestrial	2022-01-04	2023-12-31	Zone	Not active	Yes
Japan	EDFZ	Terrestrial	2021-07-06	2021-09-05	Zone	Not active	Yes
Japan	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2021-06-30	2021-11-09	Country	Not active	No
Indonesia	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2021-01-29		Compartment	Not active	No
Japan	EDFZ	Terrestrial	2019-08-01	2019-08-23	Zone	Not active	Yes
Korea (Rep. of)	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2018-07-12	2020-11-26	Country	Not active	No
Japan	Avian influenza (including infection with high pathogenicity avian influenza viruses)	Terrestrial	2018-04-15	2020-11-10	Country	Not active	No
Indonesia	EDFZ	Terrestrial	2018-02-15	2018-09-30	Zone	Not active	Yes
Malaysia	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2017-07-01	2018-07-30	Country	Not active	No
Korea (Rep. of)	EDFZ	Terrestrial	2014-05-22	2014-10-05	Zone	Not active	Yes
Malaysia	Rabies virus (Inf. with)	Terrestrial	2013-07-17	2015-07-27	Country	Not active	No
Brunei	White spot syndrome virus (Inf. with)	Aquatic	2010-01-01	2011-02-25	Country	Not active	No
Pakistan	High pathogenicity avian influenza viruses (poultry) (Inf. with)	Terrestrial	2008-07-01	2021-09-08	Country	Not active	No

Official Status vs Self-declarations:

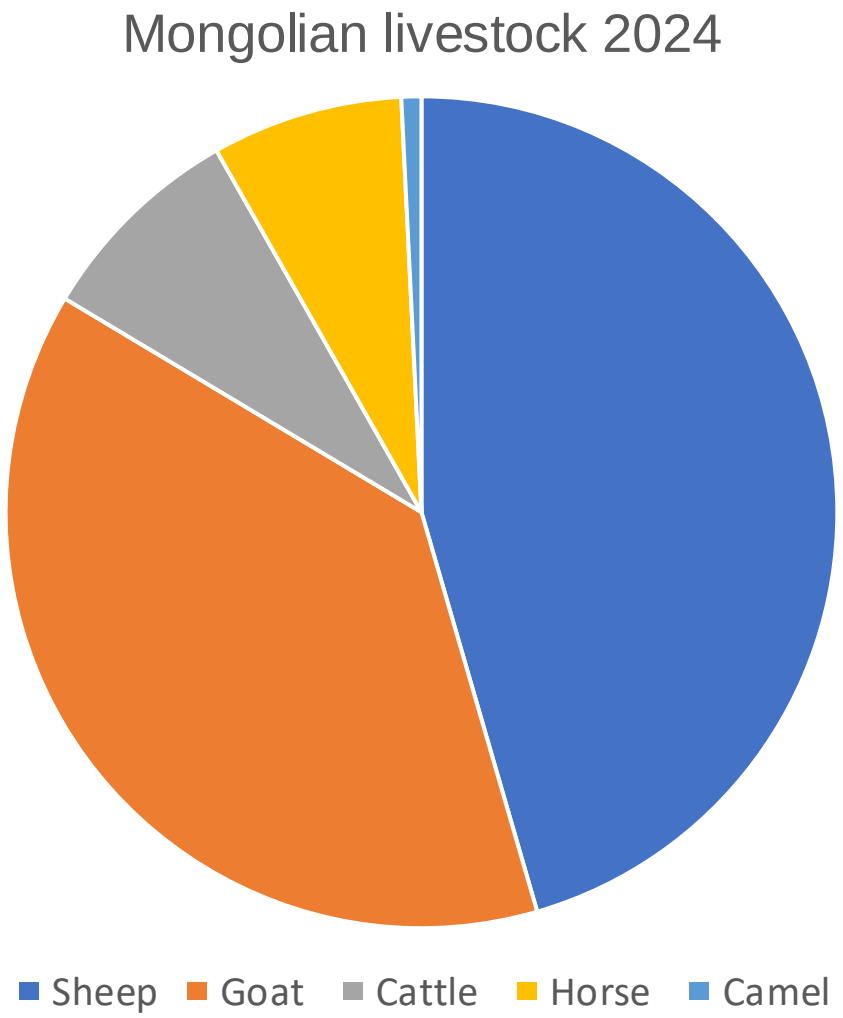
Official status	EDFZ-self-declaration	Self-declarations
For equine diseases, only for AHS	Diseases selected based on risk approach	Any diseases except AHS, listed or not
Country, or zone	Zone	country, zone or compartment
Pre-screening by Status Department	Revision by Status Department	Revision by Status Department
Evaluation by the ad hoc Group	Validation by WOAH DHoD/DHD and DDG	Validation by WOAH DHoD/DHD and DDG
Evaluation by SCAD/Decision by the WOAH Assembly	Responsability undertaken by the Delegate	Responsability undertaken by the Delegate
Annual reconfirmation needed	Usually Temporary (for an specific event)	Deactivation in the event of an outbreak
Compliance with the Questionnaire/SOPs, horizontal and disease-specific chapters of the WOAH *Terrestrial Code*		

Dourine and Surra: Disease situation in Mongolia; drawbacks of diagnostic tests (WOAH Manual), and development of pen-side diagnostic tests for dourine

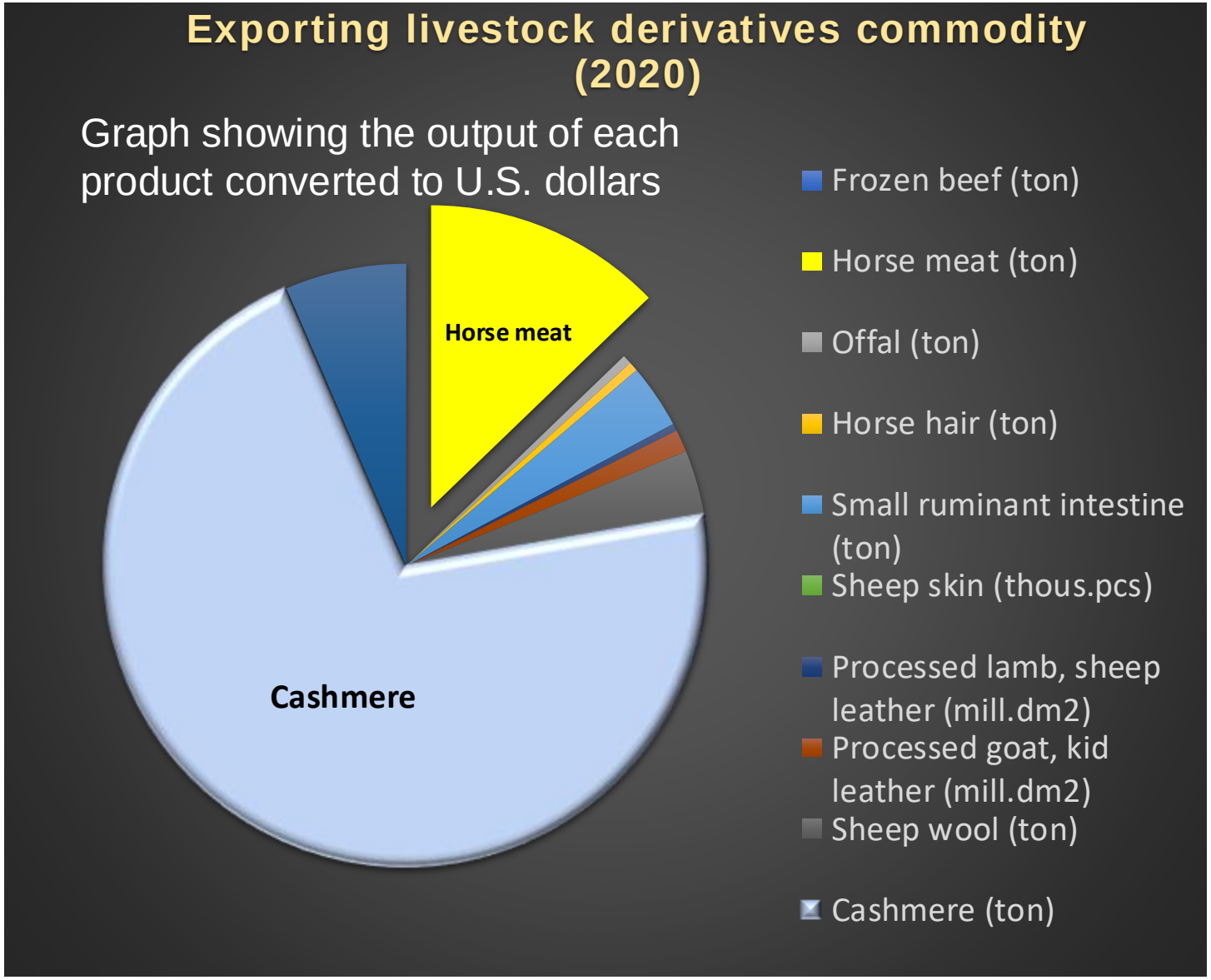
Batdorj Davaasuren[1], Banzragch Battur[1], Battsetseg Badgar[1], Keisuke Suganuma[2], Noboru Inoue[2]

[1] Institute of Veterinary Medicine, Mongolia
[2] National Research Center for Protozoan Diseases, WOAH RC for Surra, Japan

Economic Status of Horse



4.8 million horse
(7% of livestock population)



Horse breeding and herd structure in Mongolia

A stallion

Gelding
5-10

Yearling and youngster
10-20

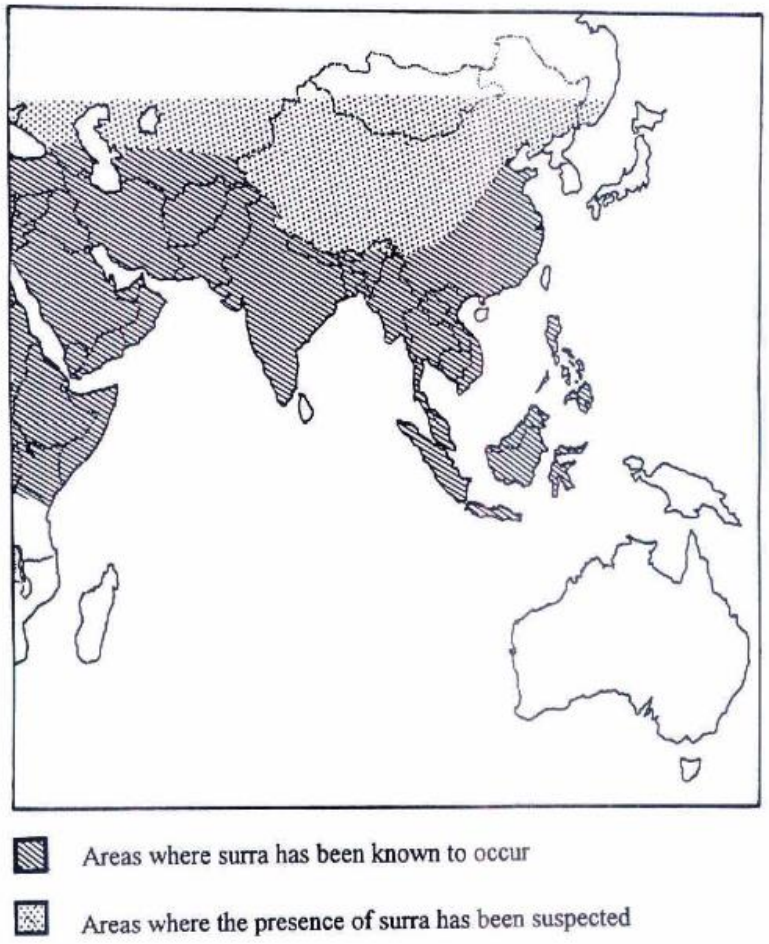
Mares with colt 10-20

- Unorganized breeding with other breed of horse
- Stallion introduction from Eastern province to other provinces

Subgenus *Trypanozoon*

	Vector	Host range
T. brucei	tsetse	wide
T. evansi	horsefly, stable fly	wide
T. equiperdum	none	equid

Surra



Y. Ozawa report (1998)

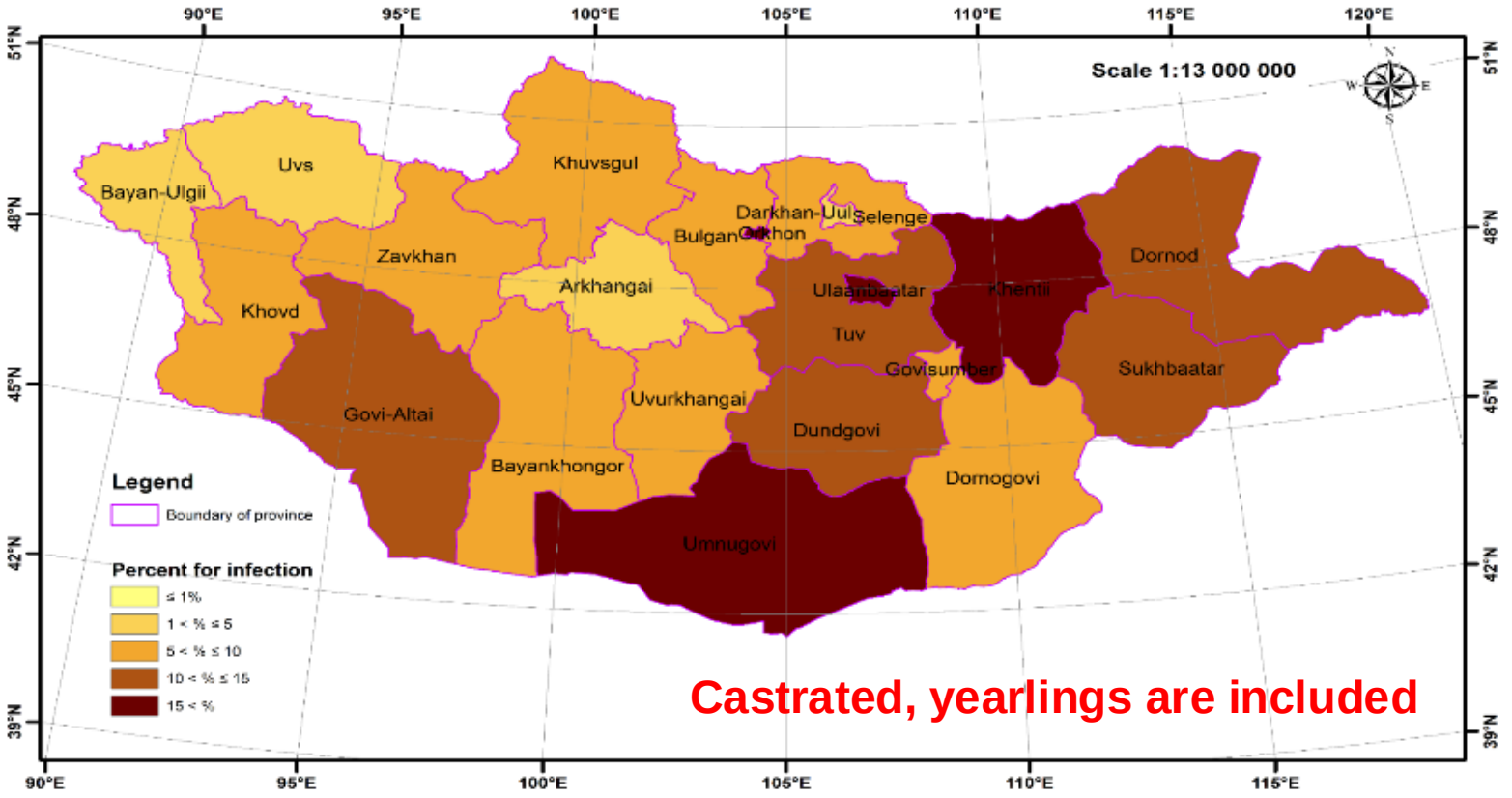
Surra outbreak reported in 1980s

Surra endemic in Xinjiang in China

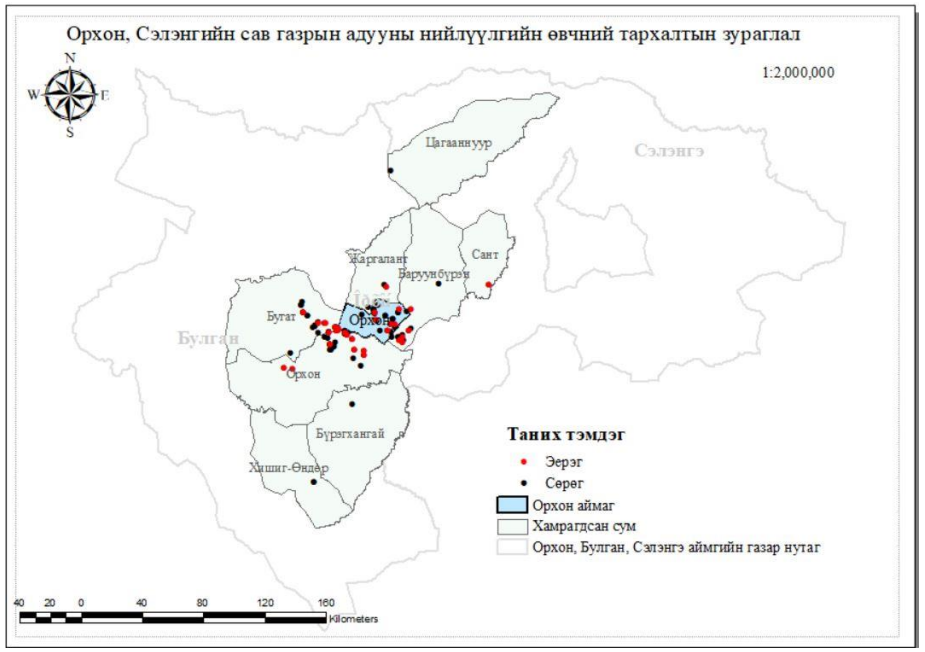
Dourine situation in Mongolia

Through SATREPS project (FY2014 to 2018) funded by JICA* and AMED*, nationwide epidemiological survey was conducted.

Epidemiological survey of dourine by Antibody detection ELISA



Castrated, yearlings are included



Out of 330 counties of Mongolia, 228 counties of 19 provinces were included in the surveillance from 2014 to 2018.

