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

Other diseases of importance and their diagnostic tests

Susanne Münstermann, WOAH consultant
s.munstermann@outlook.de

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

List of content

- Equine herpesvirus 1
- Japanese encephalitis
- West Nile fever
- Nipah virus encephalitis

https://rr-asia.woah.org/en/events/equine-rhinopneumonitis-webinar/

Courtesy of Hiroshi Bannai

Equid alphaherpesvirus 1 and 4 infection

-Equine Rhinopneumonitis
(respiratory, abortion, neurological forms)

-Virus shedding: nasopharynx, reproductive tract, aborted fetus

-Notifiable in many countries

respiratory form

abortion form

neurological form

Host range

Equids
- -domestic horses
- -zebras
- -donkeys
- -mules

Non-equid perissodactyls
- -tapirs
- -rhinoceroses

and maybe more…

Geographical distribution

Europe
North America
South America
Africa
Australia, NZ

Asia
- -Japan
- -China
- -South Korea
- -Iran
- -India
- -Mongolia
- -Kazakhstan

and more…

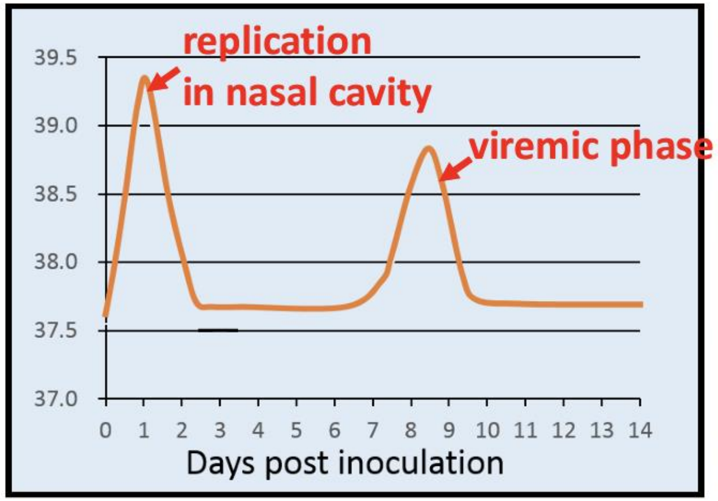
➡ **Everywhere except for Iceland**

Respiratory form

-Pyrexia, nasal discharge, swelling of submandibular lymph node, ocular discharge, etc.

-Virus shedding: 1-3 weeks from infection

-Mostly affecting young horses (<3-yo)



Abortion form

-Sudden abortion without any other signs
(sometimes stillbirth or weak foals)

-Most frequently from 9M to 11M of pregnancy

-Massive EHV-1 in fetus, placenta and amniotic fluid

➡ Transmission to other mares

Neurological form

Equine Herpesvirus Myeloencephalopathy (EHM)

-Pyrexia, followed by neurological signs

-Ataxia, recumbency, urinating incontinence

-Likely situations: event, introduction of new horses

Unique nature of EHV-1 infection

-Ubiquitous

-High incidence of respiratory infection early in life

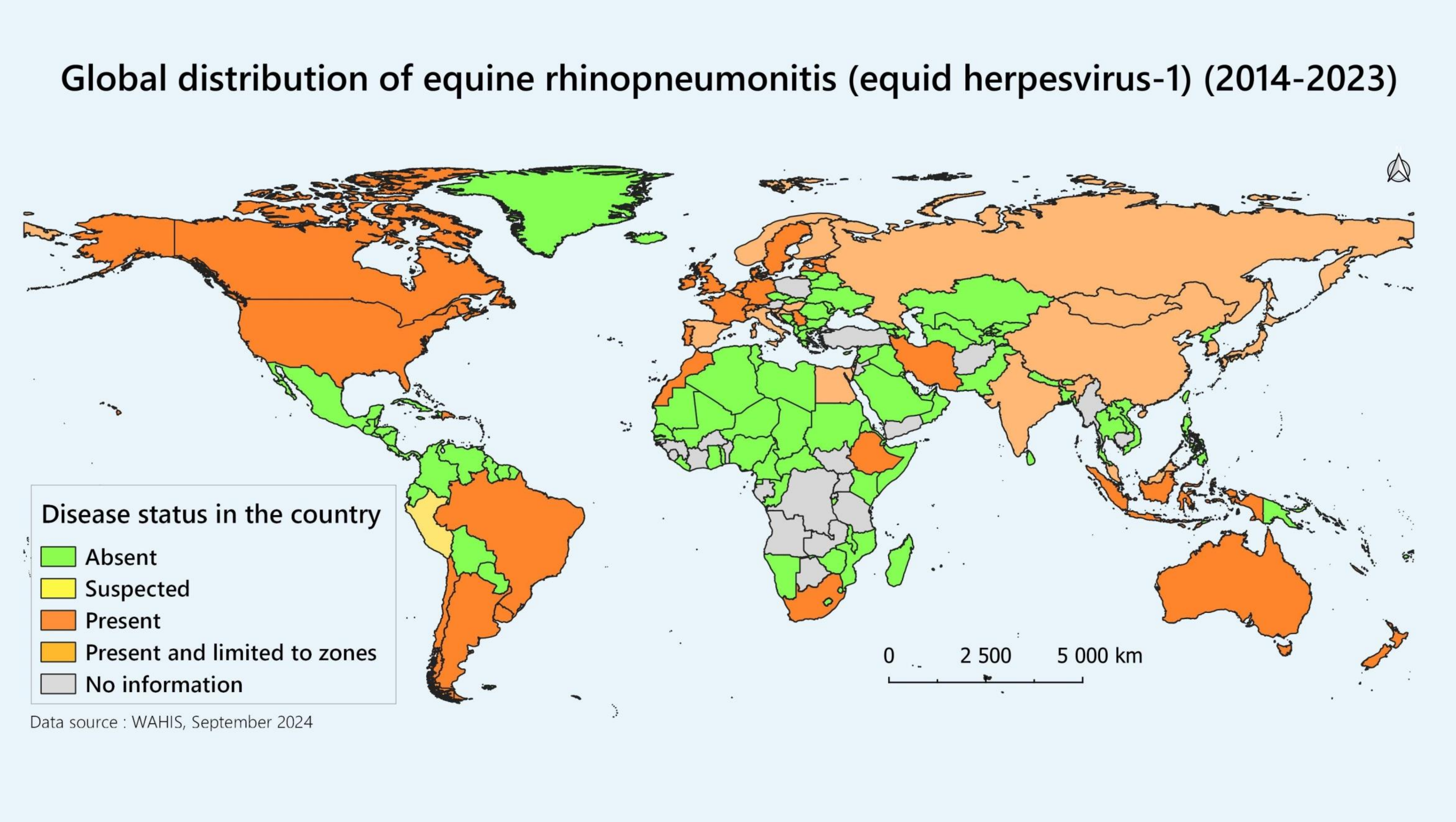
-Latency (persistence over lifetime)

-Frequent reactivation

-Same virus causes three forms of disease (respiratory, abortion, EHM)

-Asymptomatic infection

Even HHP horse is no exemption



Courtesy of Gregorie Bazimo, WAHIS

Courtesy of Ann Cullinane

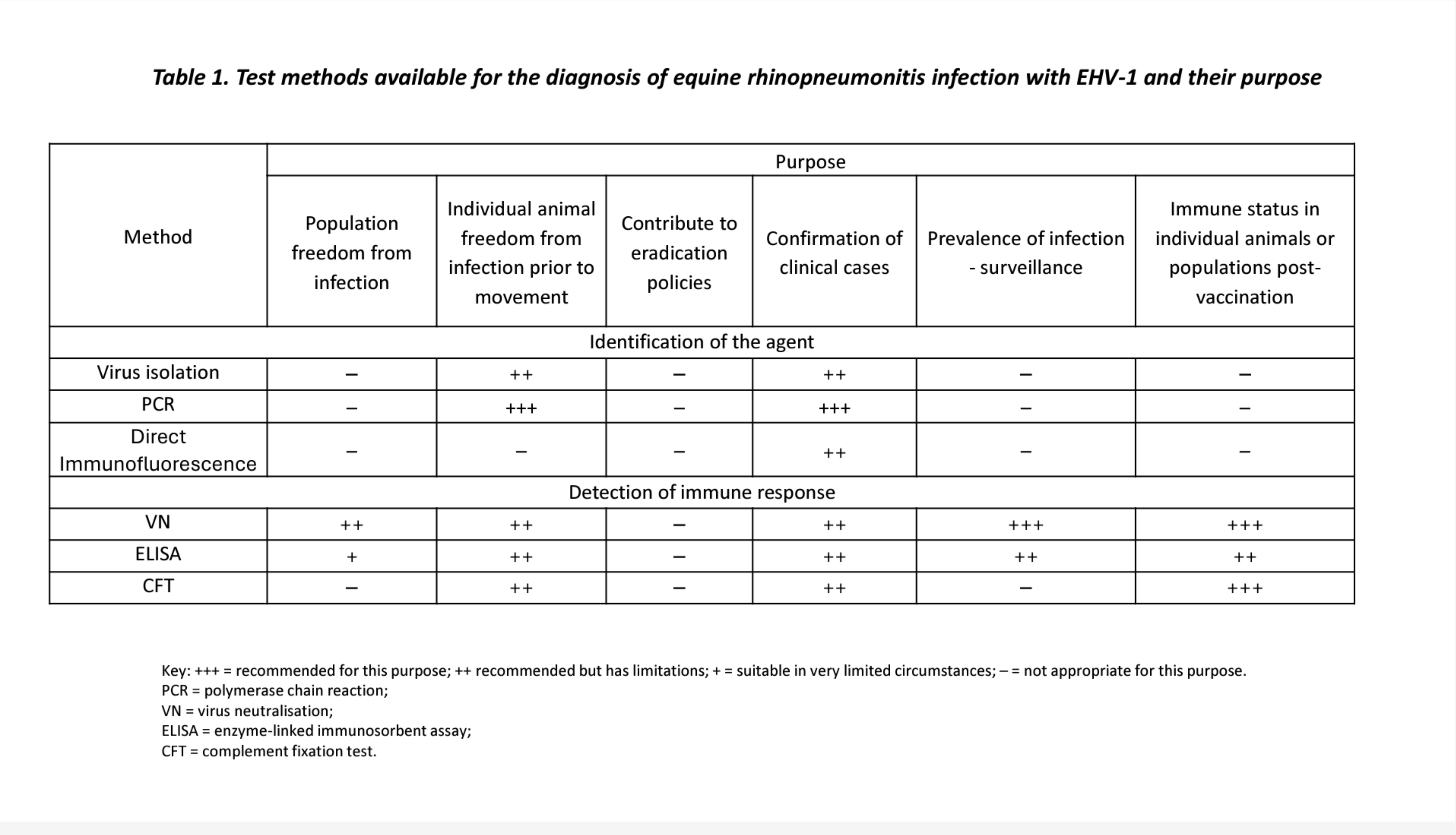


Table 1. Test methods available for the diagnosis of equine rhinopneumonitis infection with EHV-1 and their purpose

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
Identification of the agent						
Virus isolation	–	++	–	++	–	–
PCR	–	+++	–	+++	–	–
Direct Immunofluorescence	–	–	–	++	–	–
Detection of immune response						
VN	++	++	–	++	+++	+++
ELISA	+	++	–	++	++	++
CFT	–	++	–	++	–	+++

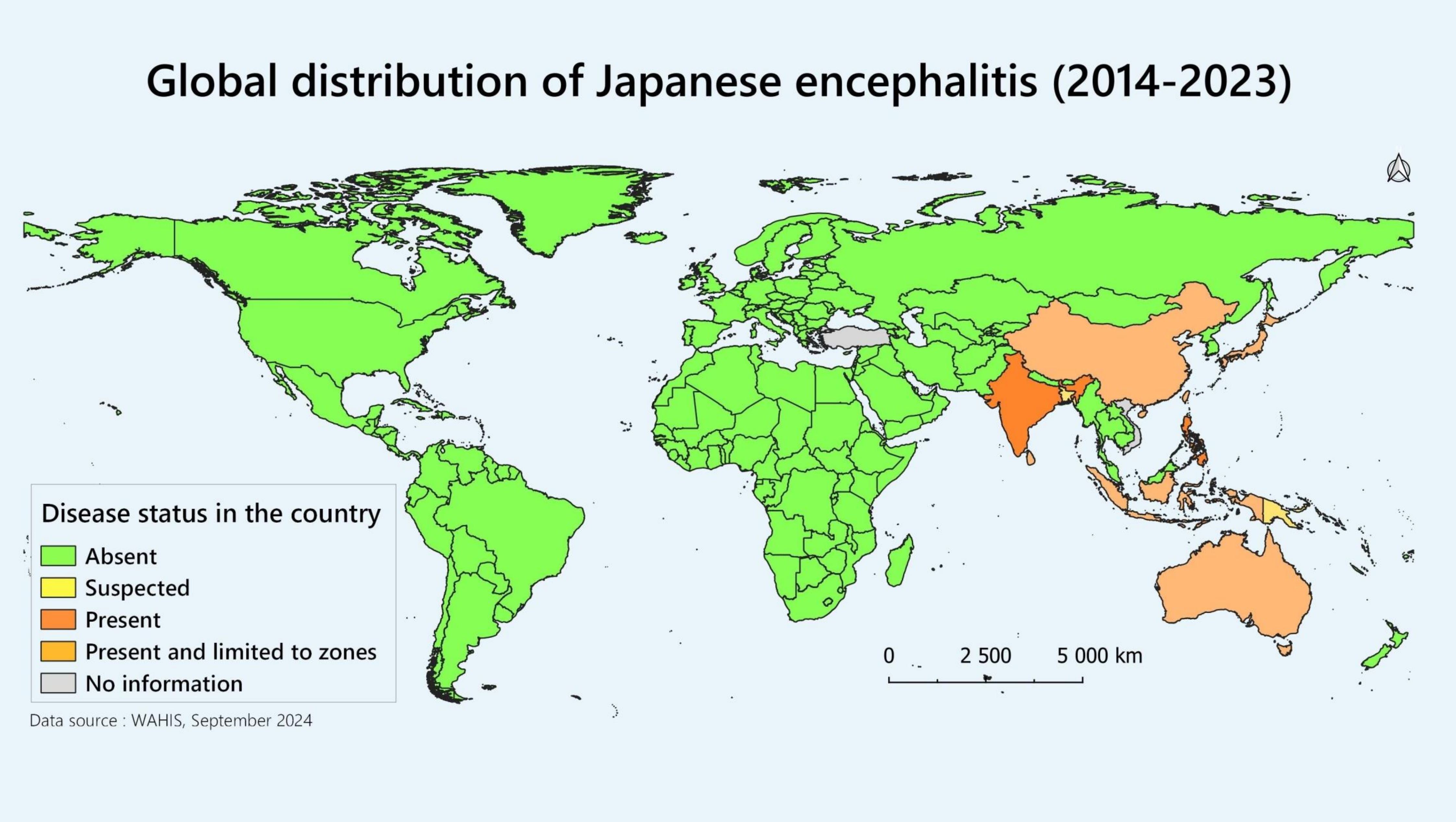
Key: +++ = recommended for this purpose; ++ recommended but has limitations; + = suitable in very limited circumstances; – = not appropriate for this purpose.
PCR = polymerase chain reaction;
VN = virus neutralisation;
ELISA = enzyme-linked immunosorbent assay;
CFT = complement fixation test.

Update on EHV Diagnostic Tests in Manual

- Added method for Complement Fixation test
- Updated PCR primer/probes
- Updated recommendations of specific tests for different purposes
- Included justification of these recommendations
- Supplied template for validation data
- Included reference to new and evolving technologies

Japanese encephalitis

https://rr-asia.woah.org/en/events/zoonoses-affecting-equines-japanese-encephalitis-west-nile-fever/

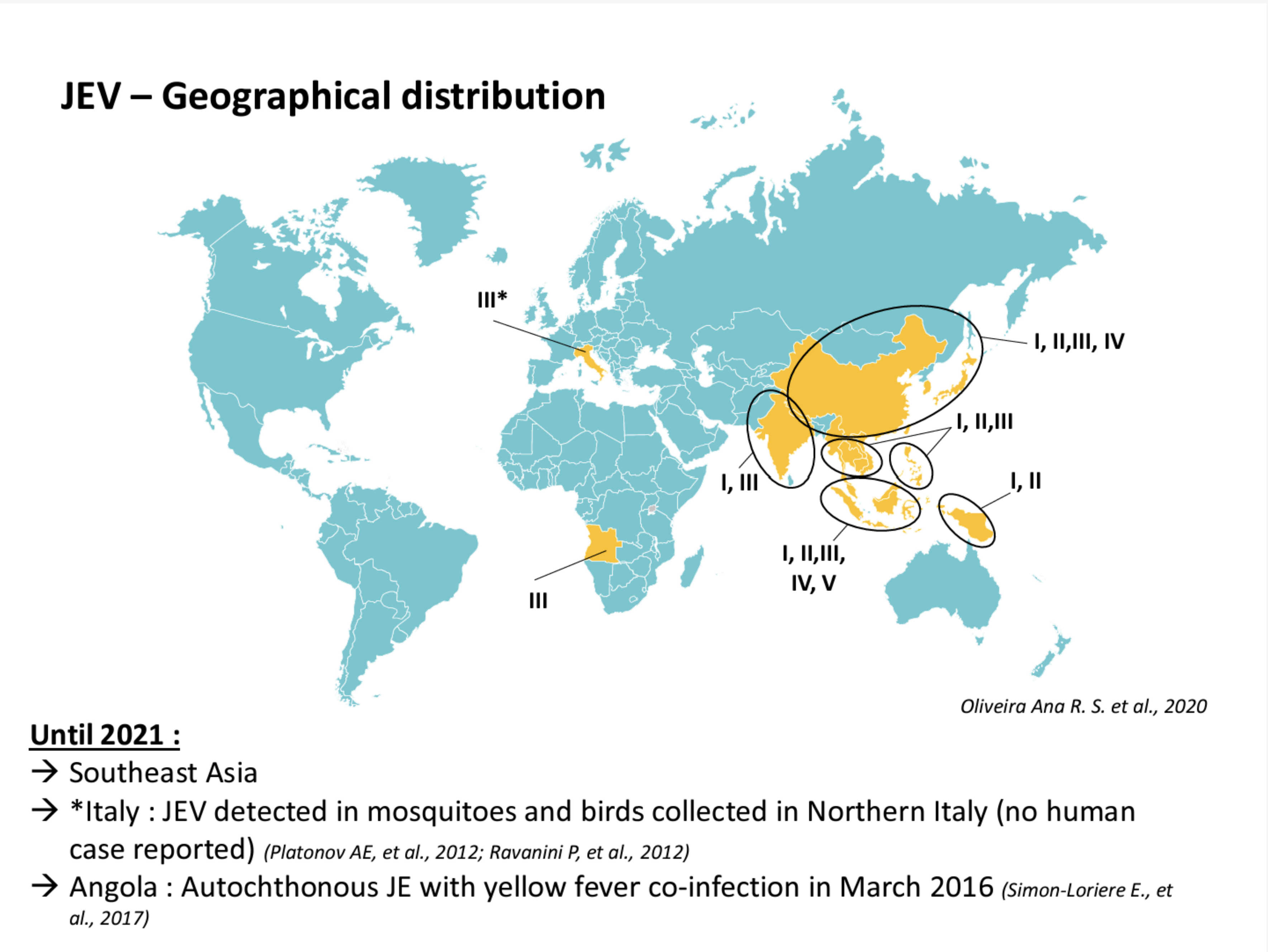


Global distribution of Japanese encephalitis (2014-2023)

Data source : WAHIS, September 2024

Courtesy of Gregorie Bazimo, WAHIS

Courtesy of Camille Migné, Anses



JEV – Geographical distribution

Oliveira Ana R. S. et al., 2020

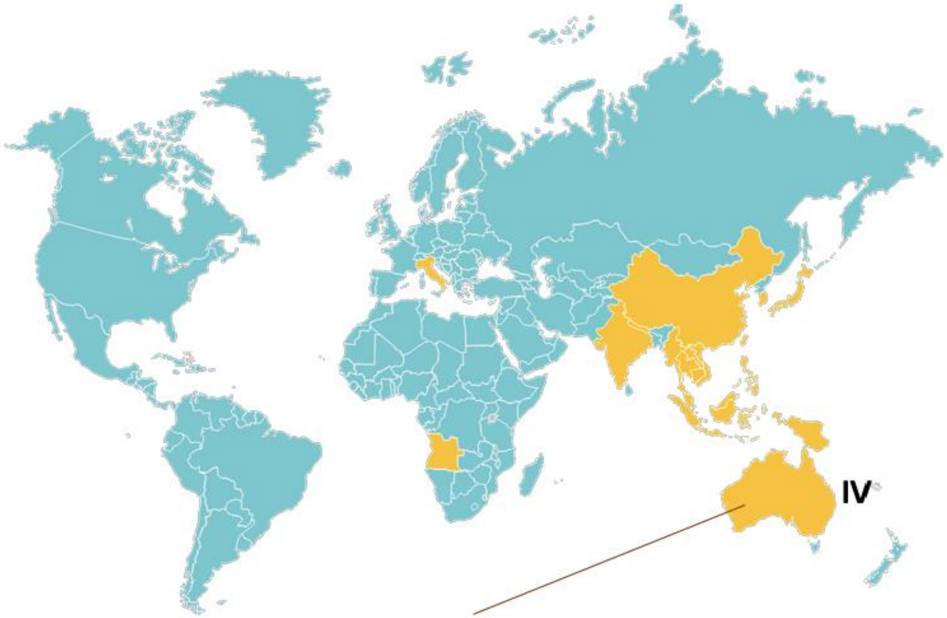
Until 2021 :

→ Southeast Asia

→ *Italy : JEV detected in mosquitoes and birds collected in Northern Italy (no human case reported) *(Platonov AE, et al., 2012; Ravanini P, et al., 2012)*

→ Angola : Autochthonous JE with yellow fever co-infection in March 2016 *(Simon-Loriere E., et al., 2017)*

JEV – Geographical distribution



JEV outbreaks in Australia in **2022**

★ Outbreaks in pigs

Mackenzie John S. et al., 2022

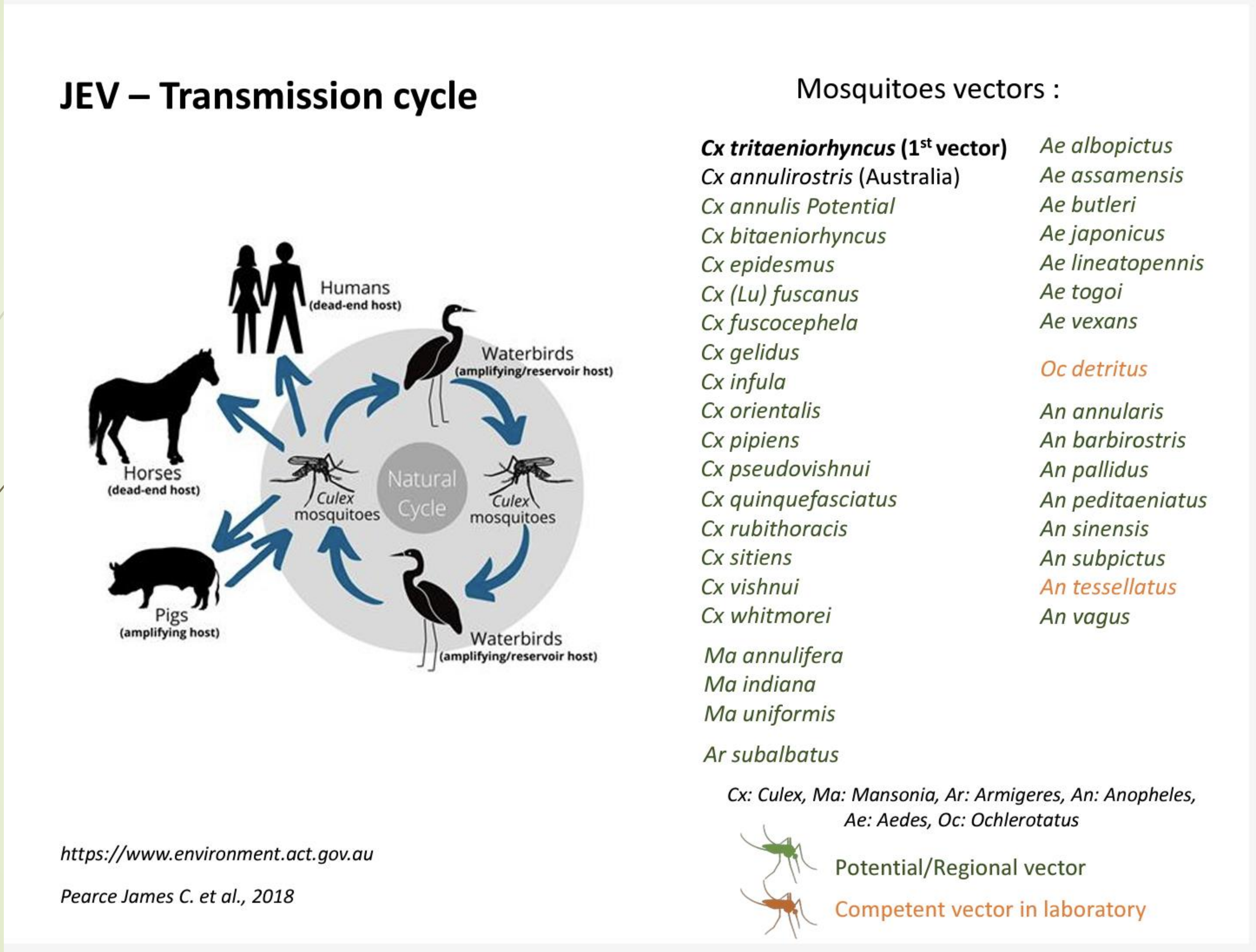
<u>Since 2022:</u>

Outbreaks in pigs reported from February 2022

→ March 30: Alpaca outbreak reported

→ May: Suspicion of equine infection (30)

No cases have been definitively confirmed despite neurological signs and tests results



JEV – Transmission cycle

https://www.environment.act.gov.au

Pearce James C. et al., 2018

Mosquitoes vectors :

***Cx tritaeniorhyncus* (1st vector)**
Cx annulirostris (Australia)
Cx annulis Potential
Cx bitaeniorhyncus
Cx epidesmus
Cx (Lu) fuscanus
Cx fuscocephela
Cx gelidus
Cx infula
Cx orientalis
Cx pipiens
Cx pseudovishnui
Cx quinquefasciatus
Cx rubithoracis
Cx sitiens
Cx vishnui
Cx whitmorei

Ma annulifera
Ma indiana
Ma uniformis

Ar subalbatus

Ae albopictus
Ae assamensis
Ae butleri
Ae japonicus
Ae lineatopennis
Ae togoi
Ae vexans

Oc detritus

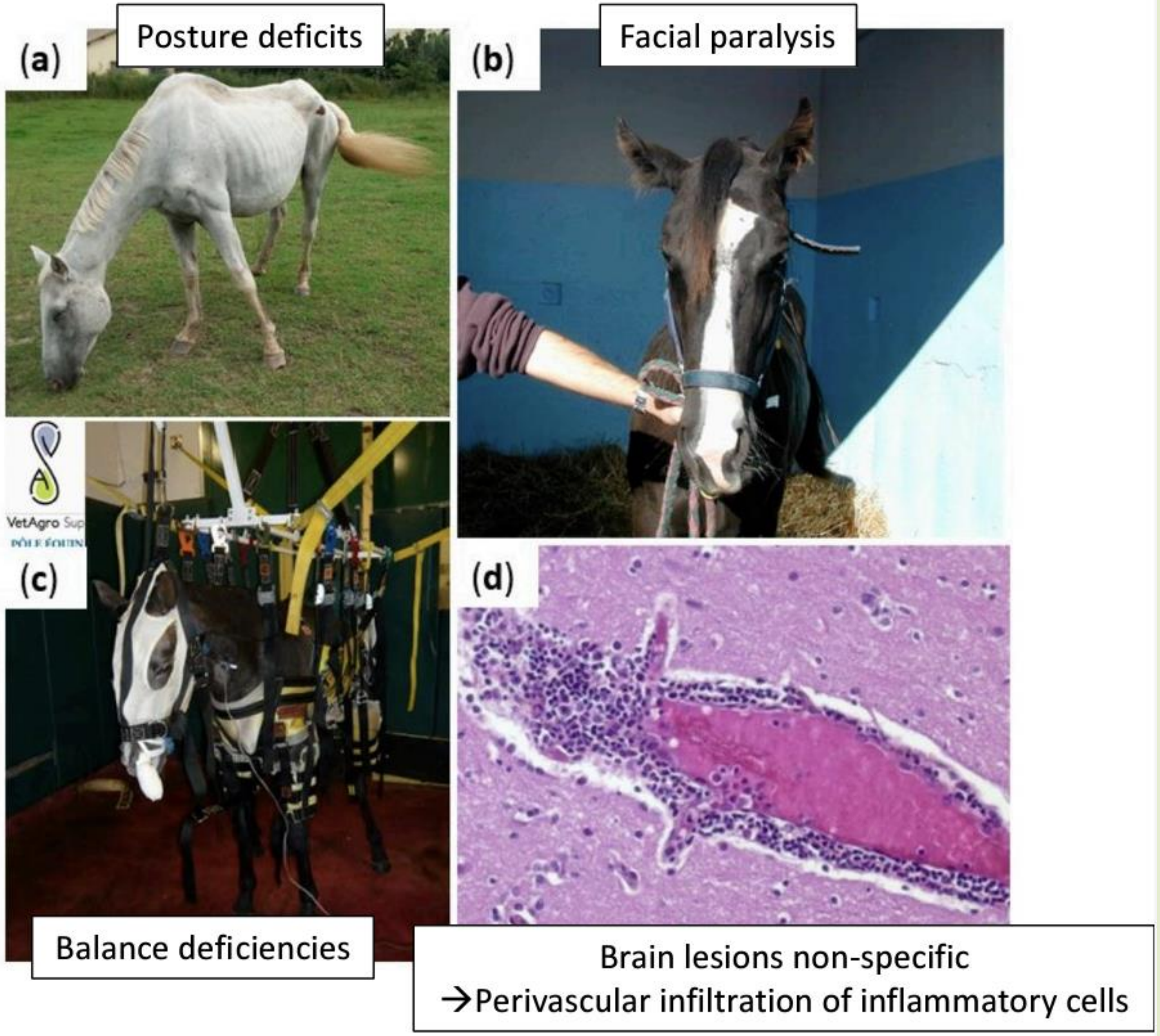
An annularis
An barbirostris
An pallidus
An peditaeniatus
An sinensis
An subpictus
An tessellatus
An vagus

Cx: Culex, Ma: Mansonia, Ar: Armigeres, An: Anopheles, Ae: Aedes, Oc: Ochlerotatus

Potential/Regional vector
Competent vector in laboratory

JEV – Disease in horses

- Most infections appear to be subclinical
- Fever, jaundice, lethargy, anorexia
- Neurological signs: ataxia, paralysis, collapse
- Death reported within 1–2 days
- **5-30% lethality** in horses

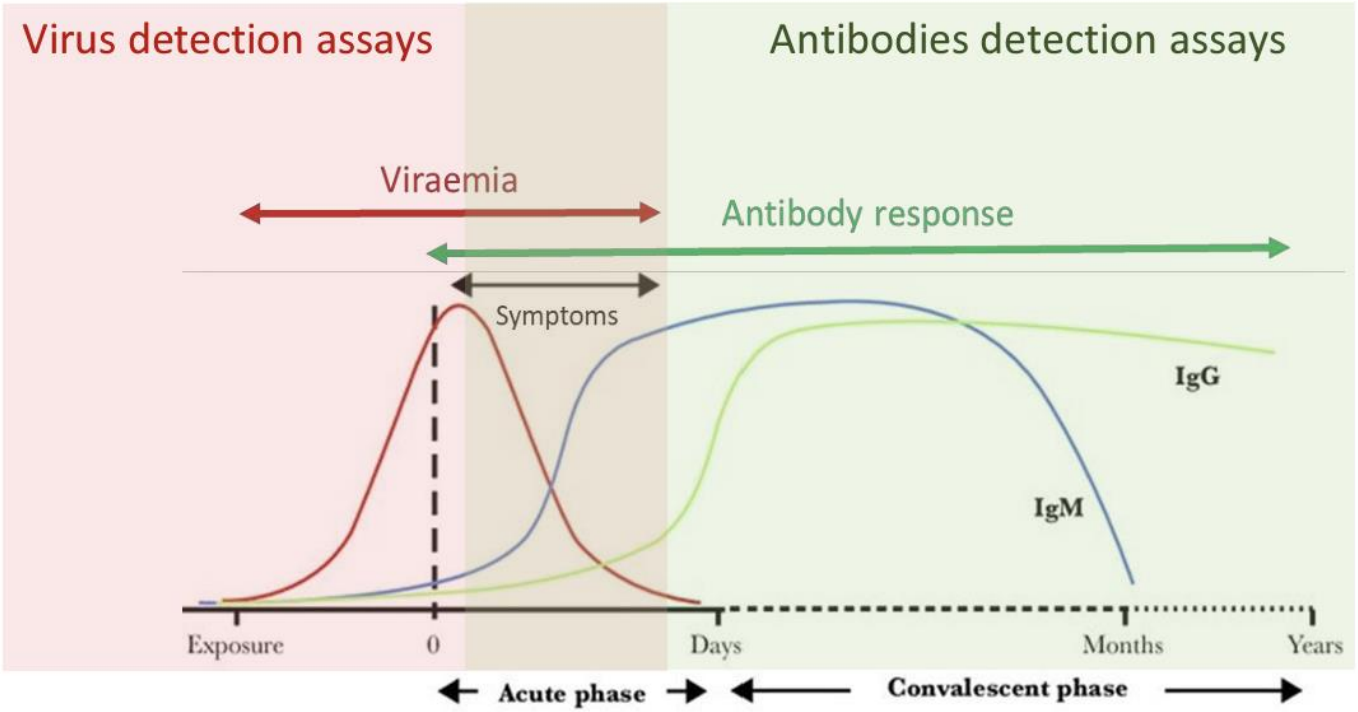


Mansfield Karen L. et al., 2017
Lecollinet Sylvie et al., 2020

JEV – Diagnosis

Diagnostic tools in laboratory

→ to be **adapted according to the kinetics of infection**



(adapted from Goncalves A. et al., 2017)

JEV – Vaccines

- In animals (available in Asia)

Nisseiken
(Japan)

https://www.jp-nisseiken.co.jp/en/products/vaccine/index.html

JEV

JEV and Getah virus

JEV, Equine Influenza and Tetanus

KM Biologics
(Japan)

https://www.kmbiologics.com/en/products/equine.html

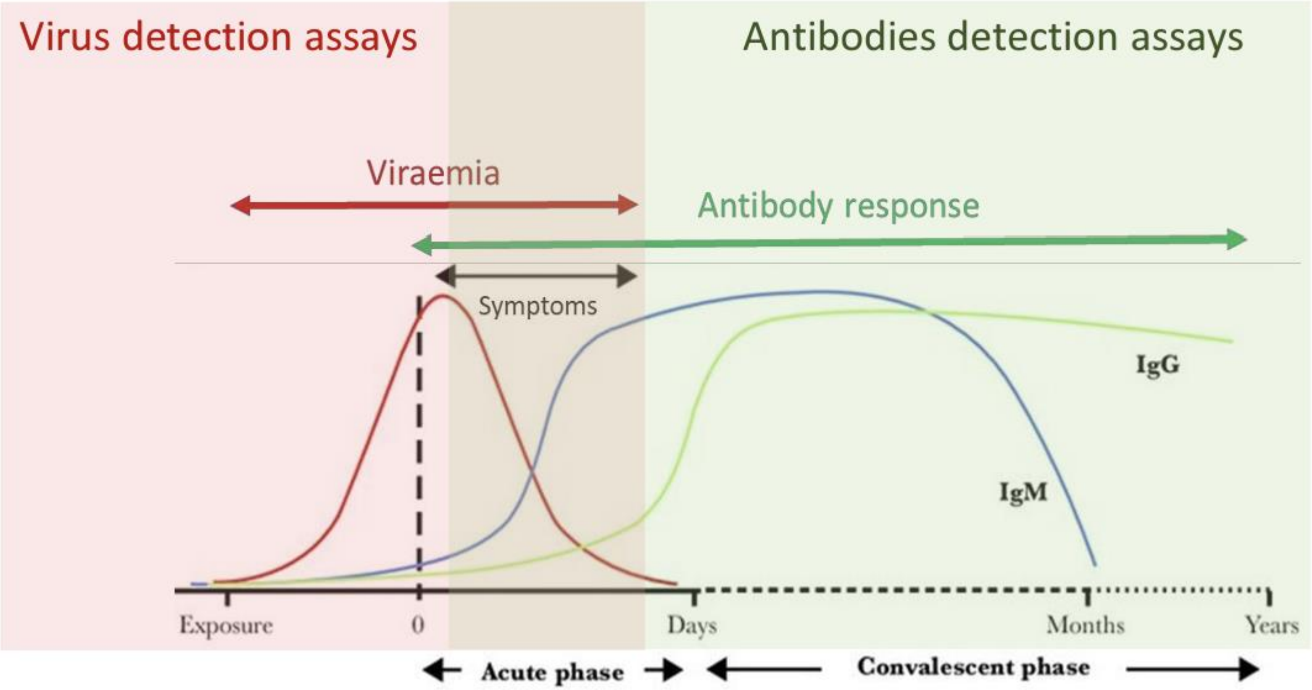
JEV

JEV, Equine Influenza and Tetanus

JEV – Diagnosis

Diagnostic tools in laboratory

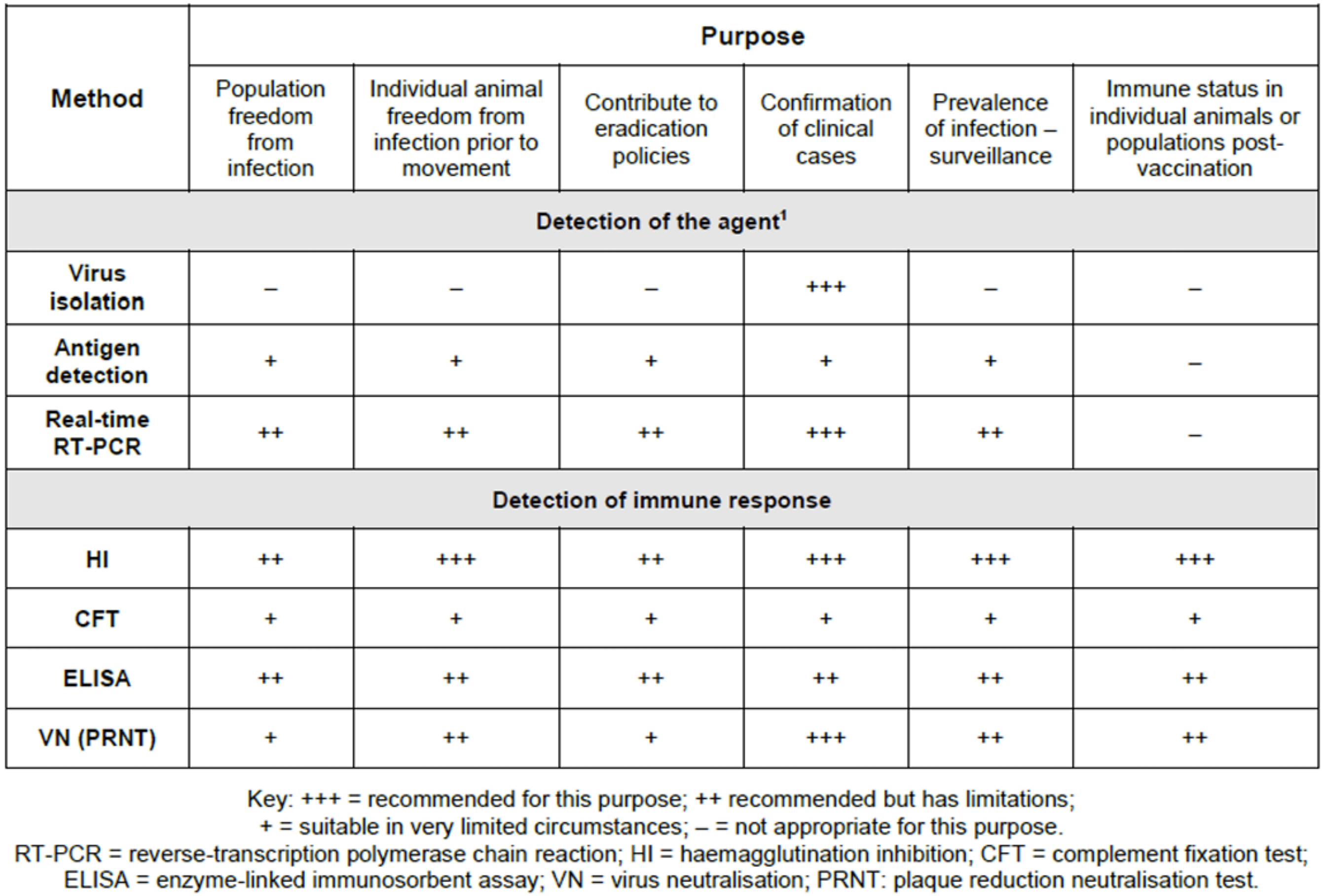
→ to be **adapted according to the kinetics of infection**



(adapted from Goncalves A. et al., 2017)

JEV – Diagnostic tools in laboratory

Test methods available for the diagnosis of Japanese encephalitis



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
Detection of the agent[1]						
Virus isolation	–	–	–	+++	–	–
Antigen detection	+	+	+	+	+	–
Real-time RT-PCR	++	++	++	+++	++	–
Detection of immune response						
HI	++	+++	++	+++	+++	+++
CFT	+	+	+	+	+	+
ELISA	++	++	++	++	++	++
VN (PRNT)	+	++	+	+++	++	++

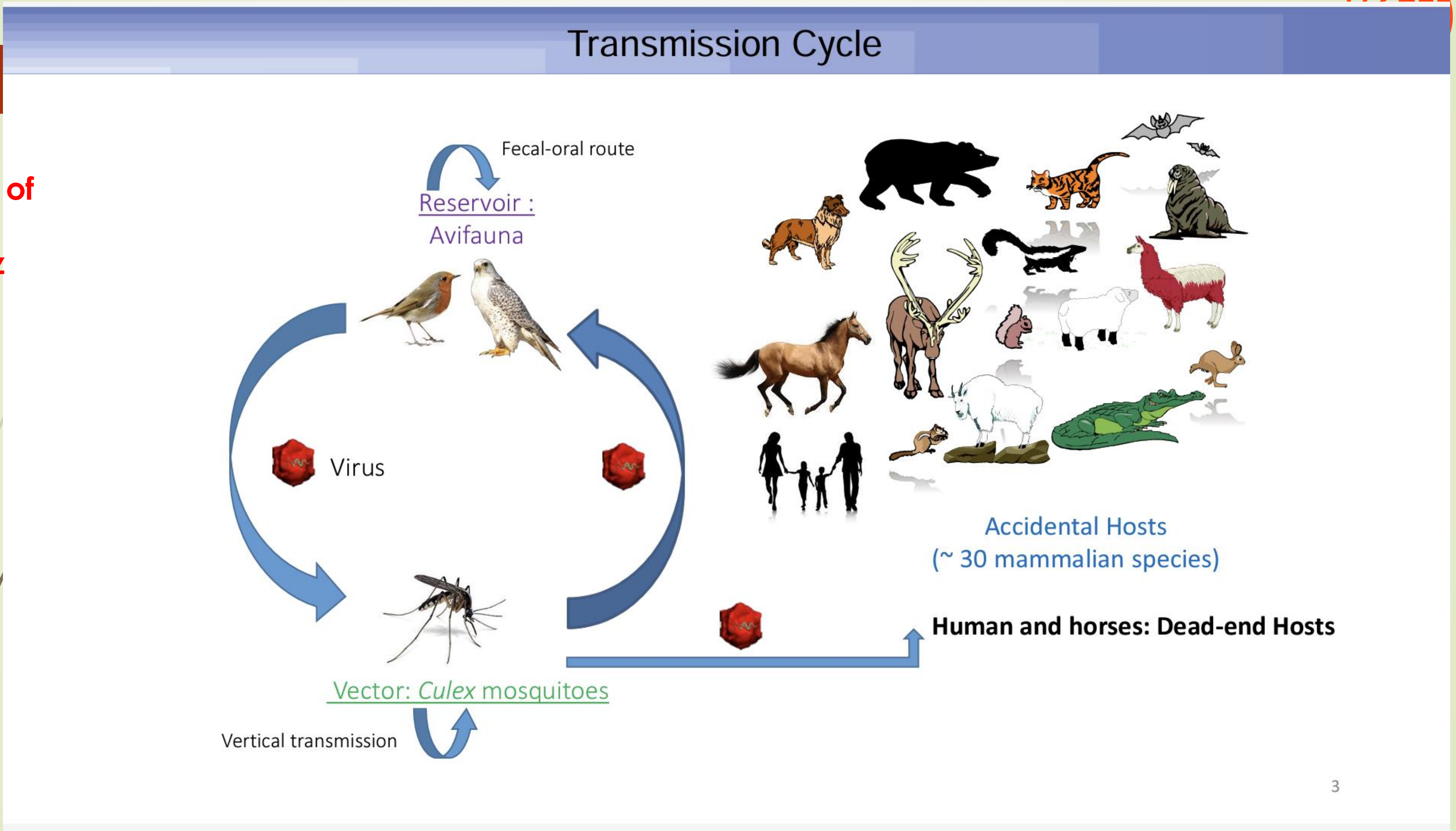
Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
RT-PCR = reverse-transcription polymerase chain reaction; HI = haemagglutination inhibition; CFT = complement fixation test;
ELISA = enzyme-linked immunosorbent assay; VN = virus neutralisation; PRNT: plaque reduction neutralisation test.

WOAH 2022, chapter 3.1.10, Japanese encephalitis

West Nile fever virus

https://rr-asia.woah.org/en/events/zoonoses-affecting-equines-japanese-encephalitis-west-nile-fever/

Courtesy of Gaelle Gonzalez



Transmission Cycle

Fecal-oral route

Reservoir : Avifauna

Virus

Vector: *Culex* mosquitoes

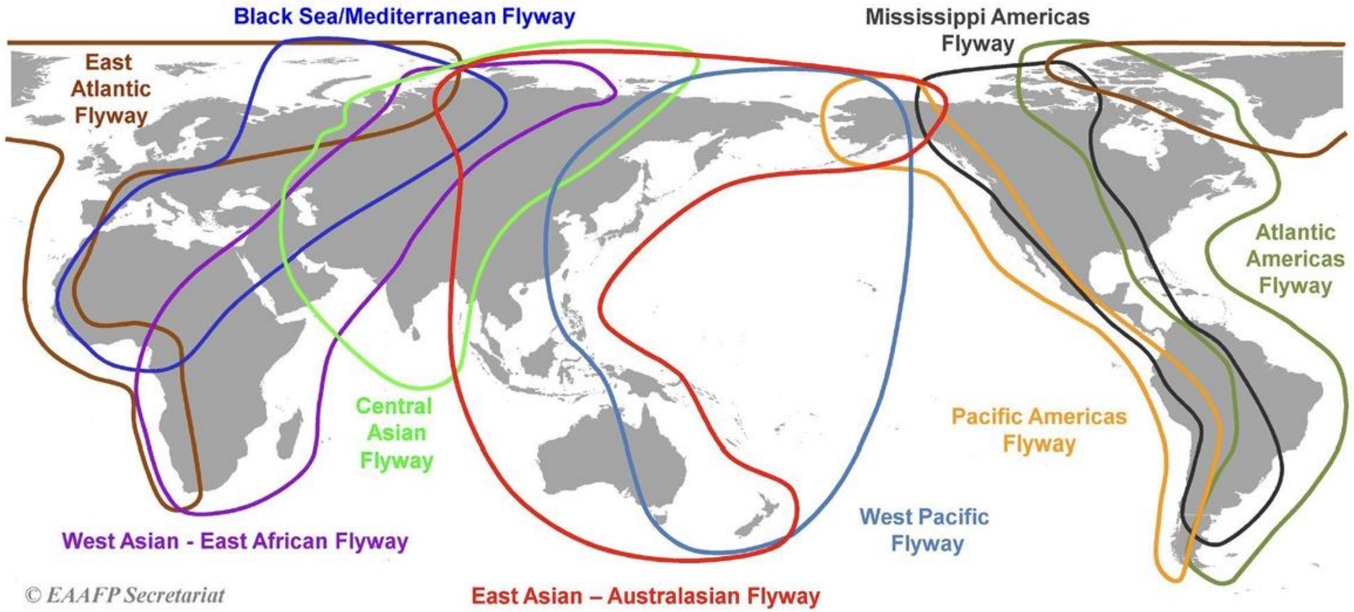
Vertical transmission

Accidental Hosts
(~ 30 mammalian species)

Human and horses: Dead-end Hosts

Avifauna: key role in long distance transport of the virus and local amplification

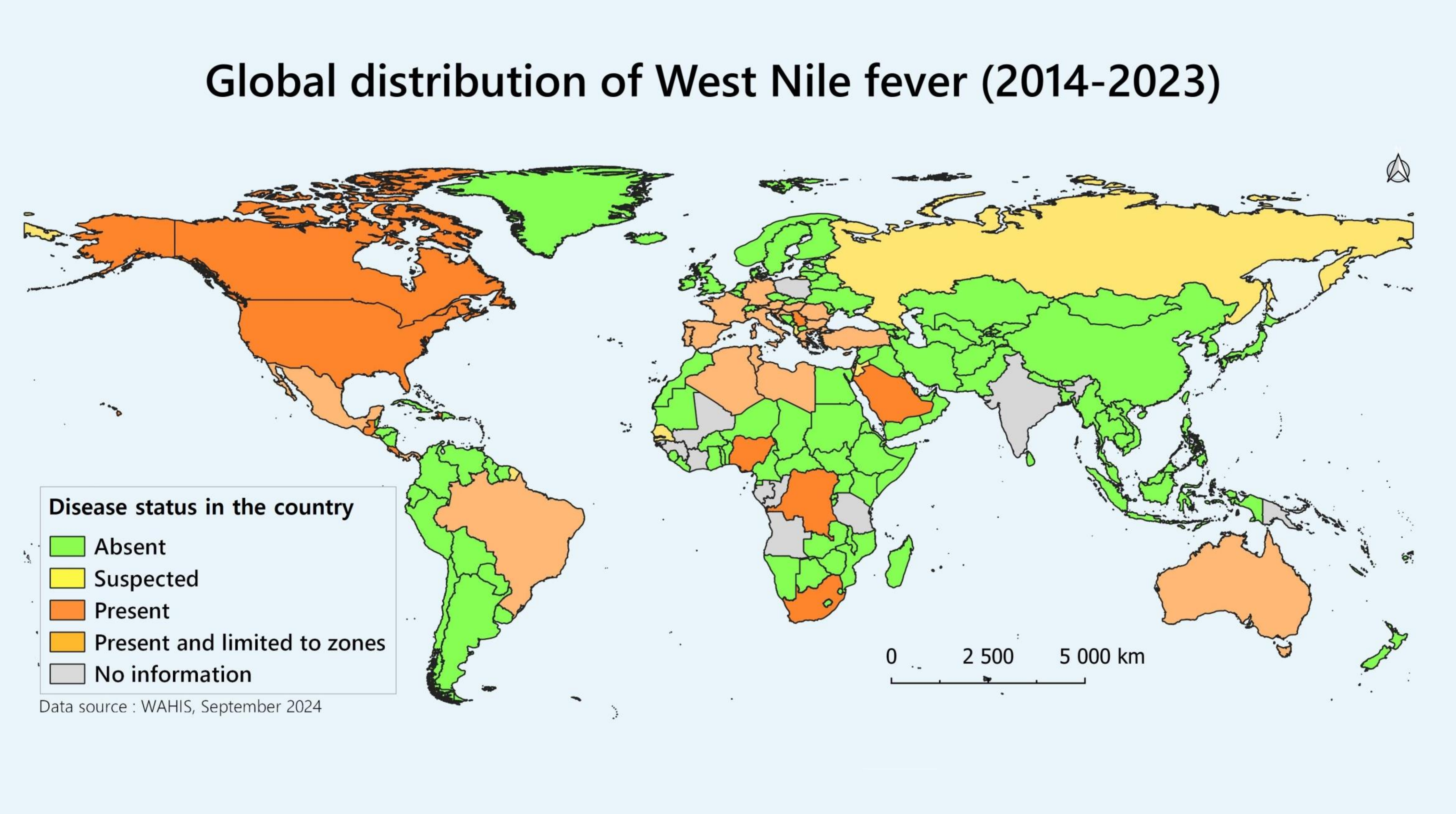
Viruses regularly introduced from the African cradle



© EAAFP Secretariat

In Europe: rare and isolated mortalities (corvids, other passerines, diurnal raptors)

Lecollinet et al, 2013



Global distribution of West Nile fever (2014-2023)

Data source : WAHIS, September 2024

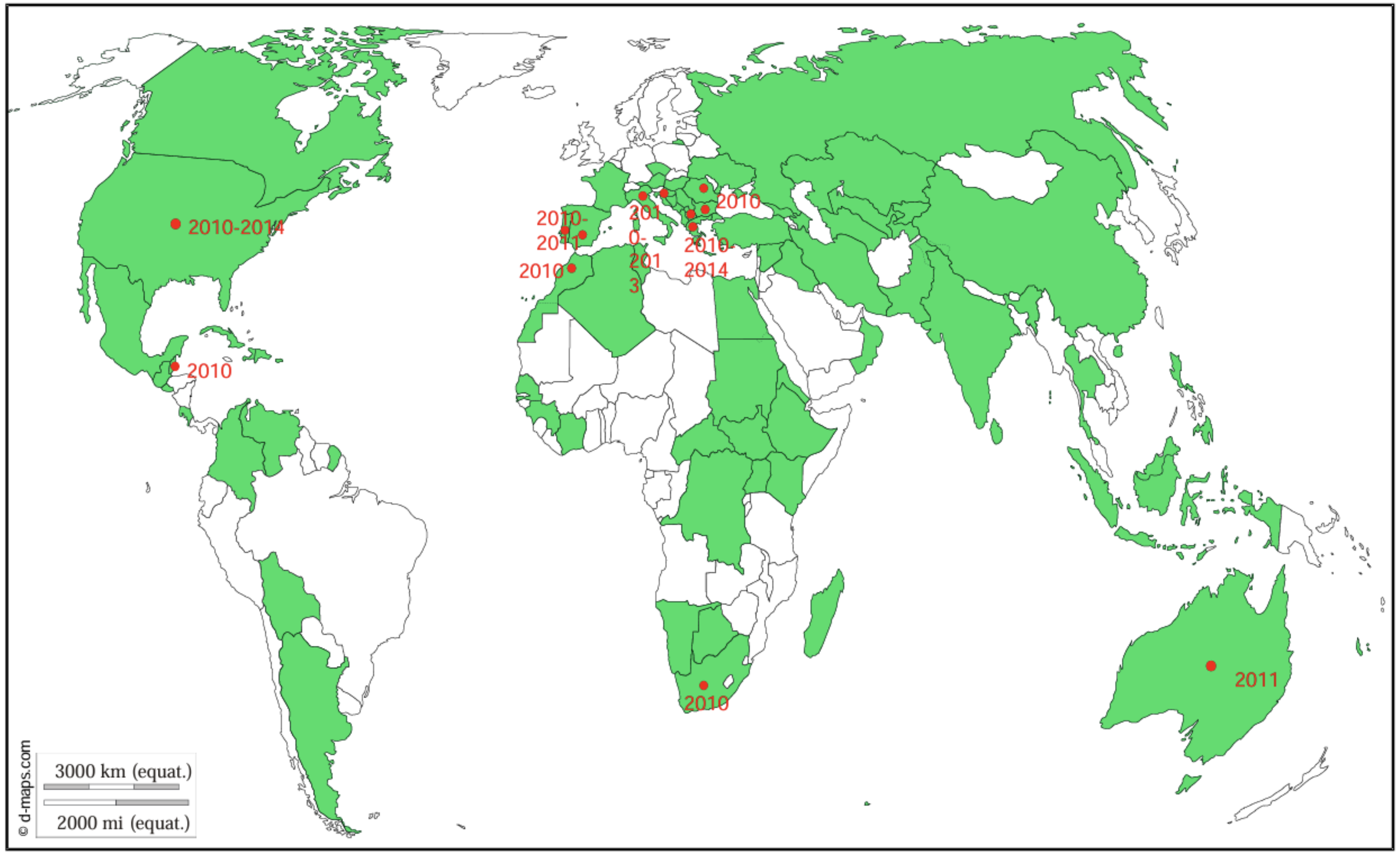
Courtesy of Gregorie Bazimo

Geographical distribution of WNV

Originating from Africa

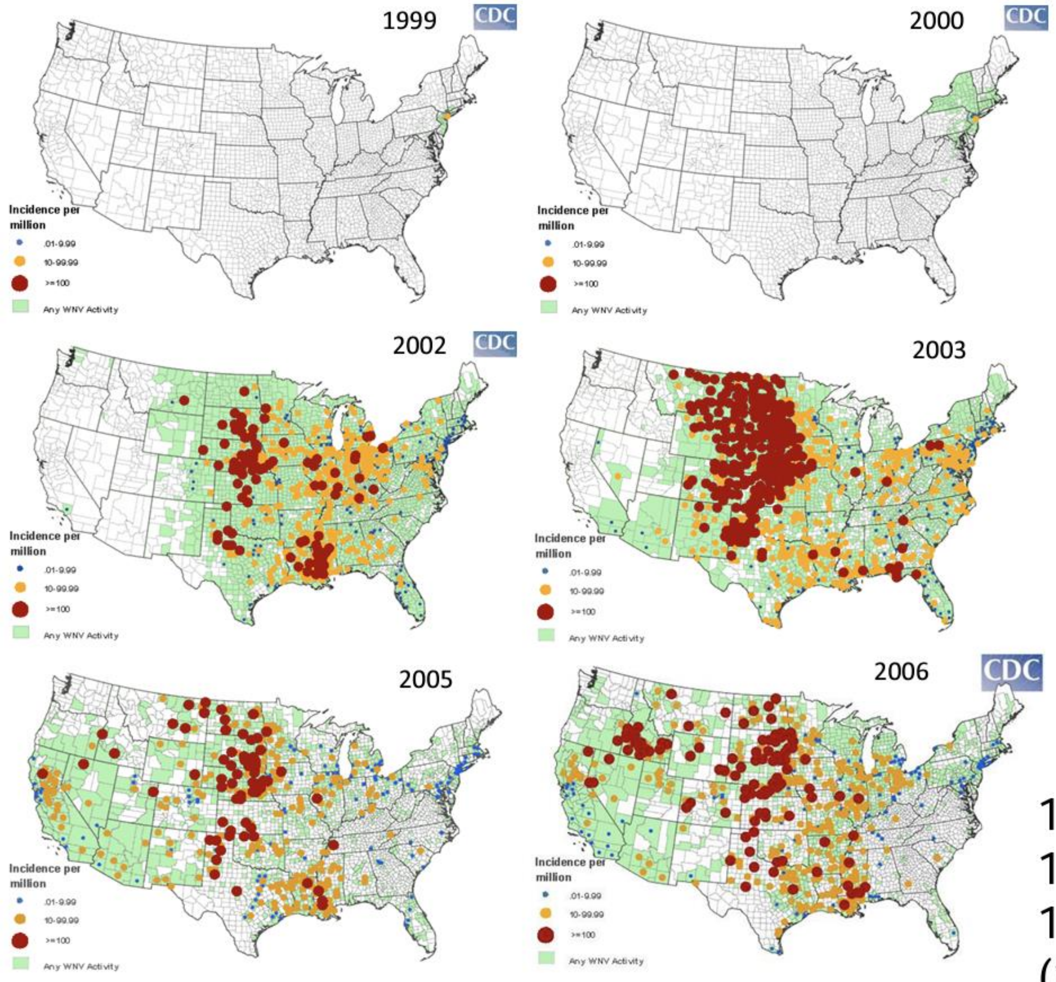
Discovered in 1937 in Uganda

Circulating on the 5 continents (except Antartica)



Lecollinet et al., 2020

WNV emergence and dissemination in the USA



1999-2010 : >30 000 human cases and 1200 deaths
1999-2002 : >57000 corvids deaths
1999-2009 : >24000 equine cases
(2002 : around 15000 cases et 4500 deaths)

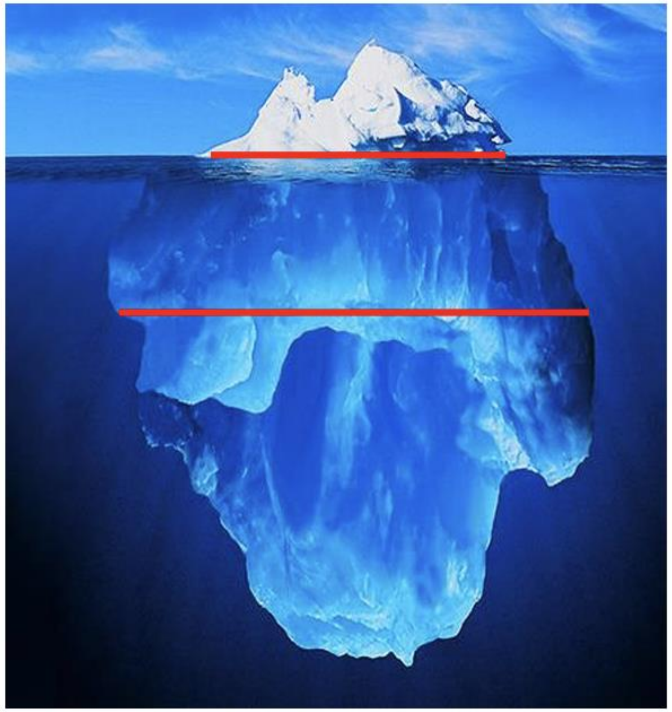
Disease in Horses

Incubation period: 3 – 15 days

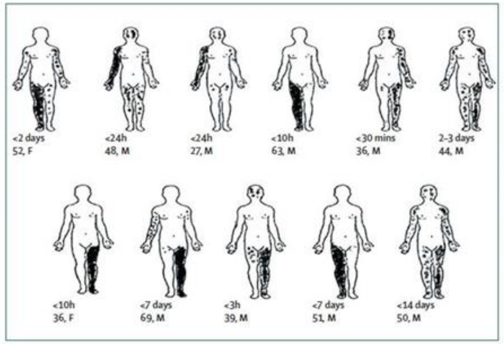
Neurological symptoms (WNND) : 1-10%
Lethality rate: 20-57% (horse), 10% (humans)
Ataxia, Paralysis, etc.

Mild illness: < 20%
Flu-like symptoms (West-Nile Fever)

Subclinical infection: 70-79%



Dr P. Garcia, 2015



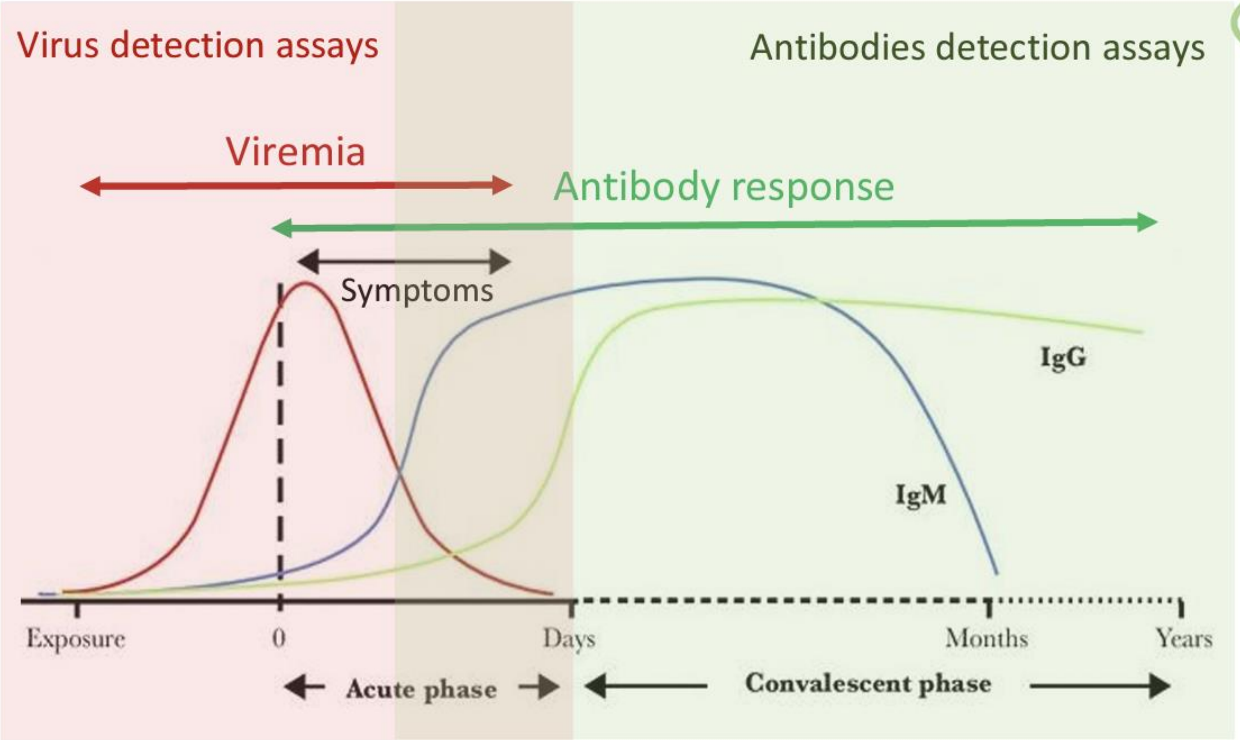
Kramer et al, Lancet, 2007

WNND: West-Nile Neuroinvasive Disease

Diagnostic of WNV infection

Blood-EDTA,
Cerebrospinal fluid
Urines
Brain

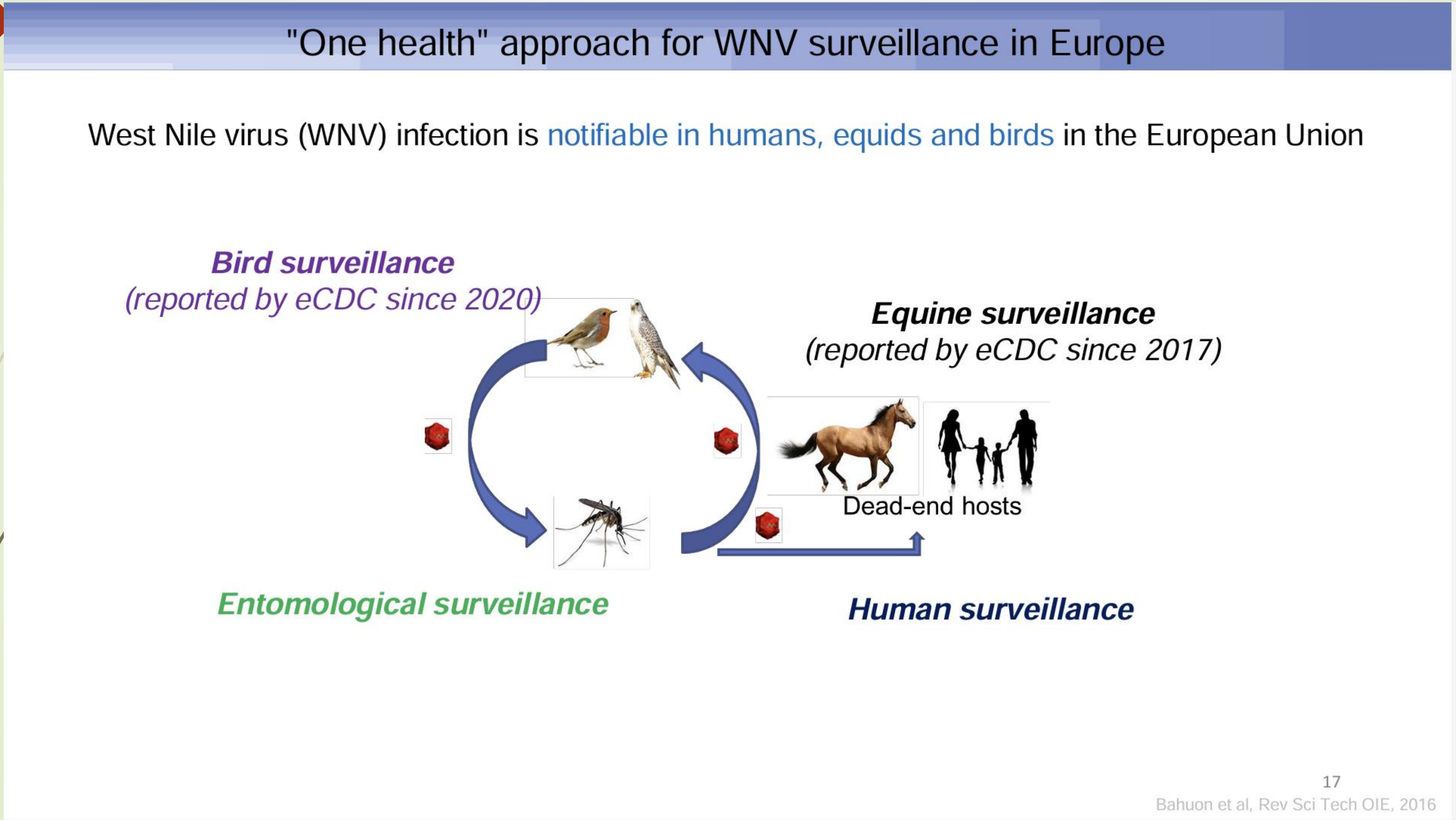
- ☐ Molecular detection of WNV genome by RT-qPCR
- ☐ Virus isolation



Sera and plasma

- ☐ MAC ELISA (IgM)
- ☐ IgG ELISA
- ☐ VNT

(adapted from Goncalves A. et al., 2017)



"One health" approach for WNV surveillance in Europe

West Nile virus (WNV) infection is notifiable in humans, equids and birds in the European Union

Bird surveillance
(reported by eCDC since 2020)

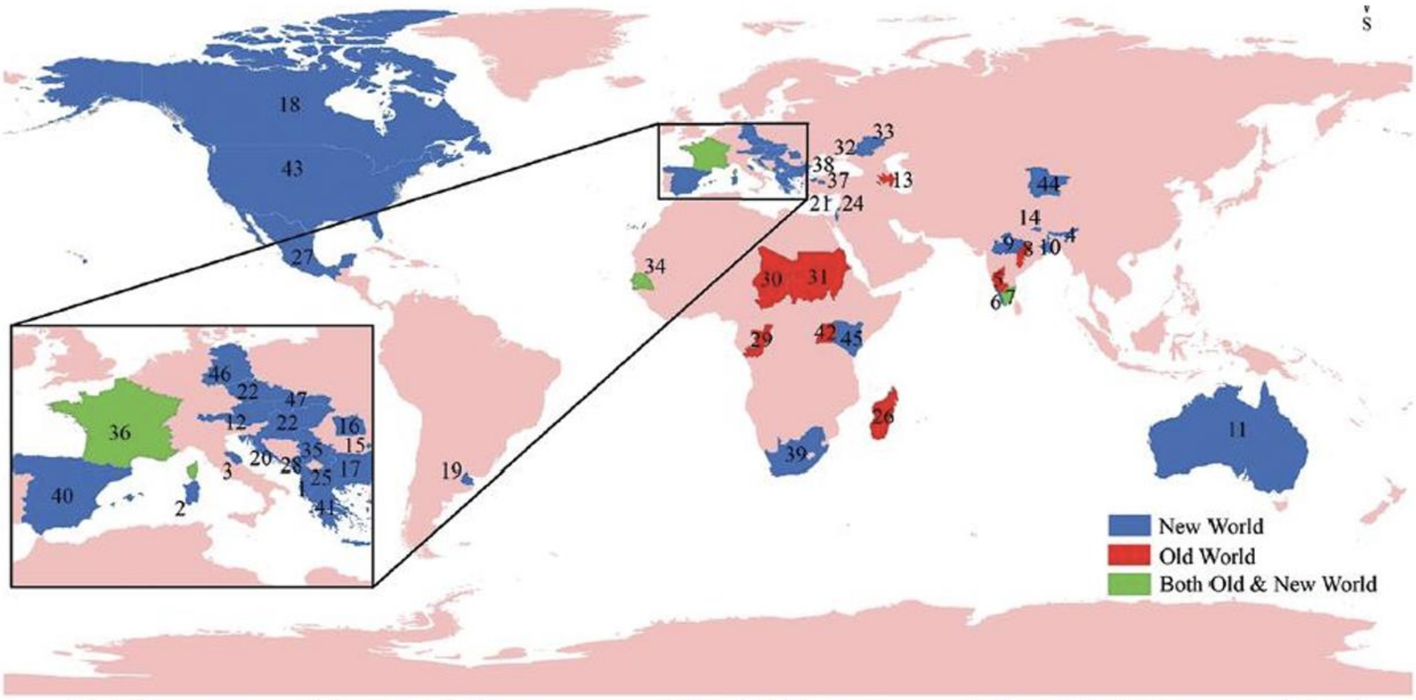
Equine surveillance
(reported by eCDC since 2017)

Entomological surveillance

Human surveillance

Bahuon et al, Rev Sci Tech OIE, 2016

WNV in Asia and Pacific regions



Sl No.	Country	Lineage	Sl No.	Country	Lineage	Sl No.	Country	Lineage	Sl No.	Country	Lineage
1	Albania	II	13	Azerbaijan	I	25	Macedonia	II	37	Turkey (Eskisehir)	I
2	Italy (Sardina)	II	14	Nepal (Bharatpur & Kathmandu)	I	26	Madagascar	II	38	Turkey (Bursa region)	II
3	Italy (Ancona)	II	15	Romania (Bucharest)	I	27	Mexico	II	39	South Africa	II
4	India (Assam)	V	16	Romania (Transylvania)	II	28	Montenegro	II	40	Spain	I & VI
5	India (Karnataka)	V	17	Bulgaria	II	29	Republic of the Congo	II	41	Greece	II
6	India (Kerala)	I	18	Canada	I	30	Chad	II	42	Uganda	II
7	India (Tamil Nadu)	I & V	19	Argentina (Chaco)	I	31	Sudan	II	43	United States	I
8	India (Chhattisgarh)	I	20	Croatia	II	32	Russia (Rostov Oblast)	II	44	China (Xinjiang)	I
9	India (Madhya Pradesh)	I	21	Cyprus	II	33	Russia (Volgograd)	II	45	Kenya	I
10	India (West Bengal)	I	22	Czech Republic	II &III	34	Senegal	VII, VIII & I	46	Germany (Eastern Part)	II
11	Australia	I	23	Hungary	II	35	Serbia	II	47	Slovakia	II
12	Austria	IX & II	24	Israel	I	36	France	II			

Chowdhury et al, 2021

- WNV detected in humans in Asia and Pacific regions
- Need to implement an integrative surveillance system

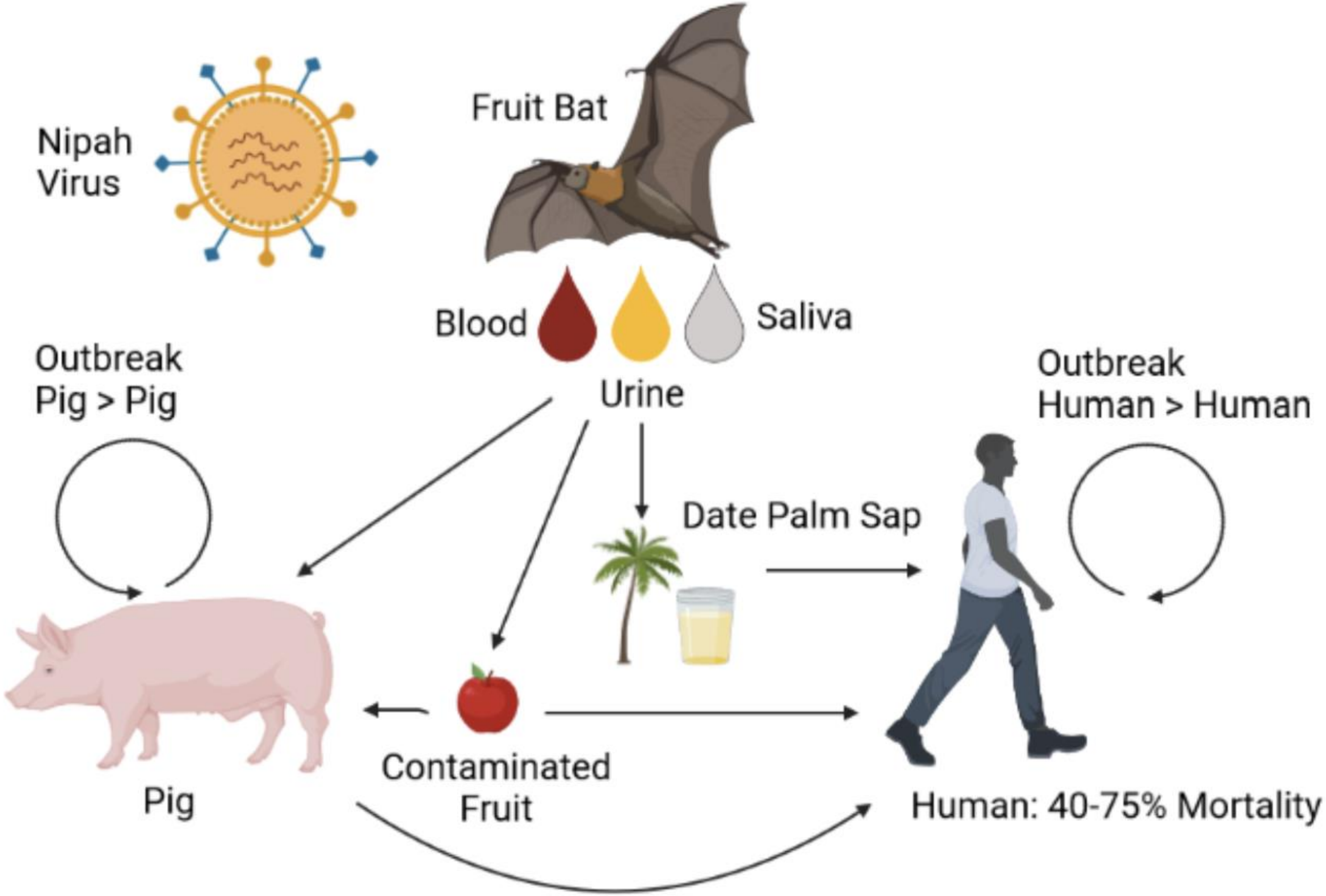
Nipah virus encephalitis

https://rr-asia.woah.org/app/uploads/2024/01/5-kim_-hendra-and-nipah-ref-lab.pdf

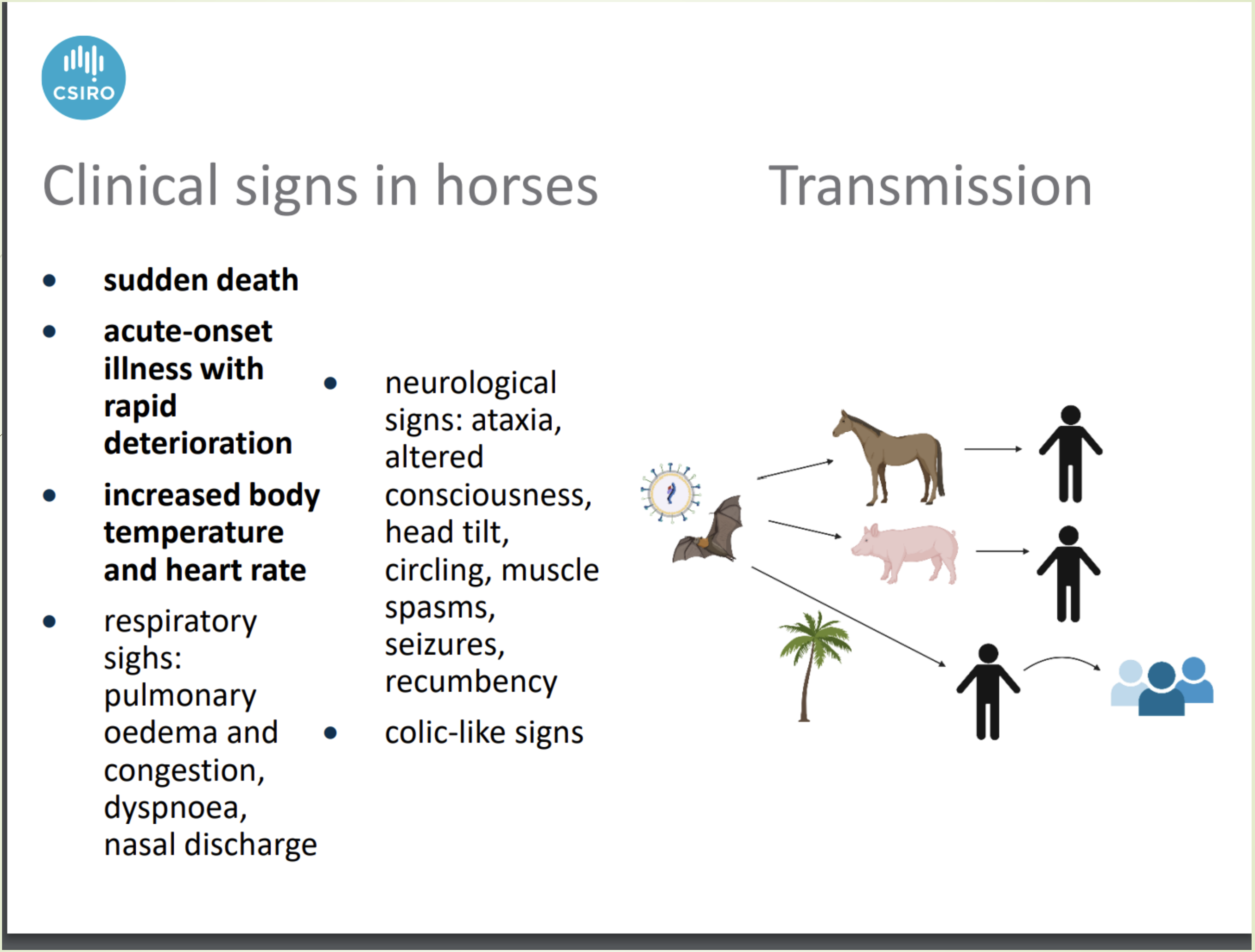
Facts about the disease

- Non-notifiable disease (except for pigs) – amplifying host is the pig
- In pigs known as porcine respiratory and neurologic syndrome, porcine respiratory and encephalitic syndrome (PRES) or barking pig syndrome (BPS)
- Zoonotic disease!!! Infection in humans
- Natural reservoir host is the flying fox /fruit bats – enzootic circulation of the virus
- RNA virus from the family Paramyxo virus
- First observerd in pigs in Malaysia and Singapore in 1998-1999; 2001 in Bangladesh and India, 2014 in Philippines
- Infection through ingestion of contaminated feed or contact with contaminated aerosols
- most of Southeast Asia, China, Madagascar, Australia, and parts of Africa are considered at-risk for NiV outbreaks
- Clinical signs: respiratory and neurological

Nipah Virus Transmission and Mortality



Courtesy of Kim Halpin, CISRO

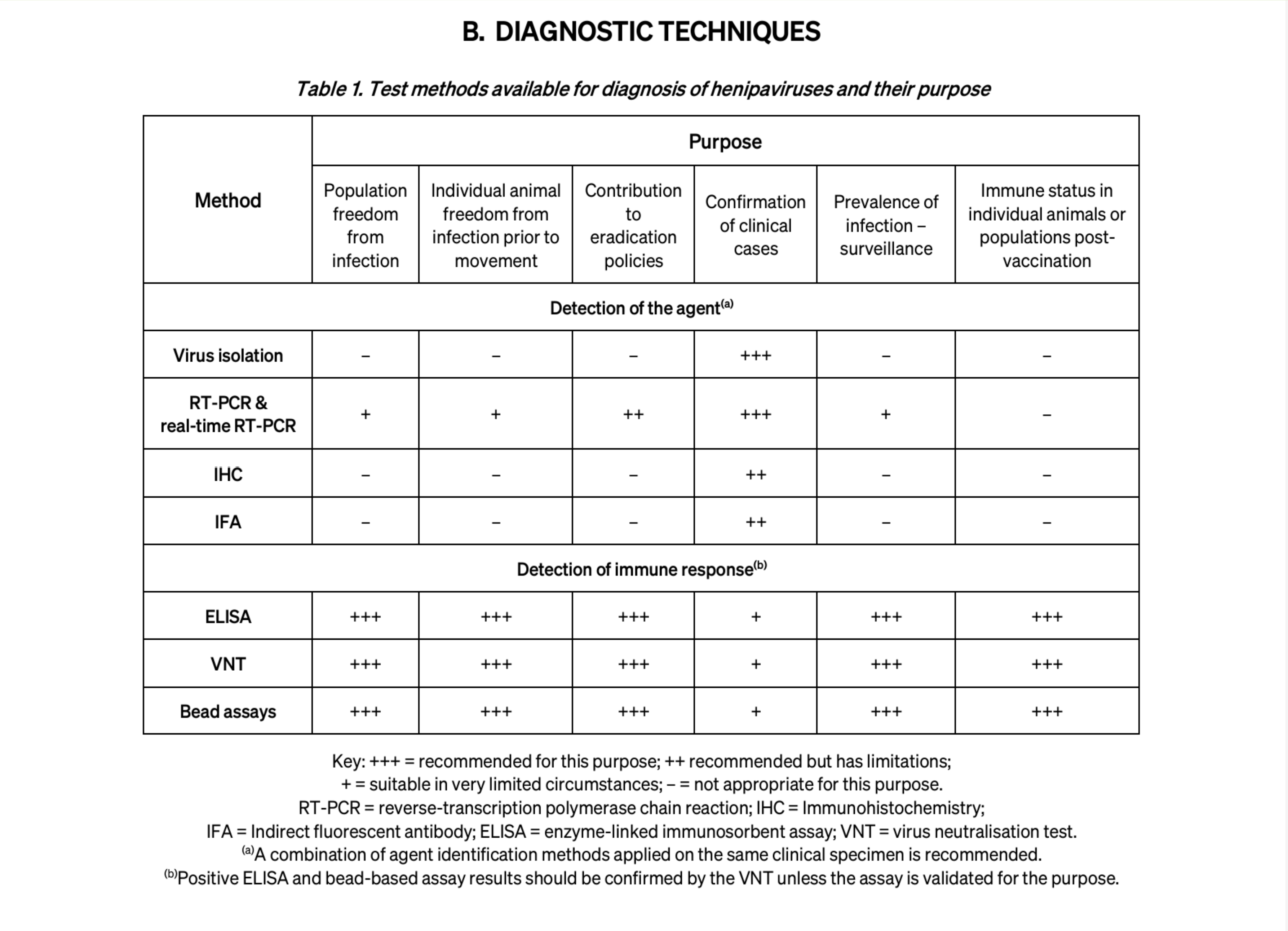


Clinical signs in horses

- **sudden death**
- **acute-onset illness with rapid deterioration**
- **increased body temperature and heart rate**
- respiratory sighs: pulmonary oedema and congestion, dyspnoea, nasal discharge
- neurological signs: ataxia, altered consciousness, head tilt, circling, muscle spasms, seizures, recumbency
- colic-like signs