Total surveyed horse serum: 4,797

Average positive = 4.8%

Survey on Selenge and Orkhon province in 2024

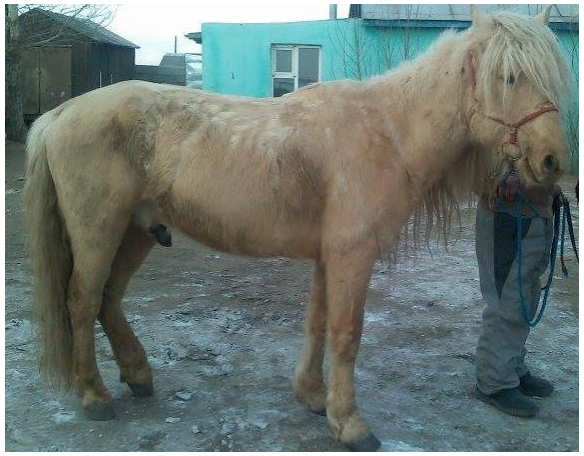
Total surveyed horse serum: 5040

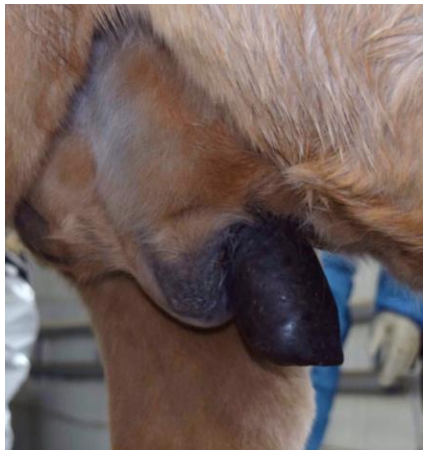
Average positive = 1.6%

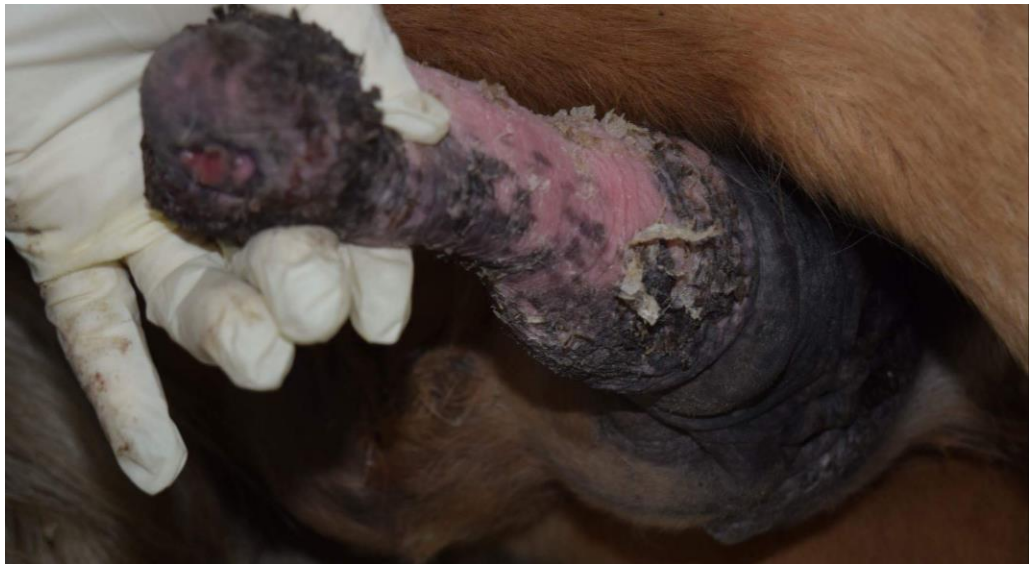
*-Japan International Cooperation Agency

*-Japan Agency for Medical Research and Development

Clinical signs







Major Symptoms:

1. Fever
2. Anemia
3. Local oedema of the genitalia
4. Paralysis
5. Depigmentation of genitalia

Drawbacks of diagnostic tests (WOAH Manual)

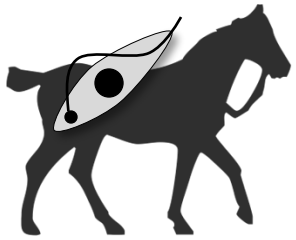
Method	Specificity	Sensitivity	Application
CATT	Good	Poor	Easy
CFT	Good	Good	Difficult
ELISA	Good	Good	Difficult
ICT	Good	Moderate	Easy
IFAT	Moderate	Moderate	Easy
PCR	High	High	Difficult

- Current diagnostic methods (CFT, CATT/*T. evansi*, ELISA and PCR) have disadvantages in either sensitivity or field application.
- **We need effective and user-friendly diagnostic tools for dourine.**

T. equiperdum isolation and whole genome analysis

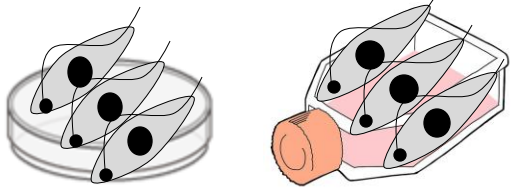
Publications

Isolation: Suganuma *et al*. (2016) Parasites & Vectors
Whole genome: Davaasuren *et al*. (2019) Microbiology resource announcements



Isolation of *T. equiperdum* from dourine horse

Culture-adapted *T. equiperdum*



Achievement!!

Applications using culture-adapted *T. equiperdum* for further research

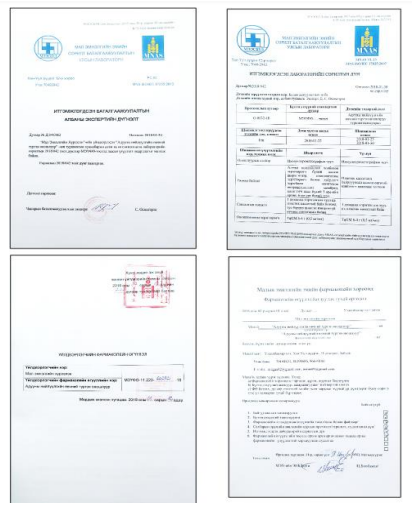
- Drug sensitivity assay → Development of trypanocidal drugs, treatment strategy.
- Experimental infection → Development of trypanocidal drugs, treatment strategy.
- Kinetoplast analysis → Clarification of species relationship and evolution among *Trypanozoon*.
- Whole genome analysis → Clarification of species relationship and evolution among *Trypanozoon*.
- Whole genome analysis → Development of novel diagnosis tools.

Culture-adapted *T. equiperdum* must be important research recourses and can be contributed for the progress of trypanosome research in future.

Development of Immunochromatographic test (ICT) for dourine (Mongolian Government Authority Accredited in 2018)

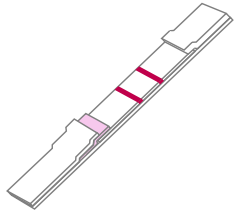
SATREPS
Science and Technology Research Partnership for Sustainable Development Program

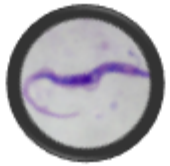
We have developed the ICT for dourine which was accredited by Mongolian Government Authority. This test is now being utilized throughout Mongolia, especially before horses are used for breeding.



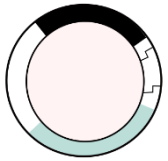
Culture obtained from infected hose (*T. equiperdum*)

Recombinant antigen for mass production for diagnostics





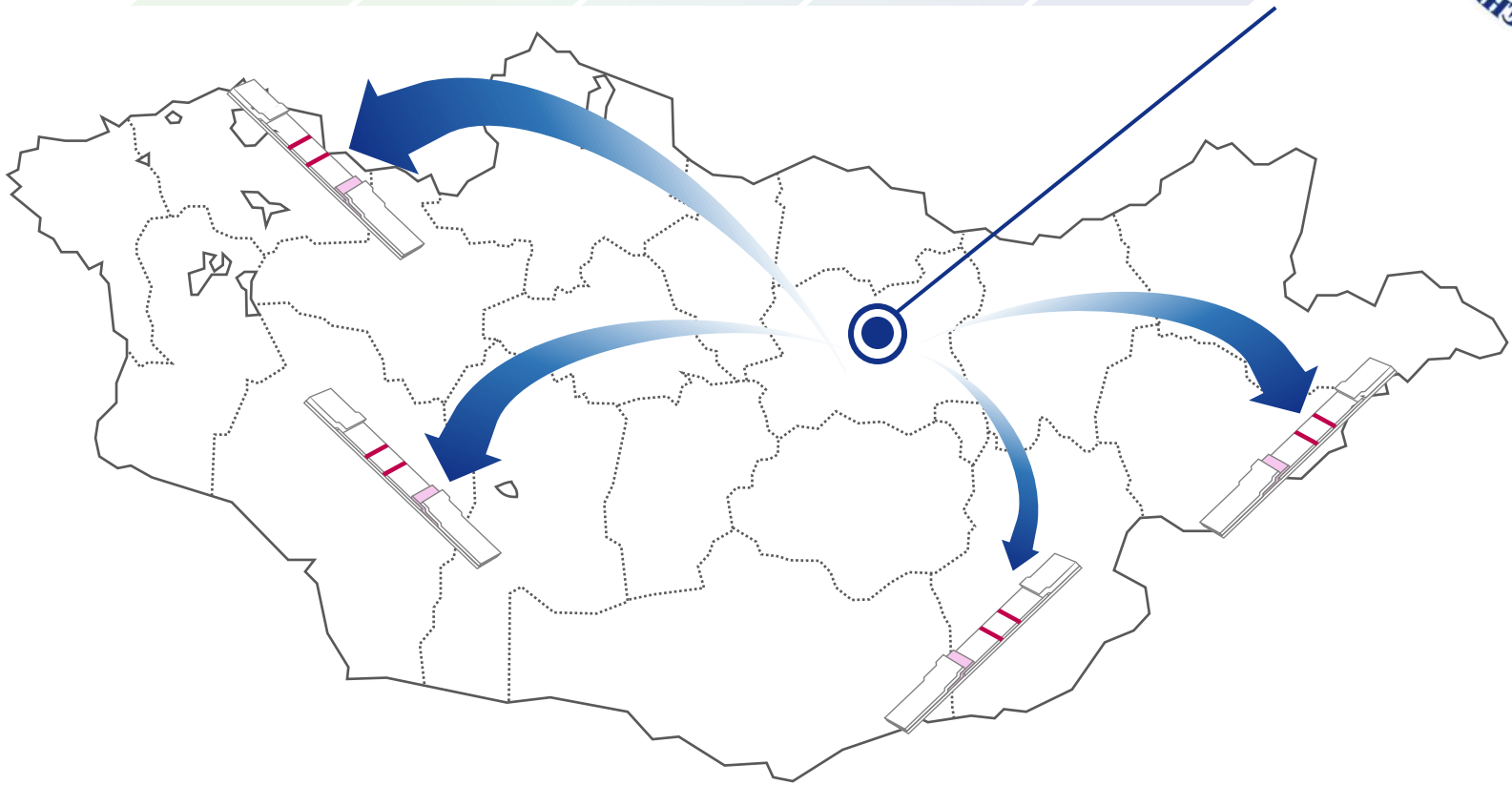
Detection of Effective antigen based on research based in on culture adapted culture.



Preparing Antibodies Against rGM6-4R Antigen of *T. equiperdum* and assembly of ICT

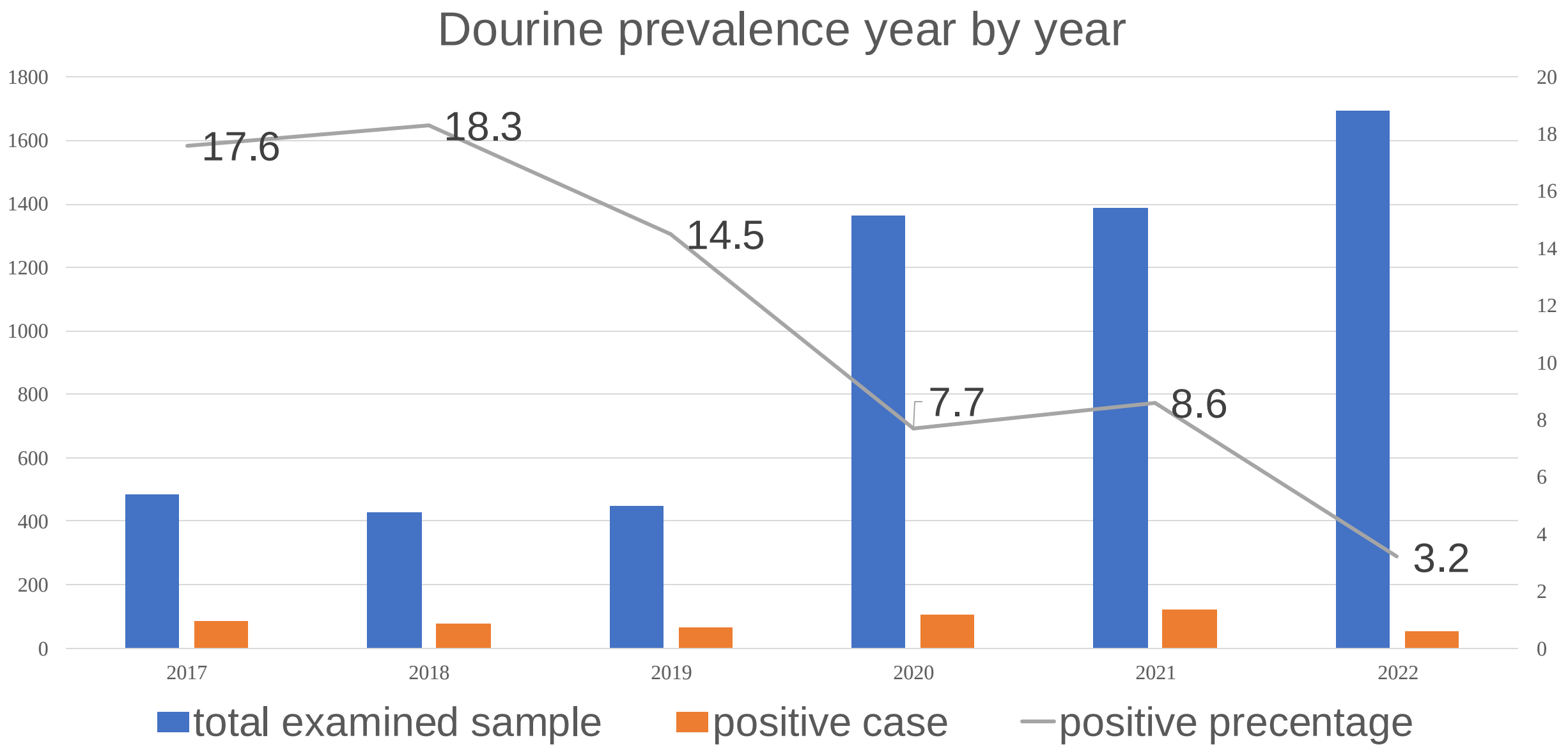
Practical use of the ICT in Mongolia

Diagnostic tool delivered to field

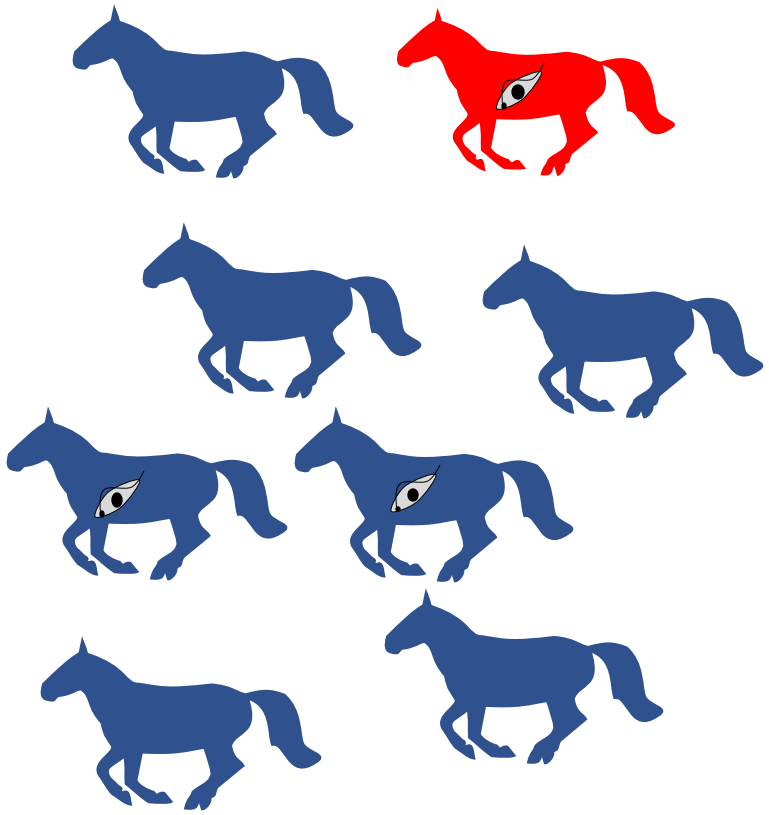


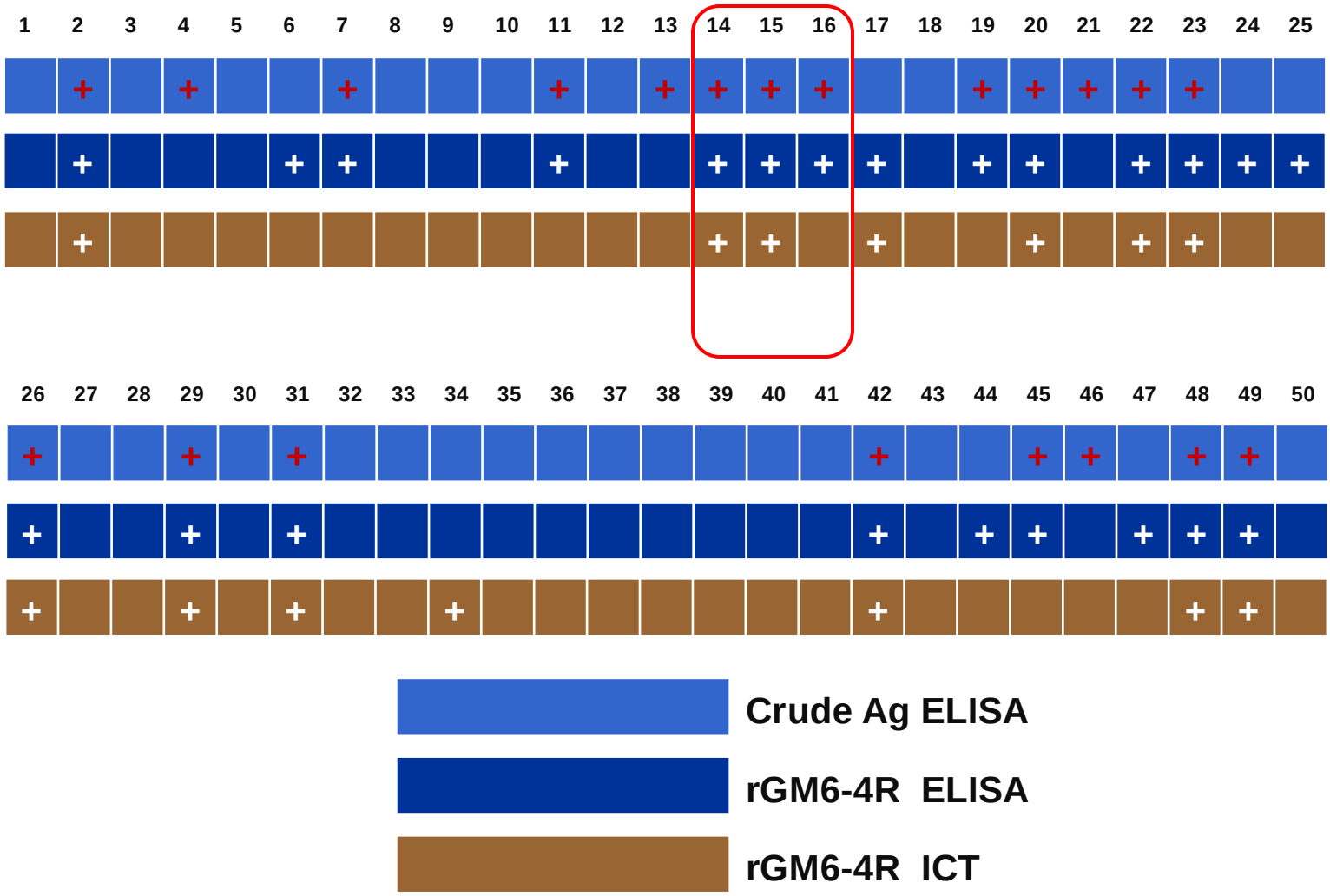
over 7,200 test strips of the ICT provided in 2024

Dourine case reduction from 2017-2022 in target group



Issue of asymptomatic case





rGM6-4R is a recombinant antigen utilized in our ICT test for dourine and ELISA

Ref.: Nguyen, T.T. *et al*. (2014) Veterinary Parasitology. 201 (1-2) 18-23.

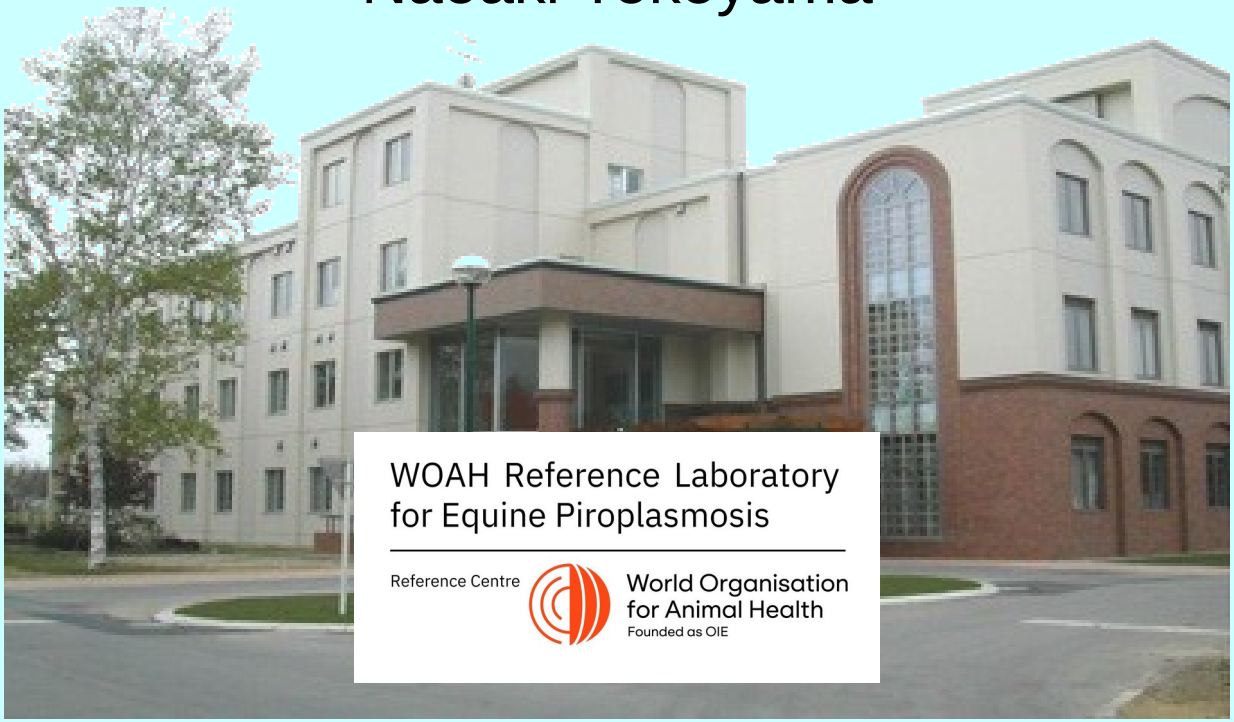
Summary of the presentation

- Dourine prevalence is decreasing in certain regions, but outbreaks remain a recurring issue.
- While Mongolia is currently free of surra outbreaks, the presence of active cases near its borders poses a significant risk.
- Implementing effective measures like culture isolation, diagnostic tool development, and prevalence surveys is crucial for mitigating the spread of these diseases.
- The availability of affordable diagnostic tools in targeted areas is contributing to reduced case numbers.
- Asymptomatic cases pose a significant challenge in disease control, as they can spread the infection without showing any symptoms.

Thank you for your attention

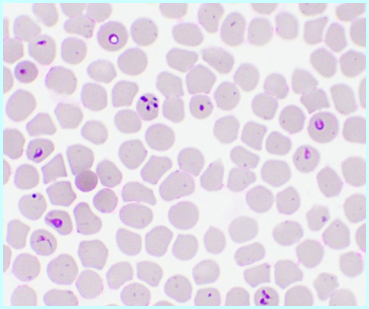
WOAH-recommended diagnostic assays for equine piroplasmosis and their limitations

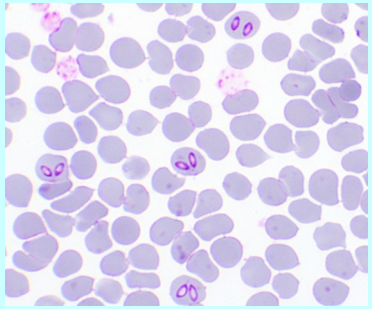
Naoaki Yokoyama



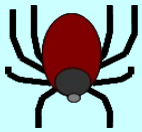
National Research Center for Protozoan Diseases
Obihiro University of Agriculture and Veterinary Medicine

Equine piroplasmosis





Theileria equi
Babesia caballi



Clinical (Anemia)

Death
Recovery

Sub-clinical

Hyalomma, Amblyomma, Rhipicephalus, and Dermacentor

Chronic carriers (Source of infection)

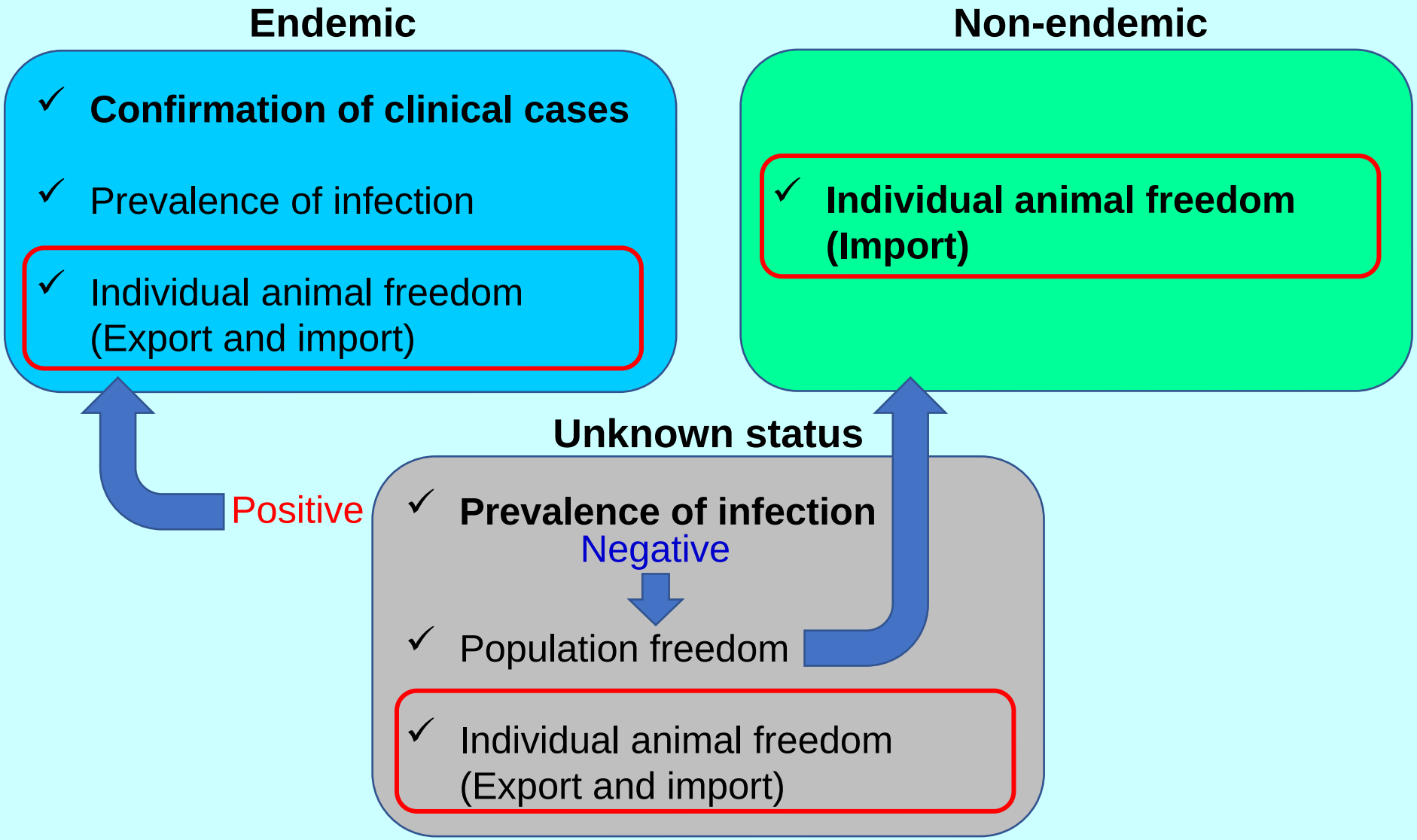
Global epidemiology of equine piroplasmosis

Choice of diagnostic assays

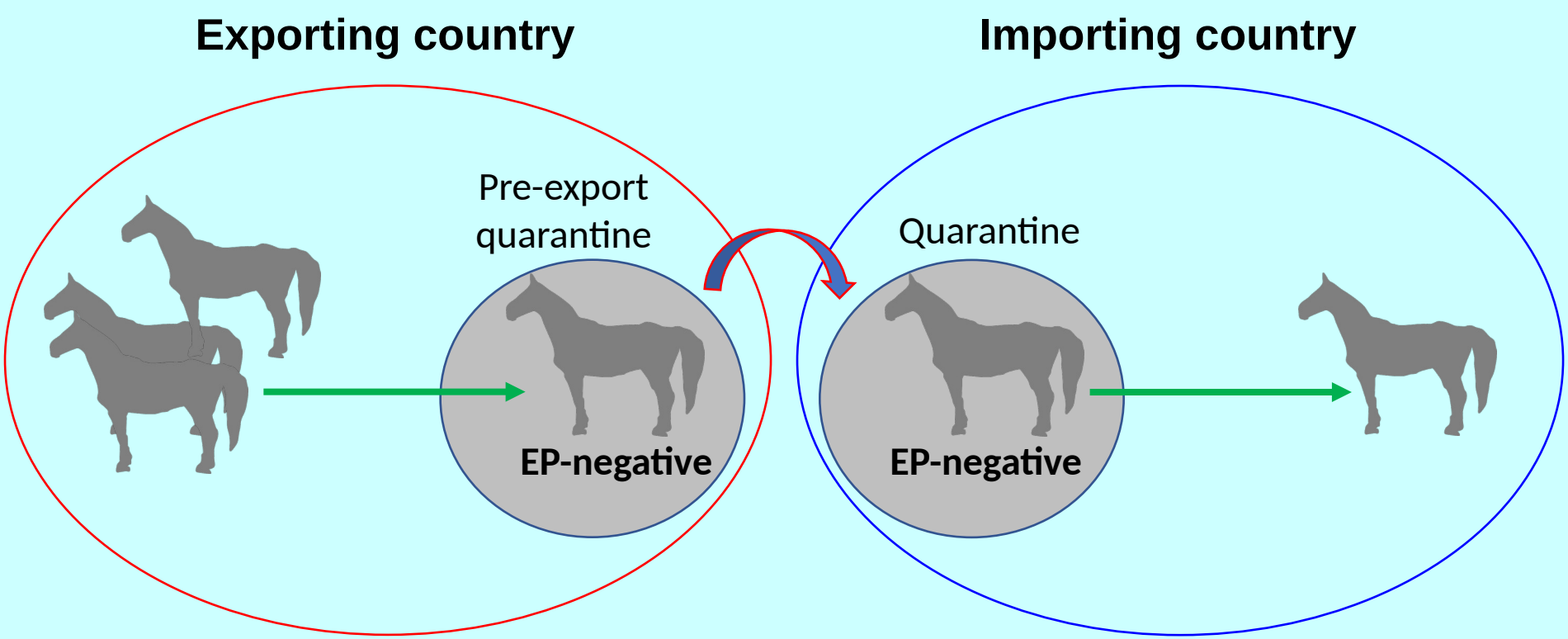
WOAH terrestrial manual

Method	Purpose				
	Population freedom from infection	Individual animal freedom from infection	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection - surveillance
Microscopy	-	+	-	++	+
PCR	+++	+++	+++	+++	+++
IFAT	++	++	++	-	++
cELISA	+++	++	+++	-	+++

Purpose of equine piroplasmosis diagnosis



Confirmation of individual animal freedom



Diagnostic tests used in international movement of horses

Importing country	cELISA	IFAT	CFT	Microscopy	Note
New Zealand	+	+			
Australia	+	+			
Canada		+			IFAT or cELISA
USA	+		+		
China (Hong Kong)		+			
Japan	+	+		+	cELISA or IFAT
Thailand		+	+	+	Microscopy or IFAT or CFT
UAE					
Singapore	+	+			cELISA or IFAT

Competitive enzyme-linked immunosorbent assay (cELISA)

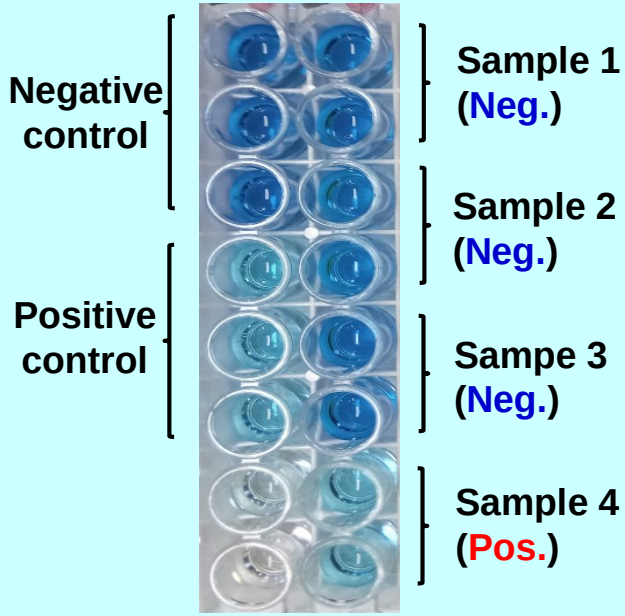
RAP1 or EMA1 Antigen

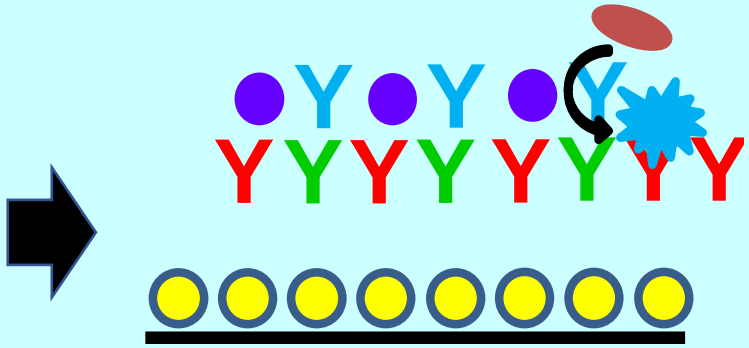
T. equi or *B. caballi* antibodies in test serum

Mouse anti-RAP1 or EMA1 Mab

Biotinylated anti-mouse IgG antibody, avidin-alkaline phosphatase complex

Substrate

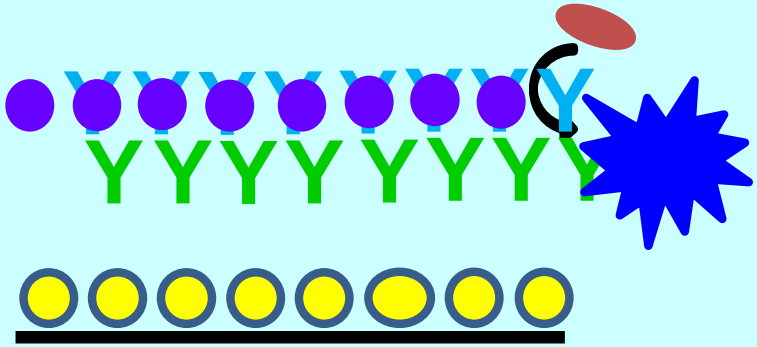




Positive (>40% inhibition)

High inhibition

Low OD

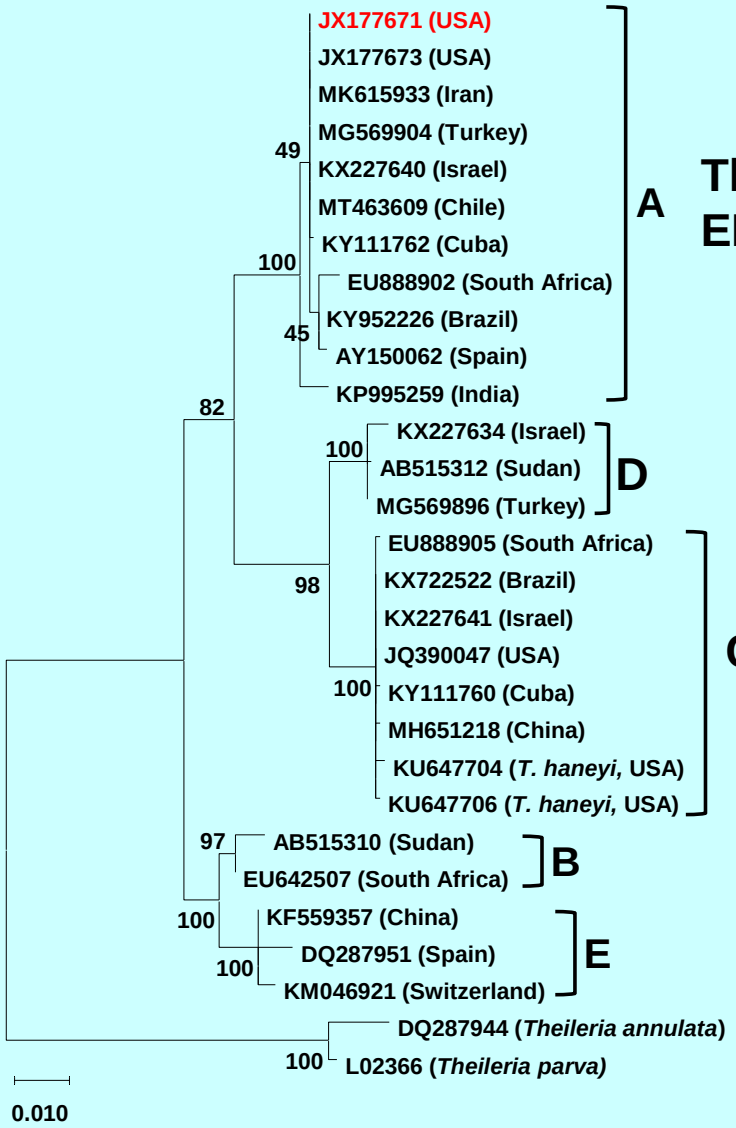


Negative (<40% inhibition)

Low inhibition

High OD

Limitation of *T. equi* cELISA

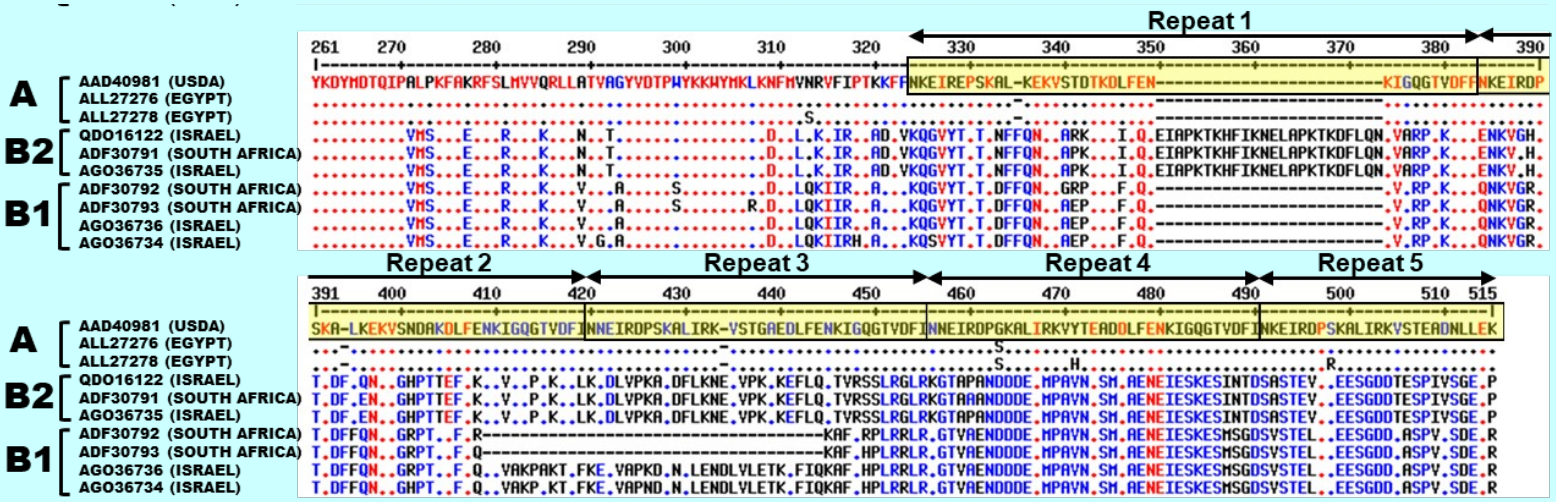


The cELISA was based on EMA1 from genotype A.