Transmission

WOAH Manual, Chapter 3.1.16

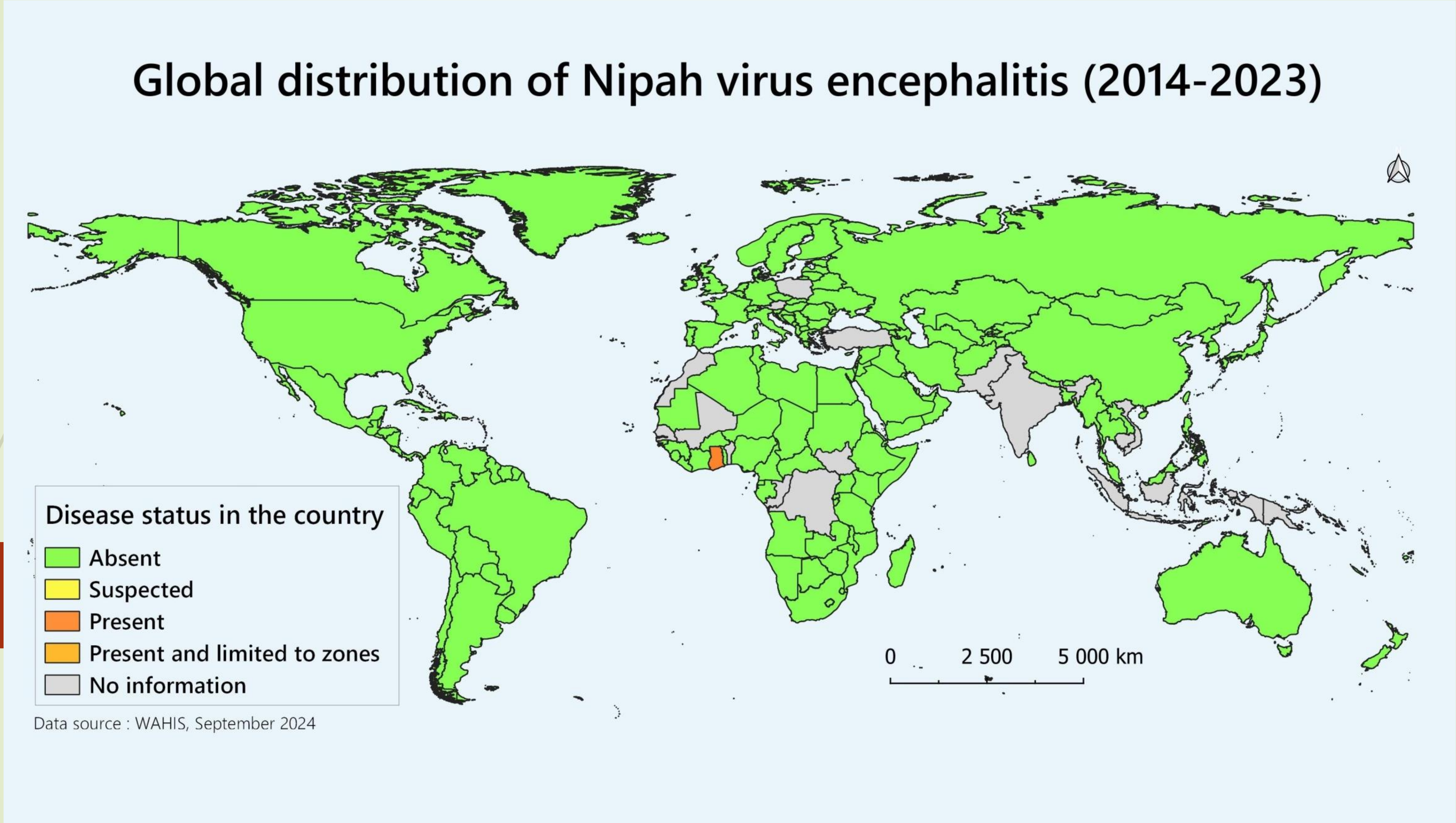


B. DIAGNOSTIC TECHNIQUES

Table 1. Test methods available for diagnosis of henipaviruses and their purpose

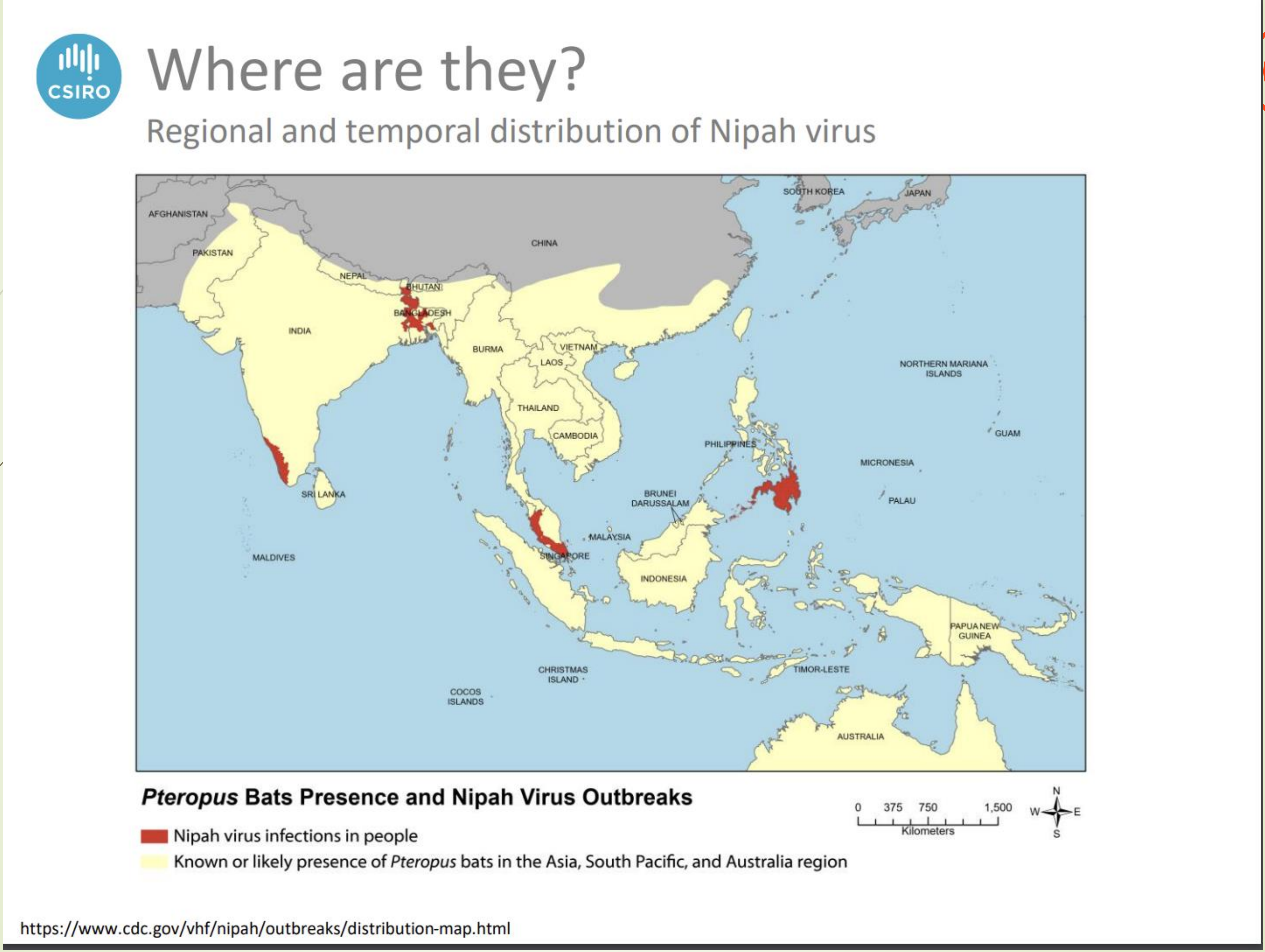
Method	**Purpose**					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribution to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent[(a)]						
Virus isolation	–	–	–	+++	–	–
RT-PCR & real-time RT-PCR	+	+	++	+++	+	–
IHC	–	–	–	++	–	–
IFA	–	–	–	++	–	–
Detection of immune response[(b)]						
ELISA	+++	+++	+++	+	+++	+++
VNT	+++	+++	+++	+	+++	+++
Bead assays	+++	+++	+++	+	+++	+++

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
RT-PCR = reverse-transcription polymerase chain reaction; IHC = Immunohistochemistry;
IFA = Indirect fluorescent antibody; ELISA = enzyme-linked immunosorbent assay; VNT = virus neutralisation test.
[(a)] A combination of agent identification methods applied on the same clinical specimen is recommended.
[(b)] Positive ELISA and bead-based assay results should be confirmed by the VNT unless the assay is validated for the purpose.



Global distribution of Nipah virus encephalitis (2014-2023)

Data source : WAHIS, September 2024



Where are they?

Regional and temporal distribution of Nipah virus

***Pteropus* Bats Presence and Nipah Virus Outbreaks**

🟥 Nipah virus infections in people

🟨 Known or likely presence of *Pteropus* bats in the Asia, South Pacific, and Australia region

https://www.cdc.gov/vhf/nipah/outbreaks/distribution-map.html

Courtesy of
Kim Halpin,
CISRO

Thank you for your attention

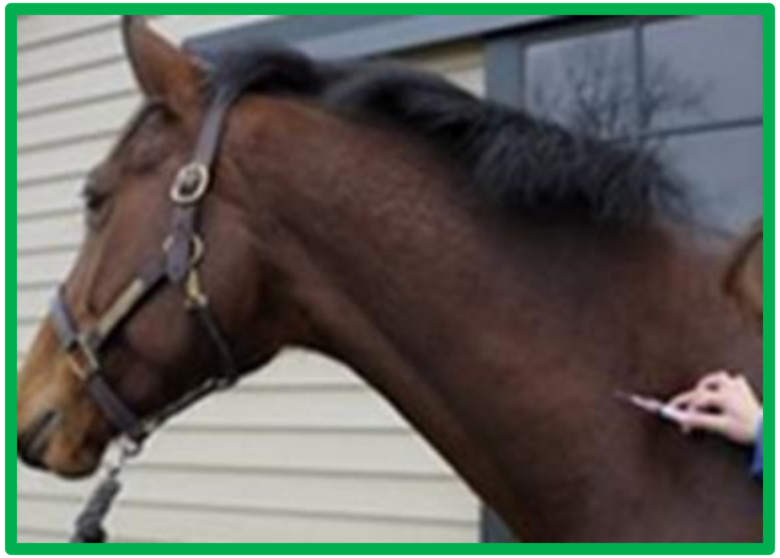
Vaccines and recommended vaccination schedules to prevent equine influenza (EI)

Manabu Nemoto

Equine Research Institute, Japan Racing Association (JRA)
WOAH Reference Laboratory for Equine Influenza

Equine influenza (EI)

- Equine influenza virus (EIV): Influenza A virus, H3N8
- Respiratory diseases: fever, cough, nasal discharge
- Significant economic losses to the horse industry
- Vaccination is an important measure to control EI



Representative examples of commercially available vaccine technologies

Vaccine type	Feature	Product
Whole inactivated	Inactivated viruses	Duvaxyn (Elanco)
Subunit vaccine	Only antigenic fragments (HA/NA)	Equip F (Pfizer)
Live-attenuated	Attenuated, cold-adapted virus	Flu Avert (Merck)
Canary pox vectored	Vector with HA gene	PROTEQ FLU (Boehringer)

(Paillot, Vaccines 2014; Oladunni et al., Viruses 2021)

WOAH Expert Surveillance Panel on Equine Influenza Vaccine Composition

- The panel started about 30 years ago
- To avoid vaccine breakdown, the meeting reviews annually the epidemiological and virological information
- The panel recommends proper vaccine strains every year

(WOAH bulletin, 2017)

WOAH releases recommendations related to vaccine strains!

World Organisation
for Animal Health
Founded as OIE

Expert surveillance panel on equine influenza vaccine composition

8th July 2021 and 7th July 2022 by Videoconference

Conclusions and Recommendations

However, many vaccine products are not updated and still use old vaccine strains…

Committee of selection on animal influenza vaccine strains in Japan

- Organized by National Veterinary Assay Laboratory, MAFF
- Reviewing avian and equine influenza vaccine strains
- If needed, the committee changes vaccine strains

  - The latest changes were in 2016

  according to WOAH recommendations

(Photo from NVAL, MAFF HP)

Vaccine schedules

Vaccination requirements of equestrian/horse racing authorities

	FEI	BHA (UK)	Japan	Summary
Primary vac. (V1 and V2)	21-60D	21-60D	14-60D	**14-60D**
First booster (V3)	Within 6M +21D	120-180D	Within 7M	**About 6M**
Booster (V4, V5…)	Within 1 yr (Minimum)	Within 6M	Within 1 yr (Minimum)	**Within 1 yr**
Booster (Competition)	Within 6M +21D	Within 6M	Within 7M	**About 6M**

Vaccination requirements of equestrian/horse racing authorities

	FEI	BHA (UK)	Japan	Summary
Primary vac. (V1 and V2)	21-60D	21-60D	14-60D	**14-60D**
First booster (V3	Within 6M			About 6M
Boos (After				Within 1 yr
Boos (Competition)	+21D	6M	[illegible]	About 6M

- V1 should start after 5-6 months of age
  - Interference by maternal antibody
  - Maturation of foal’s immune response

(Paillot, Vaccines 2014)

Vaccination requirements of equestrian/horse racing authorities

	FEI	BHA (UK)	Japan	Summary
Booster (After V4)	Within 1 yr (Minimum)	Within 6M	Within 1 yr (Minimum)	**Within 1 yr**
Booster (Competition)	Within 6M +21D	Within 6M	Within 7M	**About 6M**

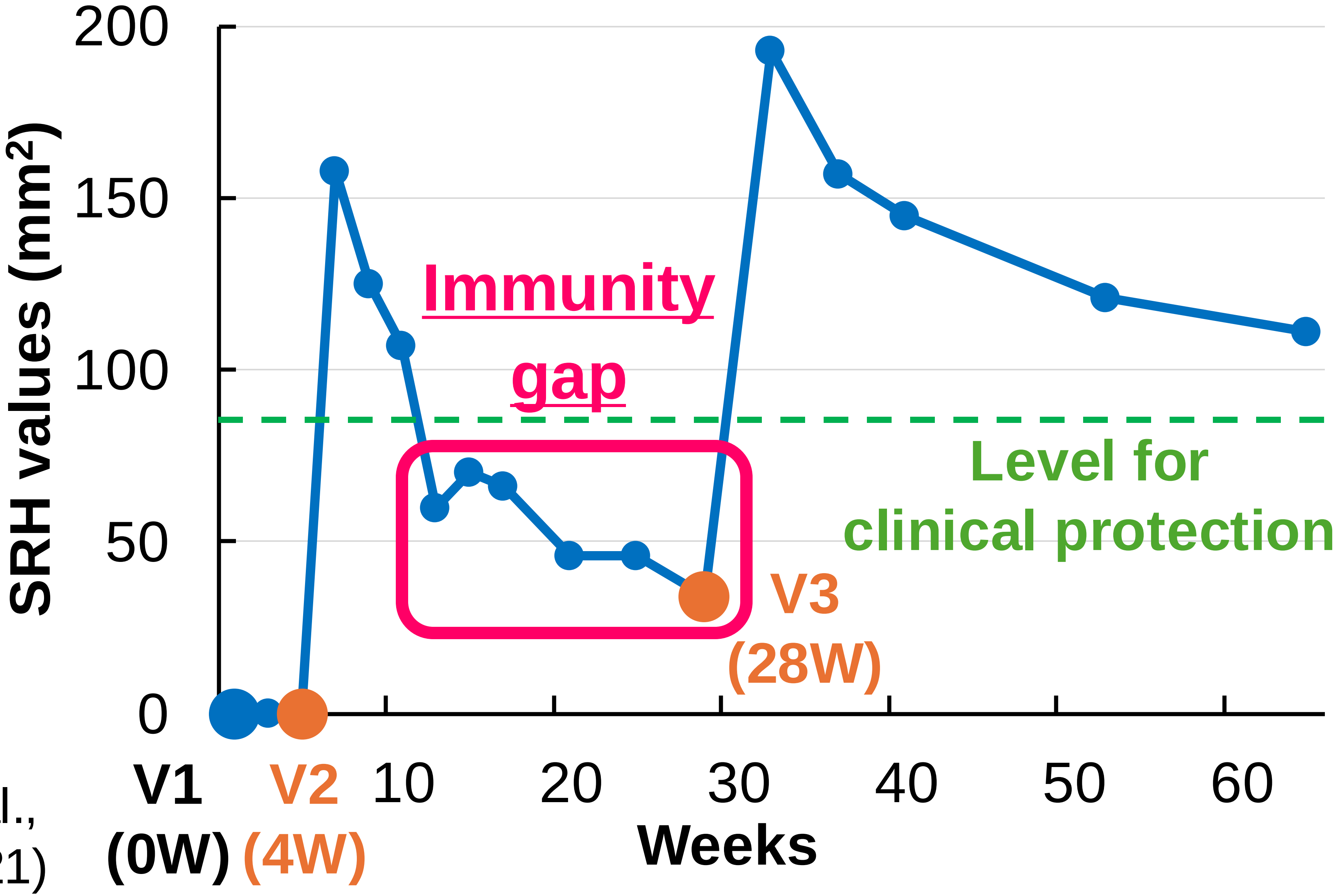
- Annual booster shot should not be relied on as the sole preventative measure (Gildea et al., Equine Vet J 2020)

→ **Biannual shot is recommended**

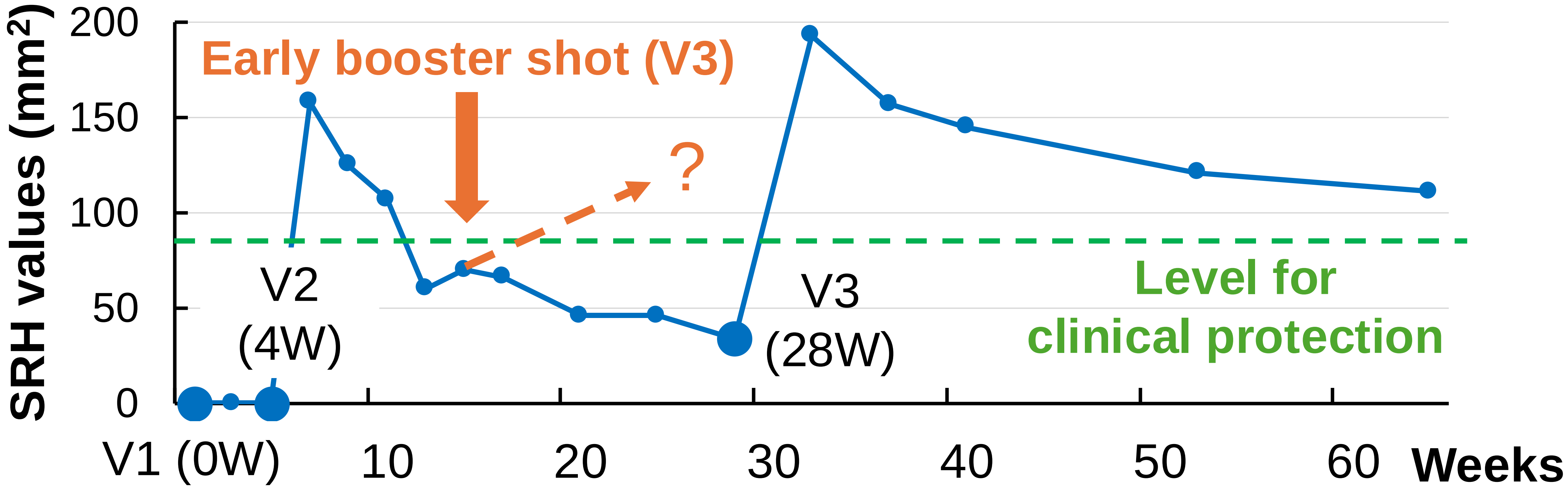
Example of SRH antibody level after vaccinations

	Summary
Primary vac. (V1/V2)	**14-60D**
First booster (V3)	**About 6M**

(Data from Dilai et al., J Equine Vet Sci 2021)



Is early booster shot beneficial to shorten the gap?



- **Beneficial**: Cullinane et al., Vet J 2001; El-Hage et al., Equine Vet J 2013
- **Not beneficial**: Heldens et al., Vet J 2007

→ **There is no clear solution for the immunity gap…**

Two training centers (TC) of JRA

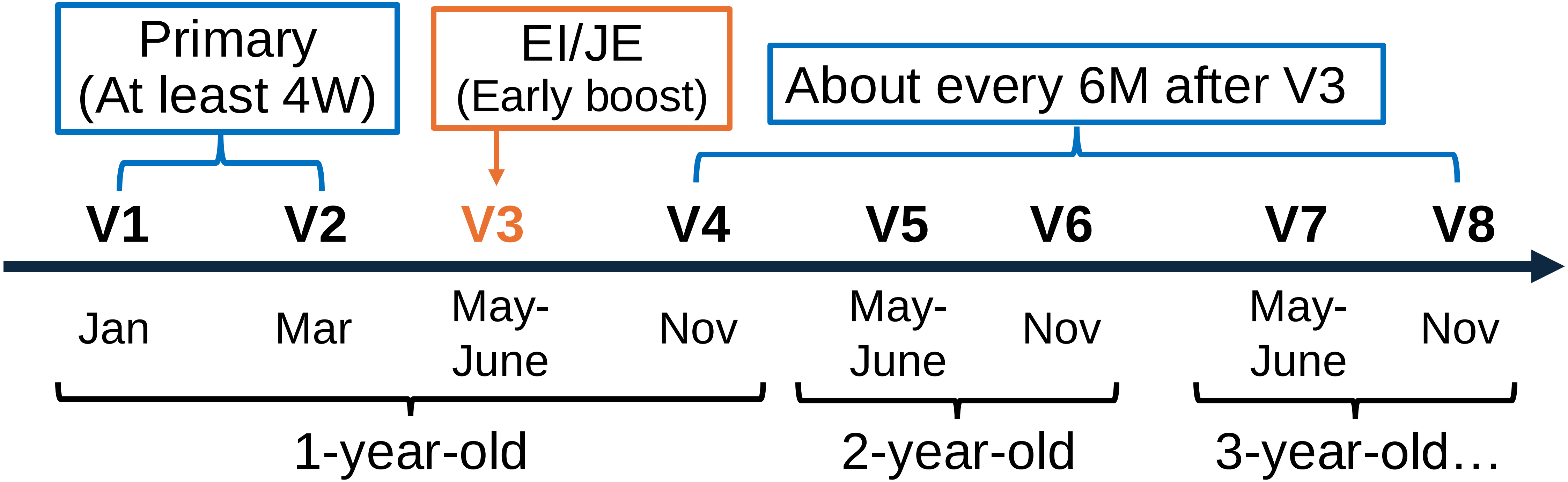
- They are in Miho (Ibaraki Pref.) and Ritto (Shiga Pref.)
- About 2,000 horses are at each TC
- Once an outbreak occurs, virus spreads quickly

→It is important to control by vaccination

Miho TC: 2,240,000㎡

Ritto TC: 1,522,000㎡

Recommended EI vaccination schedule in Japan



Primary (At least 4W)

EI/JE (Early boost)

About every 6M after V3

V1 V2 **V3** V4 V5 V6 V7 V8

Jan Mar May-June Nov May-June Nov May-June Nov

1-year-old

2-year-old

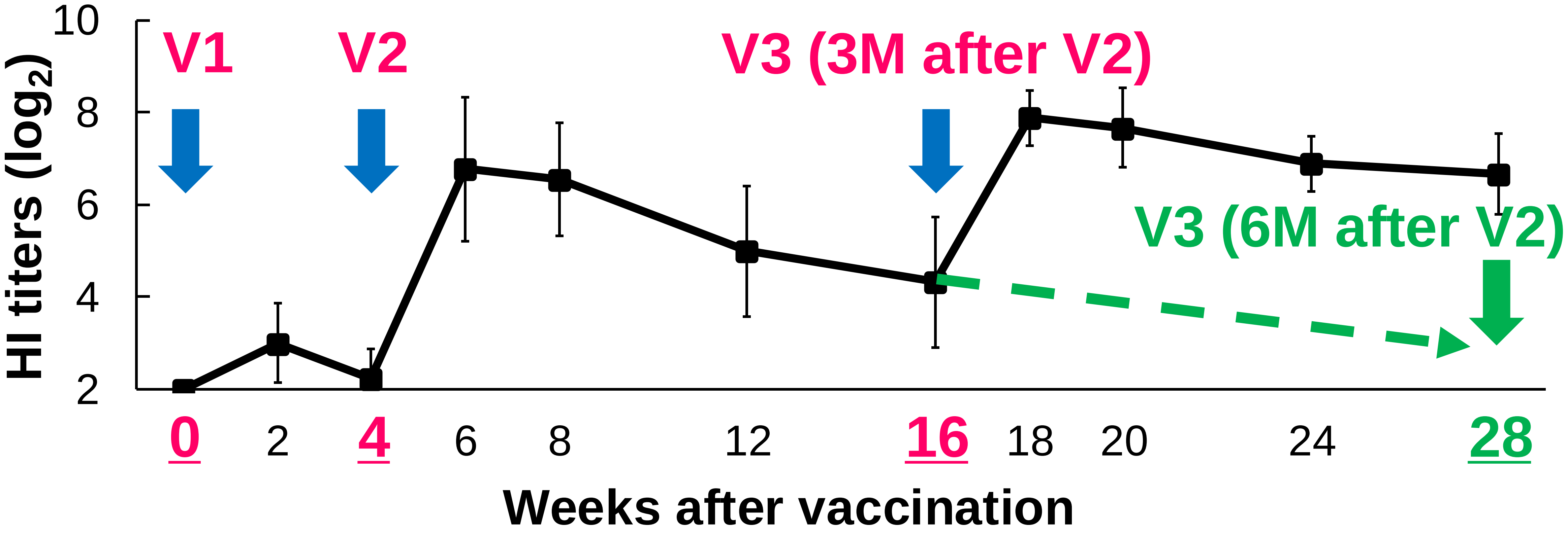
3-year-old…

Subsidies: To increase the vaccination coverage

- 1- and 2-yr-old horses before entering TC: **50% (JLIA)**
- Racehorses kept at TC: **100% (JRA)**

Immunity gap between V2 and V3 is shortened by recommended schedule in Japan

- An inactivated vaccine without adjuvant (Nisseiken, Tokyo) was inoculated into nine 1-year-old horses three times



(Data from Ohta et al., J Equine Vet Sci 2022)

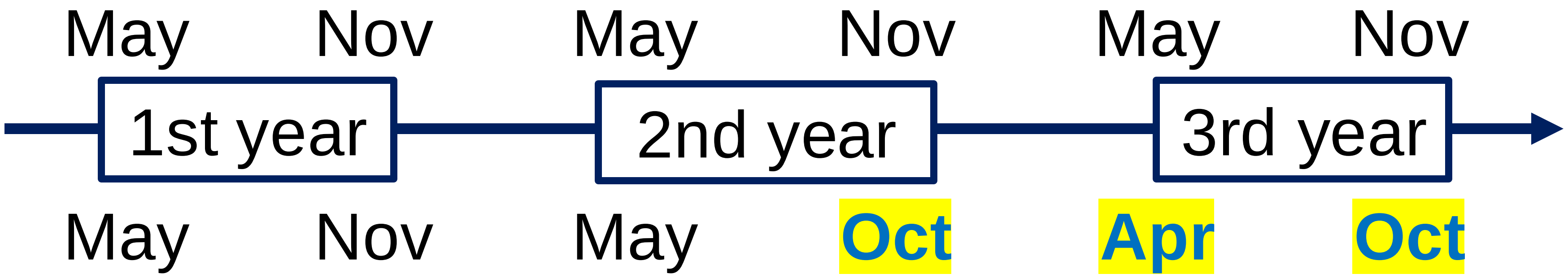
Intervals of booster vaccination for competition horses

FEI	BHA (UK)	Japan
Within 6M+21D	Within 6M	Within 7M

Within 6M+21D
or Within 7M ➡

- Vaccination months can be fixed
- It is easy to manage both EI and JE

(Example of schedule)



	1st year		2nd year		3rd year
May	Nov	May	Nov	May	Nov
May	Nov	May	**Oct**	**Apr**	**Oct**

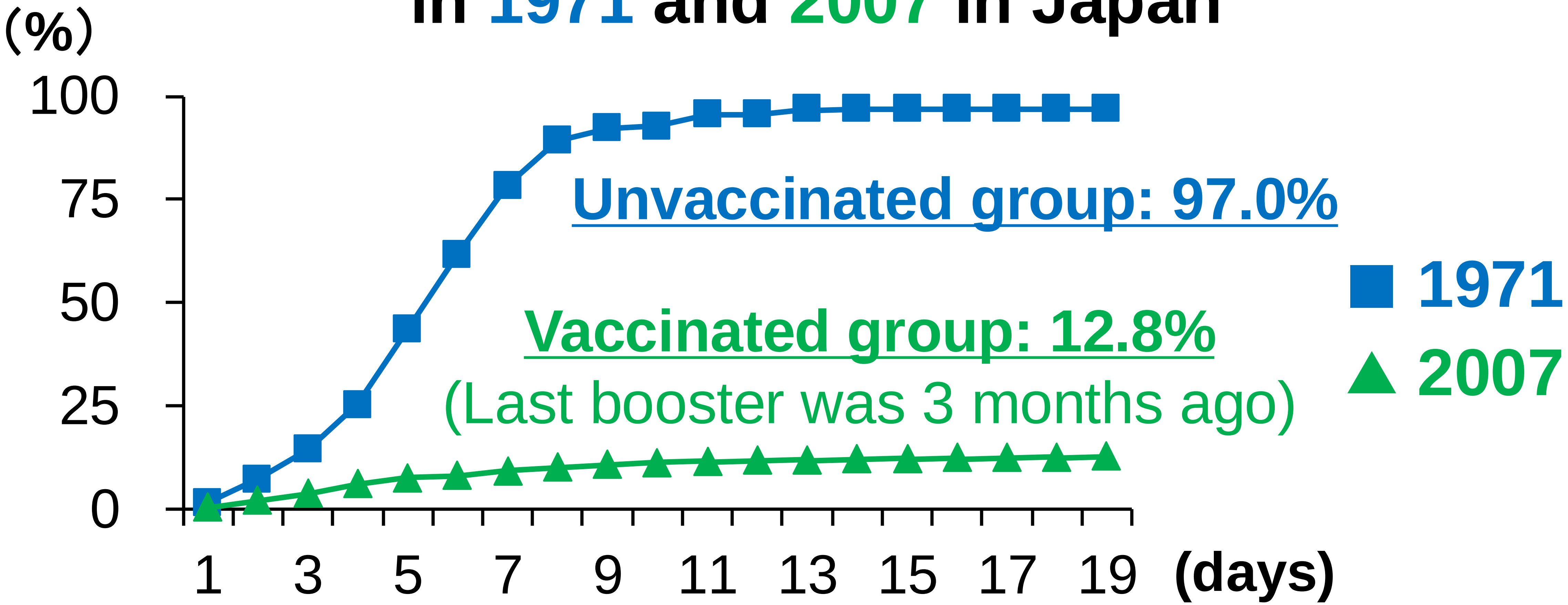
Within 6M → Vaccination months can change from year to year

Booster shot: Within 6 or 7 months?

Age	Post vaccination months	Vaccination numbers	HI titer	
2	5-6 months: n=34	4	**92**	No significant difference
2	6-7 months: n=34	4	**75**	
3	5-6 months: n=36	6	**78**	No significant difference
3	6-7 months: n=36	6	**59**	

- Although HI titers were lower in the group 6-7 months, there was no significant difference
- Within 7 months should be practical to manage many horses

Rate of febrile horses (%) in two outbreaks in 1971 and 2007 in Japan



Unvaccinated group: 97.0%

Vaccinated group: 12.8%

(Last booster was 3 months ago)

- Febrile horses dramatically declined!
- Vaccination minimized damage to industry

Conclusions

- It is important to change vaccine strains quickly according to the recommendations
- After primary vaccination, booster vaccination every about 6 months is recommended
- At a site with many horses, high coverage and systematic vaccination are important to minimize outbreaks

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

General Guidance on Disease surveillance

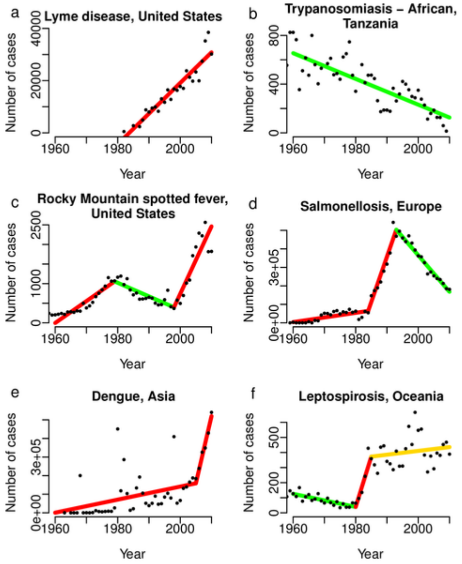
Paolo Tizzani
Veterinary epidemiologist
Data Integration Department

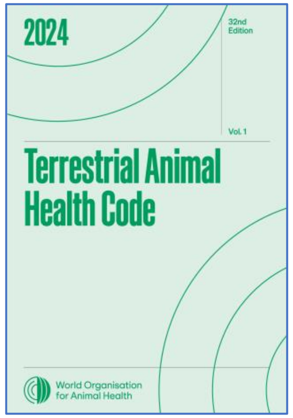
Mauro Meske
Project Liason Officer WOAH-IHSC
Disease Status Department

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

Surveillance introduction and objectives

- Disease surveillance is aimed at demonstrating **the absence** of infection or infestation, determining **the presence** or distribution of infection or infestation or **detecting as early as possible** exotic diseases or emerging diseases
- Tool to monitor **disease trends**, to facilitate the control of infection or infestation, to **provide data for use in risk analysis**, for animal or public health purposes, to **substantiate the rationale for sanitary measures**
- The type of surveillance applied depends on the **objectives** of the surveillance, the **available data sources** and the **outputs needed** to support decision-making.





Surveillance definitions

- **Confidence:** probability that the type of *surveillance* applied would detect the presence of *infection* or *infestation* if the *population* were infected and is equivalent to the sensitivity of the *surveillance*.
- **Sample:** group of elements (sampling *units*) drawn from a *population*, on which tests are performed or parameters measured to provide *surveillance* information.
- **Sampling unit:** means the *unit* that is sampled. This may be an individual or a group, such as an *epidemiological unit*.
- **Sensitivity** means the proportion of infected sampling *units* that are correctly identified as positive.
- **Specificity** means the proportion of uninfected sampling *units* that are correctly identified as negative.

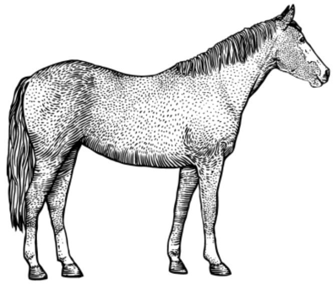
Surveillance definitions

- **Study population** means the *population* from which *surveillance* data are derived.
- **Surveillance system** means the use of one or more *surveillance* components to generate information on the health status of a *populations*.
- **Target population** means the *population* to which conclusions are to be inferred.
- **Test** means a procedure used to classify a *unit* as either positive, negative or suspect with respect to an *infection* or *infestation*.

Surveillance systems

Design of the surveillance system

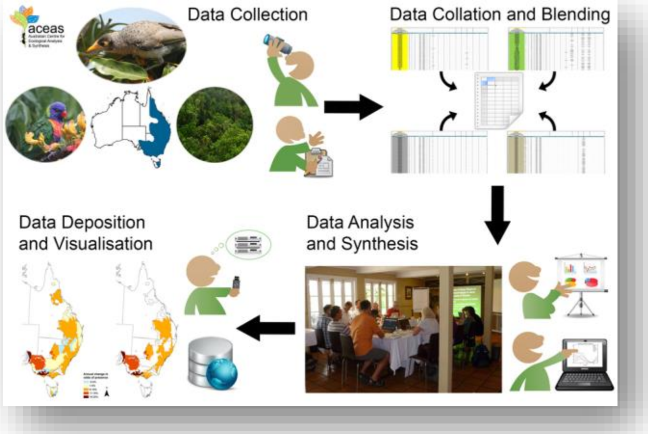
- Definitions of appropriate populations
- Timing and temporal validity of surveillance data
- Case definition
- Epidemiological unit
- Clustering
- Diagnostic tests
- Analytical methodologies
- Scope of the surveillance system
- Follow up actions



Surveillance systems

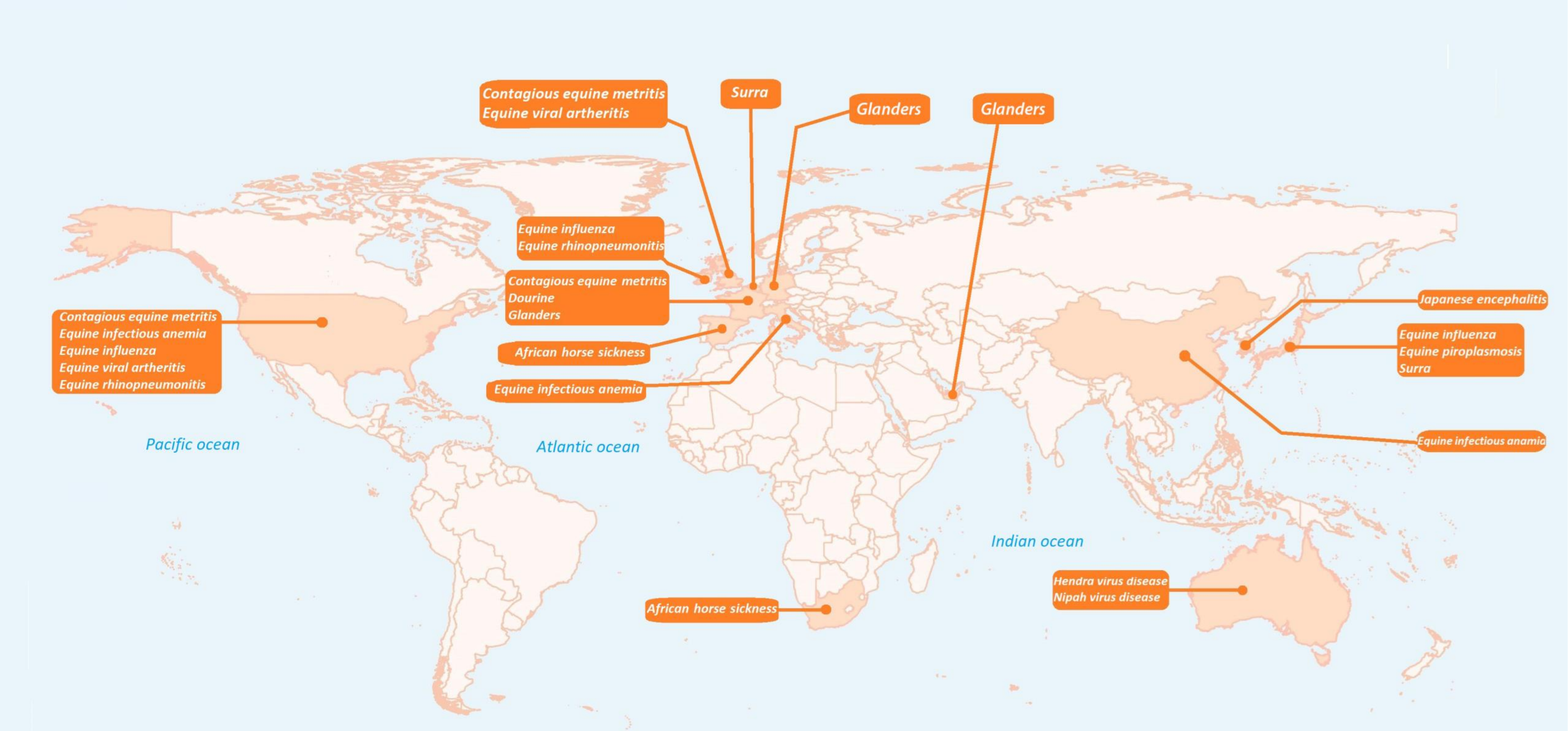
- Implementation of the surveillance system

  - Diagnostic tests (Key):
    - ✓Confirmation of suspicions by lab test
    - ✓Diagnostic capabilities in National Labs
    - ✓Agreements with Regional Labs to send samples
    - ✓Referral to WOAH Reference Labs
    - ✓Regular Proficiency tests to ensure quality



  - Data collection and management

WOAH Reference Laboratories for Equine Diseases



Comprehensive Surveillance System for Equine Diseases

01

Comprehensive Surveillance System

Formal and ongoing system for detecting and investigating outbreaks

Procedure for rapid sample collection and transport

System for recording, managing, and analyzing diagnostic, epidemiological, and surveillance data

02

Targeted Sampling and Diagnostic Approaches

Crucial for detecting and monitoring infections in equids

Effective disease detection and monitoring

Utilizes specific sampling methods and diagnostic tools

Implement **targeted surveillance strategies** based on epidemiological situation
Selecting **specific-high-risk populations**

Define a series of **complementary methods** (i.e., clinical surveillance, serological testings, agent identification) and **follow –up investigations** to confirm or exclude suspicions

03

Clinical and Pathological Surveillance

Clarifies the status of suspected cases

Confirms suspected cases through clinical signs and pathological findings

Essential for accurate disease diagnosis

May have limitations due to asymptomatic carrier animals being the main reservoir of the disease.

Ensure diagnostics tests have **high Se and Sp**, validated for equids

Ensure Quality of the test (Regular **Proficiency tests**)

04

Vector Surveillance

Identifies high-risk areas and vector species

Identifies presence and abundance of vector species

Consider biology and behavior of local vector species

Utilize traps, nets, sticky targets, or other tools

Base surveillance on scientific sampling

Consider size and ecological characteristics of the area

Animal Surveillance is always preferred rather than vector surveillance

05

Sentinel Animals in Surveillance

Provide early detection of infections

Monitor disease dynamics

Sentinel animals are often unexposed equids managed at fixed locations and regularly observed and tested.

The primary purpose of using sentinel animals is to detect infections at specific locations, such as boundaries of infected zones.

Sentinel animals are an effective tool in monitoring disease spread and assessing the risk of infection in equine populations.

Surveillance systems

- Quality assurance
  - Surveillance systems should be subjected to **periodic auditing** to ensure that **all components function** and provide **verifiable documentation of procedures** and basic checks to detect deviations of procedures from those specified in the design, in order to **implement appropriate corrective actions.**

Early warning for Early detection

Surveillance methods

An early warning system is essential for the timely detection, reporting and communication of occurrence, incursion or emergence of diseases, infections or infestations and is an integral component of emergency preparedness. It should include the following:

- appropriate access to the target populations
- access to laboratories capable of diagnosing and differentiating relevant infections or infestations;
- training and awareness programmes for detecting and reporting unusual health incidents;
- legal obligation to report
- epidemiological investigations of cases to acquire accurate knowledge of the situation for further action.
- effective systems of communication between the Health Authority and relevant stakeholders;
- a national chain of command.

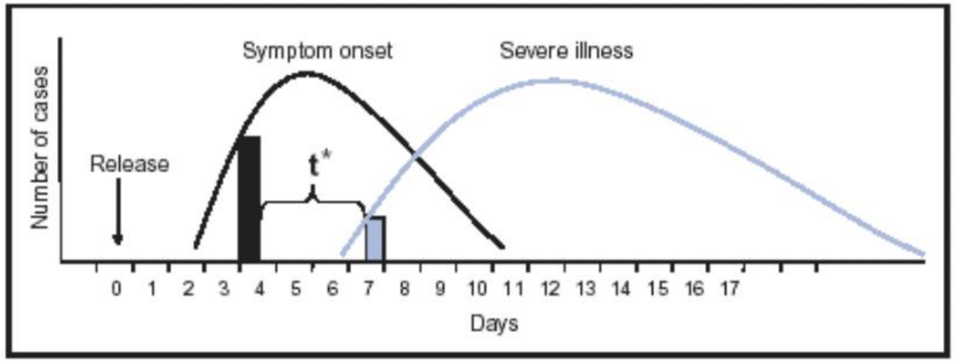
Surveillance methods

Early warning for Early detection

Syndromic surveillance.

Provides a timely system for detecting, understanding, and monitoring health events. By **tracking symptoms** of patients in emergency departments—**before a diagnosis is confirmed**—public health can detect unusual levels of illness to determine whether a response is warranted.

FIGURE. Syndromic surveillance — rationale for early detection

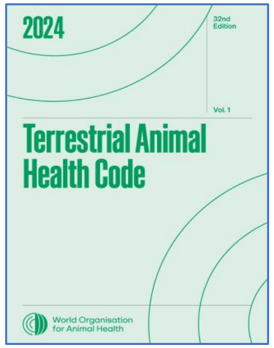


* t = time between detection by syndromic (prediagnostic) surveillance and detection by traditional (diagnosis-based) surveillance.