C (*T. haneyi*) lacks EMA1 → **False-negative cELISA results**

Limitation of *B. caballi* cELISA

- ✓ **False-negative results due to genetic diversity**



B. caballi cELISA was based on RAP1 from genotype A and cannot detect antibodies in animals infected with genotypes B1 and B2.

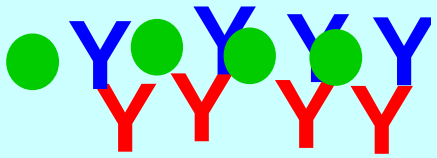
- ✓ **False-positive results potentially due to cross-reactive epitopes**

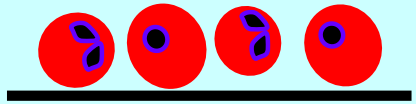
Immunofluorescent antibody test (IFAT)

T. equi- or *B. caballi*-infected RBCs

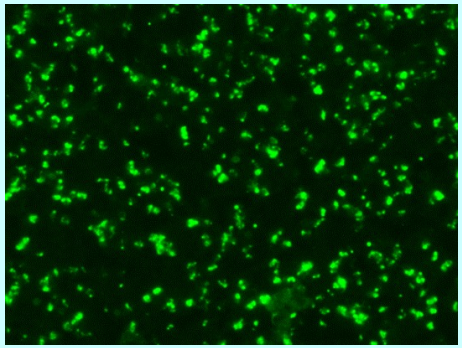
T. equi or *B. caballi* antibodies in test serum

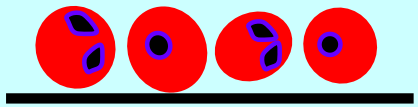
Fluorescence-labeled anti-horse IgG



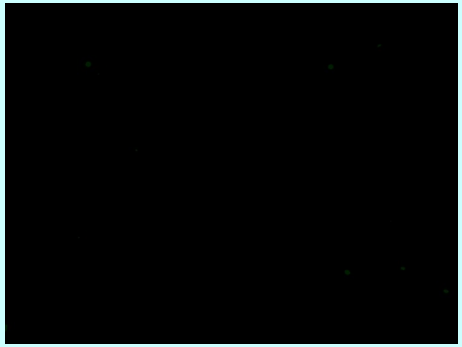


Positive





Negative



Advantages of cELISA over IFAT

	cELISA	IFAT
1. Commercial availability	Yes	No?
2. Available as kits	Yes	No
3. Interpretation of test results	Easy: Based on predetermined cut-off	Difficult: Requires expertise

Laboratories continue to use cELISAs despite their limitations.

Summary of our activities as reference lab

Provide diagnostic services
IFAT, cELISA, & PCR

Develop diagnostic assays
PCR & ELISA

Offer expert advice
Interpretation of results

Supply diagnostic materials
IFAT slides & DNAs

WOAH Reference Laboratory for Equine Piroplasmosis

Reference Centre — World Organisation for Animal Health, Founded as OIE

Provide training
Sero-diagnosis

IFAT slides supplied in 2023

France	2,400
UK	412
Ireland	40
Netherlands	680
Argentina	800
Australia	100
China	100
Singapore	100
India	60
Japan	400
Total	**5,092**

Improved diagnosis of equine piroplasmosis

World Organisation for Animal Health
Founded in 1924

Equine piroplasmosis - challenges when using WOAH-recommended tests

Sanjay Kumar
ICAR-National Research Centre on Equines, India

Global Economic Impact of the Equine Industry – 2022

Significant International Movement

- Between 2021 and 2022, the exports and imports of *Horses* grew remarkably – significant international movement of horses.
- Emphasize the need for more advancement in diagnostic development - safe cross-border movements of horses.

Involves ~ 4.22 billion US dollars ;
> 1.5 million full-time jobs

WOAH Recommended diagnostic test methods for equine piroplasmosis and their purpose

Methods	Population freedom from infection	Individual animal freedom from infection	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection - surveillance	Immune status in individual animals or populations post-vaccination
Detection of Agents – *Theileria equi* or *Babesia caballi*						
Microscopic Examination	-	+	-	++	+	-
PCR	+++	+++	+++	+++	+++	-
Detection of immune response - *Theileria equi* or *Babesia caballi*						
IFAT	++	++	++	-	++	-
cELISA	+++	++	+++	-	+++	-

+++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose

Detection of the Agent – *Theileria equi* or *Babesia caballi*

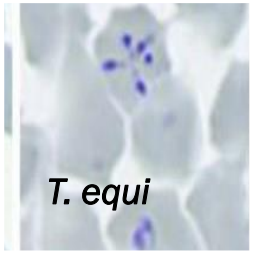
MICROSCOPIC EXAMINATION

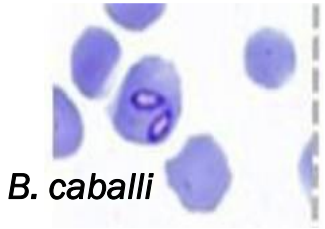
MOLECULAR METHODS – POLYMERASE CHAIN REACTION (PCR) ASSAY

Microscopic Examination

Per cent *T. equi* or *B. caballi* Parasitaemia

	T. equi	*B. caballi*	
Acute/Clinical	1 – 7 %	0.1 – 1 %	Microscopy is useful
Chronically carrier	**~0.1 %***	**Not possible**	**Microscopy is ineffective**





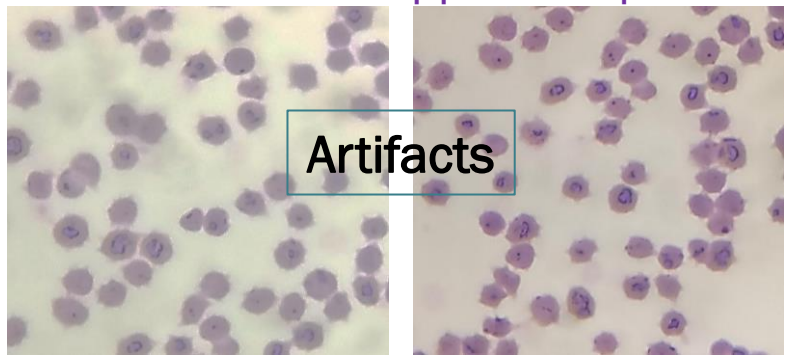
**: Horse once infected remains lifelong latently carrier to T. equi parasite*

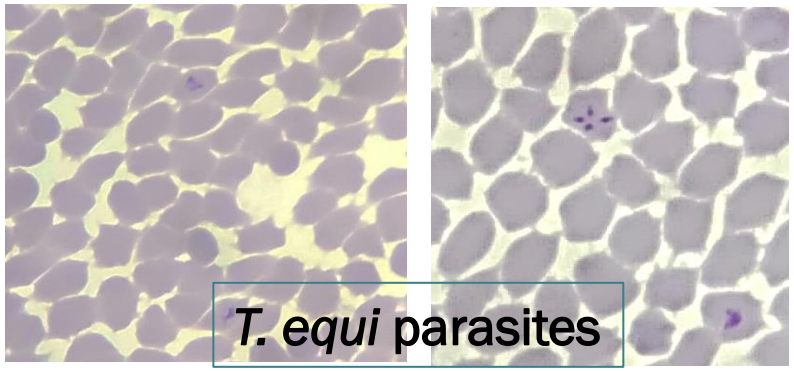
Challenges

- Blood smears should be prepared **within 2 to 3 hour of blood collection** (CDC, USA).
  - Delay may **affect the parasite morphology** and staining characteristics.
  - Rouleaux formation.
- Lab needs to **optimize** Giemsa Staining conditions.
- **Expertise is needed** to differentiate between artifacts and *T. equi* - or *B. caballi*.
- **Training is required** for quarantine veterinary officers and lab personnel.

OK

Difficult to appreciate parasite





Polymerase chain reaction (PCR) Assay

- **WOAH recommends 18S rRNA-based PCR assays for *T. equi - and B. caballi***
  - **Two specific primer sets** - Bashiruddin et al., 1999;
  - **Multiplex PCR** for simultaneous detection of *T. equi* and *B. caballi* - Alhassan et al., 2005.
- **DNA extraction methodology :**
  - Phenol Chloroform Isoamyl alcohol combination (PCI) / Commercial Kits.
- **PCR results may vary with the use of different PCR reagents/conditions:**
  - *Taq polymerase > Hot-start/ High-fidelity/ Ex Taq/ Multiplex etc.*
- ***T. equi / B. caballi* positive control DNA – no uniformity**
  - *DNA from* micro-aerophilous stationary phase *(MASP) cultured infected RBCs – USDA strain*.

PCR Assay

- **Heterogenicity in 18s rRNA from different genotypes of *T. equi* and *B. caballi*.**
  - **Requirement of validated PCR assay** having ability to detect all the genotypes of *T. equi*.
- **Sequencing of PCR amplified products** may be required for confirming the results.
- **An international consensus on PCR primer sets, other reagents, and cycling conditions is required.**
  - **WOAH manual needs to address PCR assay, keeping the present scenario of genetic diversity.**

Detection of *Theileria equi* and *Babesia caballi* antibodies

Indirect Fluorescent Antibody Test (IFAT)

Competitive Enzyme Linked Immunosorbent Assay (cELISA)

Indirect Fluorescent Antibody Test (IFAT)

- **Equipment Requirement** : Fluorescence microscope with 100x objective lens; calibrated.
- **IFAT slides preparation :** *T. equi* - or *B. caballi* infected RBCs from MASP in-vitro cultures.

Micro-aerophilous Stationary Phase - MASP in-vitro Culture system - Challenges

- **Specific requirements** : BSL-2 facilities, culture media, serum and RBCs from uninfected horse, and incubation conditions, (O_2, CO_2, and N_2).
- Not all strains of *T. equi* and *B. caballi* adapt to in vitro conditions.
- Frequent media change is necessary.
- **Time consuming** - Field strains may take 8 – 15 days to start growing in-vitro.
- **Few Laboratories maintain MASP cultures at present.**

IFAT - challenges

- **Results interpretation**:
  - **Subjective, expertise required**, especially when sera have low antibody titers.
- **Standardization Issues**:
  - IFAT kits are **not commercially available**.
  - The use of different commercial reagents for IFAT leads to inconsistent results between labs.
- **Certified Reference Materials (CRMs):**
  - Need of certified/reference *T. equi* - or *B. caballi* - **positive serum controls**.
  - **Only a few laboratories are able to share these control samples**.
- **Fluorescence** :
  - Quick observation is important to avoid false-negative result, as **fluorescent signals fade rapidly**.

cELISA – *Theileria equi* or *Babesia caballi*

- **Accessibility issues:**
  - VMRD Inc, USA, marketed globally through distributors.
- **Requirement for specific equipment** :
  - Calibrated ELISA reader, incubator, pipettes are vital for interlaboratory results consistency.
- **High cost - cELISA kits are expensive :**
  - One test kit = $1269.93.
  - Total Samples: 84 (in duplicate) = **$15.11/sample** (if full plate is used in one go), *excluding costs for lab infrastructure, instruments and man-power*.
  - Storage/Transportation : 2-8°C
- **Sero-epidemiological surveys using** cELISA are not economical.
  - **PCR assay – genetic heterogenicity – consensus or validated primer sets.**

Conclusions

Equine's Blood/serum Sample

Agent Detection

Microscopic Examination

- Acute/Clinical – Useful
- Chronic – Ineffective
- *T. equi* & *B. caballi* – together
- Expertise

PCR assay

- Multiplex PCR – simultaneous *T. equi* & *B. caballi* diagnosis
- Heterogenicity in 18s rRNA
- Optimization

Antibody Detection

IFAT assay

T. equi ***B. caballi***

- IFAT slides,
- Optimization, subjective
- No Commercial Kit

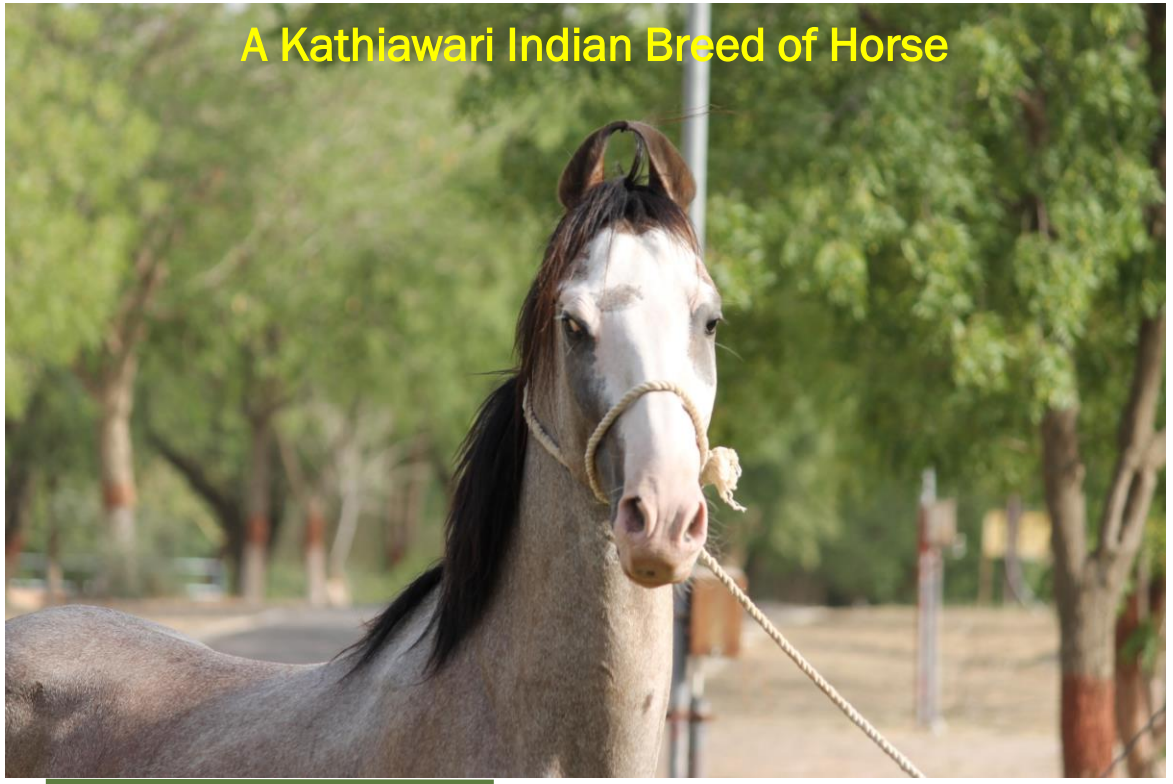
cELISA kit

T. equi ***B. caballi***

- Commercial Kit – VMRD, USA
- Expensive

Activities as National Referral Laboratory on Equine Piroplasmosis

- **Developed diagnostics assays**
  - ELISA (EMA-2 antigen based); Rapid diagnostic – LFA; IFAT; PCR and RT-PCR,
- **Performing WOAH-recommended diagnostic assays**
  - cELISA, IFAT, PCR, Microscopic examination
- **Maintain in-vitro cultures**
  - MASP in-vitro culture system for *Theileria equi* and IFAT slide production.
- **Activities**
  - Sero-epidemiological studies on Indian equines – ELISA.
  - Providing training to staff on Equine Piroplasmosis diagnostics to veterinarian and technicians.
  - Supplying diagnostic material – Reference positive *T. equi* DNA, serum samples, recombinant protein, stained–blood smears
  - Provide expert advice on treatment and diagnosis.
- **International Collaborative R&D activities**
  - Prof. N. Yokoyama, NRCPD, Japan – to investigate genotypic diversity of *T. equi* and develop improved diagnostics for equine piroplasmosis.
  - ASEAN countries – CTU, Philippines; CMU, Thailand.



Thank you

WOAH Reference Laboratory
for Equine Infectious Anaemia

Reference Laboratory

World Organisation for Animal Health

EIA: recommended diagnostic tests (WOAH Manual) and novel diagnostics tests

Dr Xiaojun Wang

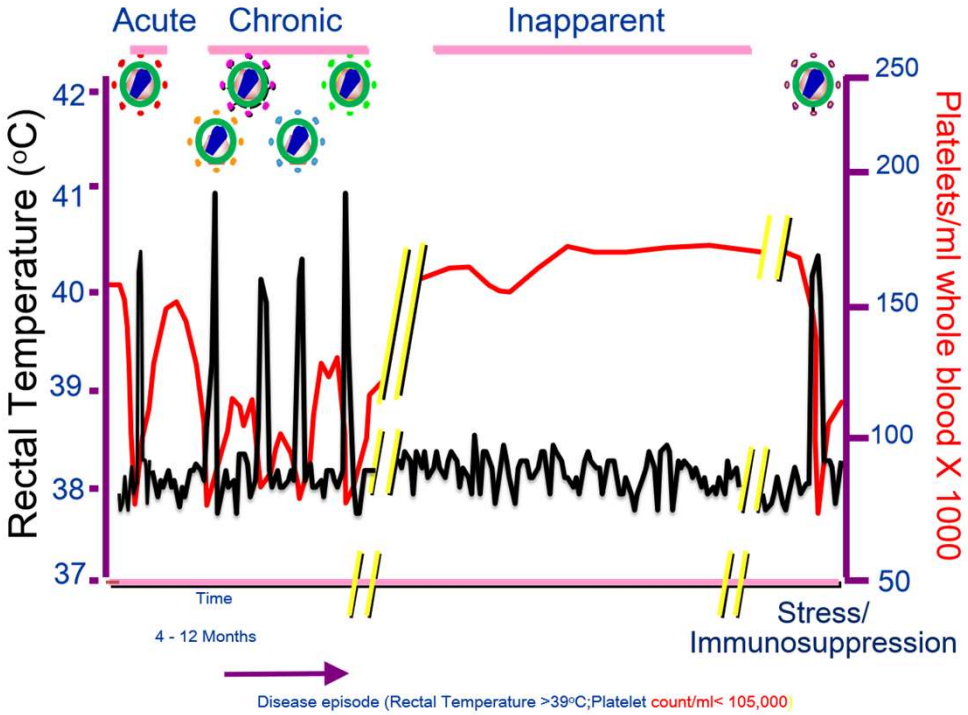
Harbin Veterinary Research Institute

the Chinese Academy of Agricultural Sciences

WOAH Reference Laboratory for Equine Infectious Aneamia

Email: wangxiaojun@caas.cn

EIA: course of the disease



✓***Acute phase:*** High fever with a severe anemia（~ 90 days）

✓***Chronic phase:*** recurrent febrile episodes (~1 year)

✓***Inapparent phase:*** long term carrier w/o any clinical signs (life-long)

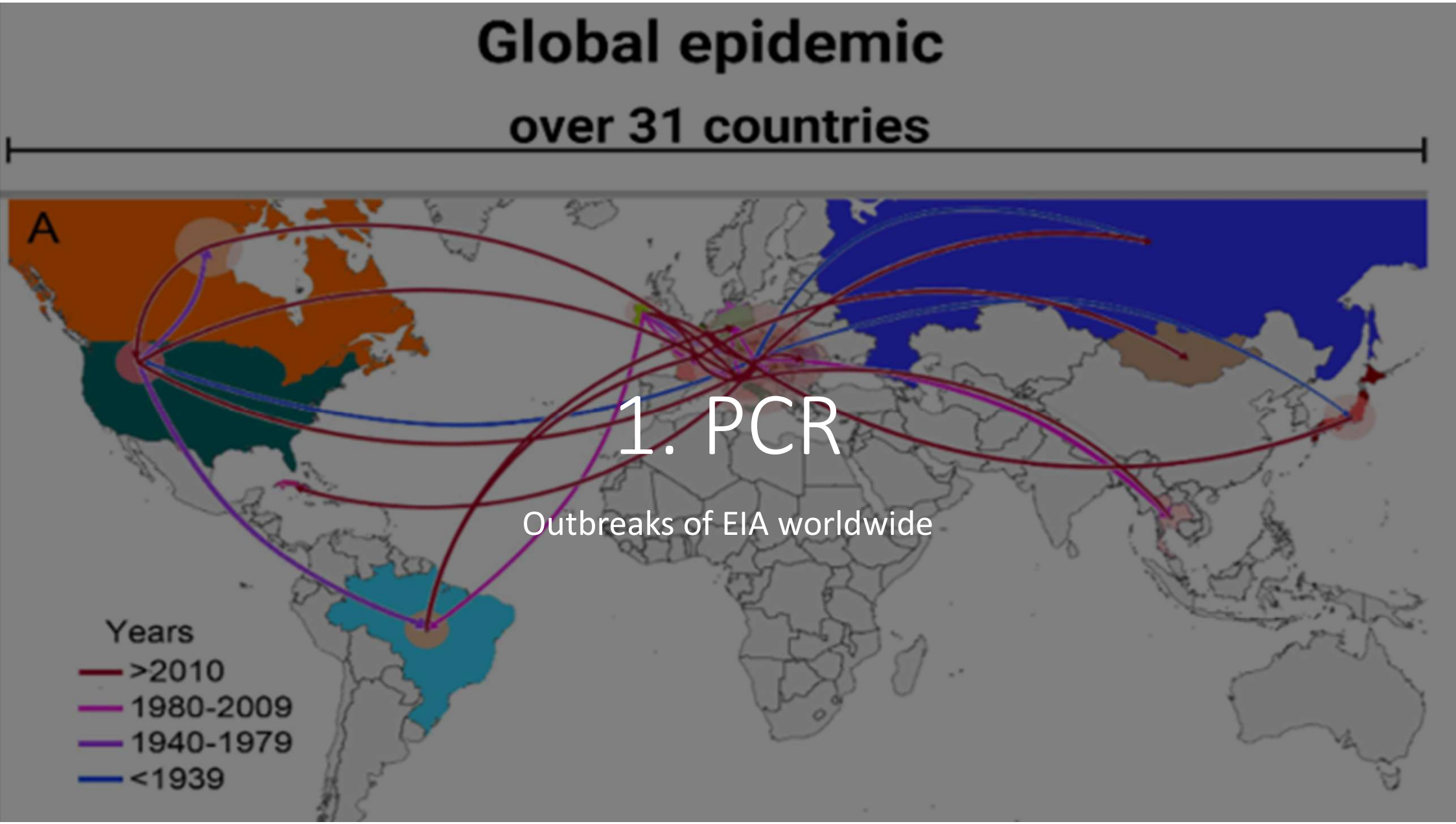
Recommended tests by WOAH

Main limitations

- Highly diverse viral genes
- Low viral load poor cell adaptation
- Low sensitivity
- Low specificity
- No commercial reagents

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Identification of the agent[1]						
PCR	–	+/–	–	+/–	–	–
Virus isolation	–	–	–	+	–	–
Detection of immune response						
AGID	++	++	++	++	++	–
ELISA	++	++	++	+	+	–
Immunoblot	–	++	++	++	–	–

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
PCR = polymerase chain reaction; AGID = agar gel immunodiffusion; ELISA = enzyme-linked immunosorbent assay.

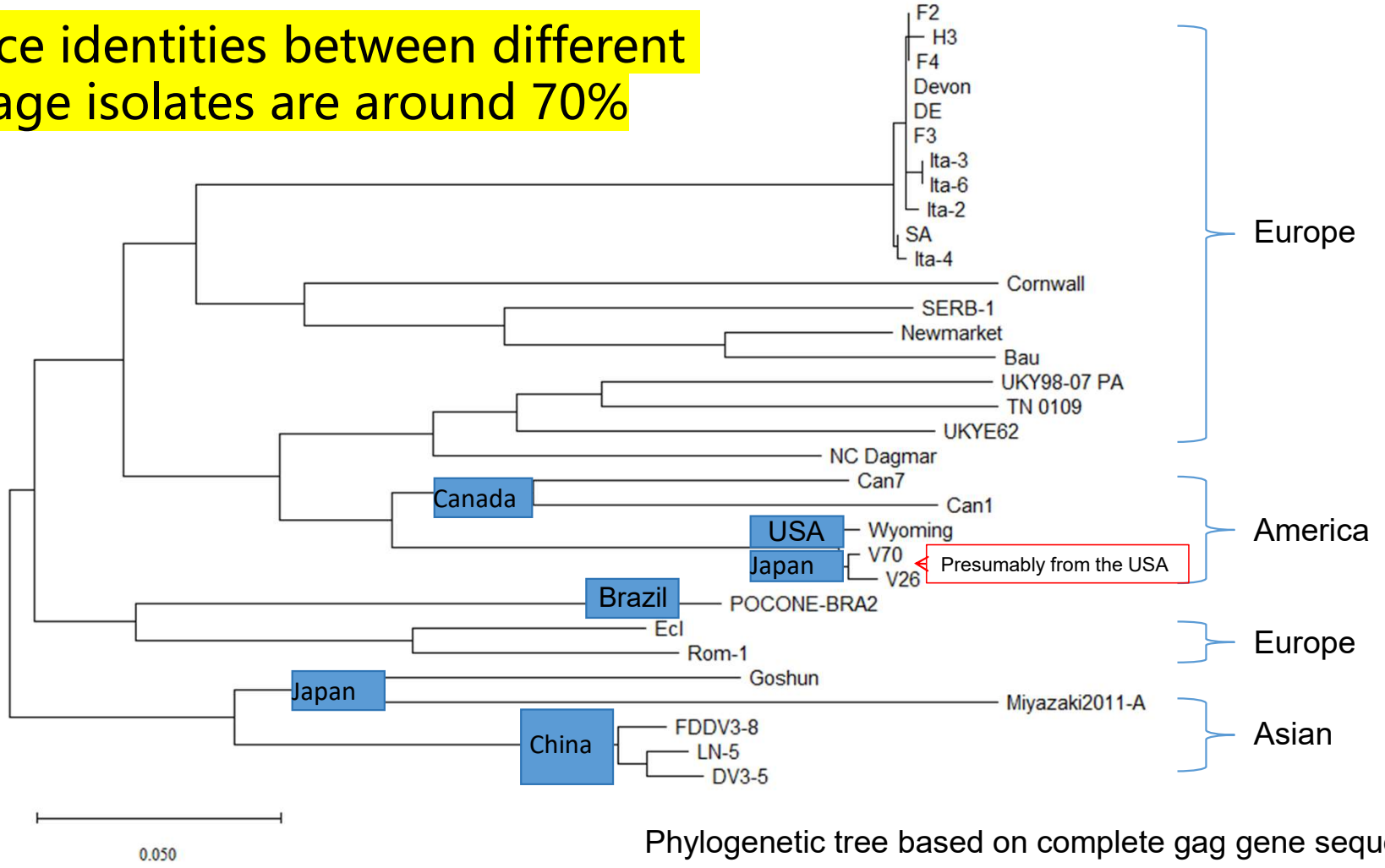


1. PCR

Outbreaks of EIA worldwide

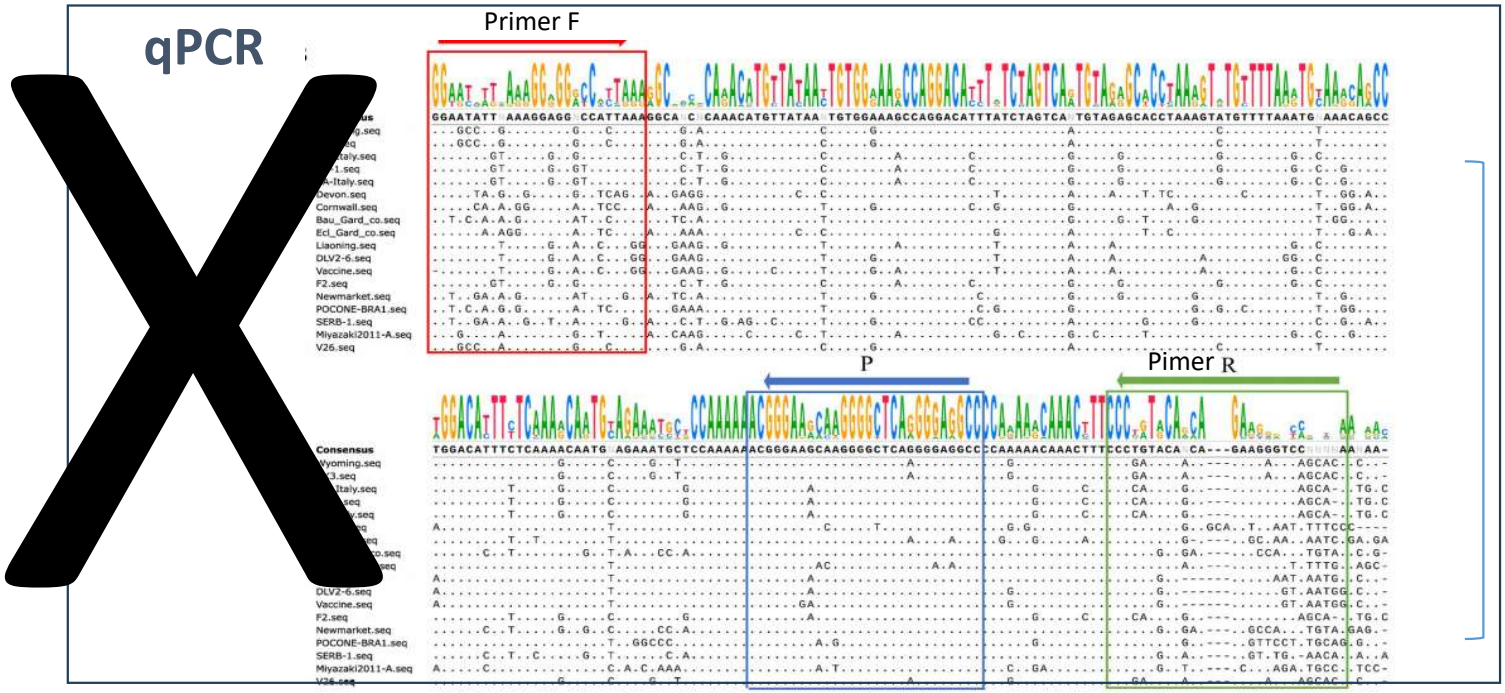
Phylogeographic patterns of EIAV

The sequence identities between different EIAV lineage isolates are around 70%



Phylogenetic tree based on complete gag gene sequences

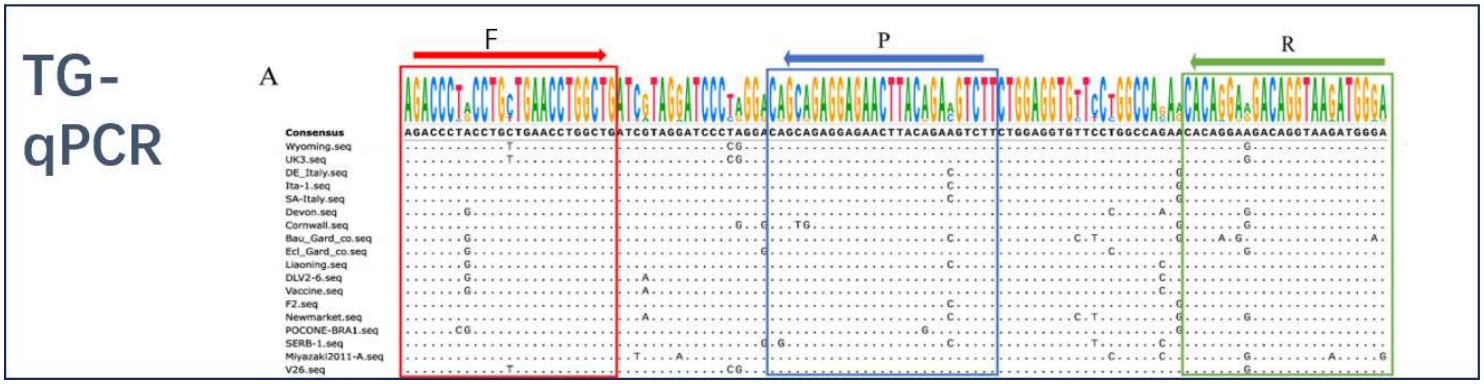
Limited usage of previous PCR method



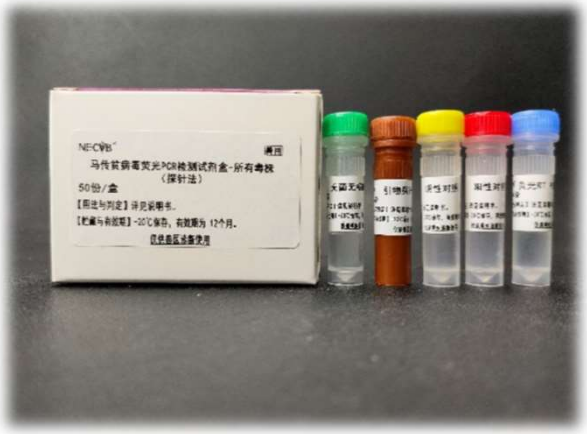
Current PCR recommended by WOAH is specific for certain strains

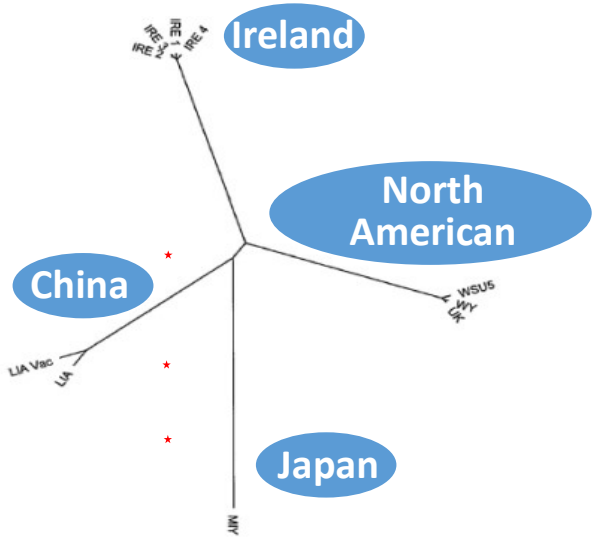
1. Newly developed TG-qPCR is suitable for more viral strains globally

tat-gag region of the EIAV genome



Designed based on conserved region of EIAV genome





TG qRT-PCR Kit

- **High sensitivity**
- **Universal detection**
- **Early diagnosis**

Shuaijie Li, et al. Microbiology Spectrum.2023

TG-qPCR is more suitable for global detection

TG qRT-PCR Kit

- Early diagnosis
- High sensitivity
- Universal detection

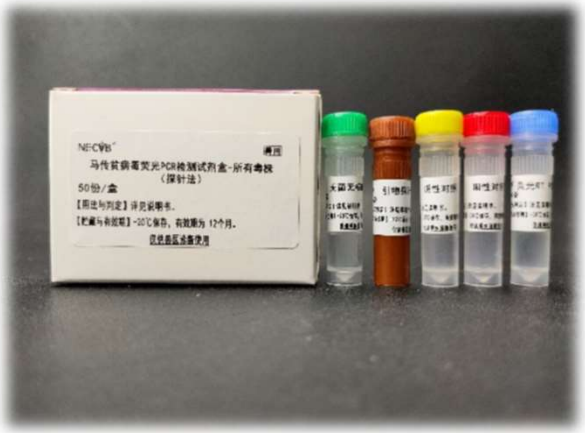


TABLE 4 Comparison of detection range between TG-qPCR and qPCR for detecting EIAVs from different geographic regions[a]

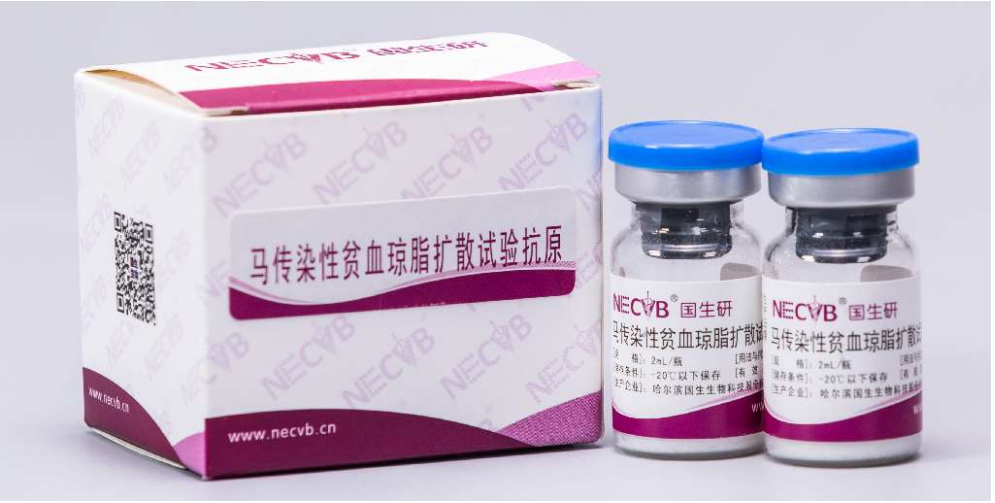
Templates[a]	TG-qPCR (Ct)	qPCR (Ct)
P-Wyoming	11.02	10.87
P-DE Italy	10.94	38.21
P-Ita-1	10.19	No Ct
P-SA-Italy	10.28	37.51
P-Devon	10.79	No Ct
P-Cornwall	10.21	No Ct
P-Bau Gard co	10.96	No Ct
P-Ecl Gard co	10.06	No Ct
P-F2	10.97	37.23
P-Newmarket	10.16	No Ct
P-POCONE-BRA1	11.23	No Ct
P-Miyazaki2011-A	11.52	No Ct
P-SERB-1	10.56	No Ct
P-V26	10.26	10.43
R-UK3	24.48	36.51
R-DLV2-6	25.71	No Ct
R-Liaoning	25.66	No Ct
R-Vaccine	29.98	No Ct

[a]"P" indicates synthetic plasmid, and "R" indicates virus RNA from EIAV strains infected cells.