Henning 2004 *MMWR*

Surveillance provisions by Disease-Code Chapter

General Principles of Surveillance (AHS, Equine Piroplamosis, Surra and Glanders)



Surveillance for these equine diseases requires:

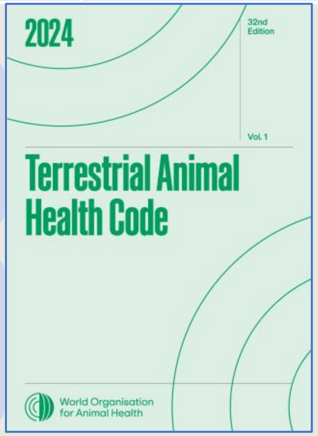
- A formal and **ongoing system for detecting and investigating outbreaks**;
- Appropriate **tools**, for collection, recording, managing and **analysis of data**; reporting and dissemination for decision making.
- **All suspected cases to be investigated by lab tests** (no pathognomonic clinical signs)
- The target population should include **domestic and wild** susceptible animals
- **An active programme of surveillance** of susceptible populations to detect evidence of disease is essential **to establish the animal health** status of a country, zone
- In a free country or zone, the surveillance programme should include an **early warning system for reporting suspected cases**

	AHS	Equine Piroplasmosis	Surra	Glanders
Clinical and pathological Surveillance (passive)	-**For horses** (likely to show clinical signs), not for asymptomatic carriers (donkey, zebras) -Suspicions should be **always confirmed by lab**	Surveillance aims at detecting clinical signs, **lab confirmation required**	Yes, but **all suspicions should be always confirmed by lab**	**Clinical:** Aims at detecting clinical signs but **of limited use** only, as **asymptomatic carrier** are the main reservoir. Systematic **pathological** surveillance is useful on deceased equids
Serological Surveillance	-For species that don't show clinical signs -**Reliable evidence of absence of AHS** in a country or zone -**Surveillance in high-risk areas**	**Active surveillance programme required to establish the status of a country or zone** (role of asymptomatic carriers) -**Surveillance in high-risk areas**	-Aims at **demonstrating individual or population freedom**; detect subclinical or latent infection; **determine prevalence- cross reactions /!\ (i.e. dourine)**	- **The preferred strategy to establish the status of a country or zone**. - **Animal identification and repeated testing** of the population are necessary
Agent Surveillance	Virological surveillance to **confirm clinical cases and follow up serological reactors** -to **determine the serotype**	Together with serological or agent identification testing with molecular techniques to **establish the status of a country or zone**	Parasitological surveillance and molecular techniques: -**To confirm clinical suspicions and serological results (**active infection), **identify parasites and subgenus**	**PCR/Culture:** suitable in very limited circumstances to confirm clinical cases **Malleinisation:** Demonstrates hypersensitivity to antigens of B. mallei. However, this method has **shortcomings**, such as **low sensitivity**, interference with other tests and **animal welfare concerns**.

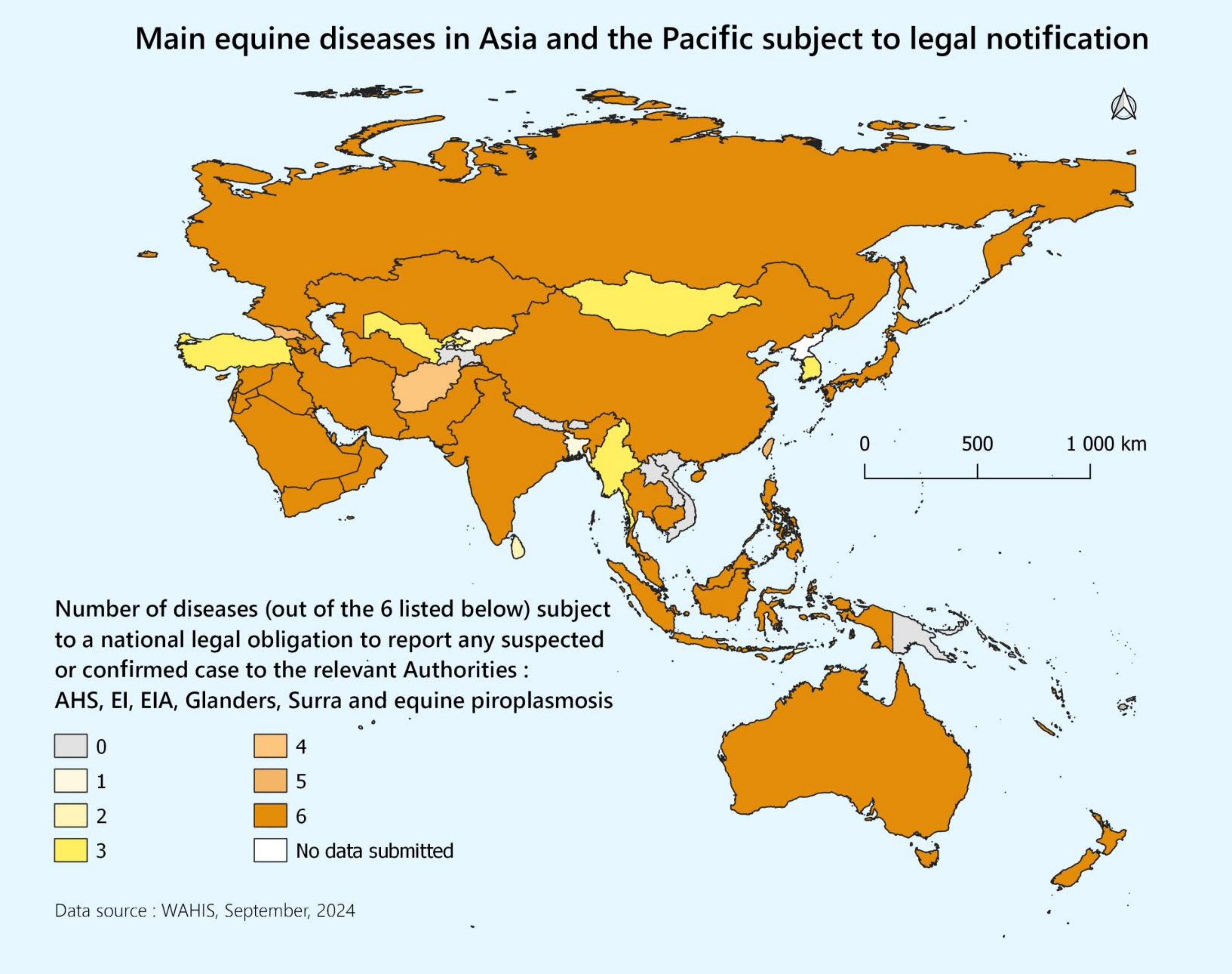
	AHS	Equine Piroplasmosis (*T. equi or B. caballi*)	Surra (*T. evansi*)	Glanders
Sentinel equids	-**To detect infections with AHSV at a particular place** (groups located on the boundaries of infected zones)	NA	-May contribute to provide **evidence of freedom** or provide data on prevalence and distribution of the infection - **Targeting highly susceptible animals** such as dogs (hunting dogs and dogs living around slaughterhouses/abattoirs), camels, donkeys or horses.	NA
Vector Surveillance	- Aims at determining different levels of risk by **identifying the presence and abundance of various vector species (Culicoides)** in an area	-Aims at determining different levels of risk by **identifying the presence and abundance of various vector species** (**competent ticks**) in an area.	- Aims at determining different levels of risk by **identifying the presence and abundance of various vector species (biting flies)** in an area	NA

Animal-based surveillance strategies are preferred to detect AHSV, *T. evansi*, *T. equi or B. caballi*

EIA, EHV, EI, JE : no specific provisions on surveillance but provisions on Code Chapter 1.4 (animal Health Surveillance) applies


2024
32nd Edition
Vol. 1
Terrestrial Animal Health Code
World Organisation for Animal Health

Surveillance measures in the region: Notifiable diseases (2019-2023)

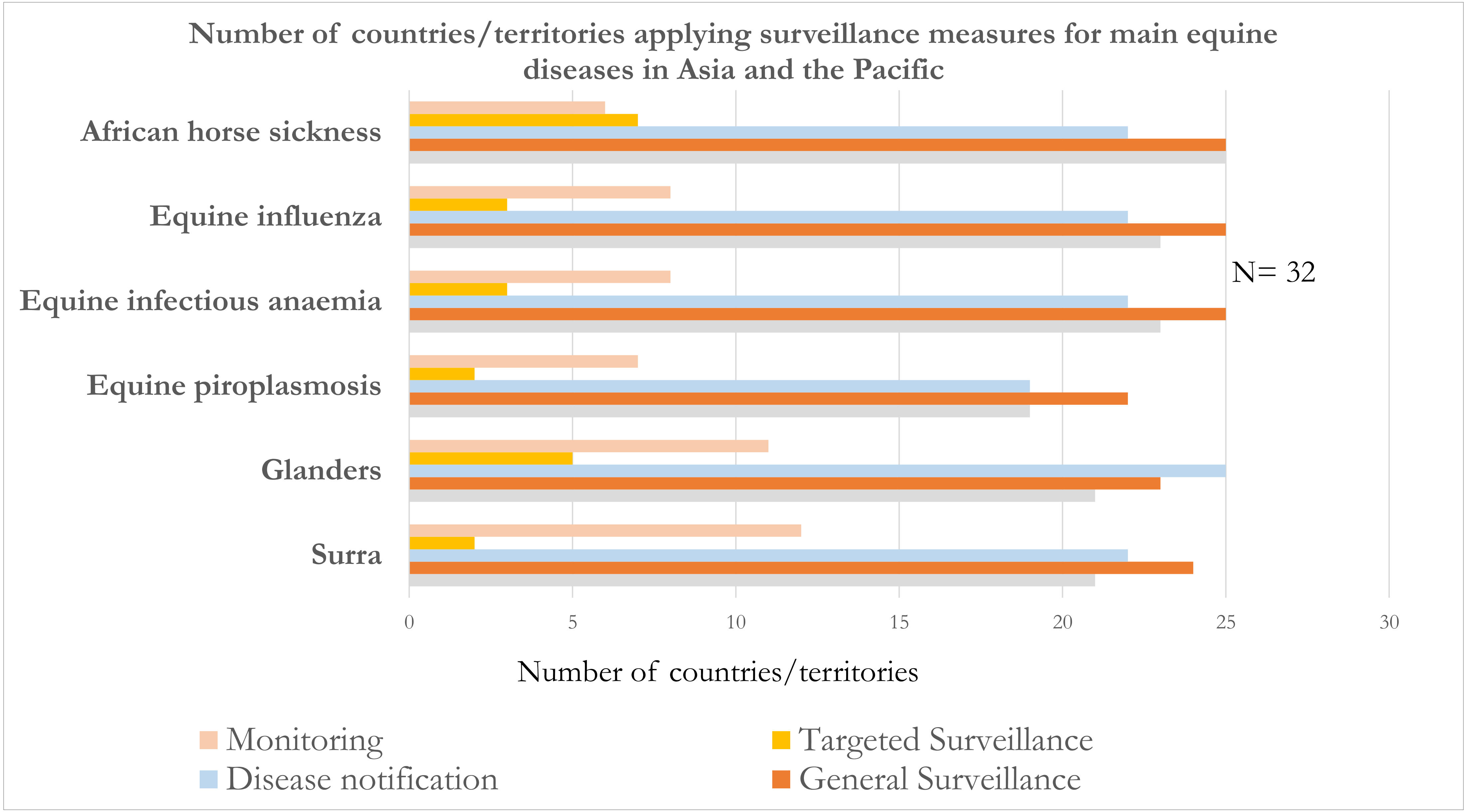


Main equine diseases in Asia and the Pacific subject to legal notification

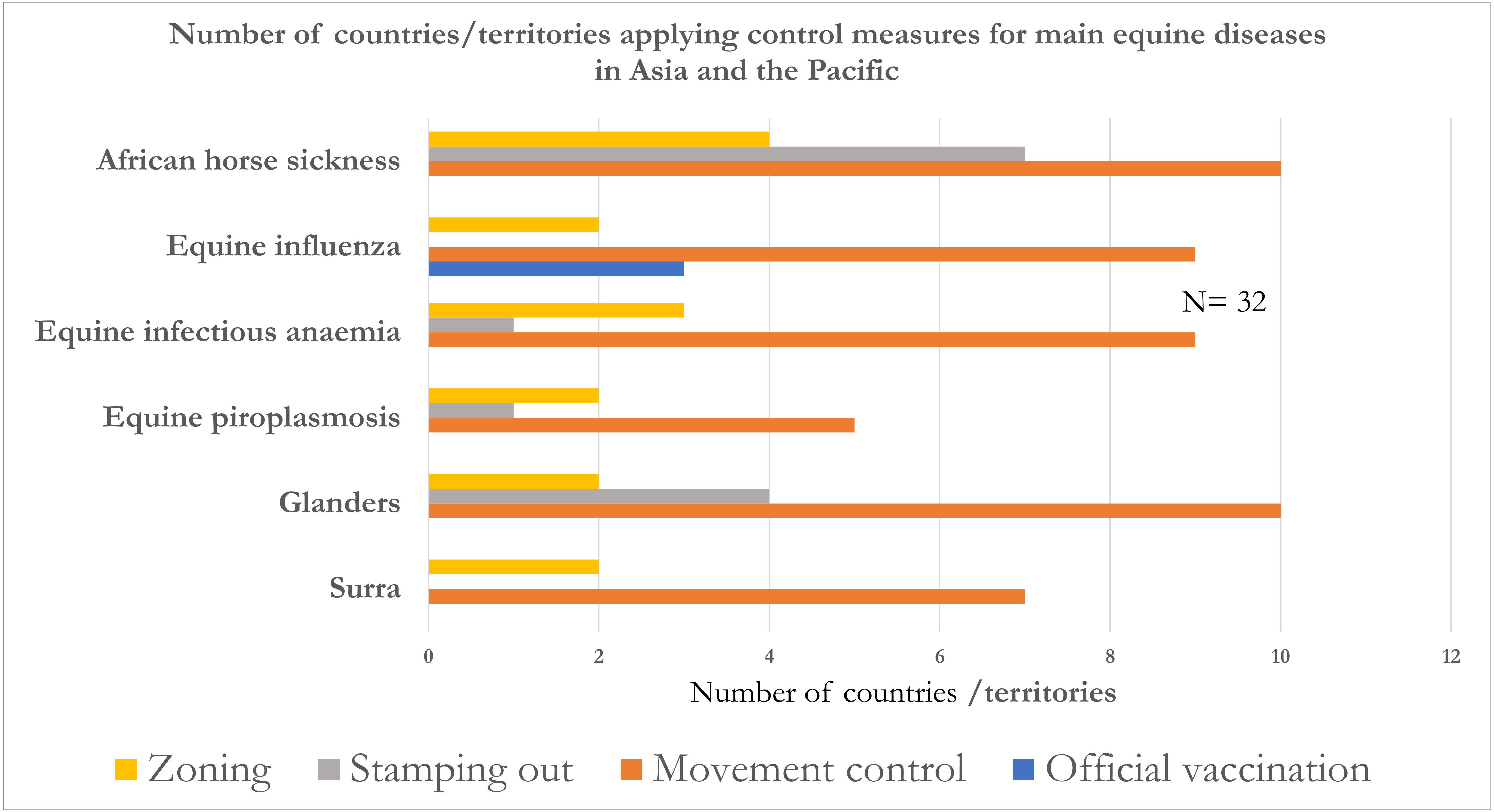
Number of diseases (out of the 6 listed below) subject to a national legal obligation to report any suspected or confirmed case to the relevant Authorities : AHS, EI, EIA, Glanders, Surra and equine piroplasmosis

Data source : WAHIS, September, 2024

Surveillance measures in the region (2019-2023)



Control measures in the region (2019-2023)

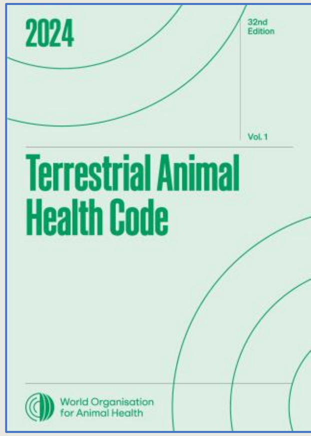


Conclusions

- Effective surveillance strategies for equine diseases require coordinated efforts, adaptable methods, **strong laboratory capabilities** and robust data management.
- Collaboration between Vet. Authorities, and **sharing of surveillance data**.
- **Timely reporting** of suspected cases- **follow up** on suspicions or positive reactors
- **Continuous monitoring and timely response** are key to controlling and preventing the spread of infections.

Useful Code Chapters on Surveillance and trade:

Chapter 1.4.	Animal health surveillance
Chapter 1.5.	Surveillance for arthropod vectors of animal diseases
Chapter 4.1.	Introduction to recommendations for the prevention and control of transmissible animal diseases
Chapter 2.1.	Import risk analysis



2024

32nd Edition

Vol. 1

Terrestrial Animal Health Code

World Organisation for Animal Health

Questions?

Thank you!

12, rue de Prony, 75017 Paris, France
T. +33 (0)1 44 15 19 49
F. +33 (0)1 42 67 09 87

woah@woah.int
www.woah.org

Facebook
Twitter
Instagram
LinkedIn
YouTube
Flickr

World
Organisation
for Animal
Health

Organisation
mondiale
de la santé
animale

Organización
Mundial
de Sanidad
Animal

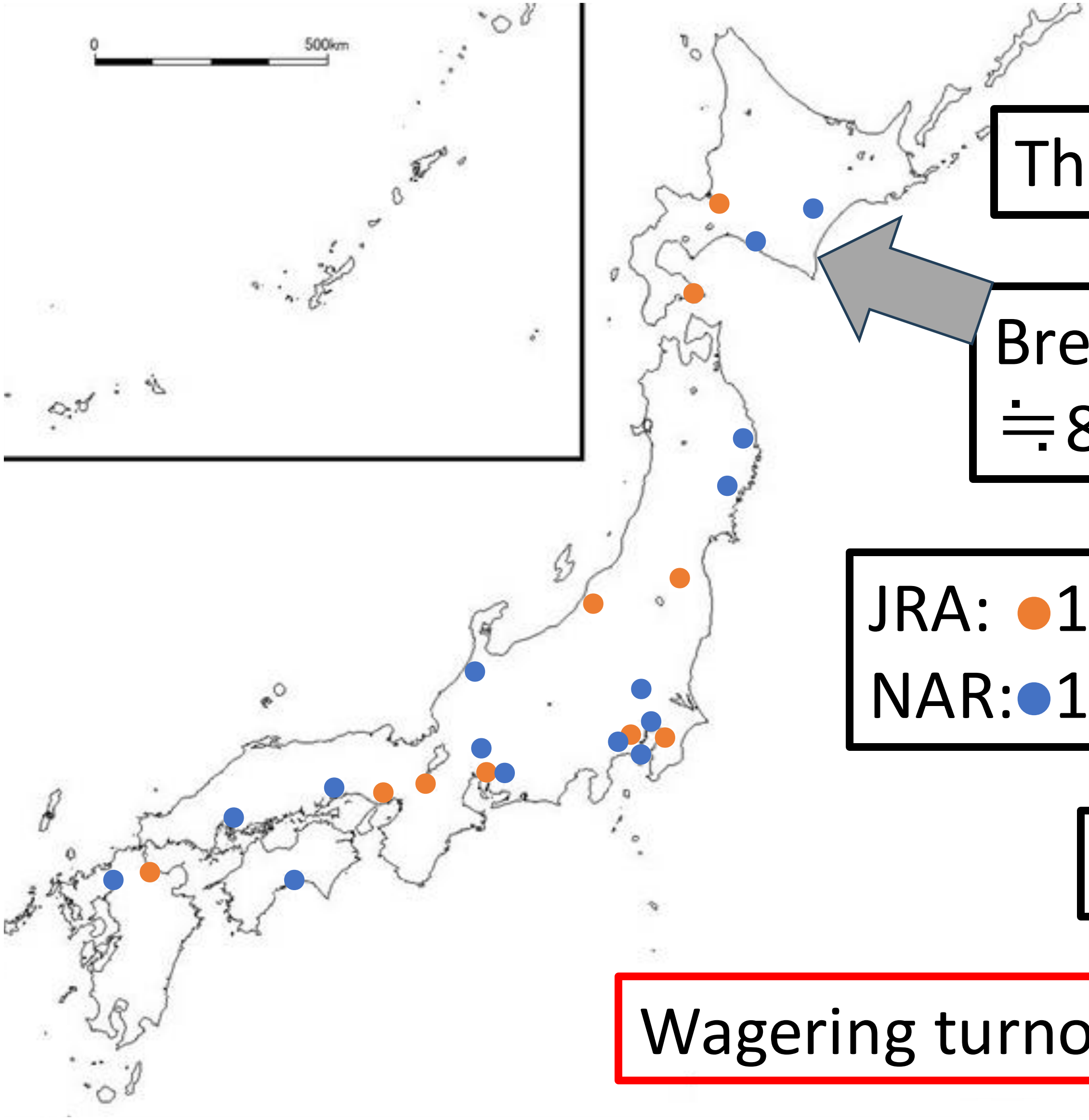
WOAH Regional Workshop on Laboratory Capacity to Diagnose Equine Diseases
Tokyo, Japan, Sep 17-18 2024

Overview of surveillance programs of equine infectious diseases in Japan

Hiroshi Bannai, DVM, PhD

Equine Research Institute, Japan Racing Association

Racing industry in Japan



Thoroughbreds: 45433

Breeding in Hokkaido:
≒8000 foals/year

JRA: ●10 RCs, 8700 horses
NAR:●15 RCs, 12300 horses

≒19000 races/year

Wagering turnover: 34 billion USD

Equine disease surveillance in Japan

Passive surveillance (notifiable diseases)

-Case detection

-Reported to local government

Active surveillance (not limited to ND)

-Case detection

-Investigation of endemic situation

-Confirmation of free status

-Operated by industrial organization or PPP

Organizing body of active surveillance

-Japan Racing Association →Racing authority

-Japan Bloodhorse Breeders’ Association →Breeding authority

-Japanese Council on Disease Prevention of Bloodhorse

-Hidaka District Council on Livestock Hygiene and Disease Prevention

→Public-private partnership

MAFF, AQS,
National Vet Lab.

Livestock Hygiene
Centers (local gov.)

JRA,
NAR

and more organizations…

Notifiable diseases in horses in Japan

Viral encephalitis
(WN, JE, VEE, WEE, EEE)
Rabies
Vesicular stomatitis
Anthrax
Equine piroplasmosis
Glanders
Equine infectious anemia
African horse sickness
Equine rhinopneumonitis
Equine influenza
Equine viral arteritis
Melioidosis
Tetanus
Surra
Dourine
Nipa virus infection
Hendra virus infection
Horse pox
Tularemia
Contagious equine metritis
Equine paratyphoid
Pseudofarcy

Active surveillance targeting…

Viral diseases:

- Equine rhinopneumonitis (respiratory, abortion)
- Getah virus infection
- Rotavirus infection
- Equine influenza
- Equine infectious anemia
- Equine viral arteritis

-case detection
-endemic situation
-genotyping

Bacterial diseases:

- Equine proliferative enteropathy
- *Rhodococcus equi* infection
- Salmonellosis
- Contagious equine metritis

Active surveillance targeting…

Viral diseases:

- Equine rhinopneumonitis (respiratory, abortion)
- Getah virus infection
- Rotavirus infection
- Equine influenza
- Equine infectious anemia
- Equine viral arteritis

-confirmation of free status

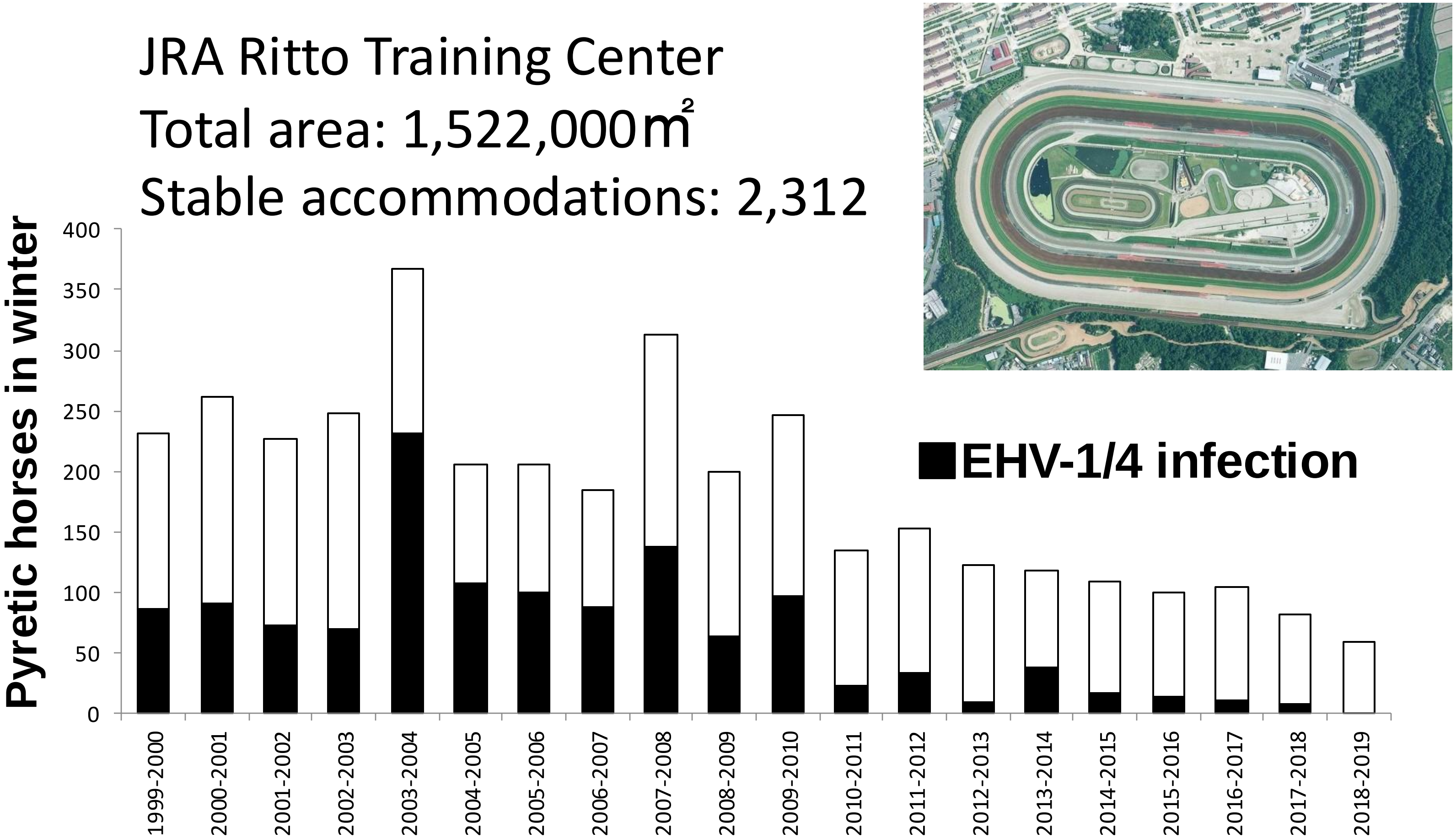
Bacterial diseases:

- Equine proliferative enteropathy
- *Rhodococcus equi* infection
- Salmonellosis
- Contagious equine metritis

Active racehorses

Equine rhinopneumonitis (respiratory form)

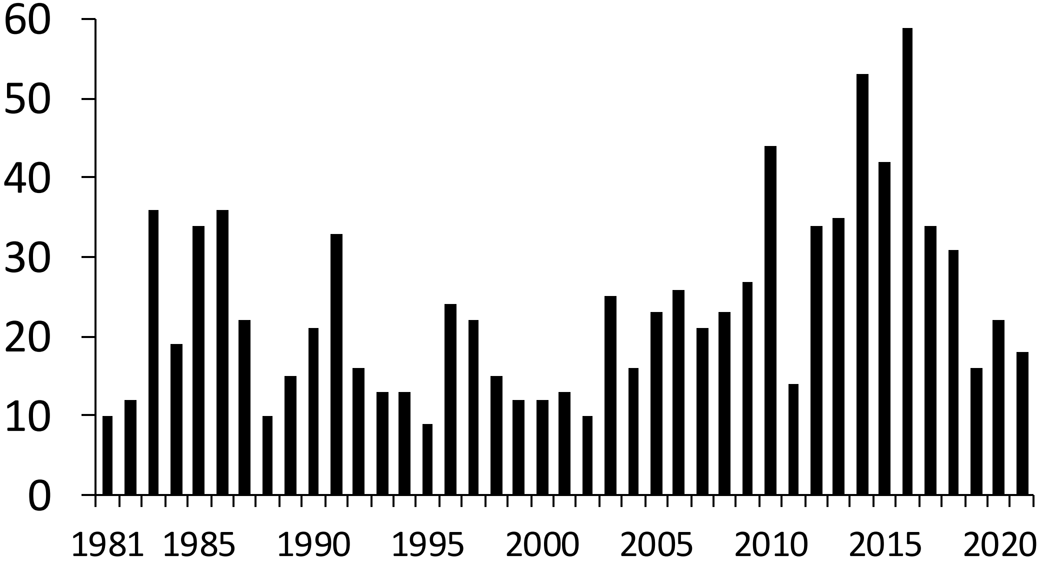
JRA Ritto Training Center
Total area: 1,522,000㎡
Stable accommodations: 2,312



-evaluation of vaccine efficacy for a better control

Breeding mares

Equine rhinopneumonitis (abortion)



-Hidaka district, Hokkaido
-case detection
-genotyping
(ORF30 polymorphism)

Number of isolates from 2010-2021

Normal strain (A2254)	**179 (97.8%)**
Neuropathogenic strain (G2254)	**4 (2.2%)**

-isolation of G2254 virus have been rare in Japan

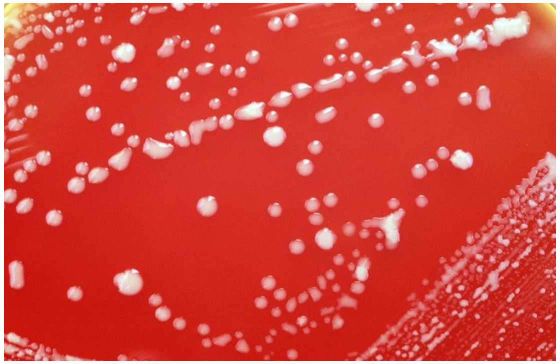
Foals

Rhodococcus equi infection
(case detection, antimicrobial resistance)

-prevalent in foals (1-3 month old）

-incursion of macrolide and rifampicin resistant strain in the USA

→detected for the first time in Japan in 2022



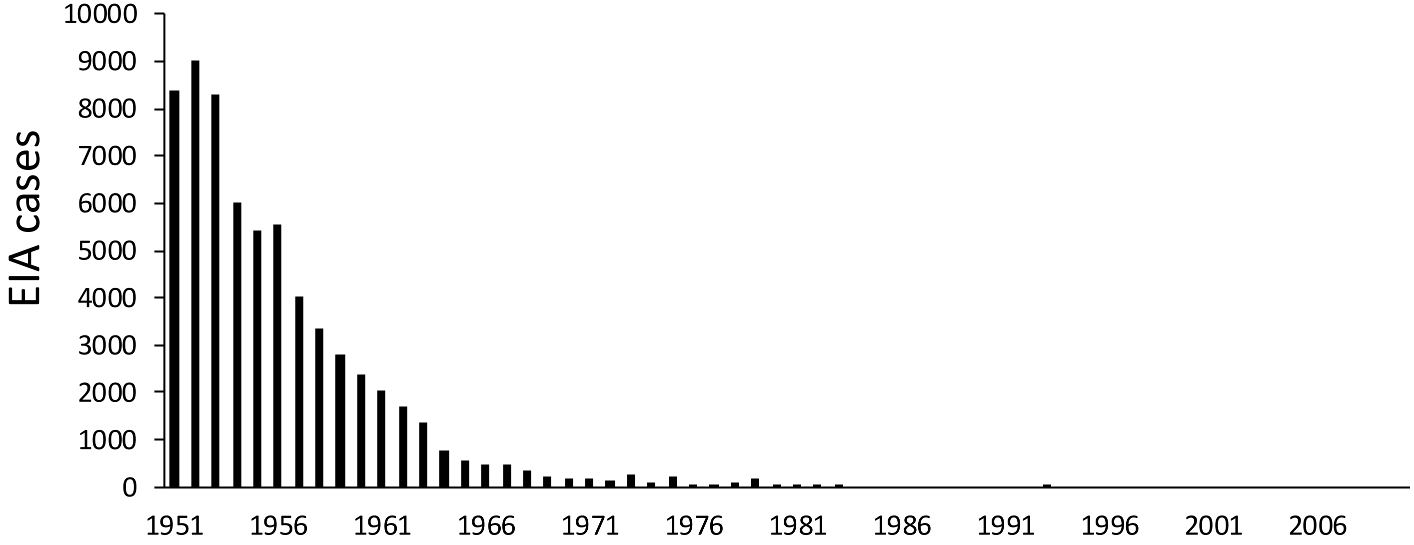
Bacterial detection: culture, PCR

Area: Hidaka district, Hokkaido

-tracheal wash

>150 cases/year including 20 deaths

Equine infectious anemia
(confirmation of free-status)



～1997	All horses tested annually
1998～	All horses tested once in 5 years
2015-17	EIA surveillance project 70133 samples (racing-, riding-, wild horses)

Self-declaration of EIA-free status

(Japanese Council of Equine Health)

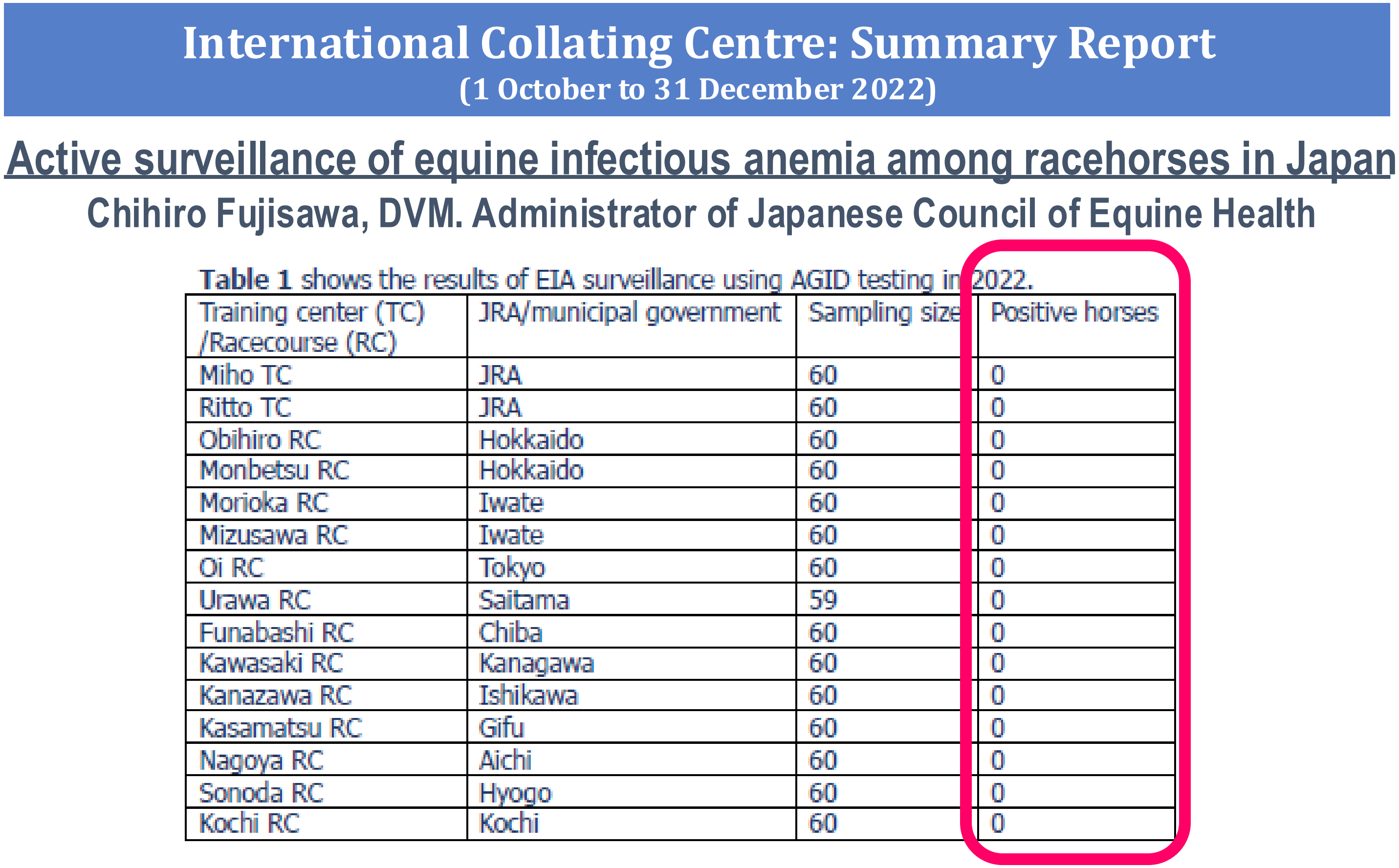
2020-present, annual EIA surveillance (random sampling)

Confirmation of free status

(less than 5% prevalence with 95% confidence level)

2 JRA training centers

13 NAR racecourses (≥58 horses each)



International Collating Centre: Summary Report

(1 October to 31 December 2022)

Active surveillance of equine infectious anemia among racehorses in Japan

Chihiro Fujisawa, DVM. Administrator of Japanese Council of Equine Health

Table 1 shows the results of EIA surveillance using AGID testing in 2022.

Training center (TC) /Racecourse (RC)	JRA/municipal government	Sampling size	Positive horses
Miho TC	JRA	60	0
Ritto TC	JRA	60	0
Obihiro RC	Hokkaido	60	0
Monbetsu RC	Hokkaido	60	0
Morioka RC	Iwate	60	0
Mizusawa RC	Iwate	60	0
Oi RC	Tokyo	60	0
Urawa RC	Saitama	59	0
Funabashi RC	Chiba	60	0
Kawasaki RC	Kanagawa	60	0
Kanazawa RC	Ishikawa	60	0
Kasamatsu RC	Gifu	60	0
Nagoya RC	Aichi	60	0
Sonoda RC	Hyogo	60	0
Kochi RC	Kochi	60	0

International Collating Centre

To keep the ball rolling

Purpose

Funding

Collaboration

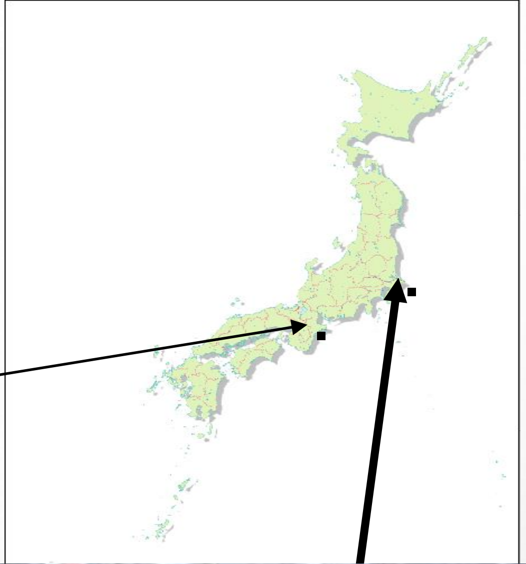
Capacity

Application

SURVEILLANCE OF EI FOR SELF-DECLARATION OF FREEDOM

TAKASHI YAMANAKA, JRA

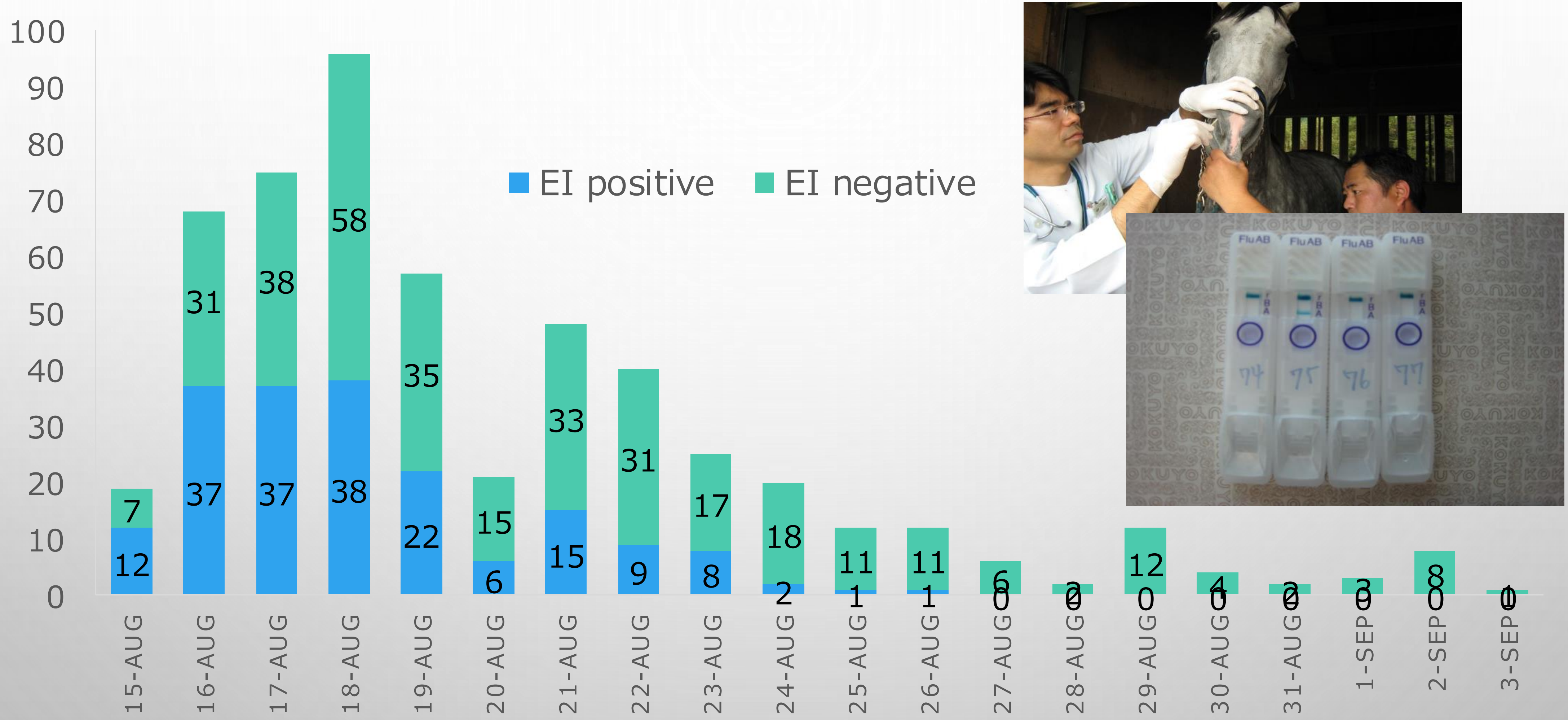
Ritto training center (Shiga)



Each training center kept about two thousands horses each.