Shuaijie Li, et al. Microbiology Spectrum.2023

2. AGID Test

- OIE recommended
- “Golden standard”
- Low sensitivity
- Time-consuming



International Comparison of AGID Tests

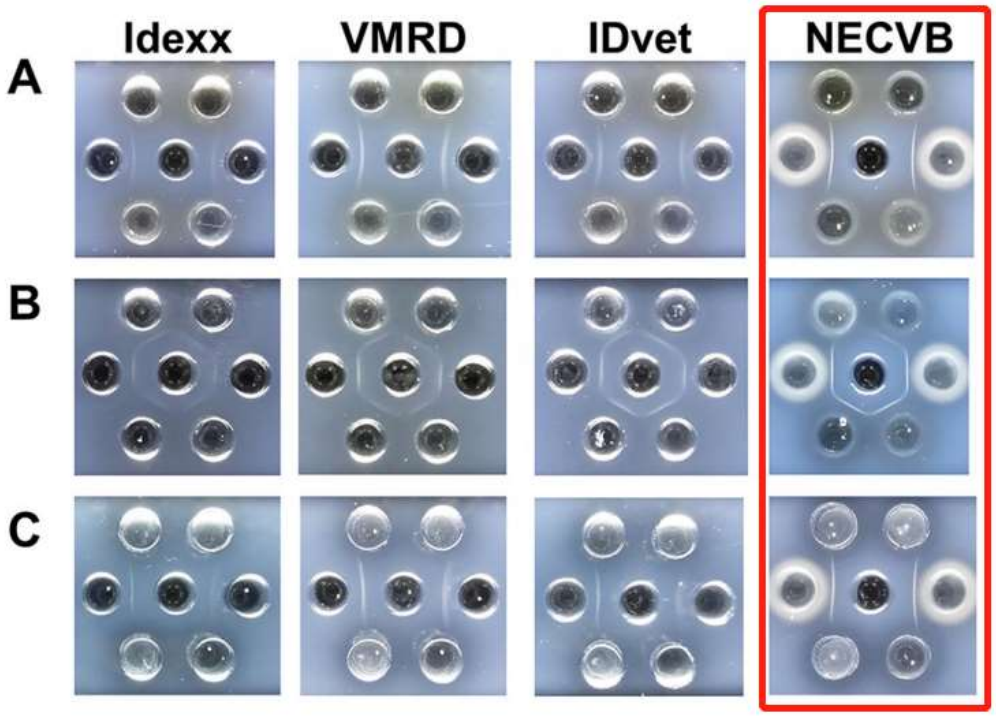


Figure 1. Results of testing for equine infectious anemia viral

Comparison of 4 agar gel immunodiffusion kits for serologic detection of equine infectious anemia virus antibodies

Hiroshi Bannai,[1] Yoshinori Kambayashi, Manabu Nemoto, Takashi Yamanaka, Koji Tsujimura

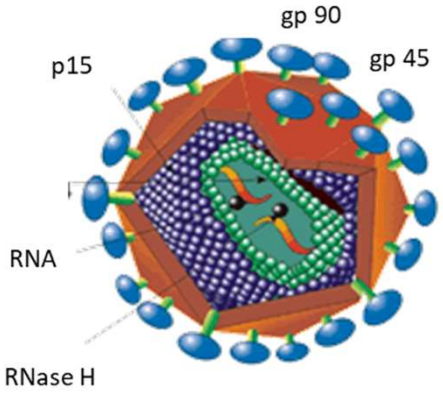
Journal of Veterinary Diagnostic Investigation
1–3

DOI: 10.1177/10406387231171567
jvdi.sagepub.com

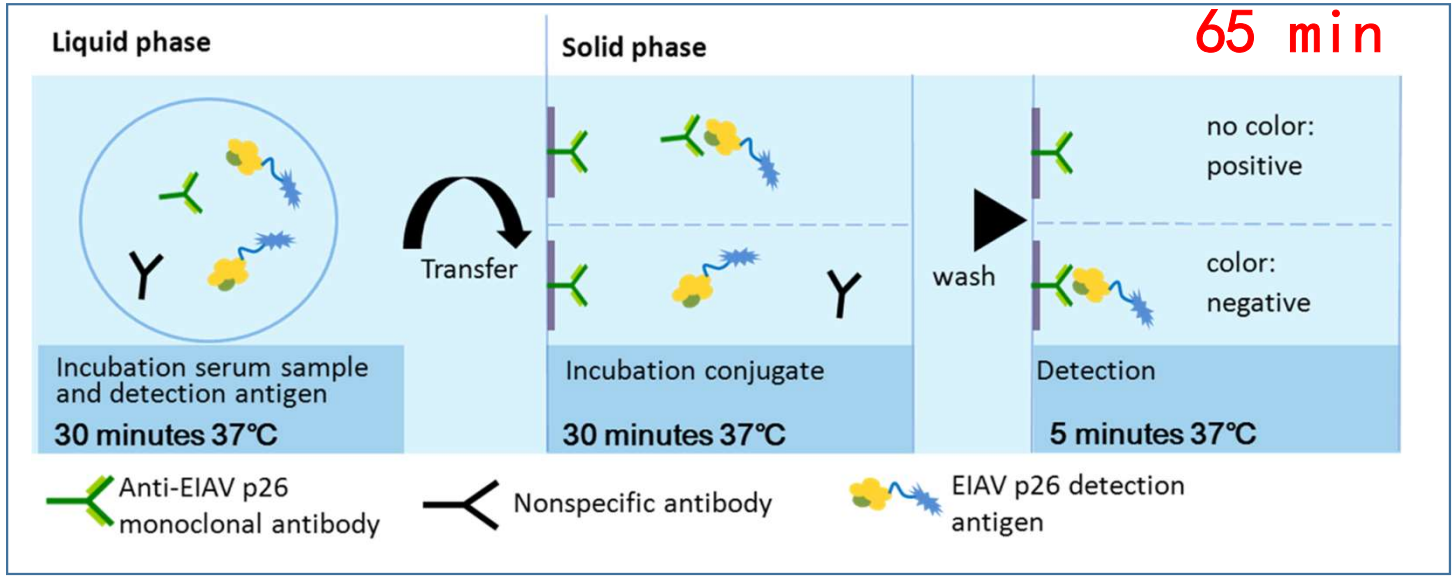
The positive control lines were sufficiently clear for evaluations to be made with all of the kits, but they showed a slight difference in sharpness: NECVB was the sharpest, followed progressively by VMRD, IDvet, and Idexx (Fig. 1A). No nonspecific lines were observed for any samples tested with the 4 kits. All of the kits detected strong-positive lines

3. cELISA

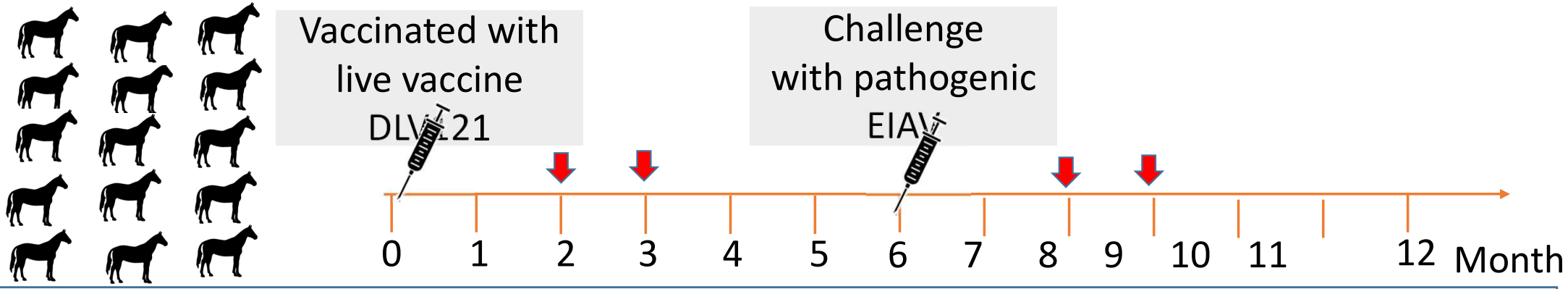
Detection of the Antibodies against EIAV



- ✓ Several commercial kits
- ✓ High sensitivity： 8-16 times higher than AGID
- ✓ High specificity: no false positive
- ✓ High throughput



Early detection: experimental infected horses



Time points	Month 2				Month 3				Month 8				Month 9			
Horse No.	IDEXX-AGID	IDEXX-cELISA	NECVB-cELISA	NECVB BP(%)	IDEXX-AGID	IDEXX-cELISA	NECVB-cELISA	NECVB BP(%)	IDEXX-AGID	IDEXX-cELISA	NECVB-cELISA	NECVB BP(%)	IDEXX-AGID	IDEXX-cELISA	NECVB-cELISA	NECVB BP(%)
#2	-	+	+	92.06	-	-	-	45.79	-	+	+	61.24	-	+	+	79.24
#3	-	+	+	74.79	-	-	-	51.34	-	-	-	21.93	+++	+	+	99.94
#5	-	-	+	96.34	++	+	+	100.00	-	+	+	93.1	-	+	+	97.44
#8	-	+	+	85.29	-	+	+	67.77	-	+	+	59.83	-	-	-	37.73
#14	-	+	+	93.71	-	+	+	95.24	-	+	+	70.63	-	+	+	68.32
#4	-	-	-	14.59	-	-	-	16.30	-	-	-	14.29	++++	+	+	100.12
#6	-	-	-	-1.77	-	-	-	44.08	-	-	-	11.36	-	+	+	55.13
#1	-	+	+	76.56	-	+	+	72.41	-	-	-	29.24	-	+	+	99.08
#21	-	-	-	17.09	-	-	-	14.77	-	-	-	10.56	-	+	+	75.34
#9	-	+	+	58.06	-	-	-	50.18	-	-	-	21.06	N/A	N/A	N/A	N/A
#7	-	+	+	68.55	-	-	-	41.24	N/A	N/A	N/A	N/A	++	+	+	98.35
#10	-	+	+	88.69	-	-	-	48.69	-	-	-	13	+	+	+	100.12
#11	-	+	+	58.79	-	-	-	36.20	-	-	-	18.8	-	+	+	98.29

cELISA: High sensitivity

The titers of antibodies against EIAV from China

Sample \ Kit	AGID			Competitive ELISA	
	IDEXX	VMRD	NECVB	IDEXX	HVRI
14EIA004	8x	8x	8x	64x	64x
Lin	8x	-	8x	64x	128x
2011	8x	4x	8x	128x	128x
Ruo	1x	-	1x	32x	32x
2009	16x	8x	8x	128x	128x

"x" stands for the most dilution fold for the detection.

Hu et al., Applied Microbiology and Biotechnology 2023

cELISA: High sensitivity

The titers of antibodies against EIAV from USA

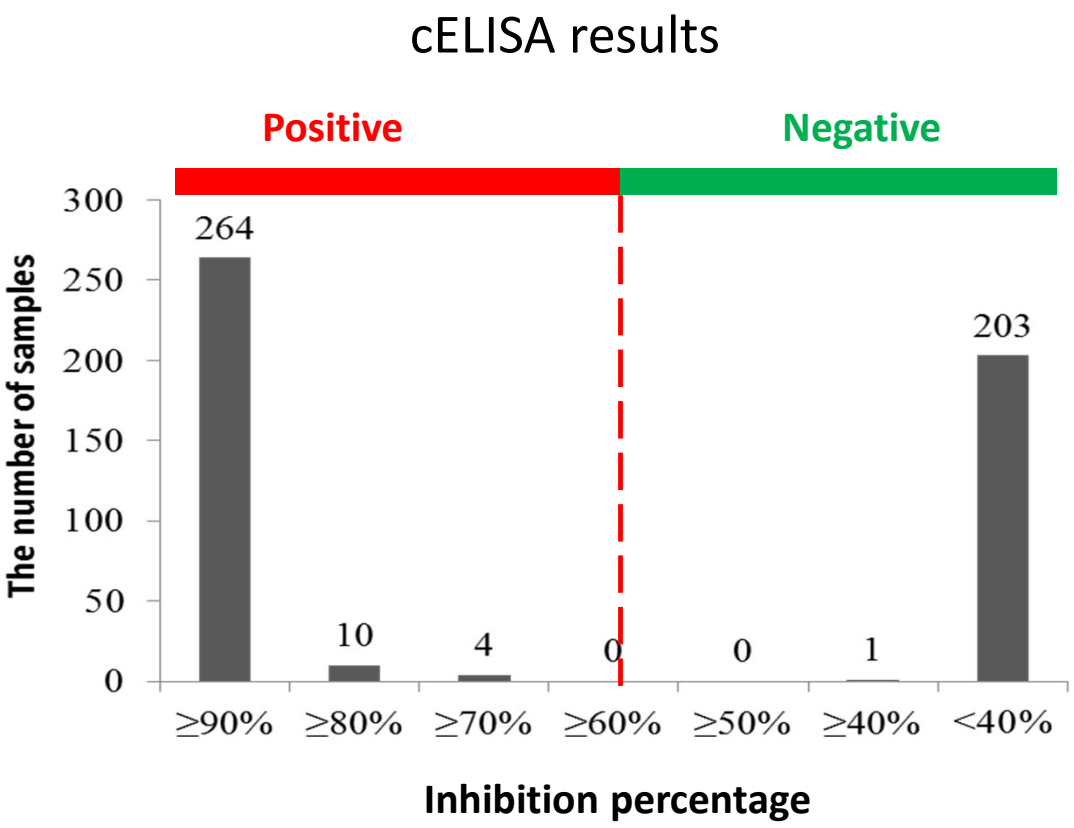
Sample \ Kit	AGID			Competitive ELISA	Indirect ELISA	Competitive ELISA
	IDEXX	VMRD	HVRI	IDEXX	VMRD	HVRI
NVSL-902	2x	N/A	2x	16x	128x	64x
NVSL-903	1x	N/A	1x	4x	64x	32x
VMRD-Strong	-	-	-	-	8x	16x
VMRD-Medium	-	-	-	-	2x	4x
VMRD-Weak	-	-	-	-	2x	2x

"x" stands for the most dilution fold for the detection; ND = Not Applicable.

Method	Kit	VMRD Anti-EIAV		
		Strong	Medium	Weak
cELISA	HVRI	+	+	+
	IP	99.39%	88.42%	67.45%
Western blot	1000 dilution of serum			

Broad spectrum: Testing samples from Argentina

481 samples from Argentina were imported in 2014



HVRI cELISA	IDEXX AGID		Total
	+	-	
+	261	18*	279
-	0	202	202
Total	261	220	481
Coincidence rate	100.00%	91.82%	96.26%

HVRI cELISA	IDEXX cELISA		Total
	+	-	
+	278	1*	279
-	0	202	202
Total	278	203	481
Coincidence rate	100.00%	99.51%	99.79%

* True positive confirmed by WB

Third-party Assessments of the cELISA Kit

Agriculture, Fisherises and Conservation Department of Hong Kong Special Administrative Region

40 samples from USA
AGID(IDEXX)
cELISA(IDEXX)
cELISA(NECVB)
Specificity: 100%
Sensitivity:100%

Year: 2018

National Engineering Research Center of Veterinary Biologics (NECVB) cELISA Assay EQUINE INFECTIOUS ANEMIA (EIA) Verification Record Sheet

1	**Description of Test** (Type of assay, whether derived from OIE prescribed or alternative test, published or in-house development) (Except sample / tissue type to be tested: preservative used, age / shelf life, temperature, transport requirements)	Evaluation of commercial cELISA test (NECVB) for the detection of EIA antibody in equine sera.
2	**References** (Provided relevant references including any that the test was based on; list publications resulting from this work if already published or submitted)	1. Kit inserts of Equine Infectious Anemia Virus cELISA Antibody Test Kit, NECVB. 2. Requirements for Equine Infectious Anemia Agar Gel Immunodiffusion (Coggins) and Enzyme-Linked Immunosorbent Assay Testing at Veterinary Services Approved Laboratories. SOP-EO-0034.02, 25 Mar 2014.
3	**Test Method Protocol** (Sufficient detail needs to be provided so that the test can be repeated by someone with experience in the basic technique) Reference to documented method	EDM-5 National Engineering Research Center of Veterinary Biologics (NECVB) cELISA Assay EQUINE INFECTIOUS ANEMIA (EIA)
4	**Prior Treatment of Specimen** (Selection of specimens, include collection, transport, method of preparation, concentration, NA extraction etc., etc.)	1-2 ml serum samples are separated from the clot and put in sterile containers as soon as possible and stored at 2-8℃, or stored frozen until testing.
5	**Reagent Selection** (Describe the controls and reagents that are being used and why they were selected for this assay development e.g. type of conjugate etc Provide details of suppliers and information on the continuous availability of supply of essential reagents)	Equine Infectious Anemia Virus cELISA Antibody Test Kit (National Engineering Research Center of Veterinary Biologics (NECVB). #NEE26300) (i) 96 well microtitration plates (stripwell format) – coated with anti-EIAV antibody. (ii) Positive control (iii) Negative control (iv) Washing solution (10x) – dilute buffer 1:10 with distilled water (v) EIAV antigen conjugate (Do not keep at room temperature for more than 2 hrs) (Ready to use) (vi) Substrate A (Ready to use) (vii) Substrate B (Ready to use) (viii) Stop solution (Ready to use)
6	**Repeatability and Reproducibility** (Repeatability; testing conducted with the same method on identical samples in the same laboratory by the same operator using the same equipment within short intervals of time. Reproducibility; testing conducted by different operators, preferably different laboratories)	**Repeatability** 1 set of samples (20 in total – QAP samples from NVSL) are tested including 7 negative sera and 13 positive sera (vary from weak to strong positive) repeated by the same operator in 3 days and the obtained results were reproducible (Refer to Table 1-3). **Reproducibility** 2 sets of samples (40 in total – QAP samples from NVSL)

		are tested including 14 negative sera and 26 positive sera (vary from weak to strong positive) repeated by 3 operators on the same date and the obtained results were reproducible (Refer to Table 4-6).
7	**Sensitivity and Specificity** (Proportion of know infected reference animals that tested positive)	20 negative, 10 weak positive and 10 strong positive sera (NVSL) are tested respectively (Refer to Table 7). Both Sensitivity and Specificity are 100%
8	**Reference Test (Gold standard)** (Justification for the reference method)	AGID is the golden method for the detection of EIA in equine serum
9	**Consistency with Reference Test**	The results obtained are 100% consistent with EDM-3 and EDM-4
10	**Controls and Reference Reagents** Describe any further controls required (positive and negative) and the reasons for their use	All controls are provided in the commercial kit
11	**Assay Interpretation** (Describe interpretation including expected results from positive and negative samples)	Based on the calculation of percentage of inhibition (I %) I% = [(OD NC - OD sample) / (OD NC - OD PC)] x 100% Negative: I% < 55% Positive: I% ≥ 55% Test valid criteria: Negative control: mean of OD_{NC} > 1.0 Positive control: mean of OD_{PC} < 0.2
12	**Measurement Uncertainty (MU)*** * It is only a preliminary MU and will be updated and reviewed annually.	Low PC serum will be made by diluting PC (kit) with negative equine sera so as to make that the I% of low PC (I%L) closed to the cut-off (55%) (Table 8). Low PC serum is tested 102 times in the 3 runs by 3 operators, the relative standard deviation (RSD) is calculated: RSD (I%L) = SD / Mean = 2.62 / 64.88 = 0.04 Expanded Uncertainty (U) is the statistic defining the interval within the value of the measure within 95% level of confidence (CI). Expanding the uncertainty is done by multiplying the RSD(I%L) by a factor 2, U (95%CI) = 2 x RSD = 0.08 This estimate can then be applied at the threshold level 95% CI = 55 ± (55 x 0.08) = 55 ± 4.4 Interpretation of MU Any result of I% falls between 50.6% to 59.4% is regarded as indeterminate within 95% confidence.
13	**Quality Assurance**	Quality assurance program from NVSL is joined on 6-month basis.

International Comparison of cELISA Tests

No.	Sample ID	Store No.	Harbin-ELISA	AGID	Idexx-ELISA	Eradikit-ELISA
158	SE 15/20	S135-58	Negative	Negative	Negative	Positive
159	SE 17/20	S136-9	Negative	Negative	Negative	Positive
160	SE 91.2/20	S09-39	Negative	Negative	Negative	Doubtful
161	SE 135.1/20	S06-2	Negative	Negative	Negative	Doubtful
162	SE 211.14/20	S08-58	Negative	Negative	Negative	Doubtful
163	SE 284.2/20	S10-45	Negative	Negative	Negative	Positive
164	Muneca	-	Positive 98.1%	Positive	Positive	Positive
165	EQC 17/7839	-	Positive 99.1%	Strong Positive	Positive	Positive
166	EQC 17/7840	-	Negative	Negative	Negative	Negative
167	EQC 17/7841	-	Positive 99.4%	Positive	Positive	Positive
168	IdVet Ref.sera Neat	-	Positive 98.6%	Positive	Positive	Positive
169	IdVet Ref.sera 1:4	-	Positive 90.5%	Negative	Negative	Positive

True Negatives: 163 (equine samples); 1 (EQC Negative)

Specificity

Harbin ELISA	100%
AGID	100%
Idexx ELISA	100%
Eradikit ELISA	77.30%

True Positives: 1 (Field case); 2 EQC (positive); 2 (Reference sera)

Sensitivity

Harbin ELISA	100%
AGID	83.30%
Idexx ELISA	83.30%
Eradikit ELISA	100%

Year: 2020

CENTRAL VETERINARY RESEARCH LABORATORY, DUBAI, UAE

Priv. Doz. Dr. Dr. habil. U. Wernery

Scientific Director

Central Veterinary Research Laboratory,
P.O. Box 597, Dubai, UAE,

VETQAS APHA SCIENTIFIC

Proficiency testing for veterinary laboratories

Year: 2019

Animal & Plant Health Agency

Results tabulation for 13982/SE PT0046: Equine Infectious Anaemia (EIA)

Distribution Date: 22/10/2019

AGIDT			19/7896	19/7897	19/7898	19/7899	19/7900
	Kit Manufacturer	Kit Batch Number	Results	Results	Results	Results	Results
Intended			Negative	1/8 dilution	1/8 dilution	Negative	1/4 dilution
			-ve	+ve	+ve	-ve	+ve
1007	VMRD	P181215-001	-ve	W+ve	W+ve	-ve	+ve
1056	Idvet	C45	-ve	M+ve	M+ve	-ve	S+ve
1113	IDVet	C45	-ve	+ve	+ve	-ve	+ve
1141	IDEXX	KP995	-ve	M+ve	M+ve	-ve	S+ve
1246	idexx	LOT CP 050	-ve	+ve	+ve	-ve	+ve
1300	VMRD	P180110002	-ve	W+ve	W+ve	-ve	W+ve
1391	Nil Return						
1438	Idexx	KP995	-ve	M+ve	W+ve	-ve	M+ve
1499	VMRD	P181215-001	-ve	W+ve	W+ve	-ve	M+ve
1686	VMRD	P190315-001	-ve	+ve	+ve	-ve	+ve
1749	IDEXX	KP995	-ve	W+ve	W+ve	-ve	+ve
1787	VMRD	P181215-001	-ve	W+ve	W+ve	-ve	+ve
1835	VMRD-EIA Veterinary Medical Research&Development	P-181215-001	-ve	+ve	+ve	-ve	+ve
1883	VMRD	P181215-001	-ve	+ve	+ve	-ve	+ve
1929	VMRD	P180417-002	-ve	+ve	+ve	-ve	+ve
1946	ID VET	C45	-ve	W+ve	-ve	-ve	S+ve
1992	ONE IDEXX DRIVE	CP050	-ve	-ve	-ve	-ve	M+ve
2025	ID.vet	F01	-ve	+ve	+ve	-ve	+ve
2123	ID Vets	D99	-ve	+ve	+ve	-ve	+ve
2443	IDEXX	KP995	-ve	W+ve	-ve	-ve	W+ve
2453	IDEXX	FR519	-ve	+ve	-ve	-ve	+ve
2507	IDEXX	KP995	-ve	+ve	+ve	-ve	+ve
2535	VMRD	P181215-001	-ve	+ve	+ve	-ve	+ve
2716	NECVB	21907220011	-ve	+ve	+ve	-ve	+ve
3143	VMRD	P181215-001	-ve	W+ve	W+ve	-ve	M+ve
3176	VMRD	P181215-001	-ve	+ve	+ve	-ve	+ve
3232	VMRD	P190315-001	-ve	W+ve	W+ve	-ve	M+ve
3248	Nil Return						

NECVB 哈尔滨国生生物科技股份有限公司

马传染性贫血病毒 cELISA 抗体检测试剂盒使用说明书

【产品货号】 NEE26300

【产品名称】 马传染性贫血病毒 cELISA 抗体检测试剂盒

【作用与用途】

本试剂盒采用竞争 ELISA 技术，用于检测马血清中马传染性贫血病毒抗体，检测结果可用于马传染性贫血感染的辅助诊断。

Testing carried out to determine the intended results of the samples using Agar Gel Immunodiffusion Test (AGIDT), was subcontracted to an Animal and Plant Health Agency (APHA) laboratory.

Sample 19/7896 = 19/7899, sample 19/7897 = 19/7898.

Comments provided by Jean-Pierre Frossard, APHA Weybridge

Laboratories 1007, 1056, 1113, 1141, 1246, 1300, 1438, 1499, 1686, 1749, 1787, 1835, 1883, 1929, 2025, 2123, 2507, 2535, 2716, 3143, 3176 and 3232, using a variety of different test kits, reported the intended result for all samples tested.

For laboratories 1007, 1056, 1141, 1300, 1499, 1749, 1787, 3143 and 3232, where the strength of the reaction was reported, it was consistent for the replicate samples (19/7897 & 19/7898), and was higher for the less dilute sample (19/7900).

Laboratory 1438 reported a medium positive result for sample 19/7897, and a weak positive result for sample 19/7898.

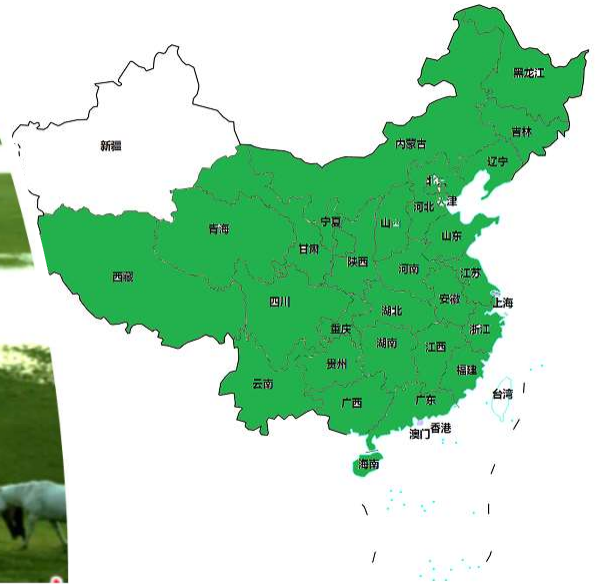
Laboratories 1946, 2443 and 2453 reported a negative result for sample 19/7898, whereas the expected result was positive.

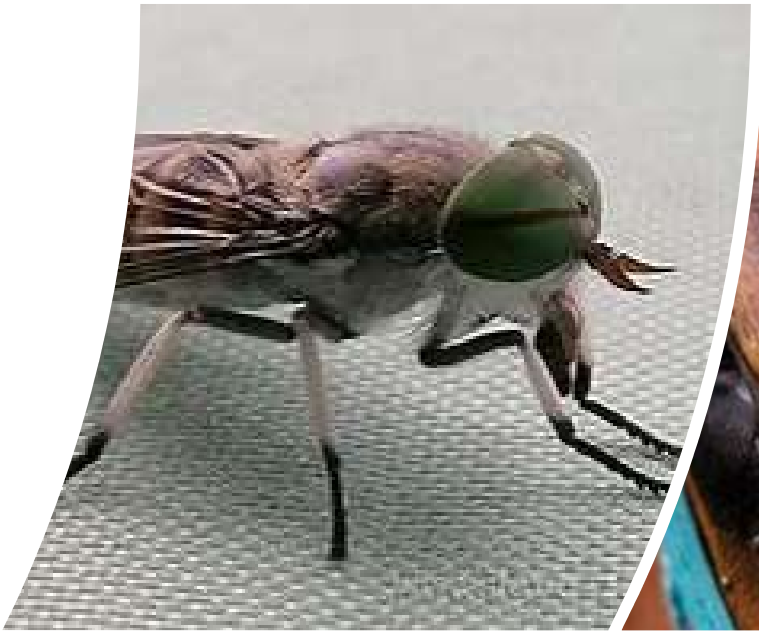
Printed: 7/12/2019

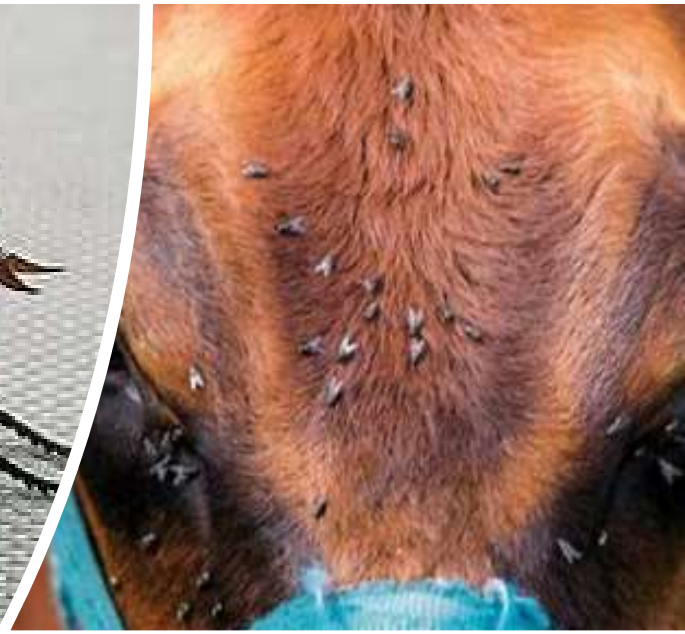
cELISA played key role in eradication of EIA in Bayanbulak, Xinjiang

- The last area where EIA was eradicated (2023) in China
- ~50,000 horses
- Grassland area 23,835 km²
- 320,000 tests every year
- No positive cases detected after 2021

马传贫cELISA抗体检测试剂盒







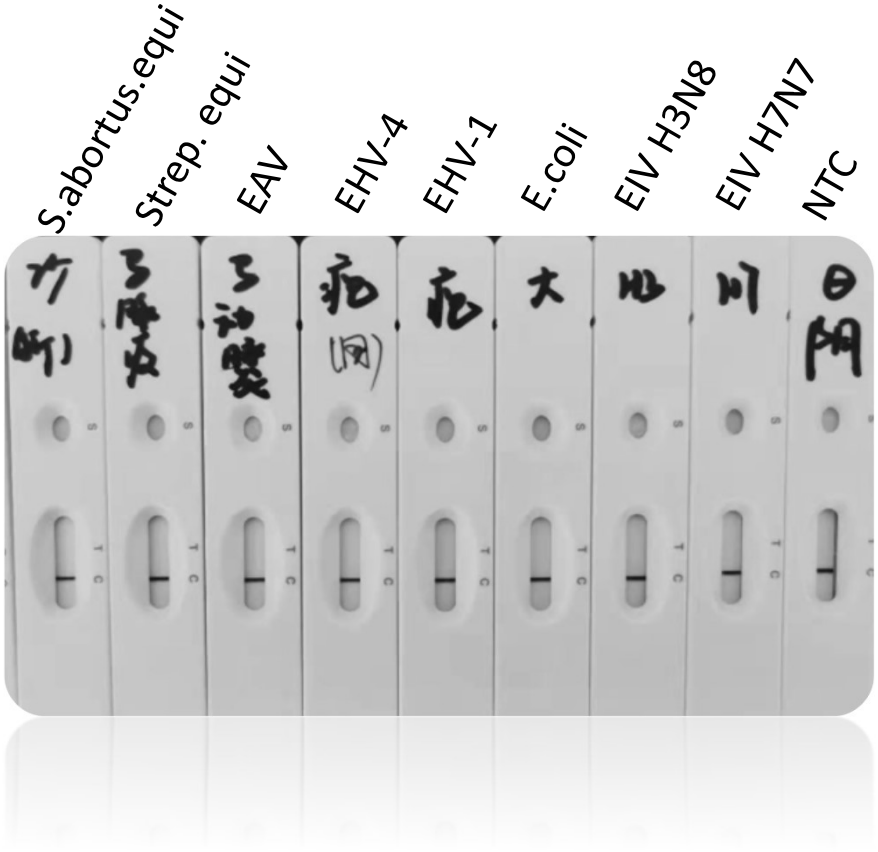
4. EIA Rapid test strip

- Onsite detection
- 10 minutes
- High specificity
- High sensitivity

Developed by Xiaojun Wang's lab

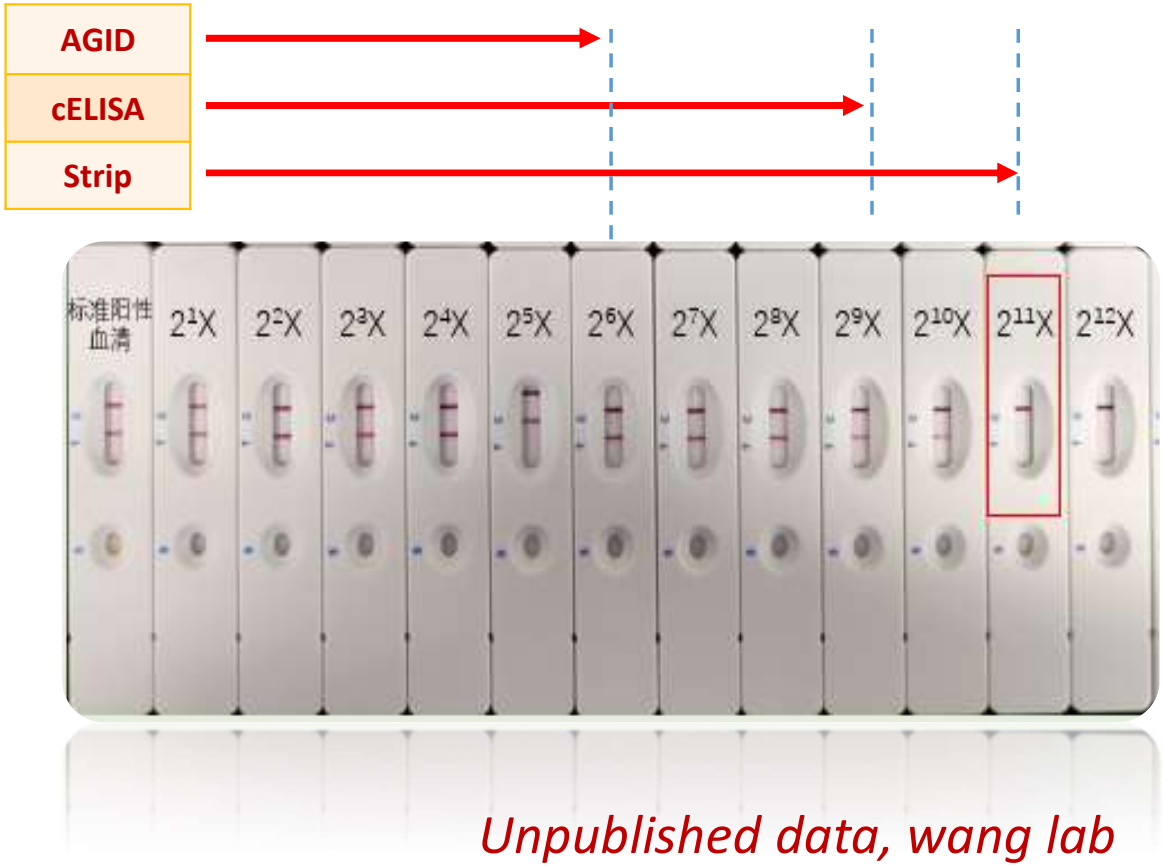
Good specificity

Anti-serum



Good sensitivity

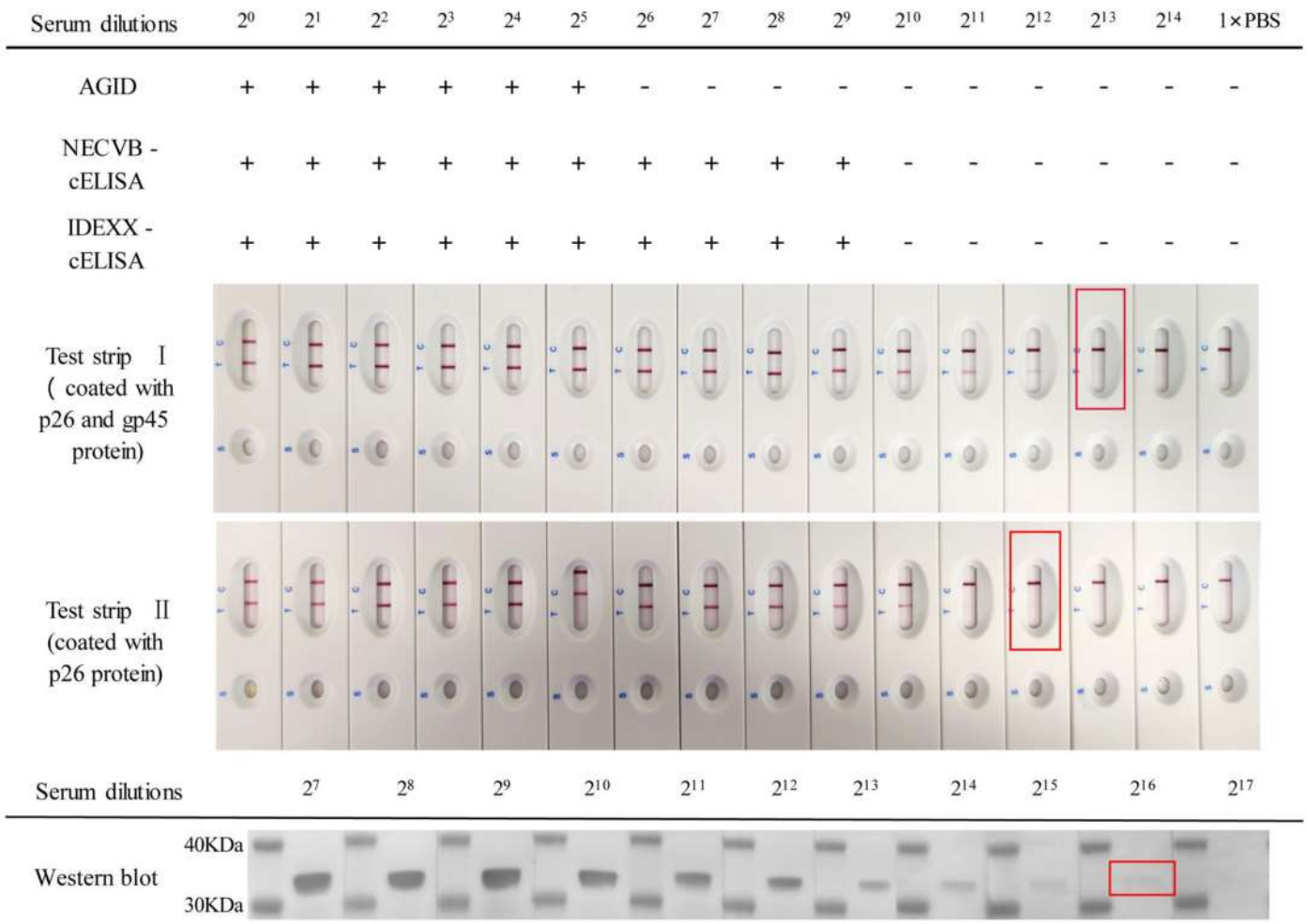
EIA positive serum



Unpublished data, wang lab

EIA Rapid test strip (NECVB)

- Onsite detection
- 10 minutes
- High specificity
- High sensitivity



Zenan Zhang, et al. Appl Microbiol Biotechnol .2024

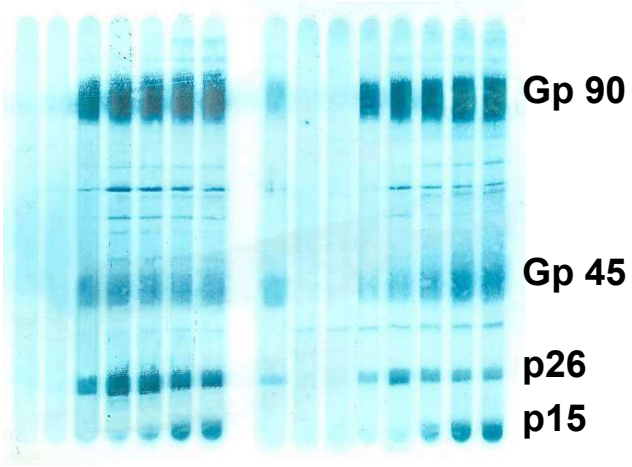
Comparation of AGID, cELISA, and Gold Strip

Sample No.	AGID	cELISA	IP	Gold Strip	Sample No.	AGID	cELISA	IP	Gold Strip
2	−	+	99.80%	+	7	−	+	100.18%	+
9	−	+	100.47%	+	9	−	+	100.03%	±
3	+	+	100.50%	+	19	−	+	95.45%	+
10	+	+	100.30%	+	31	−	+	100.42%	+
11	+	+	100.44%	+	33	−	+	99.48%	+
16	+	+	100.47%	+	36	−	+	100.52%	+
17	−	+	99.94%	±	37	−	+	99.97	+
19	±	+	100.18%	+	40	±	+	100.19%	+
20	+	+	100.68%	+	42	−	+	99.71%	+
10	±	+	100.47%	+	43	±	+	100.39%	+
11	−	+	100.33%	+	46	−	+	99.84%	+
5	−	+	99.62%	±	56	−	+	100.45%	+

Note: The result of AGID "±" stands for weak positive; the result of gold strip; "±" stands for very shallow line. Samples were from experimental infected horses.