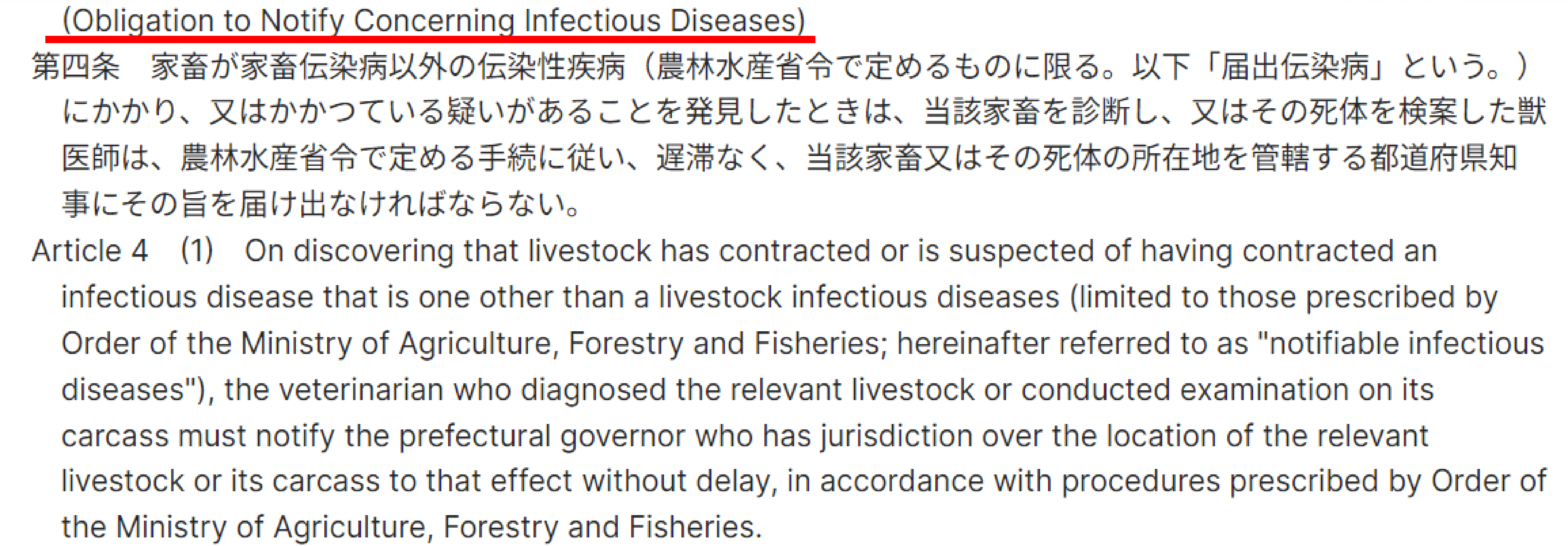
Miho training center (Ibaraki)

Daily new febrile cases in 2007 in JRA



Yamanaka et al. J. Vet. Med. Sci. 2008

NOTIFICATION ON LEGAL BASIS



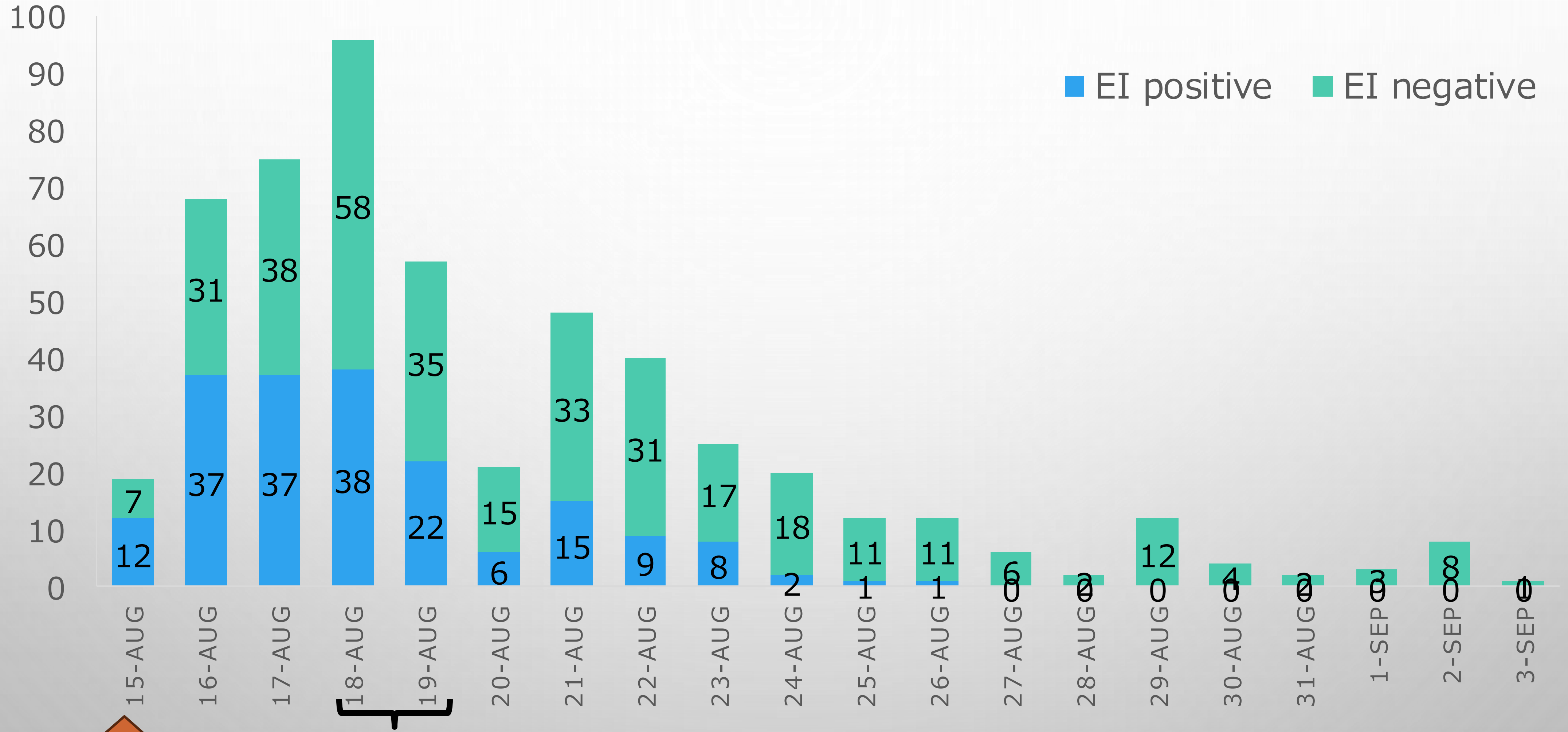
(Obligation to Notify Concerning Infectious Diseases)

第四条　家畜が家畜伝染病以外の伝染性疾病（農林水産省令で定めるものに限る。以下「届出伝染病」という。）にかかり、又はかかつている疑いがあることを発見したときは、当該家畜を診断し、又はその死体を検案した獣医師は、農林水産省令で定める手続に従い、遅滞なく、当該家畜又はその死体の所在地を管轄する都道府県知事にその旨を届け出なければならない。

Article 4　(1)　On discovering that livestock has contracted or is suspected of having contracted an infectious disease that is one other than a livestock infectious diseases (limited to those prescribed by Order of the Ministry of Agriculture, Forestry and Fisheries; hereinafter referred to as "notifiable infectious diseases"), the veterinarian who diagnosed the relevant livestock or conducted examination on its carcass must notify the prefectural governor who has jurisdiction over the location of the relevant livestock or its carcass to that effect without delay, in accordance with procedures prescribed by Order of the Ministry of Agriculture, Forestry and Fisheries.

Act on Domestic Animal Infectious Diseases Control

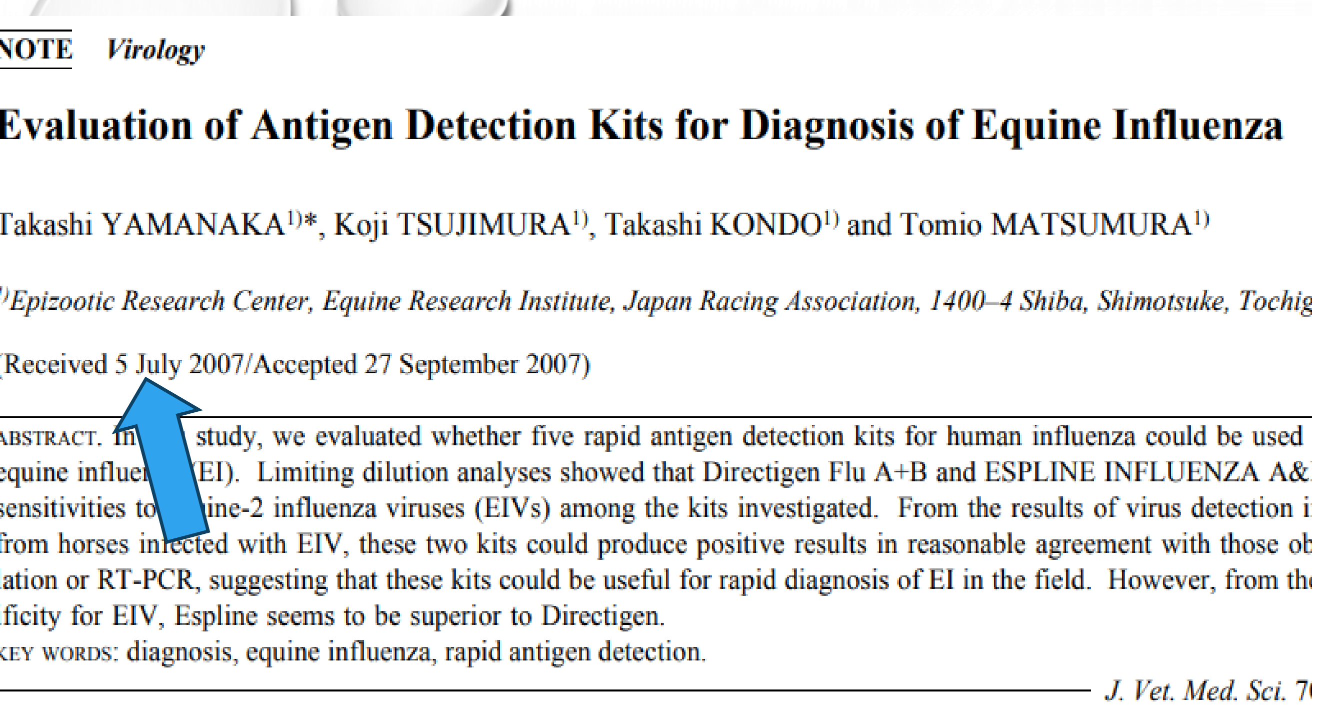
Daily new febrile cases in 2007 in JRA



Notification,
Movement banned

Races cancelled

Yamanaka et al. J. Vet. Med. Sci. 2008



NOTE *Virology*

Evaluation of Antigen Detection Kits for Diagnosis of Equine Influenza

Takashi YAMANAKA[1)]*, Koji TSUJIMURA[1)], Takashi KONDO[1)] and Tomio MATSUMURA[1)]

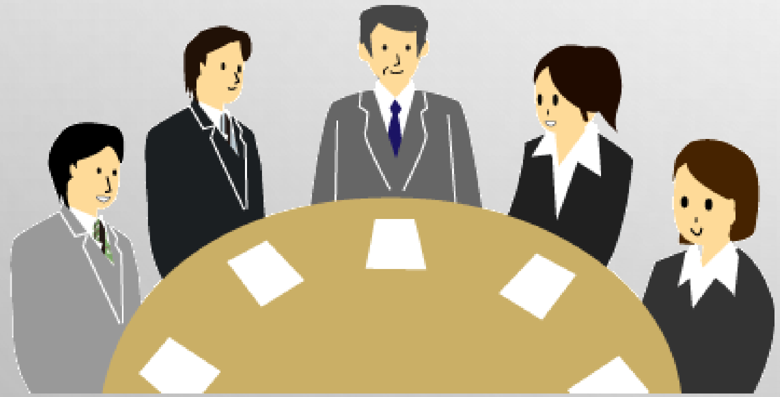
[1)]*Epizootic Research Center, Equine Research Institute, Japan Racing Association, 1400–4 Shiba, Shimotsuke, Tochig*

(Received 5 July 2007/Accepted 27 September 2007)

ABSTRACT. In study, we evaluated whether five rapid antigen detection kits for human influenza could be used equine influe (EI). Limiting dilution analyses showed that Directigen Flu A+B and ESPLINE INFLUENZA A& sensitivities to ine-2 influenza viruses (EIVs) among the kits investigated. From the results of virus detection i from horses infected with EIV, these two kits could produce positive results in reasonable agreement with those ob ation or RT-PCR, suggesting that these kits could be useful for rapid diagnosis of EI in the field. However, from th ficity for EIV, Espline seems to be superior to Directigen.

KEY WORDS: diagnosis, equine influenza, rapid antigen detection.

J. Vet. Med. Sci. 7



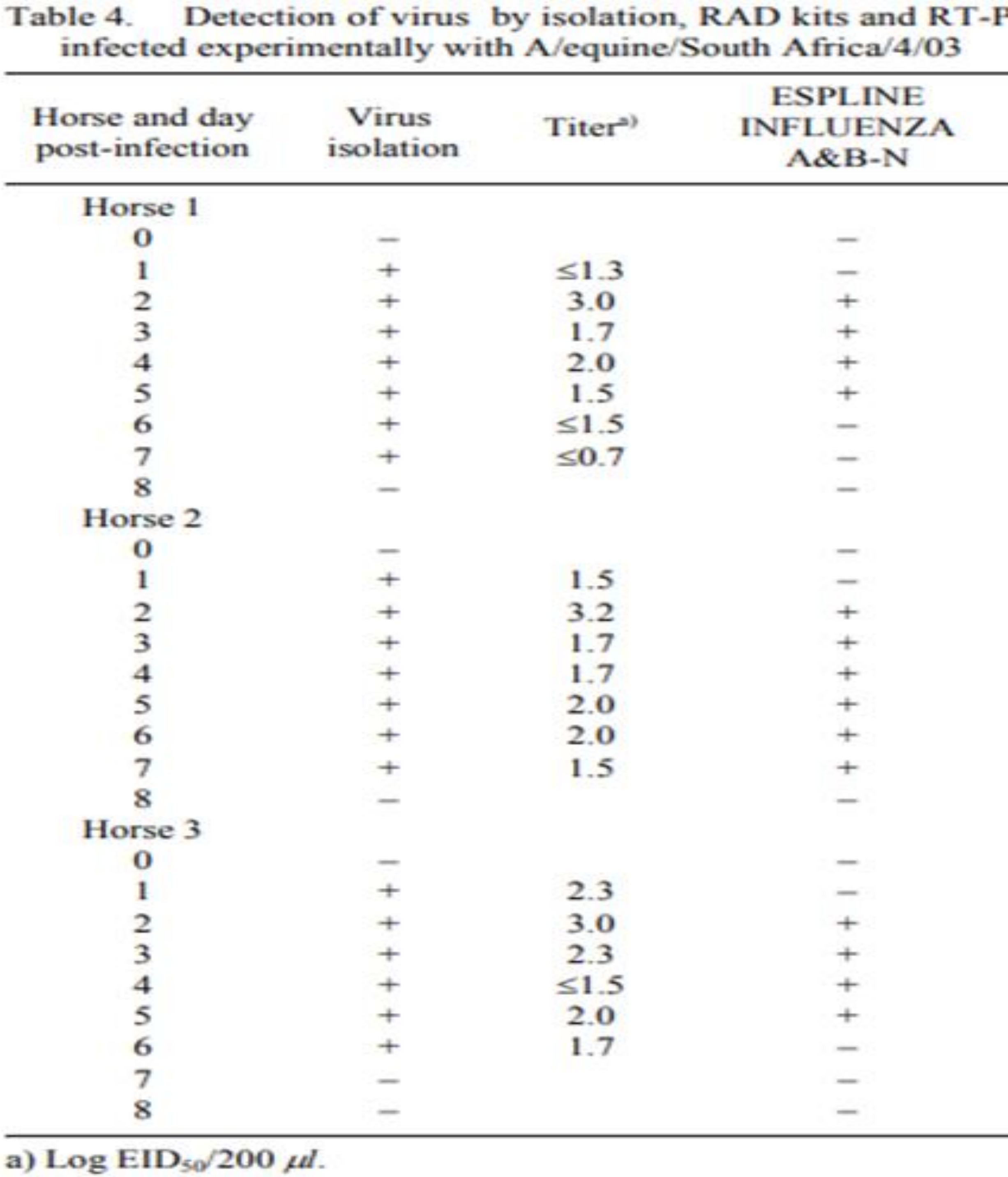
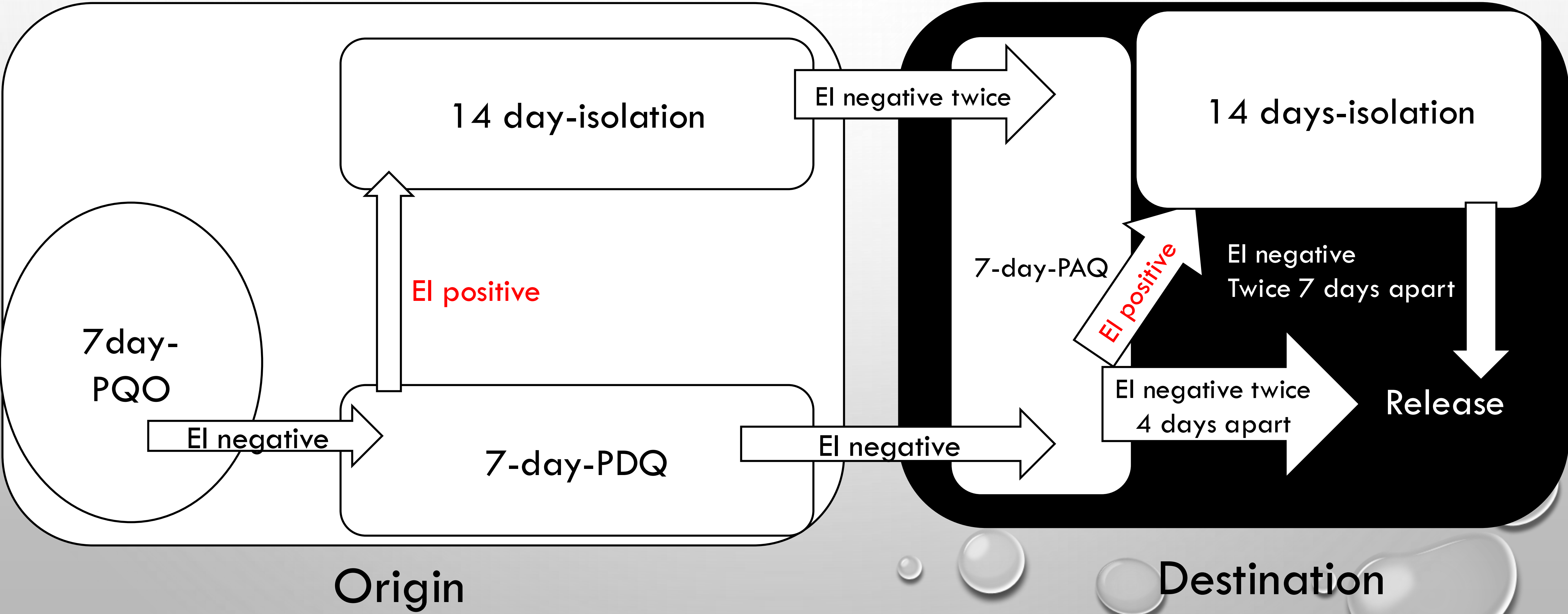


Table 4. Detection of virus by isolation, RAD kits and RT-P infected experimentally with A/equine/South Africa/4/03

Horse and day post-infection	Virus isolation	Titer[a)]	ESPLINE INFLUENZA A&B-N
Horse 1			
0	–		–
1	+	≤1.3	–
2	+	3.0	+
3	+	1.7	+
4	+	2.0	+
5	+	1.5	+
6	+	≤1.5	–
7	+	≤0.7	–
8	–		–
Horse 2			
0	–		–
1	+	1.5	–
2	+	3.2	+
3	+	1.7	+
4	+	1.7	+
5	+	2.0	+
6	+	2.0	+
7	+	1.5	+
8	–		–
Horse 3			
0	–		–
1	+	2.3	–
2	+	3.0	+
3	+	2.3	+
4	+	≤1.5	+
5	+	2.0	+
6	+	1.7	–
7	–		–
8	–		–

a) Log EID_{50}/200 μl.

GUIDELINES FOR CONTROL MEASURES AGAINST EQUINE INFLUENZA (STIPULATED ON 3RD SEP)



7day-PQO

EI negative

7-day-PDQ

EI positive

14 day-isolation

EI negative twice

EI negative

7-day-PAQ

EI positive

14 days-isolation

EI negative
Twice 7 days apart

EI negative twice
4 days apart

Release

Origin

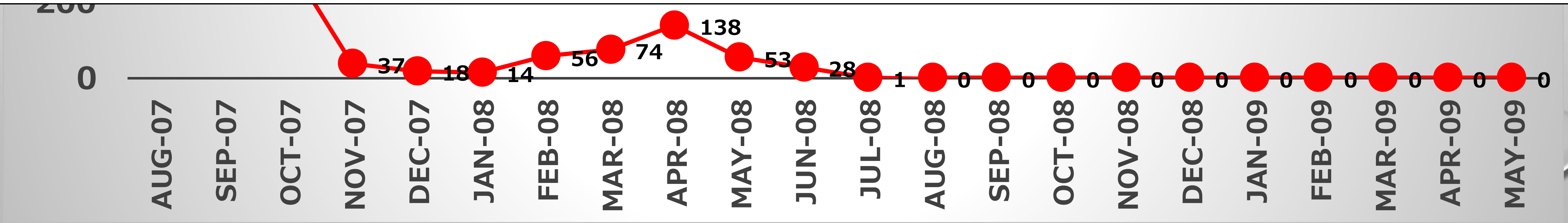
Destination

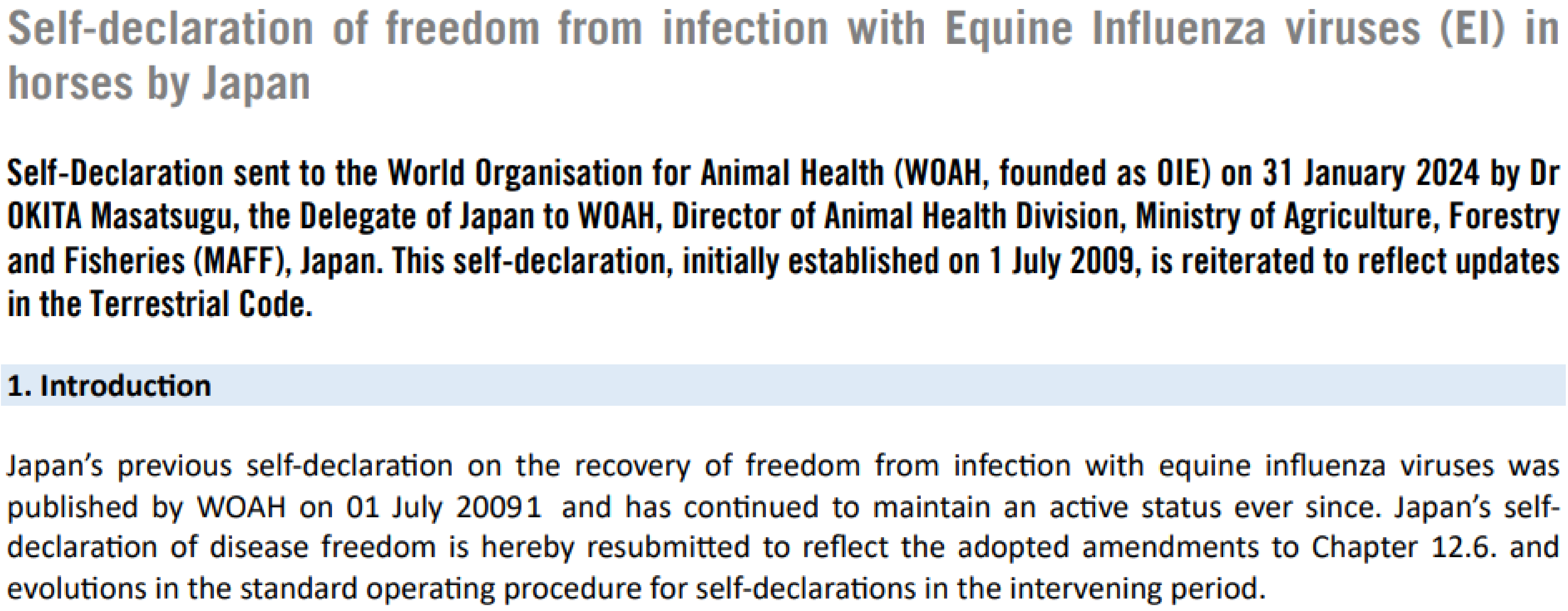
Monthly change of new EI cases

Article 12.6.5

Recovery of free status

If a case of infection with EIV occurs in a previously free country, zone or compartment, free status can be regained 12 months after the last case, provided that outbreaks were managed in accordance with Chapter 4.19. and that surveillance, in accordance with Article 12.6.4., has been carried out during that 12-month period, with negative results.





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.

1. Introduction

Japan's previous self-declaration on the recovery of freedom from infection with equine influenza viruses was published by WOAH on 01 July 20091 and has continued to maintain an active status ever since. Japan's self-declaration of disease freedom is hereby resubmitted to reflect the adopted amendments to Chapter 12.6. and evolutions in the standard operating procedure for self-declarations in the intervening period.

SUMMARY

- EQUINE H3N8 IS NOT LONG PERSISTENT IN HORSES
- PUBLIC (AUTHORITY)-PRIVATE (INDUSTRY) PARTNERSHIP PLAYED A KEY ROLE
- MOVEMENT PROTOCOL BASED ON THE RAPID ANTIGEN DETECTION TESTS WORKED

Public-Private-Academic partnership is a key role!!

Response to emergence of *Salmonella* Abortusequi Infection in Asia
---surveillance approaches and diagnostic tests

Xiaojun Wang

WOAH reference laboratory on equine infectious aneamia
Harbin Veterinary Research Institute
The Chinese Academy of Agricultural Sciences

中国农业科学院哈尔滨兽医研究所

Harbin Veterinary Research institute, CAAS

SPF Animals Facilities
(8,000 m²)

R&D Department
(8,000 m²)

Vaccine Production
Workshops
(35,000 m²)

Scientific Complex
(46,000 m²)

Supporting Facilities

Animal Facilities
(13,000 m²)

Biology Safety Laboratory
(20,000 m²)

Visiting Center
(18,000 m²)

Veterinary Graduate School (20,000 m²)

马传染病与慢病毒病研究团队
Equine Disease Group in HVRI

国家马传染性贫血
参考实验室
National Equine Infectious Anemia Reference Laboratory

2018-

国家马鼻疽
参考实验室
National Glanders Reference Laboratory

2018-

World
Organisation
for Animal
Health

马传染性贫血参考实验室
Reference Laboratory
for
Equine Infectious Anemia

2011-

中哈农业科学联合实验室
SINO-KAZAKHSTAN JOINT LABORATORY
FOR AGRICULTURAL SCIENCES
Ауыл шаруашылығы ғылымдарының Қазақст
ан-Қытай бірлескен зертханасы

2019-

- 70+ years research on equine infectious diseases
- State Key Laboratory for Animal Disease Control and Prevention
- National Reference Laboratory for Glanders
- National Reference Laboratory for Equine Infectious Anemia
- WOAH Reference Laboratory for Equine Infectious Anemia

Research Interests

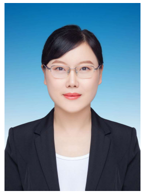
1. The mechanism of virus-host interaction
   Lentiviruses, Influenza virus
2. Epidemiology, pathogenesis, immunology of equine infectious diseases
   EIA, EHV, EI, Glanders, Strangles, Equine paratyphoid…
3. Diagnostic methods and vaccine development
   EI, EHV, Strangles, Equine paratyphoid

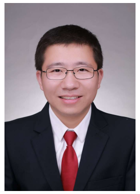

王晓钧
Xiaojun WANG


林跃智
Yuezhi LIN


郭巍
Wei GUO


王雪峰
Xuefeng WANG


胡哲
Zhe HU


杜承
Cheng DU


刘荻萩
Diqiu LIU


孙留克
Liuke SUN


任会玲
Huiling Ren


于萌萌
Mengmeng YU


李继伟
Jiwei Li


郭兴
Guo Xing

Professors

Associate Professors

Post doctoral

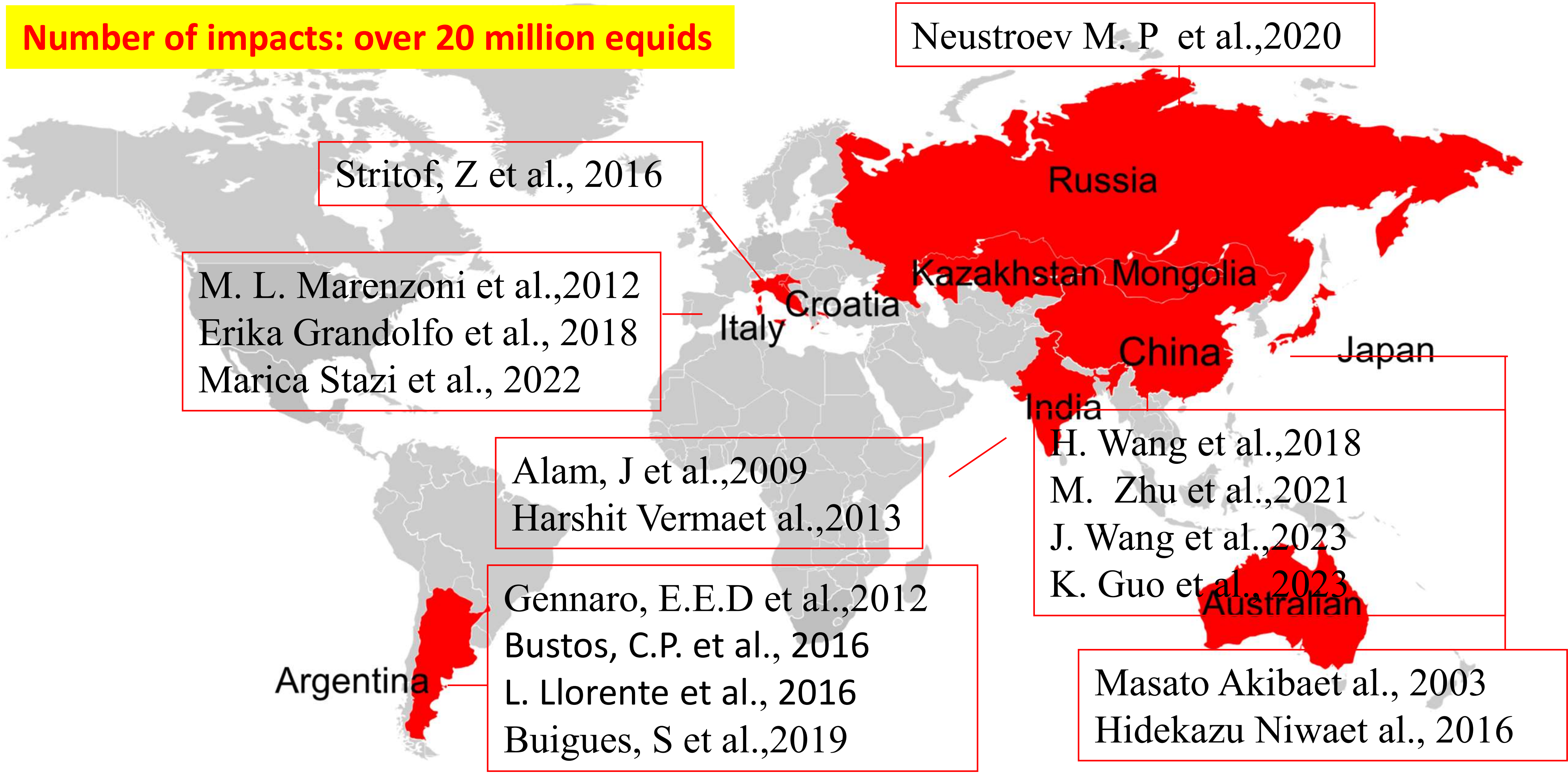
Equine paratyphoid
(*Salmonella* Abortusequi infection)

Salmonella Abortusequi (*S.* Abortusequi) is the causative agent of equine abortus salmonellosis (**Equine paratyphoid)**,

- abortion in the late stages of pregnant equids
- death in newborn foals
- arthritis in young foals.

Prevalence of equine paratyphoid in countries in the last decade

Number of impacts: over 20 million equids



Neustroev M. P et al.,2020

Stritof, Z et al., 2016

M. L. Marenzoni et al.,2012
Erika Grandolfo et al., 2018
Marica Stazi et al., 2022

Alam, J et al.,2009
Harshit Vermaet al.,2013

H. Wang et al.,2018
M. Zhu et al.,2021
J. Wang et al.,2023
K. Guo et al., 2023

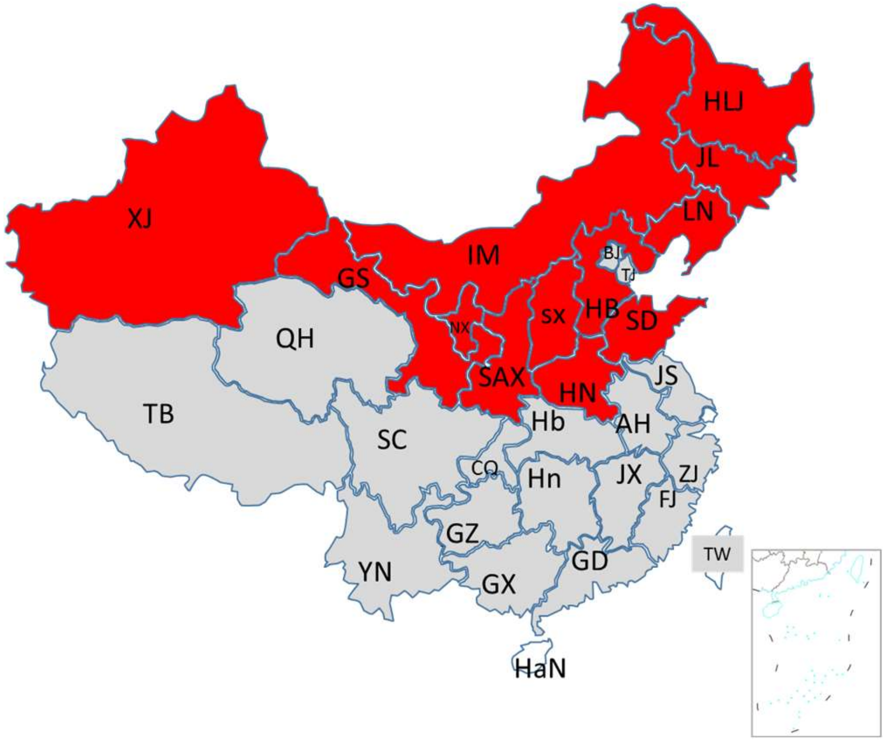
Gennaro, E.E.D et al.,2012
Bustos, C.P. et al., 2016
L. Llorente et al., 2016
Buigues, S et al.,2019

Masato Akibaet al., 2003
Hidekazu Niwaet al., 2016

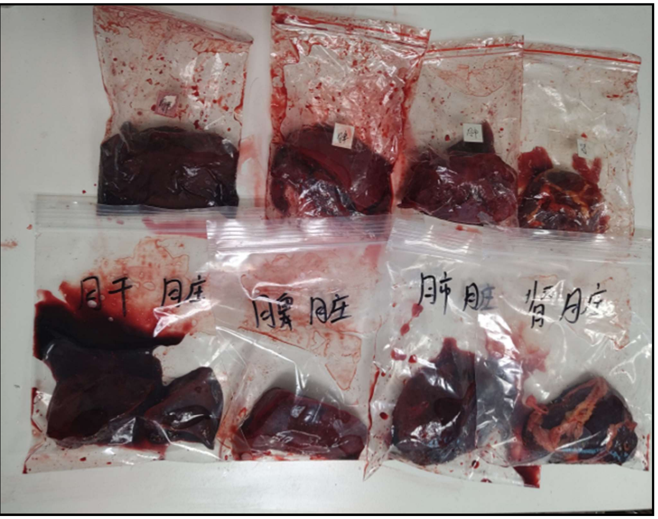
Domestic outbreak confirmed by our lab (Partial statistics)

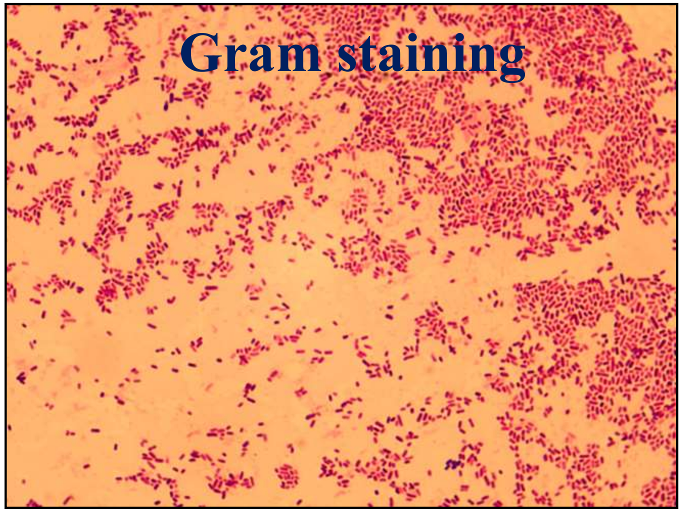
Species	Year	Area	No. of pregnancies	No. of abortions	Abortion rates
Horse	2014	IM	120	80	66.7%
	2017		48	40	83.3%
	2018		1051	751	71.5%
	2018		64	64	100.0%
	2024		150	75	50%
	2024		200	104	52%
	2024	HLJ	300	90	30%
	2024	LN	1000		20%
Donkey	2017	SD	345	93	27.0%
	2018		322	69	21.4%
	2019		300	60	20.0%
	2019		40	20	50.0%
	2020	HB	619	417	65.7%
	2021		242	102	36.3%
	2021	IM	2500	733	29.3%
	2023	XJ	460	150	32.6%
	2024	JL	50	15	30%
	2024	HLJ	2000		20%

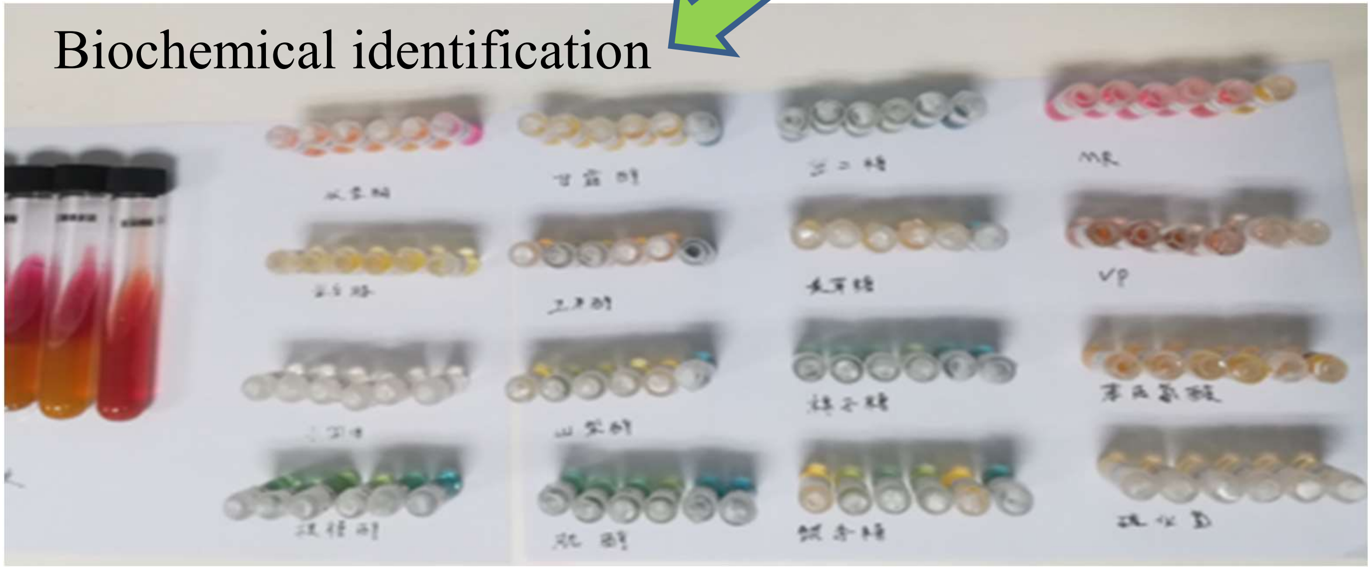
Billions of dollars losses over the past decade



Isolation and identification of pathogens: *Salmonella* Abortusequi






Biochemical identification

Serotype identification


O4/O12 H-e,n,x

Salmonella Abortusequi.