Hu et al., Applied Microbiology and Biotechnology 2023

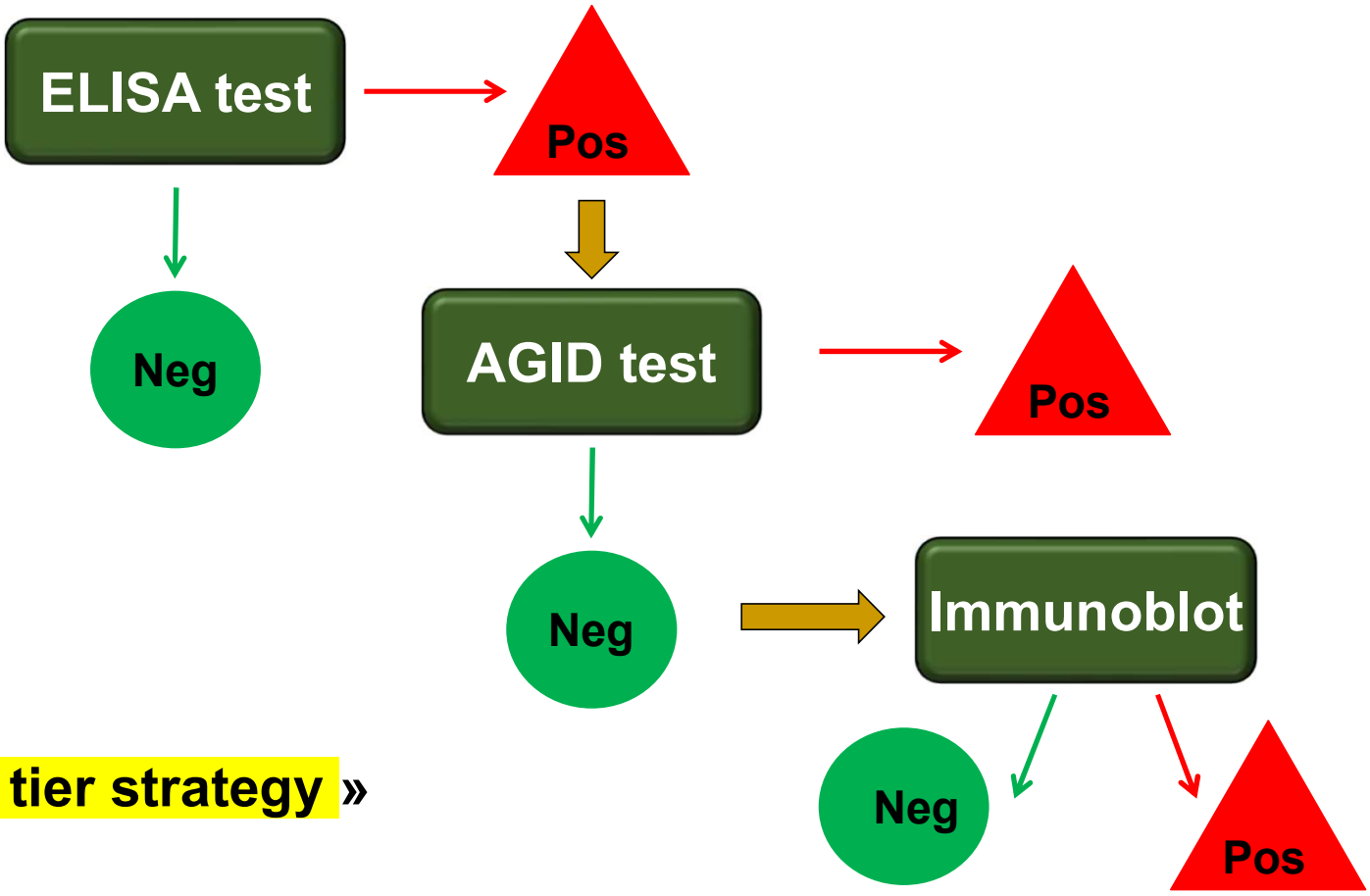
5. Immunoblot (IB)



- Gold standard serological test
- Immune response against several viral proteins is detected
- Sensitive and specific
- Ag production an issue
- Cannot be performed routinely on thousand samples

INDIRECT METHODS used in Spain

✓ *Serological diagnosis / Proposed strategy*

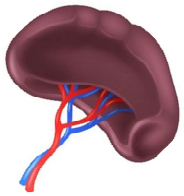


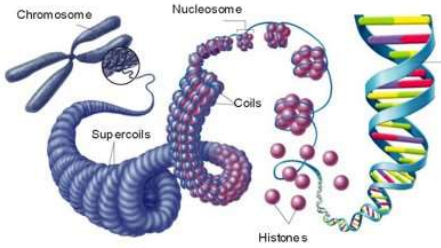
➢ **So called « 3 tier strategy »**

Reference doi.org/10.1016/j.vetmic.2013.02.027

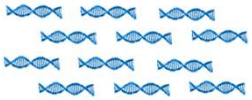
6. Next Generation Sequencing to improve EIAV diagnostics

Tissue genomic DNA extraction from spleen





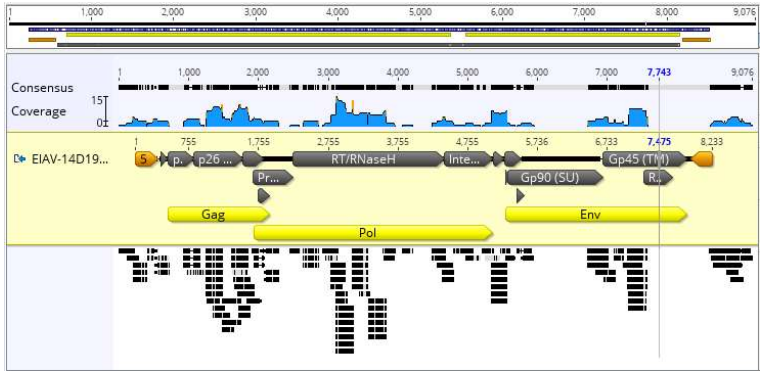
Enrichement Probes



EIAV Enrichement Step

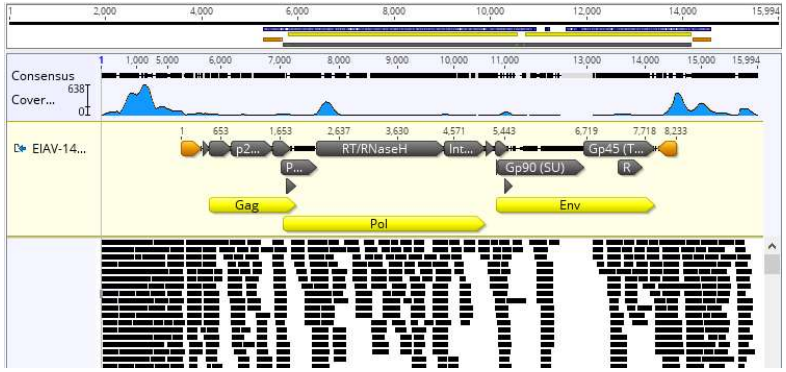
DNA library

Results : 1st Desing



45,7% coverage

Results : 2st Desing



>80 % coverage

Sanger Sequencing

100% coverage

3st Desing

Test On Going

Conclusion & Perspectives

1. AGID is the Gold Standard for confirmation, but with less sensitivity
2. iELISA is good for screening, but with limited specificity, need more confirmation tests
3. cELISAs, with good sensitivity and specificity.
4. Immunoblot, is complicated to use for large numbers of samples.
5. qPCR can be used in certain cases with proper disease course and sampling
6. Application of new sequencing technologies to the diagnosis of the EIAV infection

World Organisation for Animal Health
Founded as OIE

Acknowledgements

WOAH Reference Laboratory for Equine Infectious Anaemia
Reference Laboratory
World Organisation for Animal Health

中国农业科学院哈尔滨兽医研究所
HARBIN VETERINARY RESEARCH INSTITUTE (HVRI), CAAS

anses

European Union Reference Laboratory for Equine Diseases

https://eurl-equinediseases.anses.fr/

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri*
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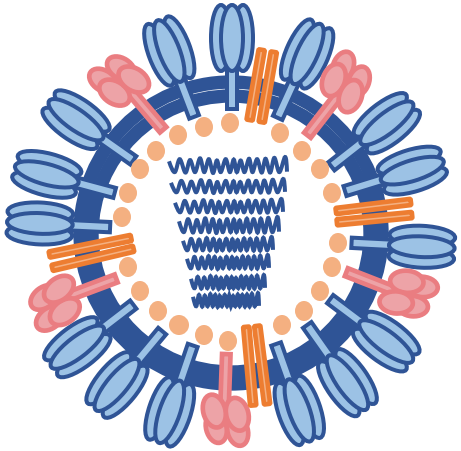
Recommended diagnostic tests (WOAH manual) and novel diagnostic tests for equine influenza

Nanako Kawanishi

Equine Research Institute
Japan Racing Association

Equine influenza (EI)

Pathogen



- Influenza A viruses
  (Family: *Orthomyxoviridae*)
- Subtypes: H3N8 and H7N7
- H7N7 has not been isolated since the late 1970s

(Webster, Equine Vet. J., 1993)

Equine Influenza (EI)

Epidemiology

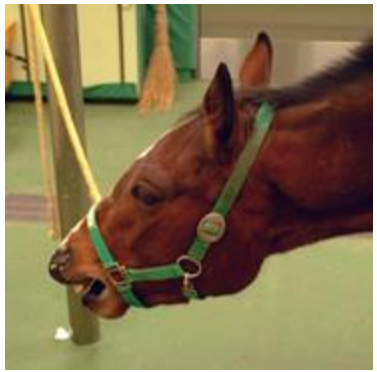
- Endemic in the world except for New Zealand and Iceland

(Cullinane and Newton, Vet. Microbiol., 2013)

- In Japan, outbreaks occurred in 1971 and 2007

Clinical signs

- Fever (≧ 38.5°C)
- Harsh dry cough and nasal discharge
- Morbidity rate in naïve horses: up to 100%



(Cullinane and Newton, Vet. Microbiol., 2013)

Equine influenza (EI)

Prevention and control

- Rapid diagnosis
- Movement restrictions
- Vaccination

Test methods for the diagnosis of EI

Virus detection

- Virus isolation
- Conventional and real-time RT-PCR
- Rapid antigen detection (RAD) kits

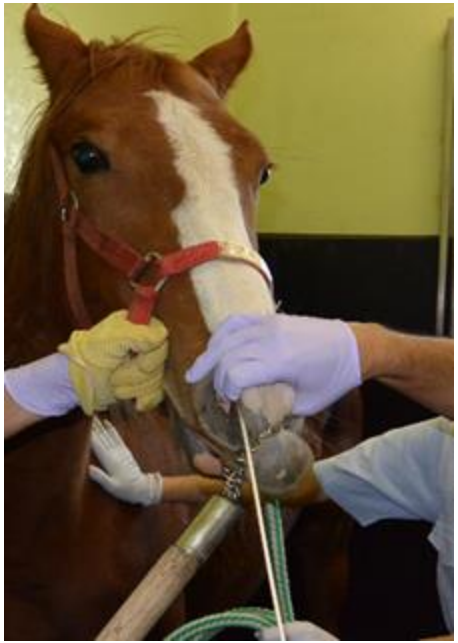
Serological diagnosis

- Hemagglutination inhibition (HI)
- Single radial haemolysis (SRH)
- Enzyme-linked immunosorbent assay (ELISA)

Sample collection

Nasopharyngeal or nasal swabs

Should be collected from acute cases



Serum

Paired serum samples (acute and convalescent phases)

Virus isolation

Gold standard for EI diagnosis

Embryonated hens' eggs

- Widely used in research labs and vaccine production
- Able to yield a robust amount of virus
- Time and money intensive

Madin-Darby canine kidney (MDCK) cells

Less permissive than embryonated hens' eggs

Reverse transcription PCR (RT-PCR)

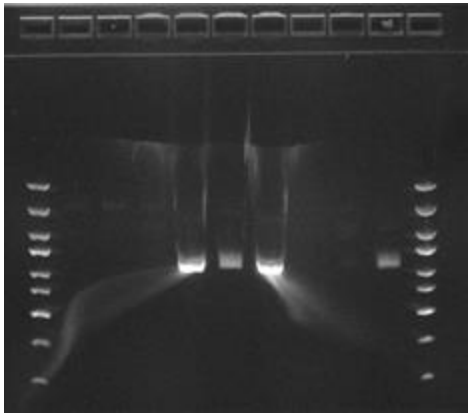
More sensitive than virus isolation

Conventional RT-PCR

Visualized by the presence of the target band

Target: HA1 subunit gene

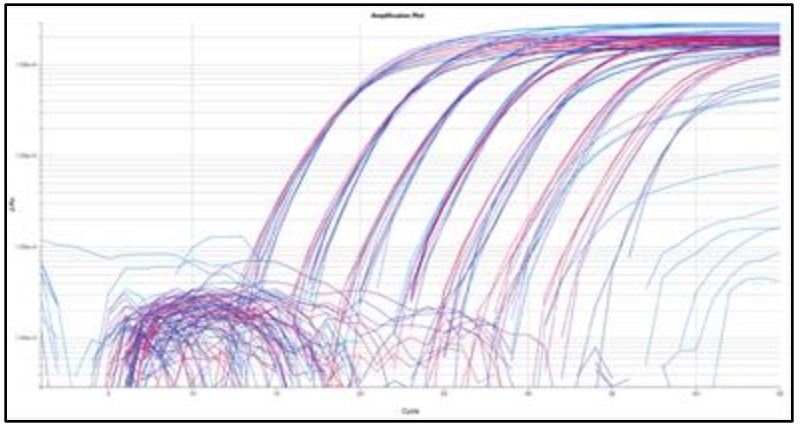
(Newton *et al*., Vet Rec, 2006)



Reverse transcription PCR (RT-PCR)

More sensitive than virus isolation

Real-time RT-PCR



- Pan-reactive influenza type A real-time RT-PCR

  Target: Matrix gene (avian influenza virus) (Heine *et al*., Avian Dis., 2007)

- Developed for detecting equine influenza viruses

  Target: Nucleoprotein gene (Lu *et al*., J. Clin. Microbiol., 2009)

Rapid antigen detection (RAD) kits

User-friendly and fast

- The first EI case in Japan in 2007 was identified
- Less sensitive than real-time RT-PCR

(Yamanaka *et al*., Influenza Other Respir. Viruses, 2016)

Quick Chaser Flu A, B

Immunochromatography

Quick Chaser Auto Flu A, B

Silver amplification immunochromatography

Hemagglutination inhibition (HI) test

Widely used for HA subtyping, surveillance, vaccine testing

Agglutination
Antibody (+)

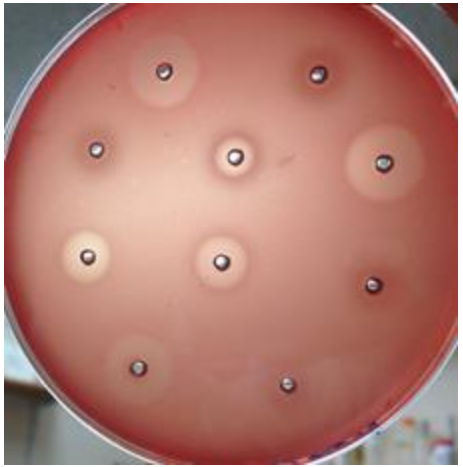
No agglutination
Antibody (-)

- Pretreatment of equine sera is needed to remove non-specific agglutinin
- The HI results may vary between laboratories

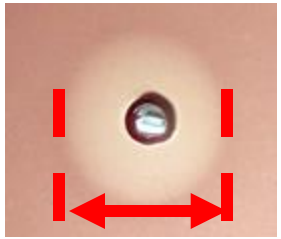
(Daly *et al*., Pharmeuropa Bio. 2007)

Single radial haemolysis (SRH)

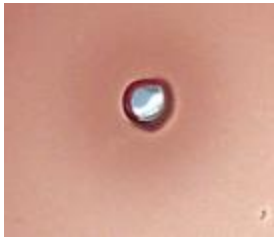
Ideal for vaccine efficacy studies



Haemolysis
Antibody (+)



No haemolysis
Antibody (-)



Zone of haemolysis

- 25mm^2 or 50% increase in zone of haemolysis: seroconversion (Cullinane and Newton, Vet. Microbiol., 2013)
- SRH antibody levels post-vaccination correlate well with protection (Mumford and Wood, Dev. Biol. Stand., 1992)

Enzyme-linked immunosorbent assay (ELISA)

Ready-to-use ELISA kits are available

- Competitive and blocking ELISA

IDEXX AI
Multi-Screen Ab Test

- Commercialized ELISAs originally developed for avian influenza are available

- Shown to be effective in the detection of influenza A antibodies in horses (sensitivities: 96-99%)

(Kittelberger *et al*., Vet. Microbiol., 2011)

Virus neutralization assay

Essential for vaccine efficacy studies and understanding immune protection

- Determines the neutralizing capability of antibodies against EIV

- The growth of the virus will be inhibited if neutralizing antibodies are present

Recent research on RAD kits for EI

- In JRA racehorse hospitals, commercialized RAD kits developed for human influenza are used
- Newly launched and updated RAD kits need to be evaluated for equine influenza viruses (EIVs) regularly

Seven RAD kits* were evaluated for EI

*Available in Japan, 2023-24

The 50% detection limits for each assay

Product name	**Detection limit** (EID_{50}*/200 µL) A/equine/Tipperary/1/2022
Alsonic Flu	2300
Check Flu A·B	2300
Immunofine FLU II	2300
Quick chaser Flu A, B	2300
Finevision Influenza	460
RapidTesta Flu·NEXT	460
Quick chaser Auto Flu A, B	66
Real-time RT-PCR	1.0

*50% egg-infectious dose (Manuscript submitted)

Take-home messages

- Because of its high sensitivity, real-time RT-PCR should be performed to detect EI cases

- Definitive diagnosis should be made by virus isolation

- Serological diagnoses are not applicable for rapid EI detection but are useful for retrospective diagnosis and surveillance

Combining several diagnostic assays is essential to control EI