Research on vaccine

Research progress of inactivated vaccines

Year	Country/ person	efficacy	Exist problems
1950s	Mohler / Traum	Low efficacy	
1950s	Mgood / Dimock	Effective（The details are Unknown）	Multiple injections of each vaccination（Twice a year, three injections each time）
1962	China	Effective（The details are Unknown）	Not promoted and applied
1966	China		Large immune dose, frequent, and heave side effects.
2015	China	Low efficacy	
2020	Russia	Low efficacy	

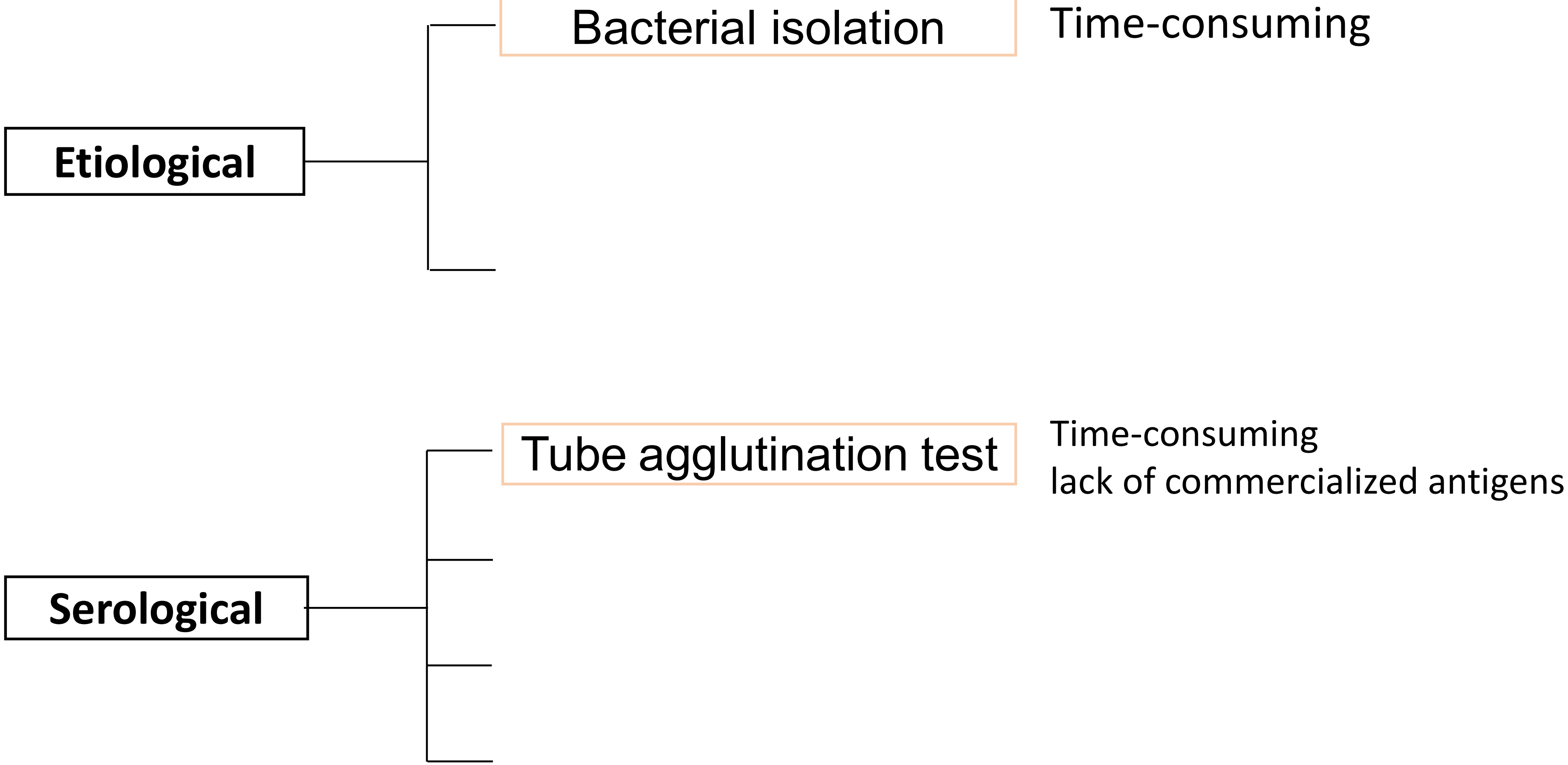
Attanuated vaccines

Year	Country	Mode of attenuation	Characteristics of strains	Immune dose	Current situation and existing problems
2013	India	Gene deletion	Not completely attenuated	420 billion	Not commercialized
1970s	China	Passage in non-susceptible (C39)	effective	5 billion	Not available
1970s	China	Chemical (C355)	effective	10 billion	Available since 2019

Challenging for disease prevention and control

- Persistent infection with long latency period
- Low efficacy of antibiotics treatment
- No accurate method for diagnosis and surveillance

Research on diagnostics



Etiological

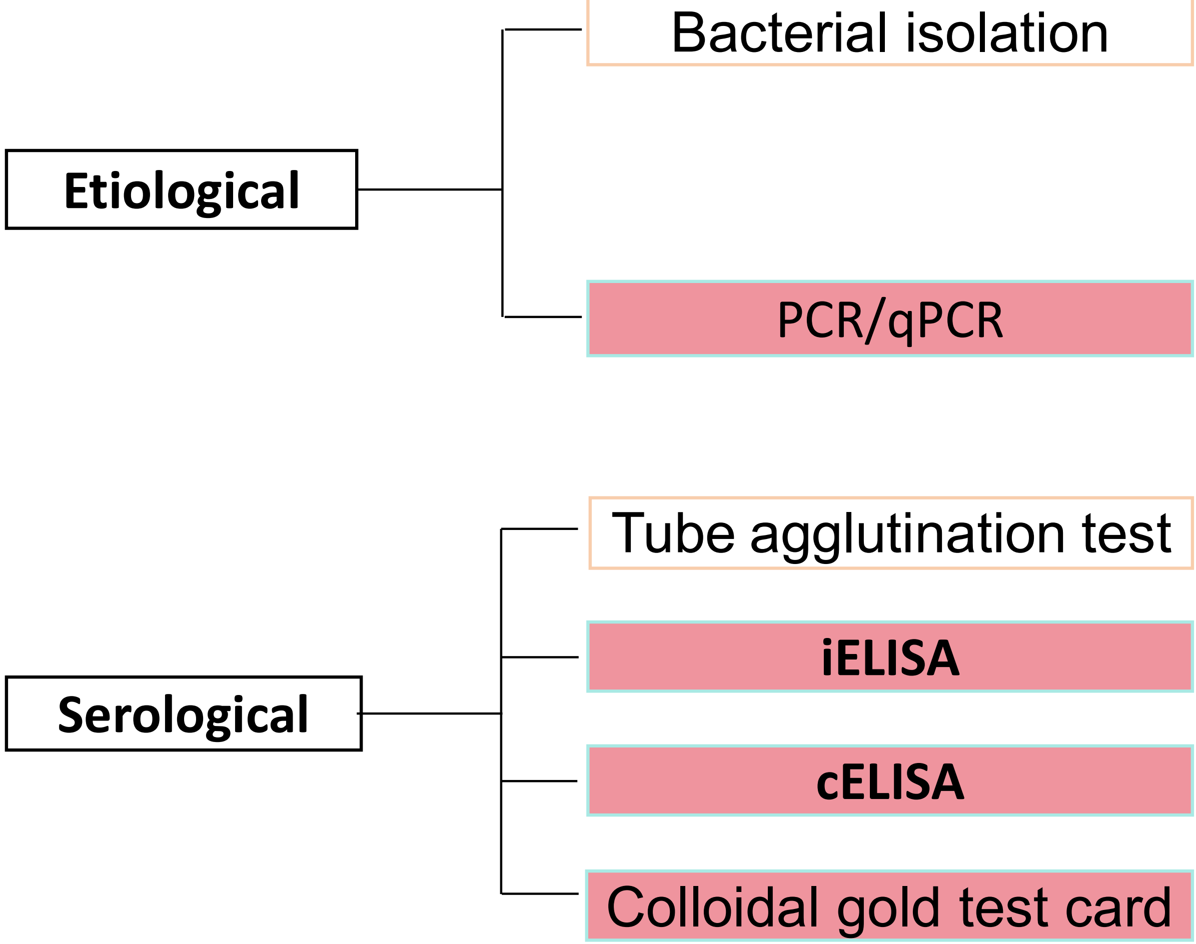
- Bacterial isolation — Time-consuming

Serological

- Tube agglutination test — Time-consuming
lack of commercialized antigens

☐ Traditional detection methods used globally

Research on diagnostics

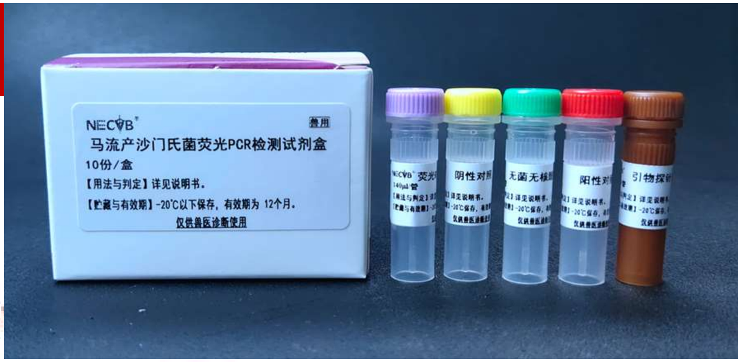


- **Etiological**
  - Bacterial isolation — Time-consuming
  - PCR/qPCR — *Wang et al., journal of clinical microbiolog*
    2023
- **Serological**
  - Tube agglutination test — Time-consuming
    lack of commercialized antigens
  - **iELISA** — *Guo et al., Transboundary and emerging diseases, 2023*
  - **cELISA** — *Guo et al., journal of clinical microbiology, 2023*
  - Colloidal gold test card — *Manuscript under review*

☐ Traditional detection methods used globally

🟥 Newly developed methods by our lab

1.*Salmonella* Abortusequi real time PCR detection kit



AMERICAN SOCIETY FOR MICROBIOLOGY
Journal of Clinical Microbiology®

CLINICAL VETERINAR

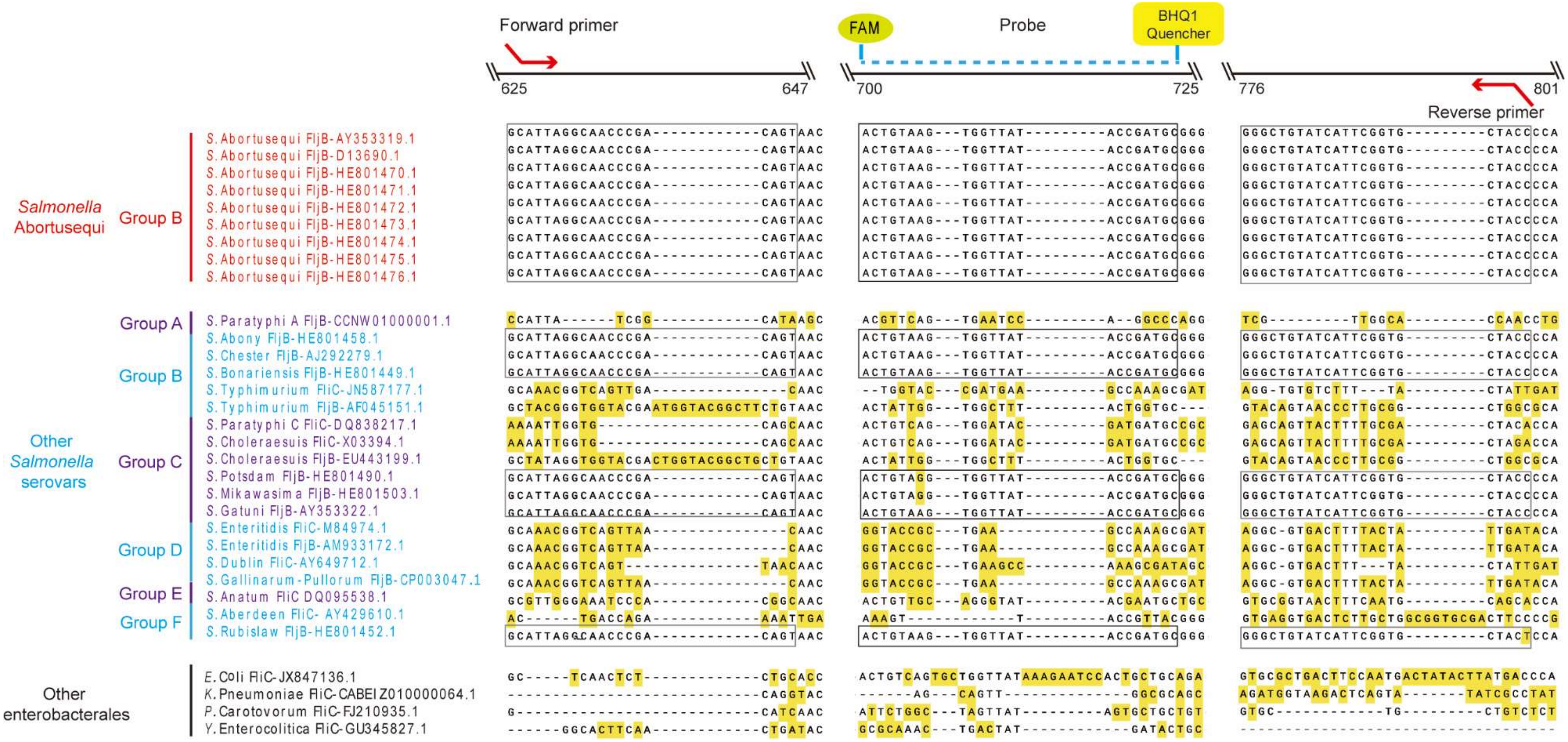
Development and Application of Real-Time PCR Assay for Detection of *Salmonella* Abortusequi

Jinhui Wang,[a] Kui Guo,[a] Shuaijie Li,[a] Diqiu Liu,[a] Xiaoyu Chu,[a] Yaoxin Wang,[a] Wei Guo,[a] Cheng Du,[a] Xiaojun Wang,[a] Zhe Hu[a]

[a]State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China

Jinhui Wang, Kui Guo, and Shuaijie Li contributed equally to this work. Author order was determined by drawing straws.

1.1 Design of the primers and probe for real time PCR based on FljB gene of *S.*



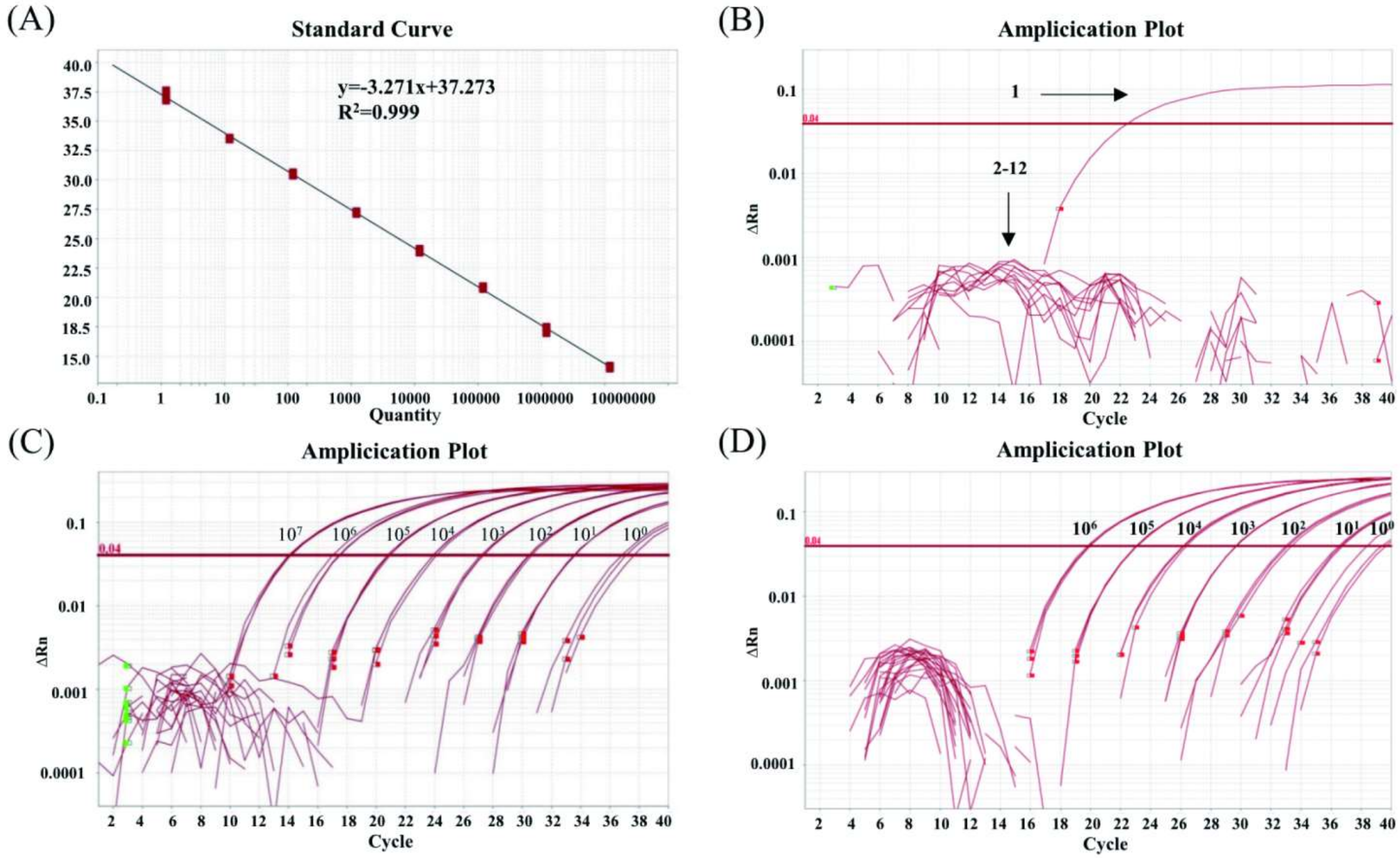
The primers and probe sequences used in our assay could distinguish *S.* Abortusequi from *S.* Typhimurium, *S.* Enteritidis, *S.* Dublin, and most *Salmonella* serotypes from Group A to Group F

1.2 Analysis specificity and sensitivity of the PCR

Detection limit:

3 copies/μL of the standard plasmid

10 CFU/μL of bacterial DNA



1:*S.* Abortusequi,
2:*S. equi*,
3:EIV,
4:EHV-1,
5: EHV-4,
6:EIAV,
7:EAV,
8:*E. coli,*
9:*S.* Typhimurium,
10:*S.* Enteritidis,
11:*S.* Dublin
12: Negative

1.3 Comparison of detection results between real-time PCR assay and bacteria islolation

Real-time PCR showed better detection rate than bacteria isolation

Types	Real time PCR	Culture		Detection rate (%)	PAP (95% CI)	NAP (95% CI)
		Positive	Negative			
Tissue	Positive	102	34	83.95	100.00 (96.37-100.00)	43.33 (31.57-55.90)
	Negative	0	26			
Plasma	Positive	2	0	1.04	100.00 (17.77-100.00)	100.00 (98.02-100.00)
	Negative	0	190			
Vaginal swab	Positive	7	41	25.81	100.00 (64.57-100.00)	77.09 (70.41-82.64)
	Negative	0	138			

2. *Salmonella* Abortusequi iELISA antibody test kit

Hindawi
Transboundary and Emerging Diseases
Volume 2023, Article ID 1403180, 11 pages
https://doi.org/10.1155/2023/1403180

WILEY | Hindawi

Research Article

Development and Application of an iELISA for the Detection of Antibody against *Salmonella* Abortusequi

Kui Guo, Zenan Zhang, Yan Yang, Weiguo Zhang, Jinhui Wang, Shuaijie Li, Xiaoyu Chu, Wei Guo, Diqiu Liu, Yaoxin Wang, Zhe Hu, and Xiaojun Wang

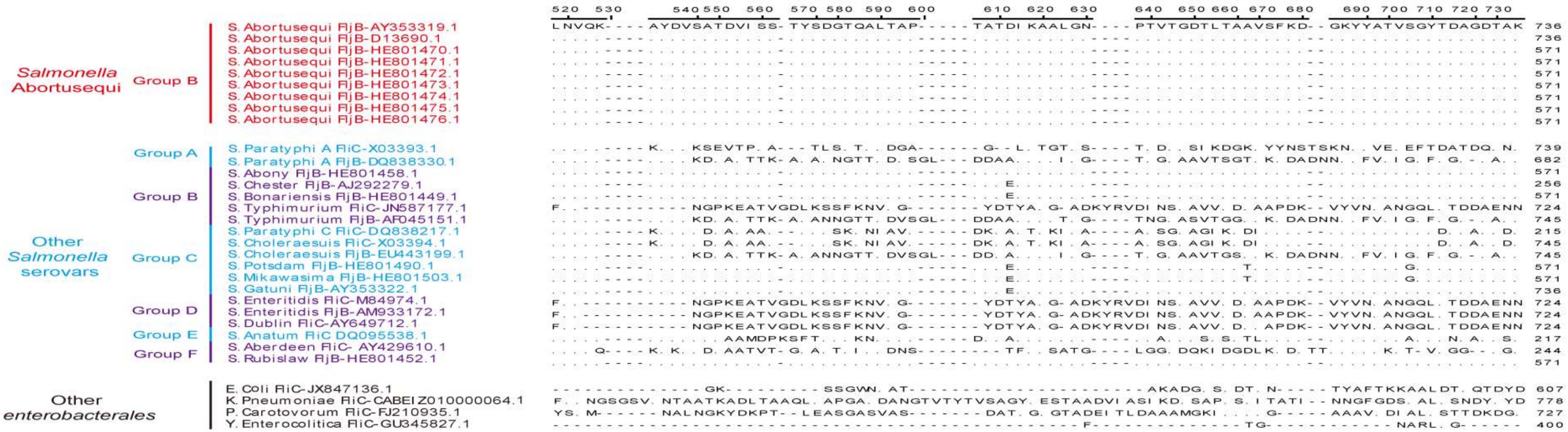
State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute,
The Chinese Academy of Agricultural Sciences, Harbin, China

Correspondence should be addressed to Zhe Hu; huzher@126.com and Xiaojun Wang; wangxiaojun@caas.cn

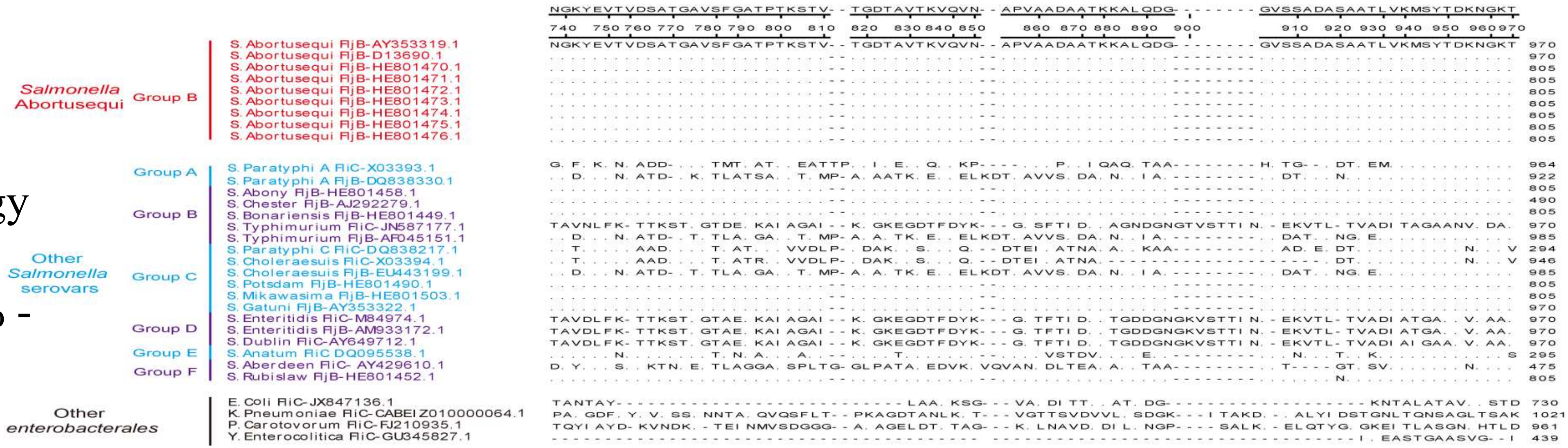
Received 27 December 2022; Revised 2 May 2023; Accepted 3 May 2023; Published 15 May 2023

2. 1Selection of target

The **FljB gene** is used as a serotype detection identifier for *Salmonella*

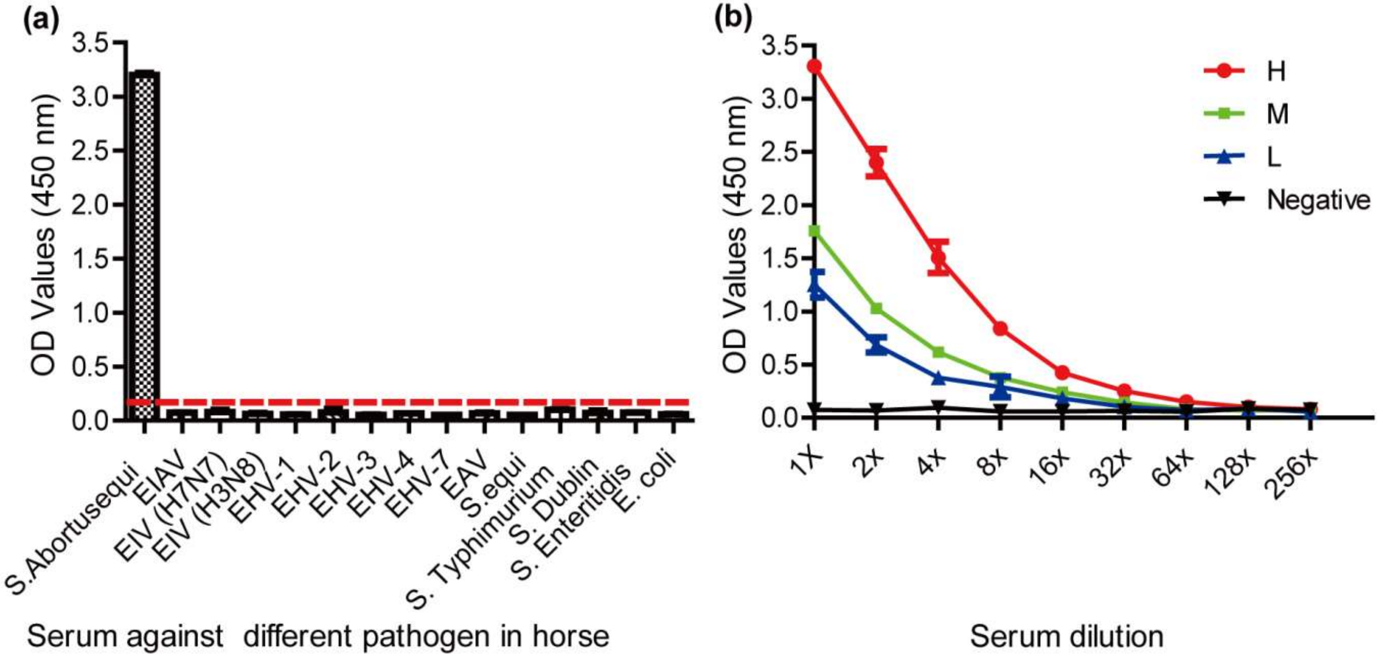


The amino acid homology of flagellin gen is 60.8% - 63.2%.



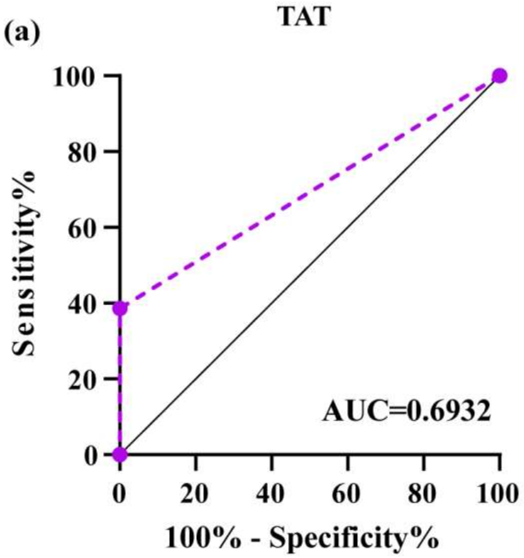
2.1 Specificity and sensitivity of antibody detection by the FljB iELISA

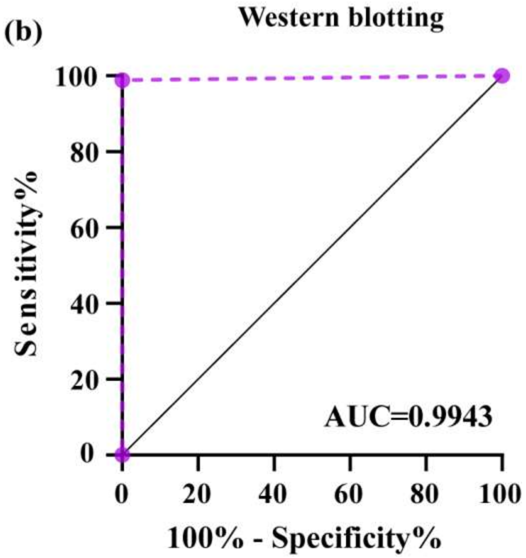
iELISA showed high specificity and 8-16 times higher sensitivity than TAT

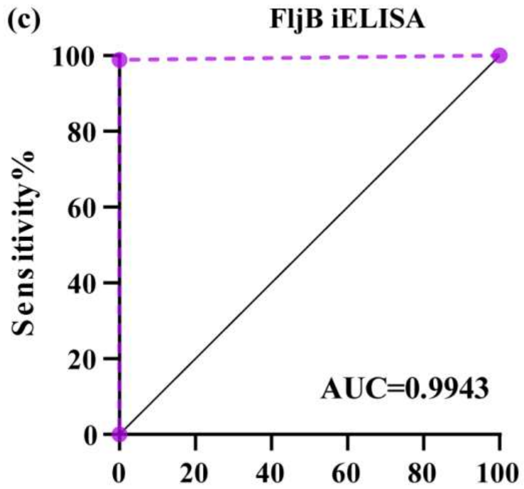


	TAT titer	iELISA titer
H	8	64
M	4	32
L	1	16

2.2 Comparison of diagnostic sensitivity (DSe) and diagnostic specificity (DSp) of iELISA, TAT and Western blotting



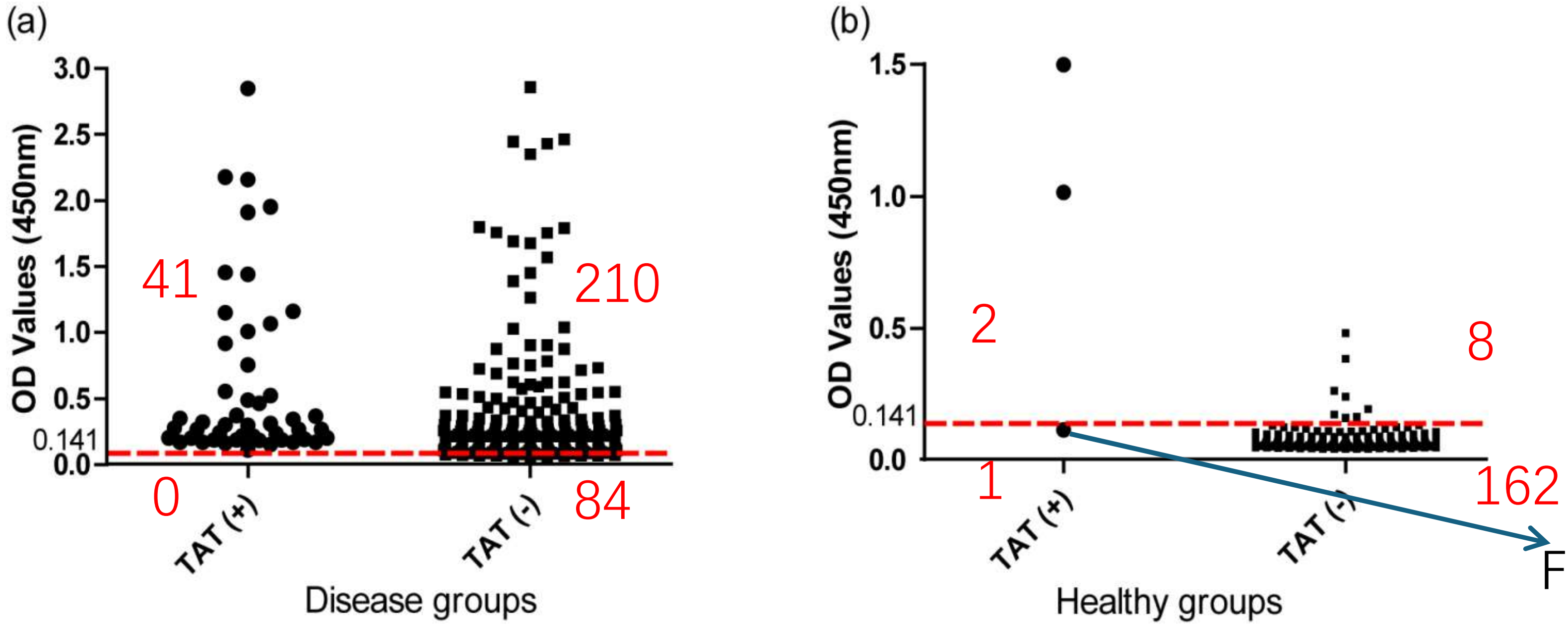




The diagnostic sensitivity (DSe) and diagnostic specificity (DSp) of iELISA are all comparable with western blot, wich is consider as the best assay.

	Accuracy	Sensitivity	Specificity
TAT	69.32%	38.6% (34/88)	100% (88/88)
iELISA	99.43%	98.86% (87/88)	100% (88/88)
Western blot	99.43%	98.86%(87/88)	100% (88/88)

2.3 Clinical detection performances of the iELISA



False positive results

	Positive detection rates	
	TAT	iELISA
Disease groups	12.2% (41/335)	74.9% (251/335)
Healthy groups	1.7% (3/173)	5.8% (10/173)

335 clinical samples from infected zones and 173 samples from healthy horses

3. *Salmonella* Abortusequi cELISA antibody test kit

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

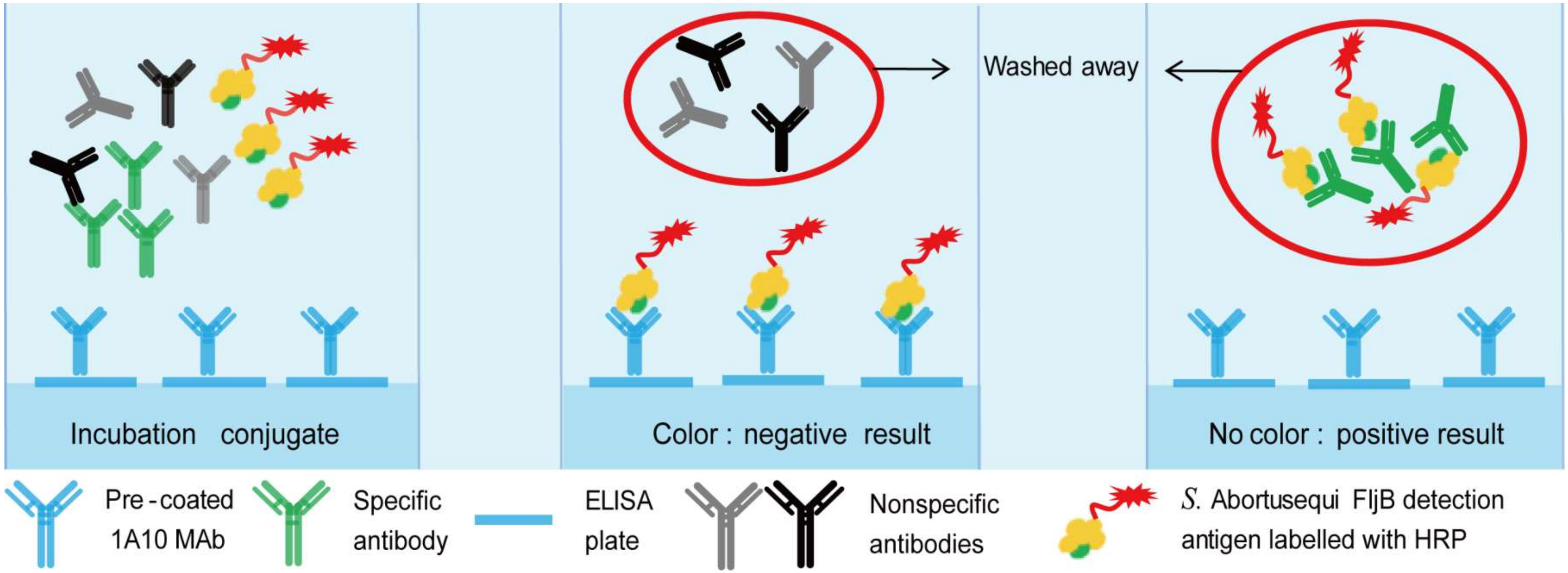
Check for updates

| Bacteriology | Full-Length Text

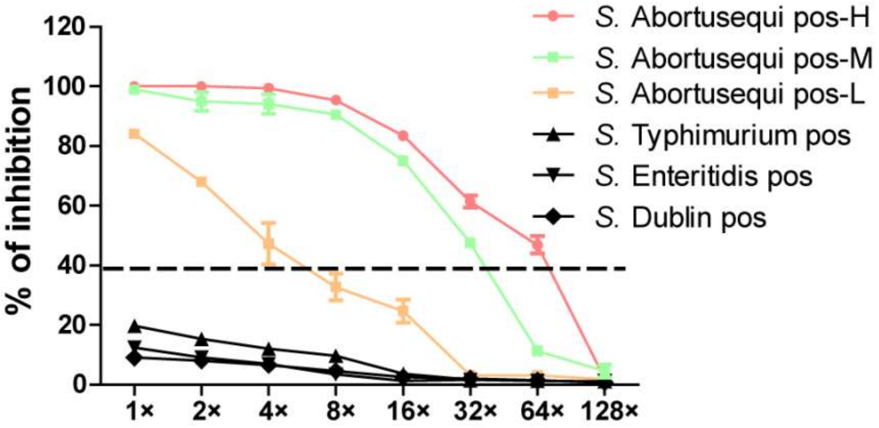
Development and application of a competitive ELISA for the detection of antibodies against *Salmonella* Abortusequi in equids

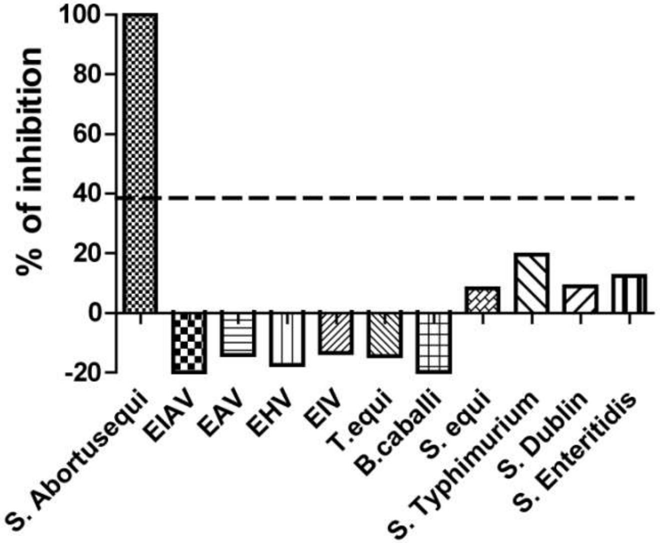
Kui Guo,[1] Wei Guo,[1] Diqiu Liu,[1] Weiguo Zhang,[1] Yan Yang,[1] Zenan Zhang,[1] Shuaijie Li,[1] Jinhui Wang,[1] Xiaoyu Chu,[1] Yaoxin Wang,[1] Zhe Hu,[1] Xiaojun Wang[1]

3.1 Schematic diagram of solid phase competition ELISA



3.2 Analytical sensitivity and specificity of the cELISA

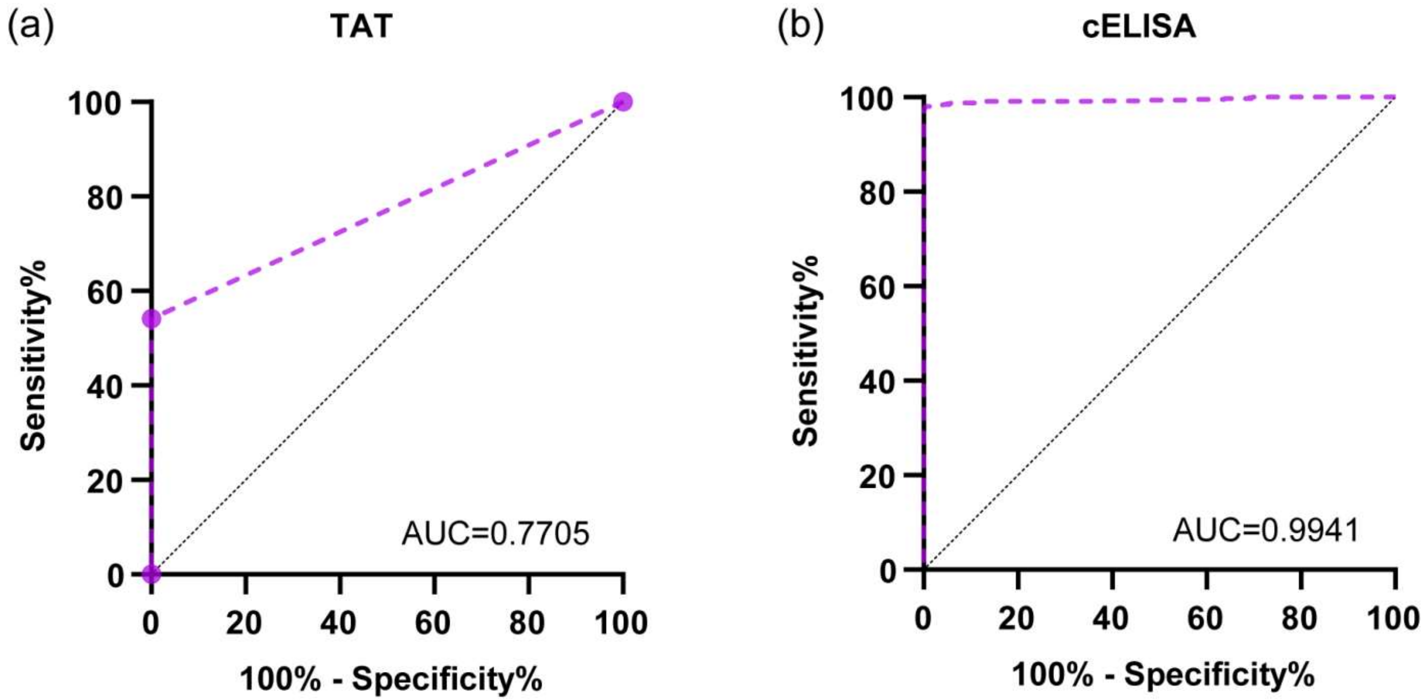




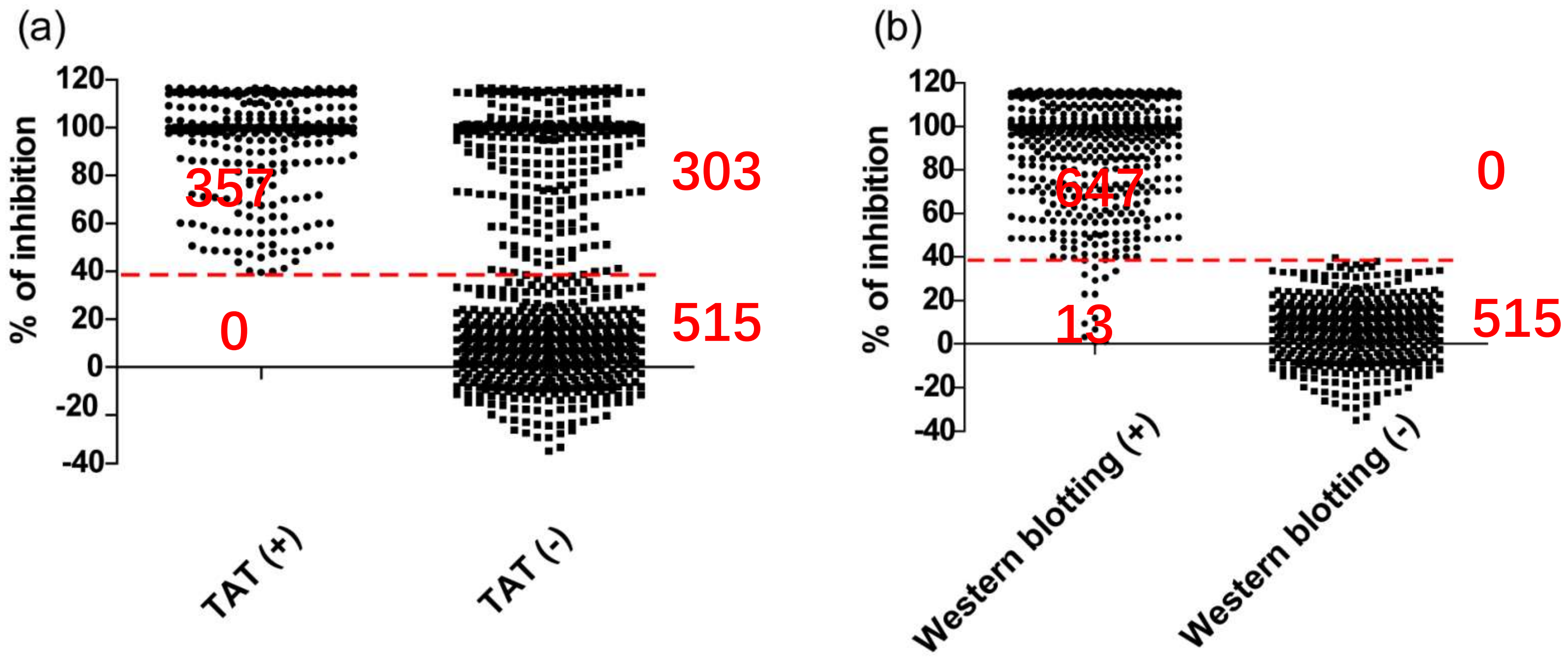
	TAT titer	ELISA titer
H	32	64
M	2	4
L	1	32

3.3 ROC curve analysis

	Accuracy	Sensitivity	Specificity
TAT	77.05% (872/1175)	53.79% (355/660)	100% (515/515)
cELISA	99.41% (1162/1175)	98.03% (647/660)	100% (515/515)



3.4 Clinical detection performances of the cELISA



1. The coincidence rate of cELISA and TAT is 75.1%（357+515）/1175）
2. The coincidence rate of cELISA and Western blot is 98.9%（(660+515）/1175）

4. *Salmonella* Abortusequi antibody rapid test kit (Colloidal Gold)

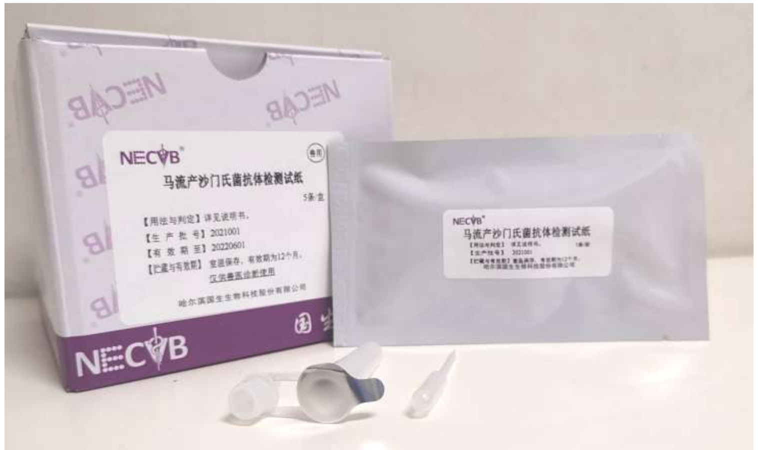
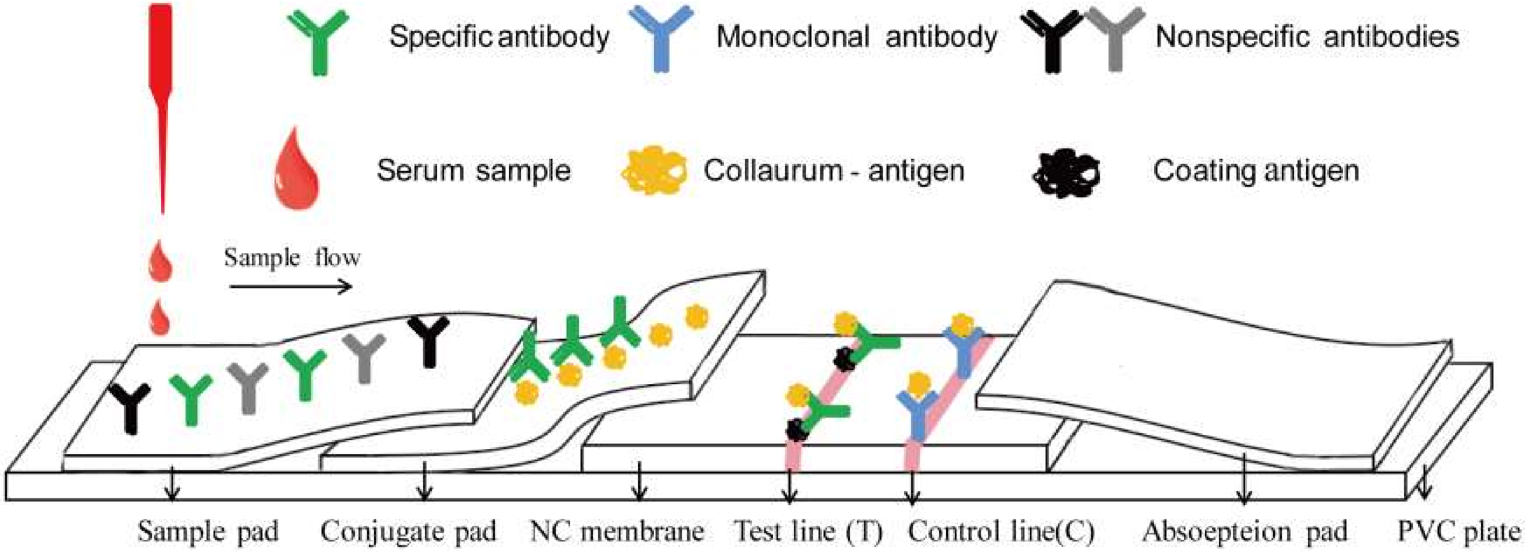


FIG 1

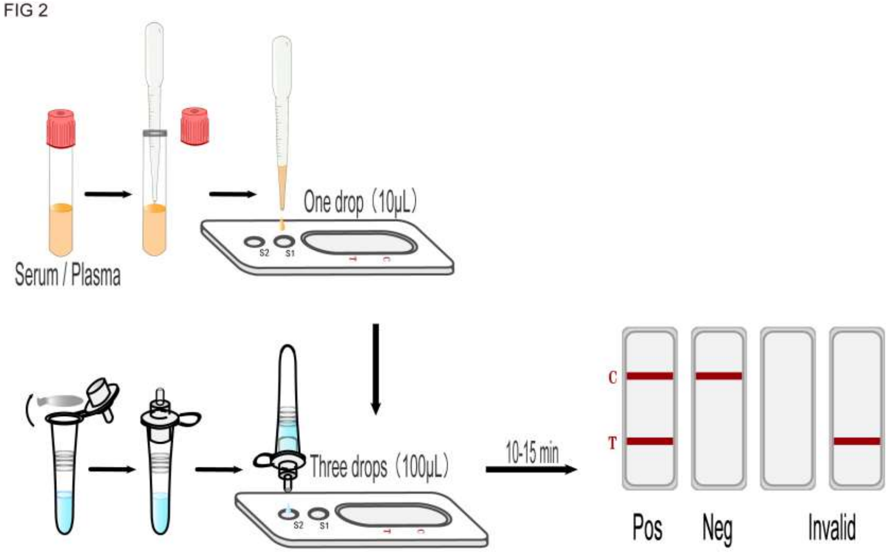


Unpublished

4. *Salmonella* Abortusequi antibody rapid test kit (Colloidal Gold)

4.1 Schematic diagram of clinical operation of the colloidal gold test card

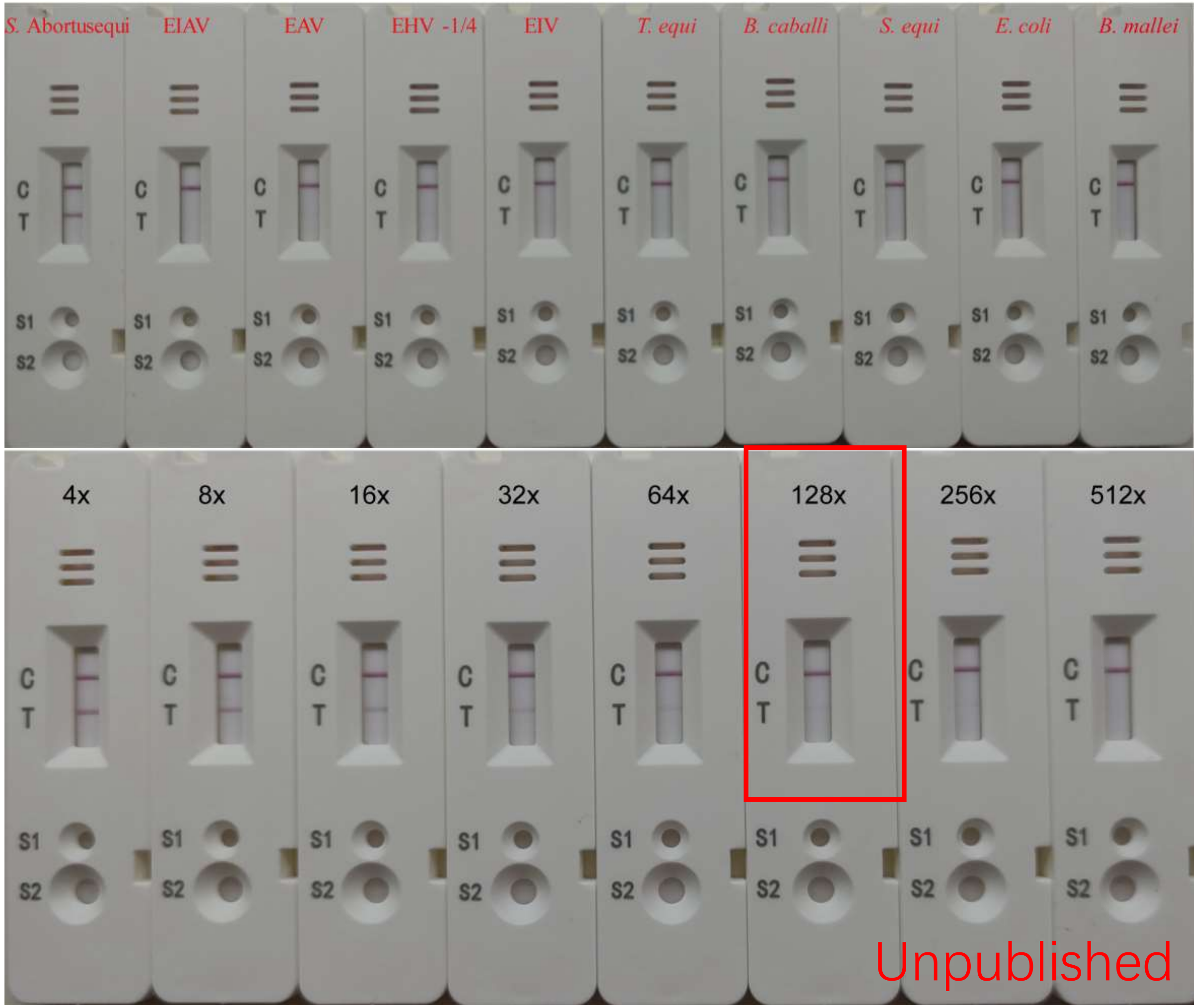
FIG 2



	Titer
iELISA	32
cELISA	32
Test card	128

4.2 Analytical specificity and analytical sensitivity of the test card

FIG 3



Unpublished

4.3Comparison of sensitivity and specificity of the test card, iELISA, and cELISA

FIG 5

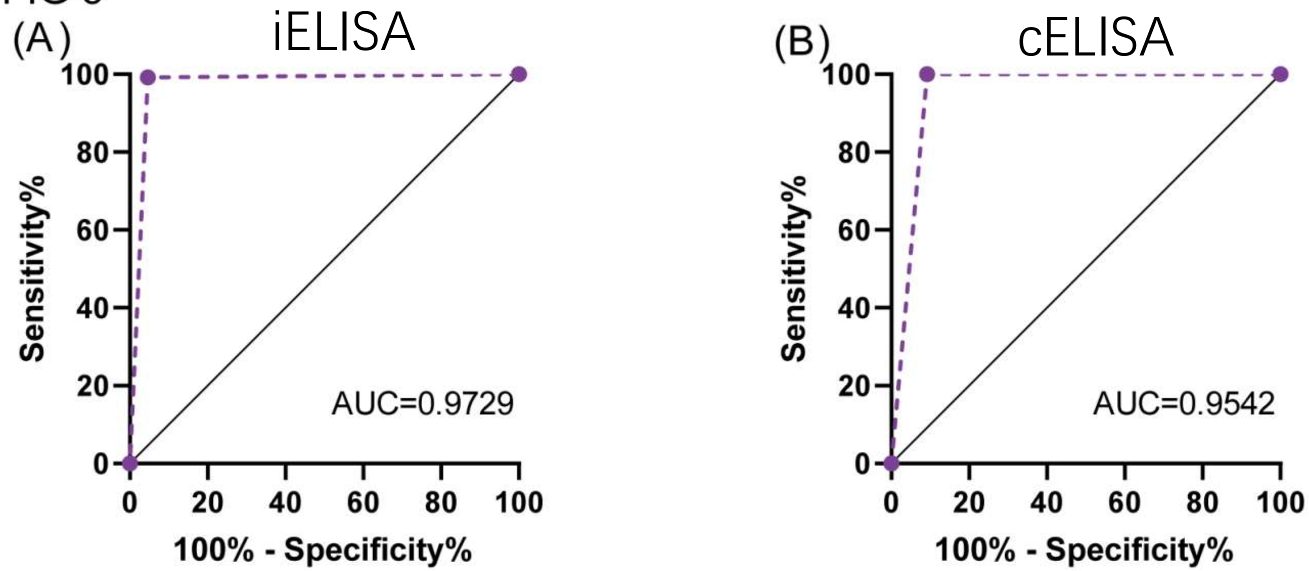
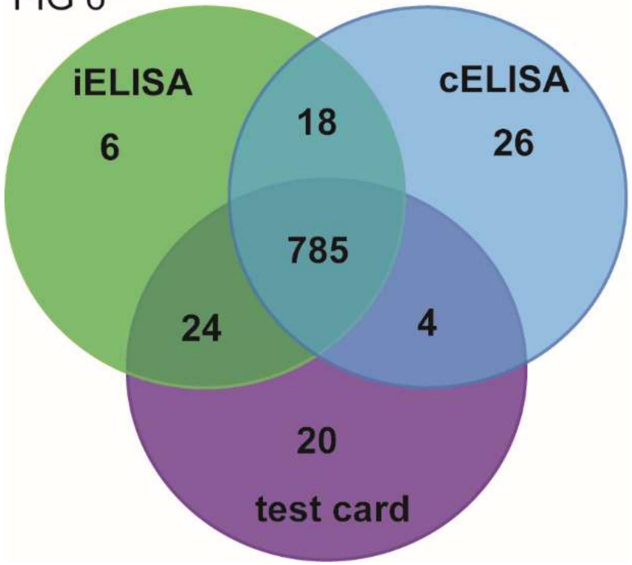


FIG 6

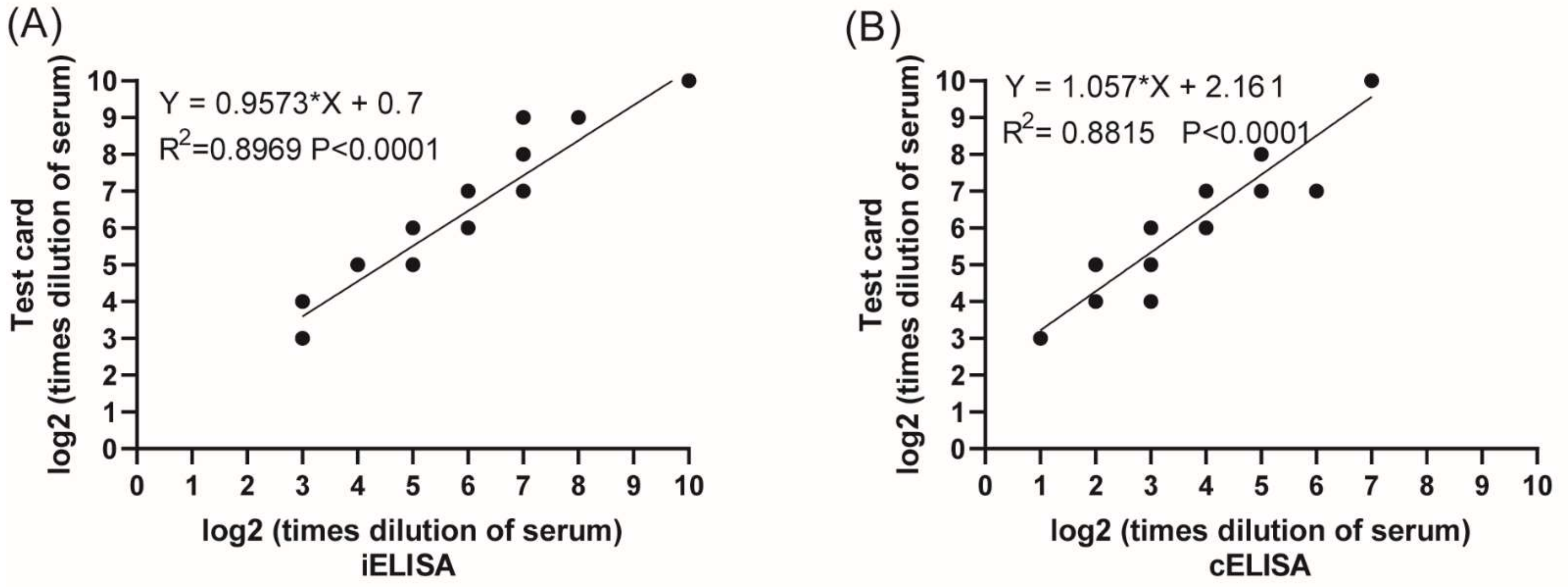


The coincidence rate of the test card with iELISA (97.12 % [809/833]) was similar to that of the test card with cELISA (94.72 % [789/833]).

Unpublished

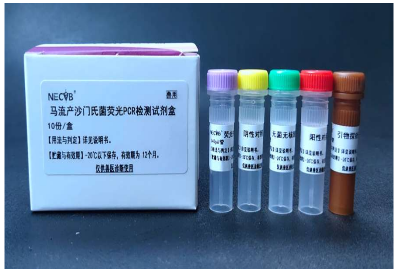
4.4 Correlation analysis of rapid test cards with iELISA and cELISA

FIG 7



Unpublished

Commercial kits for the detection of *Salmonella* Abortusequi



Real time PCR

iELISA antibodies detection kit

cELISA antibodies detection kit

Colloidal Gold card for antibody detection

1 High sensitivity

1.Fast
2.High sensitivity (3 copies/μL of the standard plasmid)

3.High specificity

2 High specificity

1.Fast： finish in 85 min (TAT ,24h)

2.High sensitivity： 8-16x higher

3.Highly specific detection of *S.* Abortusequi

3 Throughput

1.Fast： finish in 40 min
2.Highly specific detection of *S.* Abortusequi
3. Only one step response
4.Throughout: no need for dilution

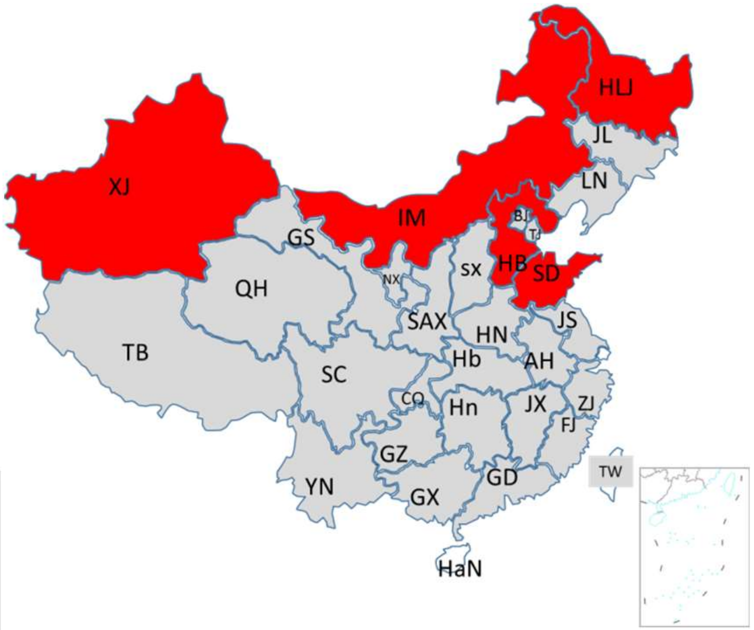
4 Field fast test

1.Fast: in 10 min
2.More sensitive than TAT,iELISA and cELISA
3.Easy to use: no need of instrument.

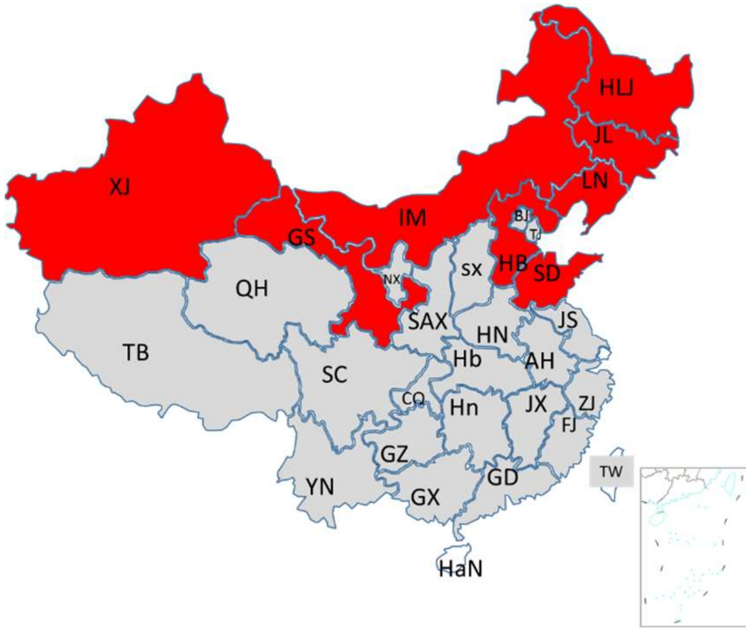
Geographical distribution of *Salmonella* Abortusequi identified by our lab

2014-2018

2019-2022



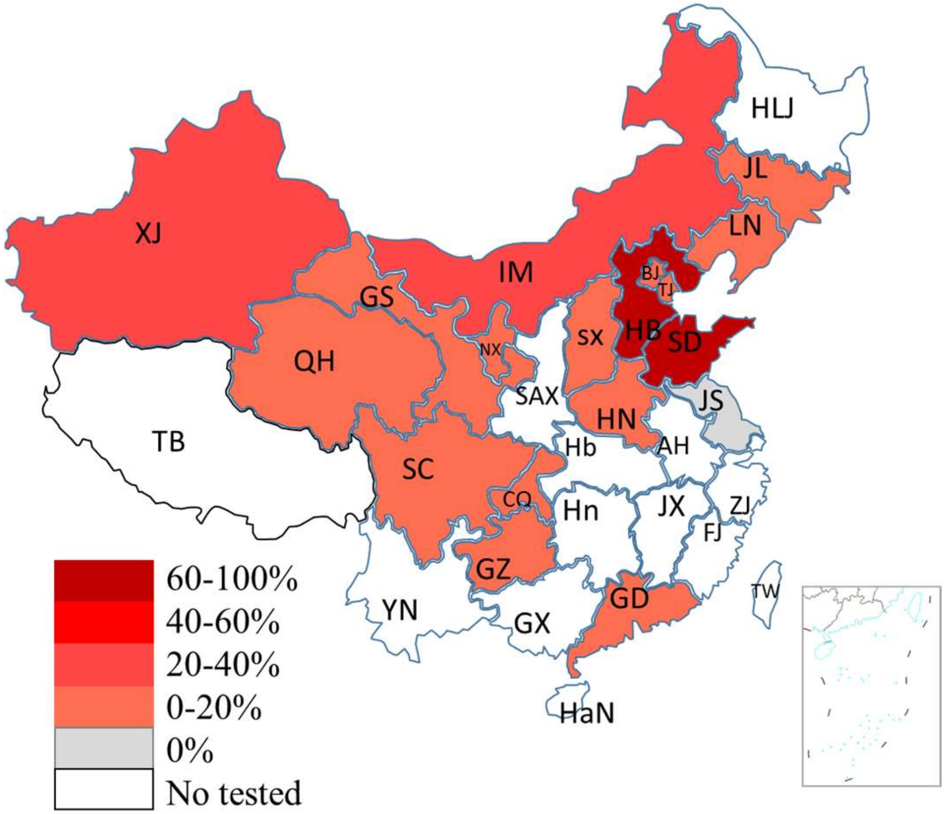
2023-2024



***Salmonella* Abortusequi** spreads epidemically and frequently causes up to 90 percent of abortions wherever it occurs.

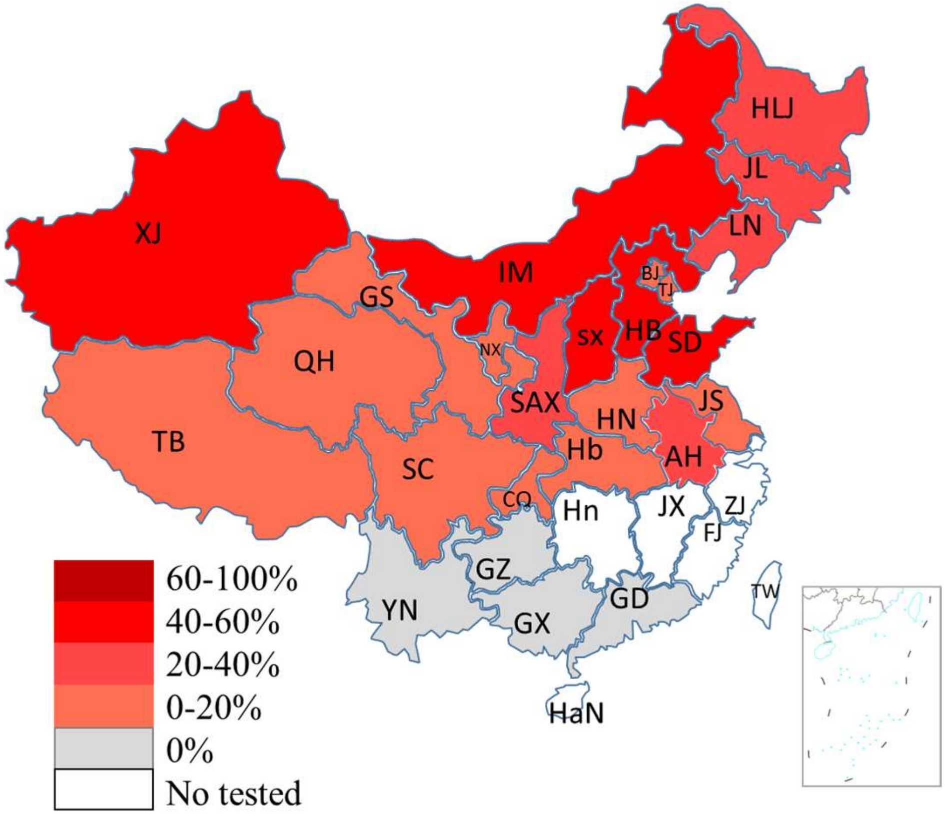
Active serological monitoring

2018-2021



Positive rate :32.6% (480/1472， 51 farms)
Samples from HB and SD were mainly from infected farms (Guo et al., 2023)

2022-2024



Positive rate :18.31% (360/1966,138 farms)
(Random salmpling)

Unpublished


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

Thank you！

World Organisation for Animal Health
Founded in 1924

National control programme for glanders in India

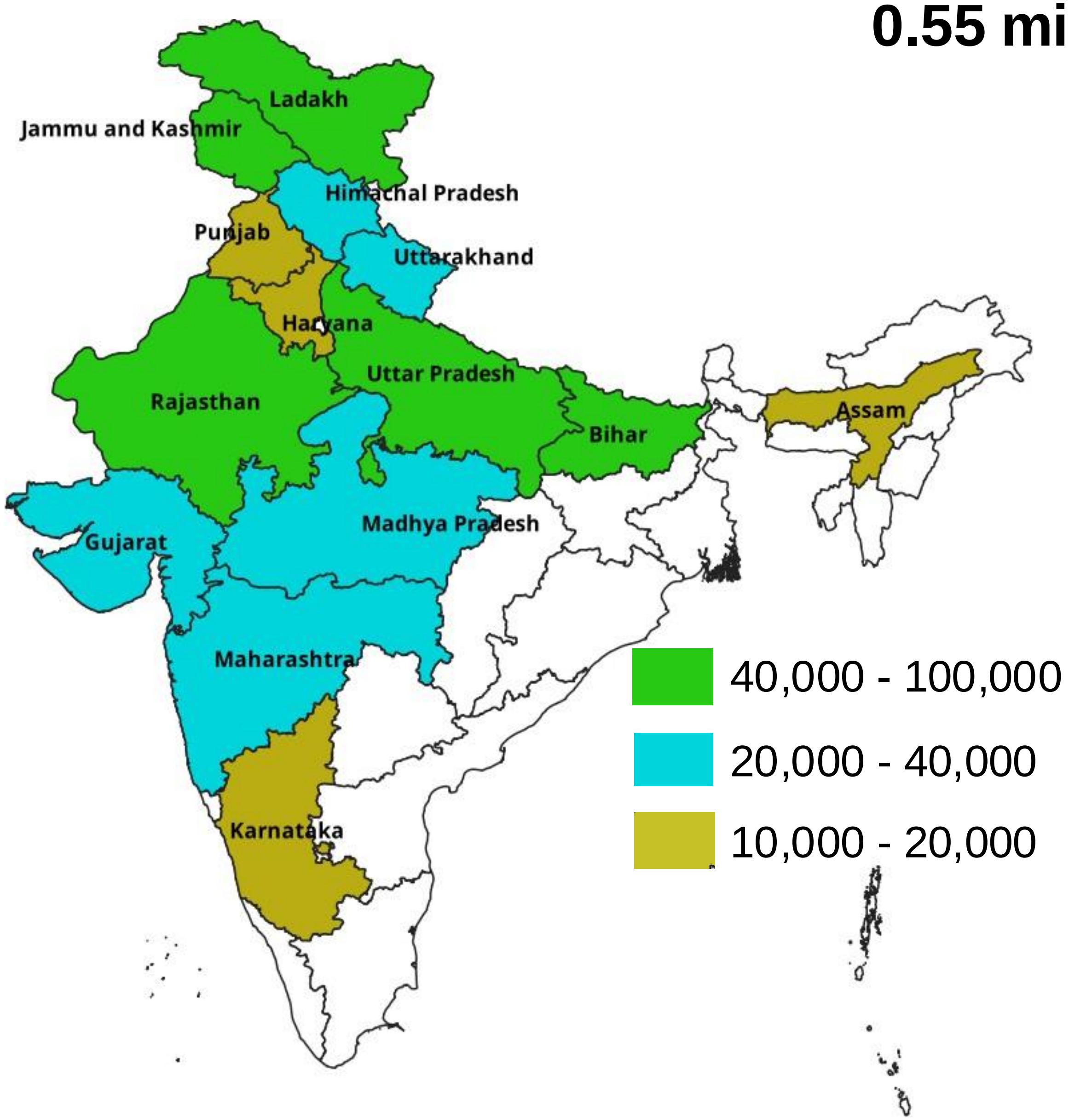
Praveen Malik
Principal Scientist, ICAR and
CEO, Agrinnovate India Limited, New Delhi (India)

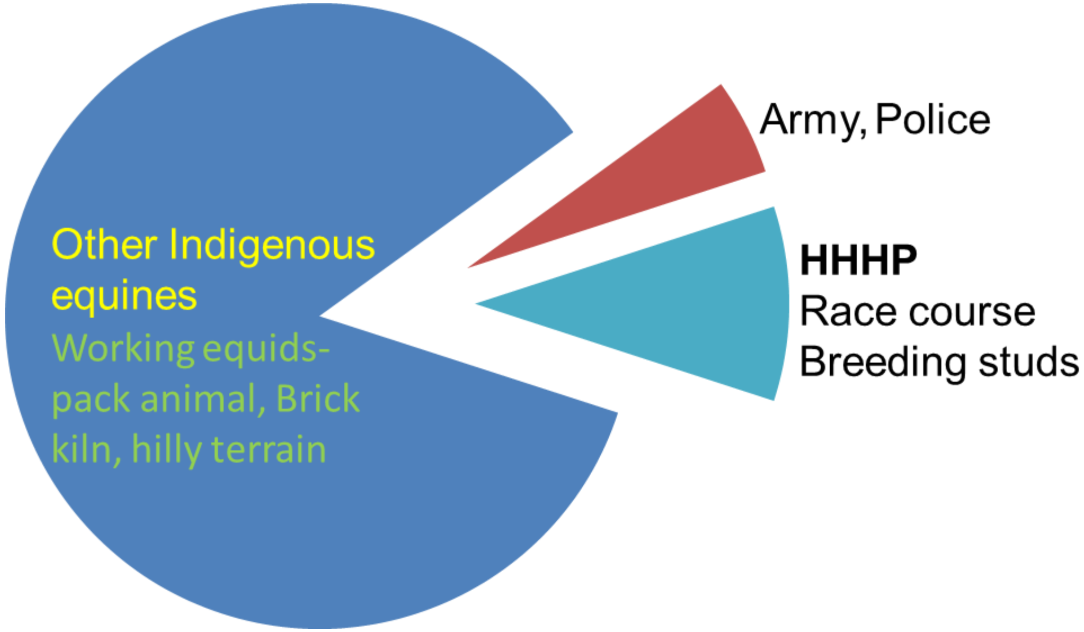
Regional Workshop on laboratory expertise for equine diseases in Asia and the Pacific, 17 – 18 September 2024, Tokyo, Japan

Distribution of equine population in India

(Livestock Census 2019)

0.55 million





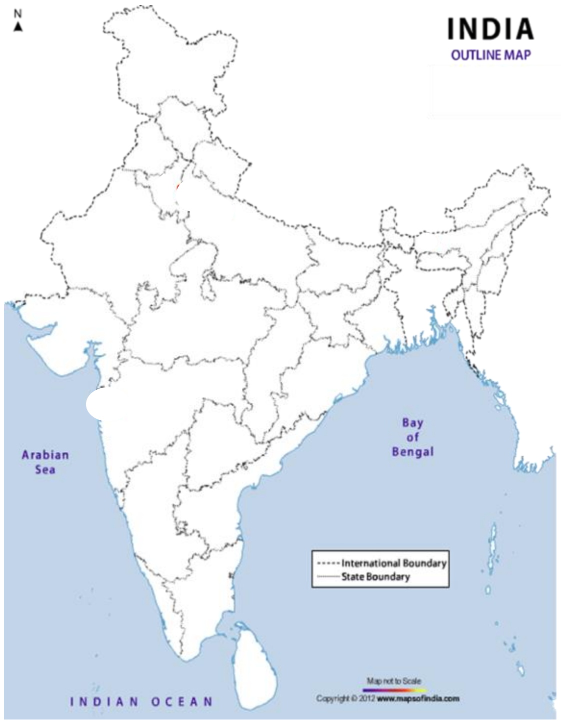
Indigenous - ~95%; Mainly unorganized

No glanders in Organized sector

Affected states – Mainly northern States (12)

Glanders outbreak status in India (Last 3 yrs WAHIS Reports)

State	Year 2021			Year 2022			Year 2023		
	No. of Outbreaks	Cases	Death	No. of Outbreaks	Cases	Death	No. of Outbreaks	Cases	Death
Haryana	2	3	1	3	9	4			
Maharashtra	2	2		1	1	1	3	4	3
Uttar Pradesh	1	9		-	-	-	-	-	-
Andhra Pradesh	-	-	-	1	1	-	-	-	-
Himachal Pradesh	-	-	-	2	5	4	2	7	5
Gujarat	-	-	-	5	6	4	2	7	0
Rajasthan	-	-	-	3	4	3	5	11	4
Jharkhand	-	-	-	1	7	3	-	-	-
Punjab	-	-	-	-	-	-	5	6	5
Total	5	14	1	16	33	19	17	35	17



- Glanders is a notifiable equine disease (since 1899) under the Prevention and Control of Infectious and Contagious Diseases in Animals Act, 2009 of Government of India
- Being a notifiable disease, DAHD issued a set of guidelines (vide letter no F. No. K-50/1/2017/LH dated 18/01/2017) that was binding on the States
- To control the outbreak of Glanders in the infected states and prevent spread of the disease to non-infected states/zones, the existing guidelines have been updated in 2019
- The National Action Plan has been framed for entire population of equids reared in different management and animal husbandry practices in India under the overall conceptual framework of the WOAH Terrestrial Code and the WOAH Terrestrial Manual 2018 (Chapters 1.4, 4.3, 12.10 and 3.5.11 respectively)
- Overall objective is surveillance, control and eradication of Glanders in equines in India

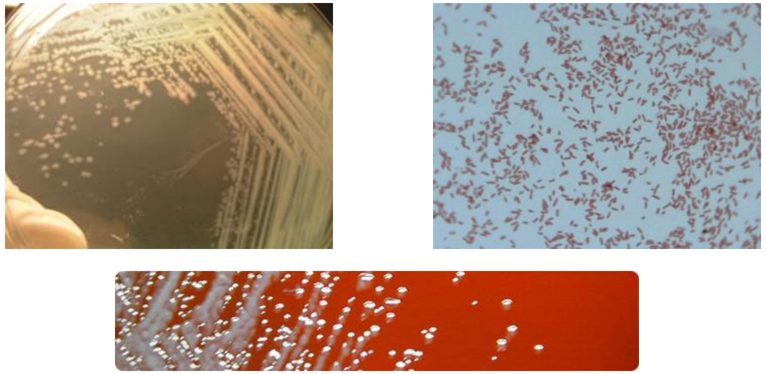
National Action Plan for Control and Eradication of Glanders in India (May 2016, Revised 2019)

- Legislative provision
- Diagnostic test, designated laboratory and National Reference laboratory
- Disease surveillance, control and eradication
- Responsibilities of State Animal Husbandry Department in the event of incidence of disease
- De-notification
- Glanders-free State/Zone/Compartment
    - Zones historically free from Glanders
    - Criteria for defining/auditing compartment
    - Criteria for attaining Glanders-free compartment
    - Movement of horses/equines between compartments for various activities
- Equine fairs/congregation/events/shows in unorganized sector
- Guidelines for pharmaceuticals, animal house, experimentation facilities, etc.
- Inter-state movement of equids
- Human Surveillance
- Compensation
- Research Priorities
- Training and Capacity Building
- Public awareness
- Surveillance Plan

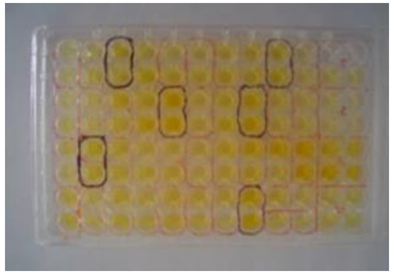
Legislative provision

- Prevention and Control of Infectious and Contagious Diseases in Animals Act, 2009
  - All States have framed rules under Section 43 of the Act for Quarantine Camps and check-posts, manner of inspection etc.
- State/ UT Animal Husbandry Department are bound to report any suspected or confirmed cases of Glanders

Diagnostic test, designated laboratory and National Reference Laboratory

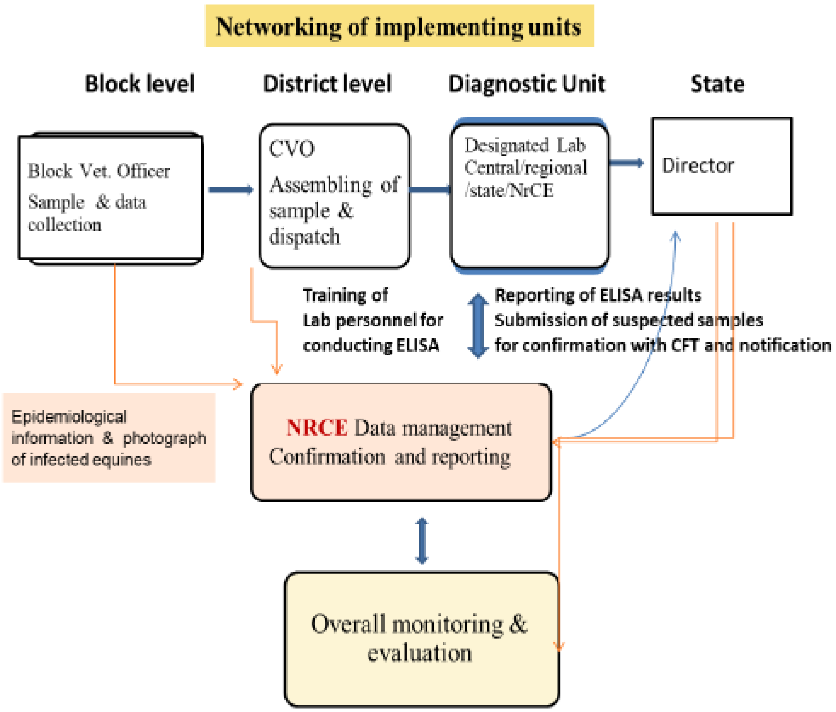


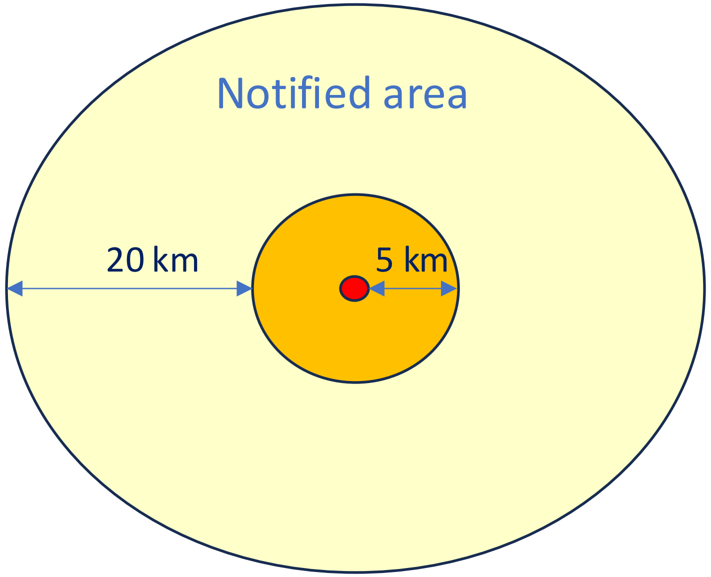
- **Two-tier sero-diagnosis approach**
  - Recombinant ELISA - screening
  - Confirmation of positive case - CFT
  - Others – Bacterial isolation, antigen and genome demonstration (PCRs)
- **National Reference Laboratory (NRL)**
  - ICAR –National Research Centre on Equines, Hisar
  - Central Military Veterinary Laboratory (CMVL), Meerut - recognized laboratory for testing of equids from the defence services and those coming in contact with their animals
- **Development of Network of Diagnostic Laboratories (DL) for glanders**
  - RDDLs/CDDL/State Diagnostic Laboratories
  - Notification of recognized/designated laboratories - by DAHD on the recommendations of a Committee and technical validation by NRL
    - Capacity building of laboratory personnel and supply of reagents
    - Verified for repeatability and reproducibility of their test results
  - All positive cases tested by the designated laboratories (DLs) and CMVL to be confirmed by the NRL



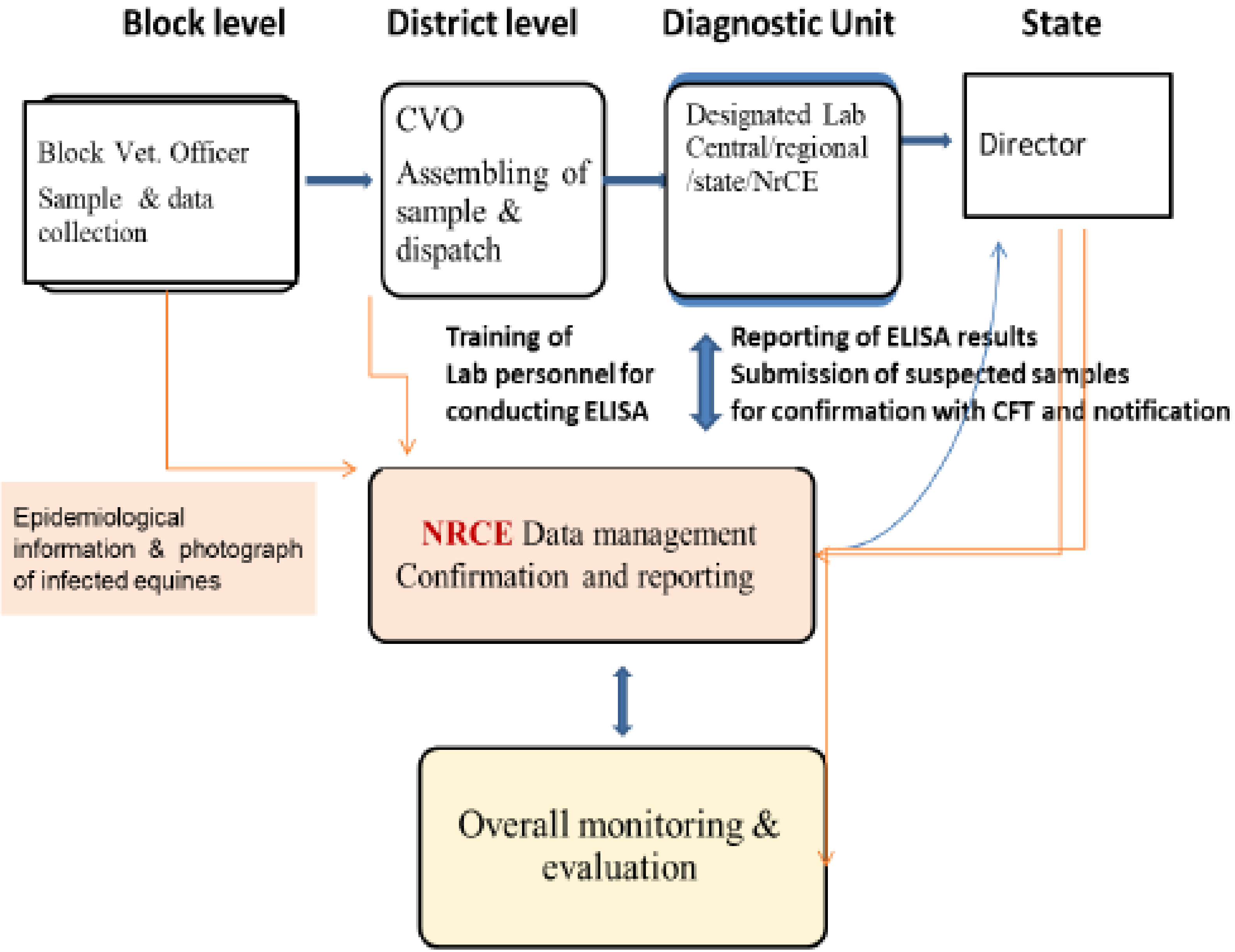
Disease surveillance, control and eradication

- Glanders surveillance programme which shall include clinical/physical, pathological and serological surveillance
- This surveillance plan is applicable to all the states in India
- Continuous, and random surveillance strategy – 10 – 20% equine population in a year
- In case of positive report – 25 km radius surrounding core infection zone is notified area
- Animal movement and equestrian events are prohibited
  - Three times surveillance around 25 km radius of the infected zone
    - 100% surveillance around 0-5 km (and epidemiologically linked area)
    - 25% surveillance around 5-25 km
  - Negative results allow denotification
- Zoos to submit biological samples from all susceptible captive wildlife species





Networking of implementing units



Block level

District level

Diagnostic Unit

State

Block Vet. Officer
Sample & data collection

CVO
Assembling of sample & dispatch

Designated Lab
Central/regional /state/NrCE

Director

Training of
Lab personnel for
conducting ELISA

Reporting of ELISA results
Submission of suspected samples
for confirmation with CFT and notification

Epidemiological information & photograph of infected equines

NRCE Data management
Confirmation and reporting

Overall monitoring & evaluation

ea

Responsibilities of State Animal Husbandry Department in the event of incidence of disease

- Notification
- Restrictions on movement of equines
- Screening of equines for glanders (physical / sero- surveillance based on risk assessment)
- Destruction of positive cases
- Disinfection of notified areas
- Sample collection and submission to designated laboratories
- Communication of status with relevant stakeholders
- Awareness and community engagement

De-notification

- By State government following post-outbreak surveillance (three tests in first three months, at minimum 21 days apart)
- Regulated equine movement to be withdrawn for restoring equine activities
- Surveillance to continue for another 9 months, with another sample being taken within 3 months of denotification

Glanders free State / Zone / Compartment

- Historical freedom – no reports for at least 10 years
    - All equids in the zone/region shall have identification system, movement records and health card
- Eradication has been achieved by active surveillance in past 3 years
- Infection is not known to be established in wildlife within the state or zone
- Glanders / *B.mallei* infection surveillance for at least past 3 years
- Appropriate bio-security and sanitary measures

Defining/auditing compartment

- Existence of an animal id^n system, bio-security and surveillance mechanism
- To be certified by the attending veterinarian/District Chief Veterinary Officer /Director General, RVS and notified by DAHD
- To be reviewed every 5 years by an expert team constituted by DAHD

The term “infected” shall be defined as the infection of *B. mallei* confirmed either by bacterial isolation or demonstration of antigen/genomic DNA or antibody to *B. mallei* demonstration and epidemiologically linked to a confirmed or suspected case of infection with *B. mallei*, or giving cause for suspicion of previous contact with *B. mallei*

Glanders-free compartment

- Naturally or artificially separated premises/areas with restricted entry of man, materials and animal
- Management of animals/stud under the supervision of qualified veterinarian
- Proper documentation, including records of treatment, vaccination, testing for various diseases, movement records
- Epidemiological separation of the compartment from other equids (unorganized sector)
- Any other factors preventing risk of *B. mallei* infection

Criteria for attaining and maintaining

- Evidence of absence of Glanders / B.mallei infection either historically or based on past and ongoing Glanders surveillance
- Equids in the compartment have not shown clinical signs & pathology consistent with Glanders during last 10 years
- Evaluation of risk management
- Newly inducted equids to be quarantined (21 days minimum) and tested
- Appropriate biosecurity and sanitary measures
- Physical screening
- A testing program for demonstration of infection (bacterial culture) or DNA / antibody to B. mallei at least during last three years has been established
- Mixing of animals of other compartments having similar management practices shall be permitted.
- Restricted contact with personnel having attended equids of different immune status and management practices.
- Private establishments to be registered with DAHD
    - State Veterinary Authority to carry out documented periodic inspection of facilities, biosecurity, records and surveillance procedure

Movement of horses/equines between compartments for various activities

- Movement of equines shall be permitted between various Glanders-free compartments
- Any equestrian event (horse shows, race, polo, etc.) can be organized for participating horses from disease free compartments; even if it falls in the notified areas, following all biosecurity measures
- Equids have been tested negative
  - within 30 days of moving out of the compartment
  - within 21 days of the return to the original compartment

- **Equine fairs/congregation/events/shows in un-organized sector**
- Equine fairs, congregation, shows, or any equestrian events in which equids from unorganized sector take part shall not be permitted to be held in 25 Km of radius of the notified area/focus of infection
- Veterinary authority to take decision not to permit any events of equines in unorganized sector where adjoining districts have reported Glanders
- Equestrian events shall be permitted provided:
    - No case of Glanders during the past one year and ongoing surveillance is in place
    - Equines from notified area/district/zone which fall within 25 km radius from infection source shall NOT be permitted to participate
    - A certificate of a Glanders' test with negative results within 30 days
    - In equine fairs, blood samples from at least 30% population of participating equines shall be collected randomly for testing
    - In such events, common watering and feeding not to be permitted
    - Equine owners to be encouraged to get their animals physically and serologically examined within one month of return

For pharmaceuticals, animals house, experimentation facilities holding equines

- To be free from Glanders/ *B. mallei* infection - equines should be tested twice every year
- Any new incumbent should be quarantined and
  - tested negative once, if introduced from non-infected states/zones
  - twice within three months with second test carried out within 21 days before entrance into the facility
- DAHD may arrange inspection of these facilities for compliance of guidelines

Inter-state movement of equids

- To be regulated by State Veterinary Authority
- Equids with prescribed “Health Card” only be permitted to move between states.
- Animals from infected states (having incidence of Glanders during the last 3 years) shall be permitted to enter in non-infected states by the State Veterinary Authority upon verification of the certificate of a Glanders‟ test carried out within 30 days and
- Movement between non-infected states or historically Glanders-free states shall be permitted with one Glanders test result carried out within 30 days before the date of inter-state migration
- Equines do not show signs and pathology consistent with Glanders on the day of entry

Human Surveillance

- Following confirmation of Glanders in equines, human health authorities to be intimated for testing of all in-contact personnel including the owners
- CMVL will undertake testing of human samples specifically from defense service personnel, if needed

Compensation

- State / UT Veterinary Authority are responsible for paying compensation to *bonafide* owners for eliminating Glanders/*B. mallei* infected animals in accordance with DAHD notification
- Paid under ASCAD on a 50:50 basis (as on date)
- Compensation shall also be paid for animal suspected to be infected with Glanders but dies before receipt of test results positive for Glanders
- Compensation amount to be revised **every three years**.

Research Priorities

- By designated laboratories in consultation with ICAR-NRCE
- Capacity building to conduct the test
- ICAR-NRCE to develop improved diagnostic methods/kits

Training and Capacity Building

- CVE Programmes on Glanders for field vets and lab personnel
- ICAR-NRCE to support Regional Disease Diagnostic Laboratories (RDDLs) and also other Designated State / UT Laboratories

Public awareness

- Public awareness by public, private and government institutions, and NGOs to sensitize all stakeholders about zoonotic significance of disease wrt equines and humans
- Regular awareness campaigns at pilgrimage/tourist places where equines are used for transportation

Other details included

- Destruction of the Infected Equines/animals
- Disinfection of the premises including the disinfectants
- SOP for serum samples collection from equines for Glanders surveillance
- Suggestive format of Equine Health Card along with the guidelines
- Details of Glanders' Surveillance Plan

Way forward

- Review of the NAP needed, mainly for
  - Notified/Infected area/zone, Surveillance zone
    - Define purpose of surveillance
    - Focus on hot spots/zones
    - Investigate risk factors for disease spread
    - Include all susceptible species in screening
    - Attempt contact tracing
  - Organization of equine congregations
  - Compensation issues
  - Community engagement and risk communication strategies

- Establishment of more DFZs/compartments for HHP equines

Thank you...

Piroplasmosis' surveillance in Mongolia

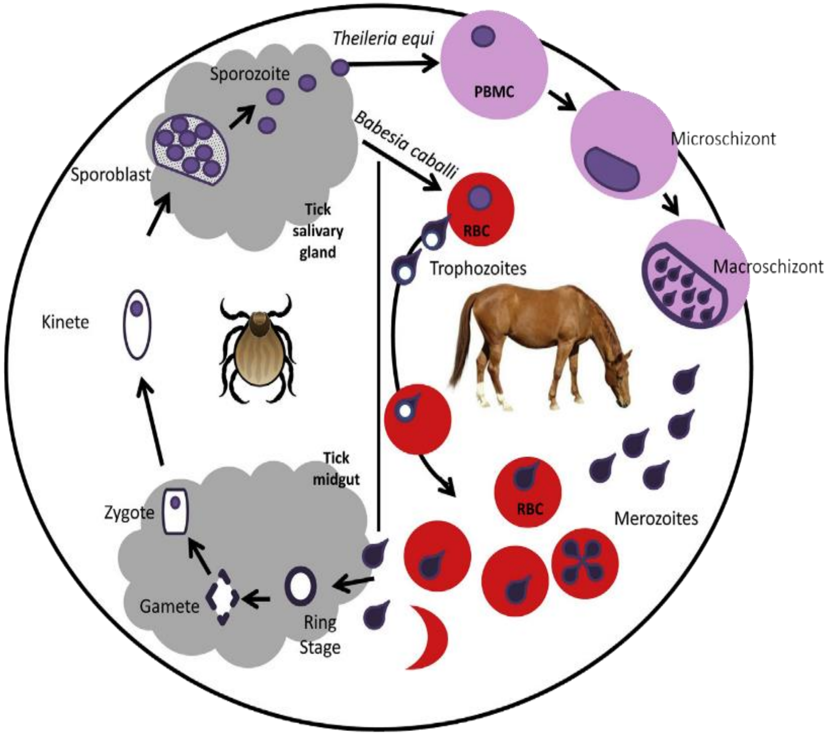
B. Enkhtaivan[1]., P. Myagmarsuren[1]., D. Otgonsuren[1]., T. Sivakumar[2]., B. Davaasuren[1]., M. Zoljargal[1].,
S. Narantsatsral[1]., B. Davkharbayar[1]., B. Battur[1]., N. Inoue[2]., B. Battsetseg[1], N. Yokoyama[2]

[1] Laboratory of Molecular Genetics, Institute of Veterinary Medicine, Mongolian University of Life Sciences.
[2] National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine.

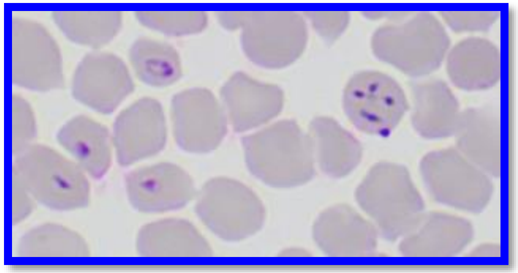
Regional workshop on laboratory expertise for equine diseases in Asia and the Pacific 17-18 September 2024.

Introduction

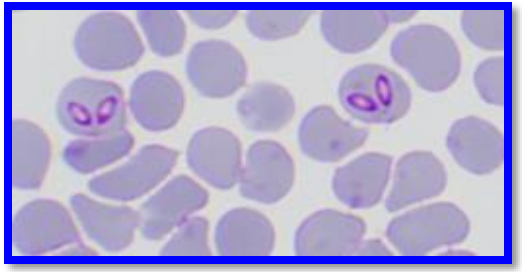
Equine piroplasmosis is caused by ***Theileria equi*** and ***Babesia caballi***.



- ***T. equi***



- ***B. caballi***



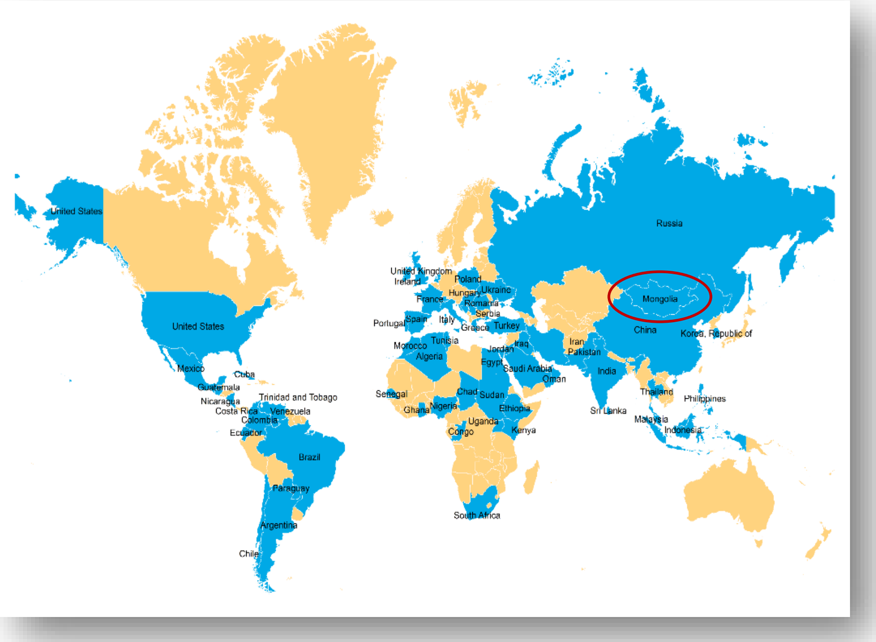
Infected animals become prolonged carriers.

(Wise at al., 2013. Equine Piroplasmosis. Vet. Clin. North Am. Equine Pract. 30: 677-693)

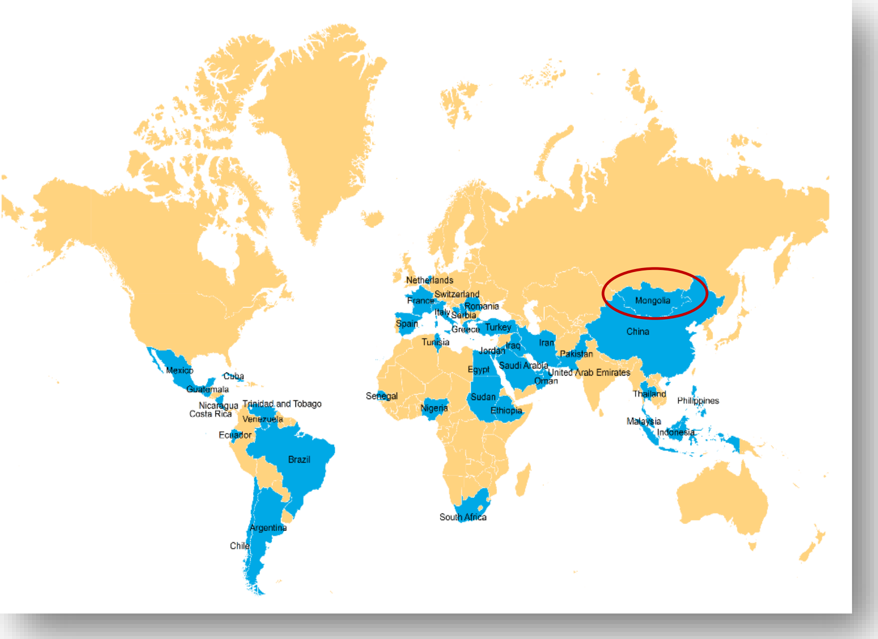
Introduction

Global distribution of equine piroplasma species

T. equi



B. caballi



https://www.obihiro.ac.jp/facility/protozoa/en/oie-rl-ep-ep

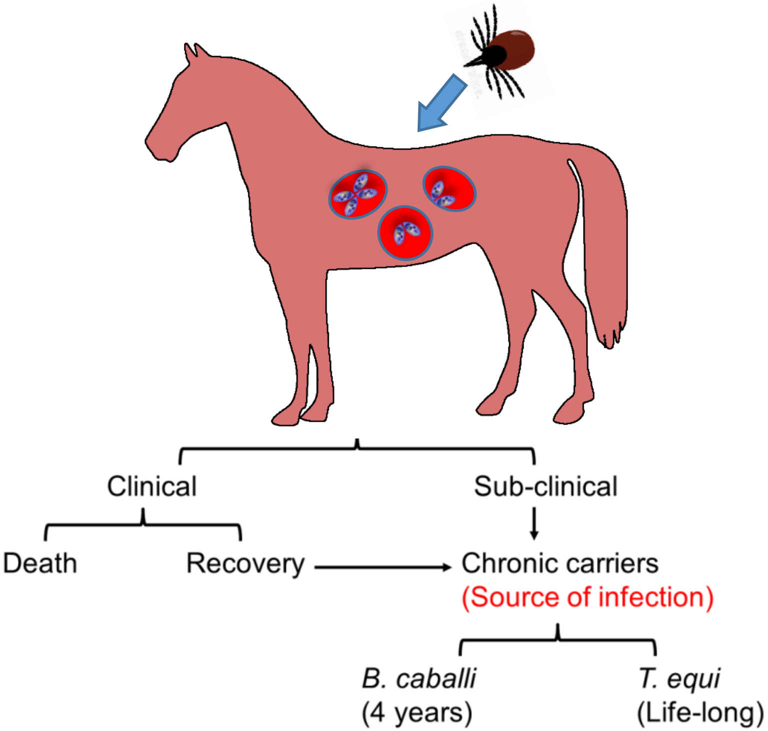
Introduction

Economical significance of equine piroplasmosis

Equine piroplasmosis

- Anaemia — Production loss
  Death
- Costs — Treatment
  Tick control
- Impaired international trade

Huge economical losses to horse industries



Clinical

Sub-clinical

Death

Recovery → Chronic carriers

(Source of infection)

B. caballi
(4 years)

T. equi
(Life-long)

Objective

- To investigate the molecular and sero-prevalance of *T. equi* and *B*. *caballi* infections in horse reared throughout the Mongolia

- To determine the parasite’s genotypes in Mongolia

- To develop distribution maps for *T. equi* and *B*. *caballi* in Mongolia

Study design

Collect samples

Extract Serum and DNA

Analyze for parasites by **ELISA** and **PCR**

Compare results

- The horses were apparently healthy and randomly selected for sampling.

- The serum was extracted in same day, while DNA extracted in one month after collect blood.

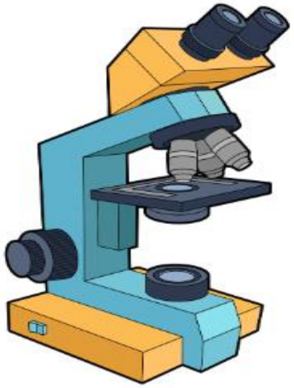
Material and methods

- Serum and Blood samples from horse

- Detection for *T. equi* and *B. caballi*

Microscopic

251 smears (2014-2015)



- Giemsa-stained
- 100x zoom
- With emersion oil

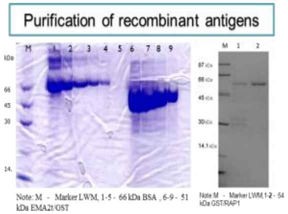
ELISA

1282 samples (2013-2017)

Antigen

EMA2t
T. equi

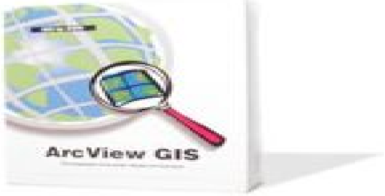
BC48
B. caballi



Purification of recombinant antigens

Note: M - Marker LWM, 1-5 - 66 kDa BSA , 6-9 - 51 kDa EMA2t/GST

Note:M - Marker LWM,1-2 - 54 kDa GST/RAP1



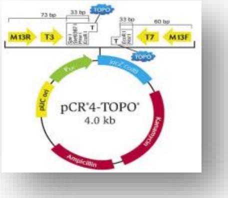
PCR

1353 samples (2014-2015)

Gene

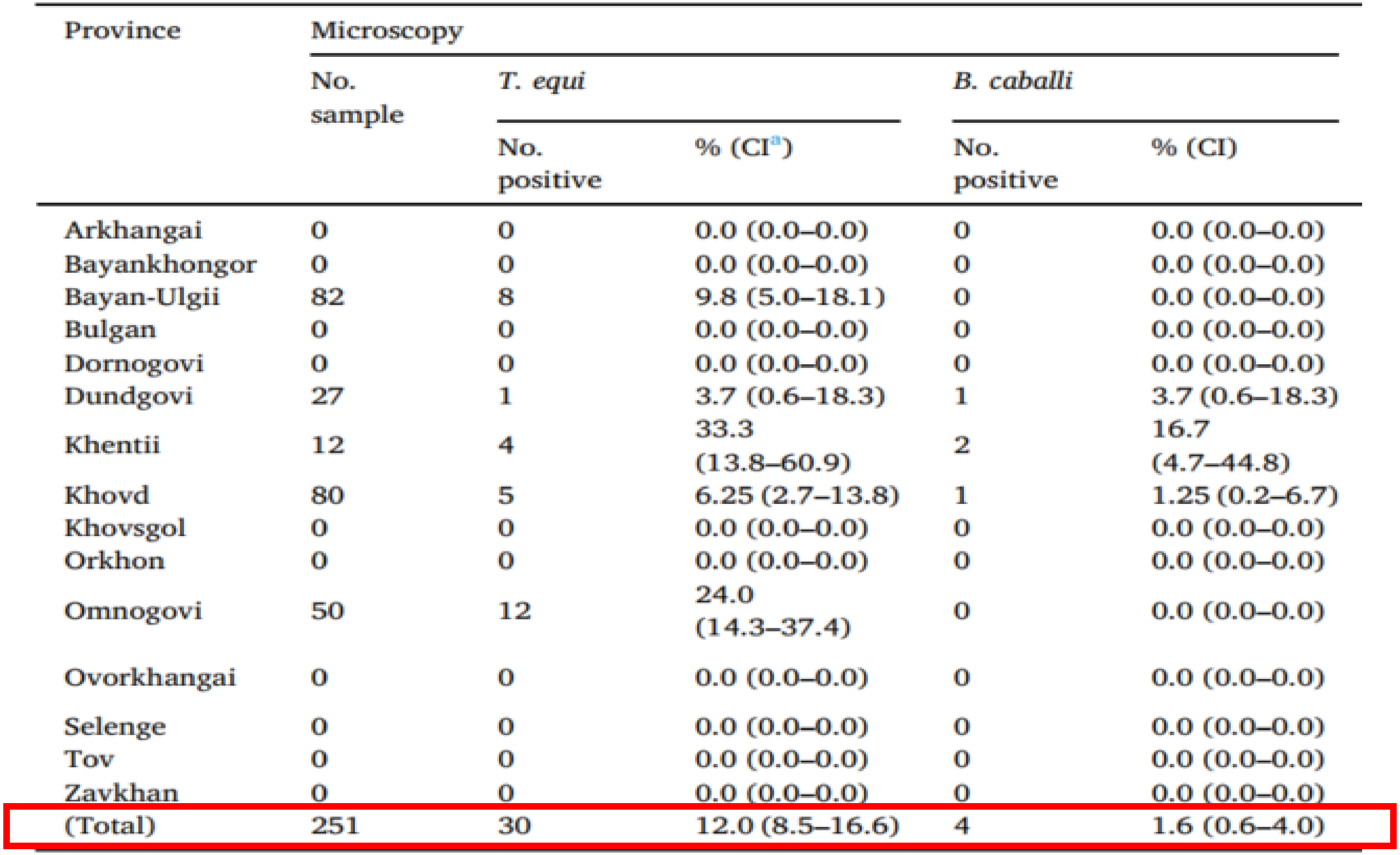
18sRNA
T. equi

Rap-1
B. caballi



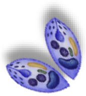
Microscopic screenings

Results…



Province	Microscopy				
	No. sample	*T. equi*		*B. caballi*	
		No. positive	% (CI[a])	No. positive	% (CI)
Arkhangai	0	0	0.0 (0.0–0.0)	0	0.0 (0.0–0.0)
Bayankhongor	0	0	0.0 (0.0–0.0)	0	0.0 (0.0–0.0)
Bayan-Ulgii	82	8	9.8 (5.0–18.1)	0	0.0 (0.0–0.0)
Bulgan	0	0	0.0 (0.0–0.0)	0	0.0 (0.0–0.0)
Dornogovi	0	0	0.0 (0.0–0.0)	0	0.0 (0.0–0.0)
Dundgovi	27	1	3.7 (0.6–18.3)	1	3.7 (0.6–18.3)
Khentii	12	4	33.3 (13.8–60.9)	2	16.7 (4.7–44.8)
Khovd	80	5	6.25 (2.7–13.8)	1	1.25 (0.2–6.7)
Khovsgol	0	0	0.0 (0.0–0.0)	0	0.0 (0.0–0.0)
Orkhon	0	0	0.0 (0.0–0.0)	0	0.0 (0.0–0.0)
Omnogovi	50	12	24.0 (14.3–37.4)	0	0.0 (0.0–0.0)
Ovorkhangai	0	0	0.0 (0.0–0.0)	0	0.0 (0.0–0.0)
Selenge	0	0	0.0 (0.0–0.0)	0	0.0 (0.0–0.0)
Tov	0	0	0.0 (0.0–0.0)	0	0.0 (0.0–0.0)
Zavkhan	0	0	0.0 (0.0–0.0)	0	0.0 (0.0–0.0)
(Total)	251	30	12.0 (8.5–16.6)	4	1.6 (0.6–4.0)

Otgonsuren et al., 2024; https://doi.org/10.1645/18-189



Sero-prevalence

Results…

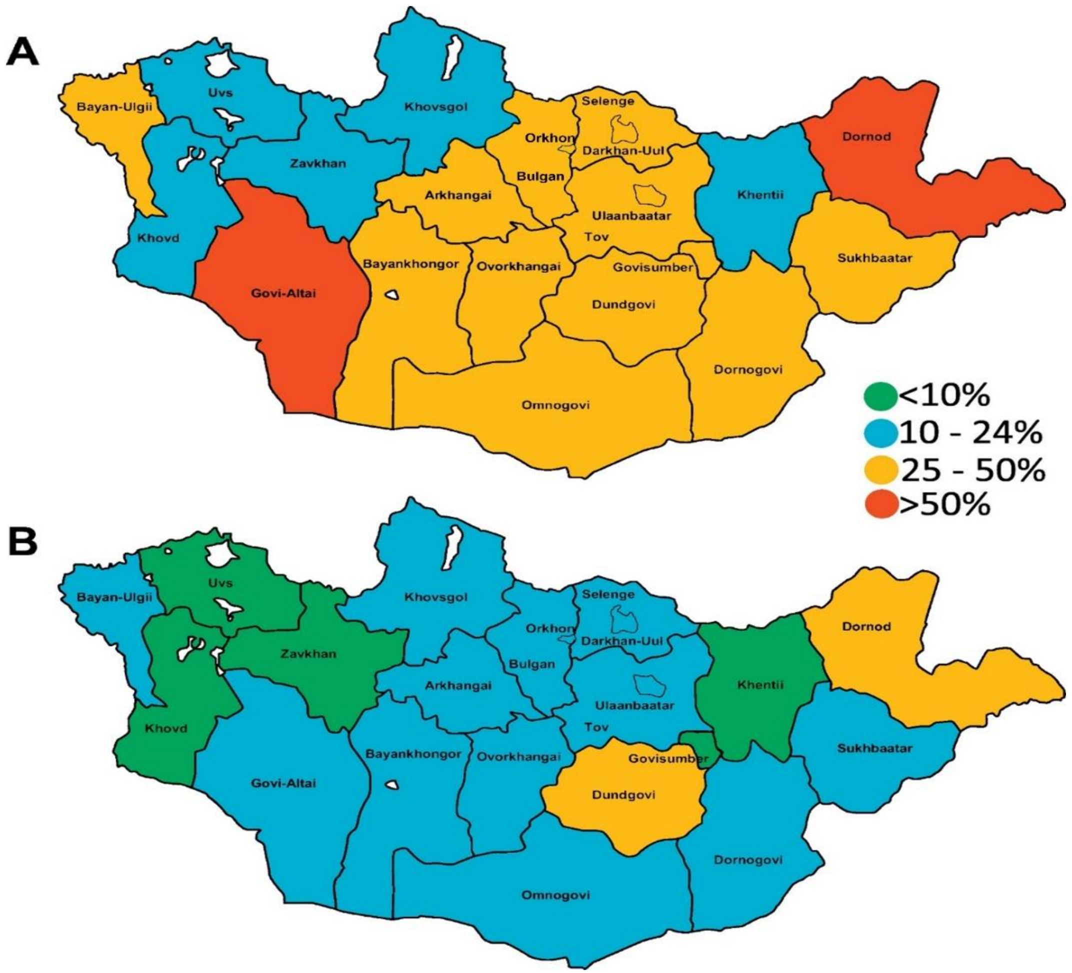
Province	No. samples	*T. equi*		*B. caballi*		*T. equi and/or B. caballi*[b]		No. co-infected (%[c])
		No. positive	% (CI[a])	No. positive	% (CI)	No. positive	% (CI)	
Arkhangai	54	20	37 (25.4-50.4)	7	13 (6.4-24.4)	22	40.7 (28.7-54)	5 (22.3)
Bayankhongor	57	18	33.3 (21-44.5)	7	12.3 (6.1-23.3)	21	36.8 (25.5-49.8)	4 (19.05)
Bayan-Ulgii	105	45	43 (33.8-52.4)	17	16.2 (10.4-24.4)	52	49.5 (40.2-58.9)	10 (19.2)
Bulgan	20	6	30 (14.5-51.9)	2	10 (2.8-30.1)	8	40 (21.9-61.3)	0 (0)
Dornod	93	69	74.2 (64.5-82)	37	39.8 (30.4-49.9)	76	81.7 (72.7-88.3)	29 (38.2)
Dornogovi	57	16	28.1 (18.1-40.8)	9	16 (8.5-27.4)	21	36.8 (25.5-49.8)	4 (19.04)
Dundgovi	33	10	30.3 (17.4-47.3)	12	36.3 (22.2-53.4)	18	54.5 (38-70.2)	4 (22.2)
Govi-Altai	29	17	59 (40.7-74.5)	7	24.1 (12.2-42.1)	20	69 (50.8-82.7)	4 (20)
Govisumber	25	7	28 (14.3-47.6)	2	8 (2.2- 24.9)	7	28 (14.3-47.6)	2 (28.6)
Khentii	98	24	24.5 (17.1-33.9)	7	7.1 (3.5-14.02)	30	30.6 (22.4-40.3)	1 (3.3)
Khovd	110	21	19 (12.8-27.4)	5	4.5 (1.9-10.2)	23	20.9 (14.4-29.4)	3 (13.04)
Khovsgol	62	15	24.1 (15.2-36.1)	9	14.5 (7.8-25.3)	21	33.9 (23.3-46.3)	3 (14.3)
Omnogovi	57	20	35 (24-48.1)	12	21 (12.5-33.3)	26	45.6 (33.4-58.4)	6 (23.1)
Ovorkhangai	26	11	42.3 (25.6-61.1)	3	11.5 (4.0-28.9)	14	53.8 (35.5-71.2)	0 (0)
Selenge	64	13	26.5 (12.3-31.7)	9	14 (7.6-24.6)	21	32.8 (22.6-45)	1 (4.8)
Sukhbaatar	136	42	31 (23.8-39.1)	15	11 (6.8-17.4)	53	39 (31.2-47.4)	4 (7.5)
Tov	48	23	48 (34.5-61.7)	6	12.5 (5.8-24.7)	26	54.2 (40.3-67.4)	3 (11.5)
Uvs	75	17	23 (14.7-33.3)	5	6.6 (2.9-14.7)	20	26.7 (18-37.6)	2 (10)
Zavkhan	133	29	22 (15.6-29.6)	11	8.2 (4.7-14.2)	39	29.3 (22.3-38)	1 (2.6)
Total	1282	423	33 (30.5-35.6)	182	14.2 (12.4-16.2)	518	40.4 (37.8-43.1)	86 (16.6)

[a] 95% confidence interval, [b] Animals infected with *T. equi* and/or *B. caballi*
[c] Expressed as a percentage of number of animals infected with *T. equi* and/or *B. caballi*

Myagmarsuren et al., 2019; https://doi.org/10.1645/18-189

Sero-prevalence based distribution map

Results…



T. equi - based on **EMA2t**

ELISA

B. caballi - based on **BC48**

Myagmarsuren et al., 2019; https://doi.org/10.1645/18-189

PCR-prevalence

Results...

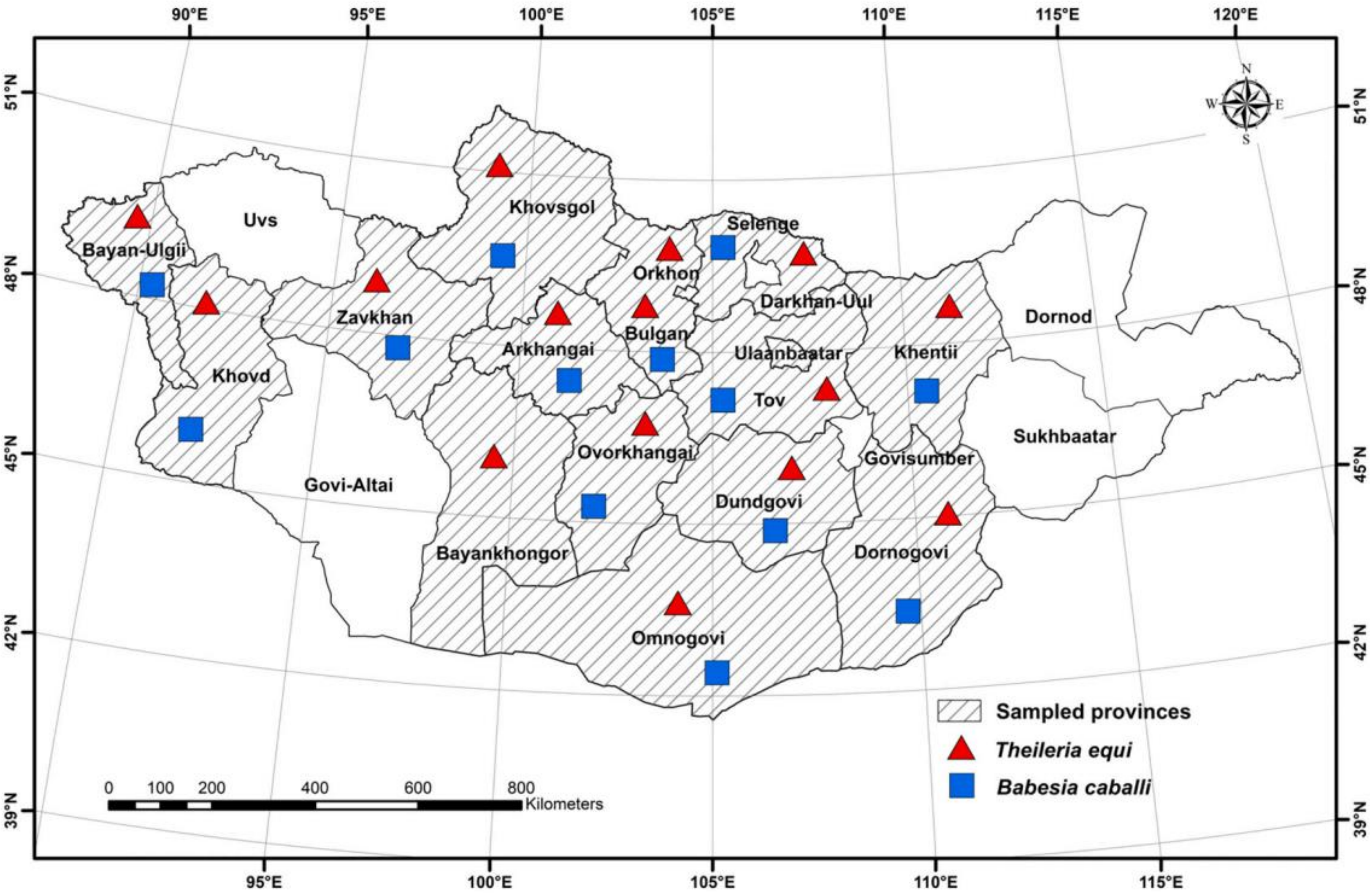
Province	No. sample	*T. equi*		*B. caballi*	
		No. positive	% (CI[a])	No. positive	% (CI)
Arkhangai	71	58	81.7 (71.2 -90.0)	2	2.8 (0.8 -9.7)
Bayankhongor	39	34	87.2 (73.3 -94.4)	0	0.0 (0.0 -9.0)
Bayan-Ulgii	145	81	55.9 (47.7 -63.7)	5	3.4 (1.5 -7.8)
Bulgan	77	61	79.2 (68.9 -86.8)	1	1.3 (0.2 -7.0)
Dornogovi	37	34	91.9 (78.7 -97.2)	2	5.4 (1.5 -17.7)
Dundgovi	86	65	75.6 (70.3 -87.5)	5	5.8 (2.7 -13.7)
Khentii	41	40	97.6 (87.4 -99.6)	5	12.2 (5.3 -25.6)
Khovd	136	98	72.1 (64.0 -78.9)	5	3.7 (1.6 -8.3)
Khovsgol	105	67	63.8 (54.3 -72.4)	6	5.7 (2.6 -11.9)
Orkhon	10	4	40.0 (18.9 -73.3)	0	0.0 (0.0 -29.9)
Omnogovi	77	64	83.1 (73.2 -89.9)	14	18.2 (11.2 -28.2)
Ovorkhangai	21	21	100.0 (84.5 -100.0)	1	4.8 (0.8 -22.7)
Selenge	93	55	59.1 (49.0 -68.6)	3	3.2 (1.1 -9.1)
Tov	236	212	89.8 (85.3 -93.0)	6	2.5 (1.2 -5.4)
Zavkhan	179	164	91.6 (87.2 -95.3)	7	3.9 (1.9 -7.9)
(Total)	1353	1058	78.2 (75.9-80.3)	62	4.6 (3.6-5.8)

[a] 95% confidence interval.

Otgonsuren et al., 2024; https://doi.org/10.1645/18-189

PCR-prevalence based distribution map

Results…



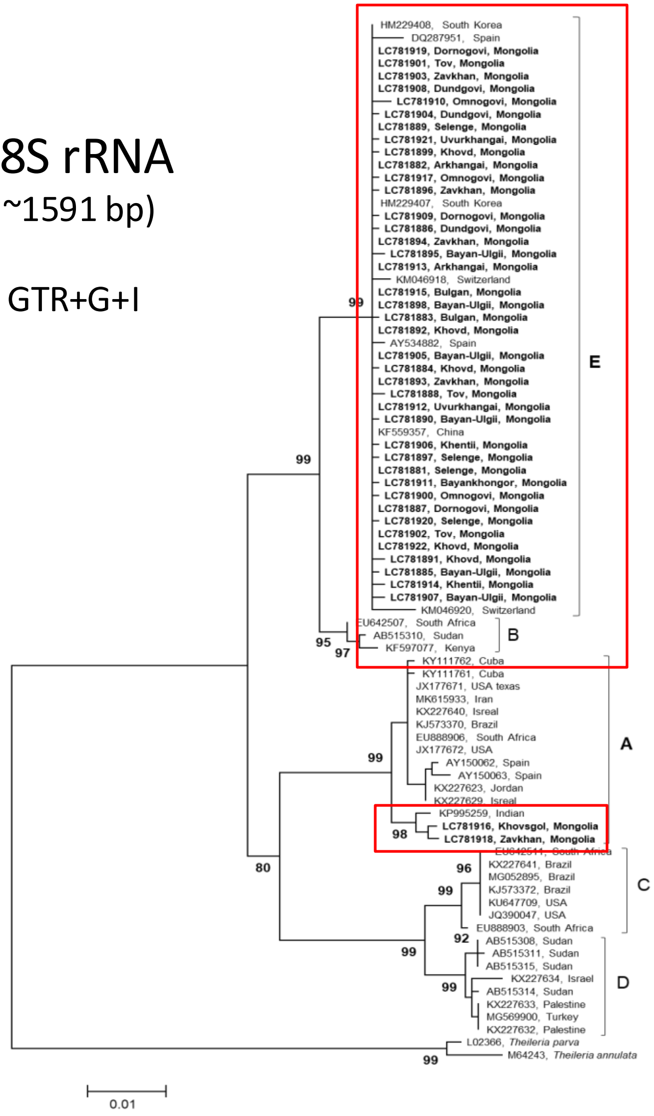
Otgonsuren et al., 2024; https://doi.org/10.1645/18-189

T. equi, 18sRNA gene sequence based **Results…**

Genotypes A and E were detected.

18S rRNA
(~1591 bp)

GTR+G+I



Otgonsuren et al., 2024; https://doi.org/10.1645/18-189

B. caballi, rap-1 gene sequence based **Results…**

A, B1 and a new clade were detected.

Rap1

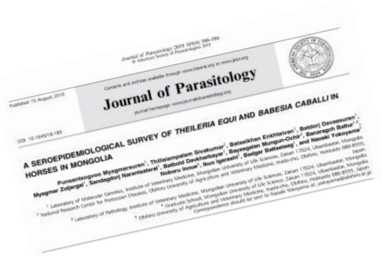
(~568 bp)

GTR+G+I

Otgonsuren et al., 2024; https://doi.org/10.1645/18-189

Equine piroplasmosis in Mongolia

Discussions



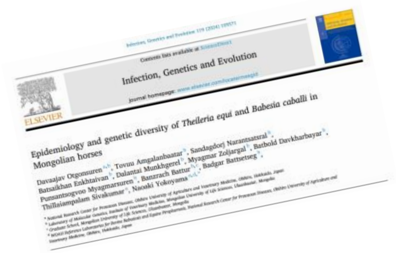
Published 15 August 2019

DOI: 10.1645/18-189

Journal of Parasitology

A SEROEPIDEMIOLOGICAL SURVEY OF *THEILERIA EQUI* AND *BABESIA CABALLI* IN HORSES IN MONGOLIA

Punsantsogvoo Myagmarsuren, Thillaiampalam Sivakumar, Batsaikhan Enkhtaivan, Baldorj Davaasuren, Myagmar Zoljargal, Sandagdorj Narantsatsral, Batbold Davkharbayar, Bayasgalan Mungun-Ochir, Banzragch Battur, Noboru Inoue, Ikuo Igarashi, Badgar Battsetseg, and Naoaki Yokoyama



Contents lists available at ScienceDirect

Infection, Genetics and Evolution

ELSEVIER

Epidemiology and genetic diversity of *Theileria equi* and *Babesia caballi* in Mongolian horses

Davaajav Otgonsuren, Tovuu Amgalanbaatar, Sandagdorj Narantsatsral, Batsaikhan Enkhtaivan, Dalantai Munkhgerel, Myagmar Zoljargal, Batbold Davkharbayar, Punsantsogvoo Myagmarsuren, Banzrach Battur, Badgar Battsetseg, Thillaiampalam Sivakumar, Naoaki Yokoyama

- A study investigated the sero-prevalence of *T. equi* in 1282 horses in various Mongolian provinces.
- Recently the PCR based-prevalence of *T. equi* in 1353 horses in various Mongolian provinces.

		Method	Positive rate	Reference
ELISA	*T. equi*	EMA-2 antigen	~ 33.0 %	(Myagmarsuren et al., 2019)
	B. caballi	Bc48 antigen	~ 14.3 %	
PCR	*T. equi*	PCR / 18sRNA gene	~ 78.2 %	(Otgonsurensuren et al., 2024)
	B. caballi	*Rap1* gene	~ 4.6 %	

Equine piroplasmosis in Mongolia

Discussions

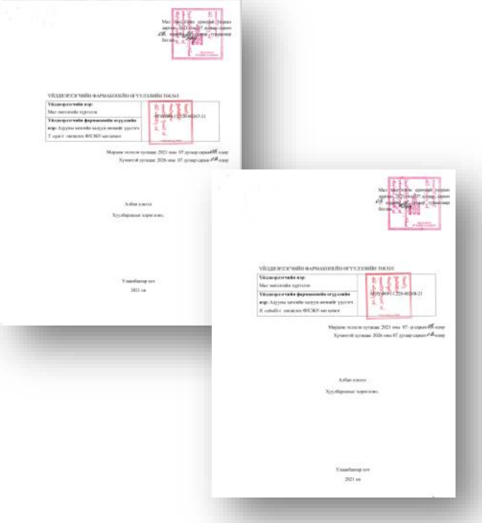
- The difference of positive rates for the parasites, specially for *T. equi's* in serological and PCR based methods may explained by following reasons.
- Because the recombinant proteins of the ELISA kit are prepared from only the parasites' A genotypes.
- However, the sequence analysis based on 18sRNA gene indicated that *T. equi*'s two different subtypes including A and E in Mongolian horses.
- Also, E type was more dominant than A subtype in Mongolian horses.
- The second possible reasons for the differential positivity's of *T. equi* and *B. caballi* may depend on their tick vectors and clearance time by host animals' immunity.

Conclusions

- All of the surveyed provinces had been exposed to both *T. equi* and *B. caballi* in Mongolia. The overall positive rate of *T. equi* was significantly higher than *B. caballi*.
- Microscopic screening reported that *T. equi* (12.0%) and B. *caballii* (1.6%), respectively.
- By ELISA, the positive rates of *T. equi* (33%) and B. *caballii* (14.2%) varied among provinces.
- By PCR, the positive rates of *T. equi* (78.2%) and B. *caballii* (4.6%) varied among provinces
- Based on 18S rRNA gene sequence, two genotypes (A and E) of *T. equi* were detected, while based on *rap-1* gene sequence there are three different genotypes (A, B1 and a new clade) for *B. caballi*.

ELISA kit for diagnosis

SATREPS project recommended diagnostic methods for effective diagnosis of piroplasmosis



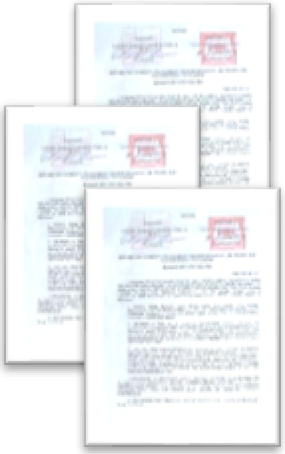
ELISA assay kit for detection of anti *T. equi antibody* MNS 6174:2010
ELISA assay kit for detection of anti *B. caballi* antibody MNS 6175:2010

These ELISA Kit are not commercial and produced in our laboratory.

Standard **MNS ISO/IEC 17025**

Diagnostic kits production and its applications

In response to a request from the General Department of Veterinary Medicine in Mongolia (GDVM), we have developed and distributed recombinant protein-based ELISA kits for the diagnosis of equine piroplasmosis to local veterinary centers throughout Mongolia in 2018-2023.



MNAS
Accreditation
system
TL 114
MNS ISO/IEC 17025

In the frame of Credentials (MNS ISO2018:17025), we have made a lot of services for equine piroplasmosis;

- Diagnosis
- Treatment
- Checking after treatment

Thank you for your kind attention!

SATREPS project all members

Equine Infectious Disease Surveillance in the Region of Hong Kong, SAR

Agriculture, Fisheries and Conservation Department
Inspection & Quarantine Branch
Equine Disease and Residues Control Division
Patrick Lau
Senior Veterinary Officer
patrick_it_lau@afcd.gov.hk

Equine Population in Hong Kong

- Around 2 040 horses in Hong Kong (including 660 in Conghua Equine Disease Free Zone)
- Approx. 900 horses in HKJC Shatin Stables, 90% horses are actively racing
- Equine animals include Thoroughbred, Pony and others but no Zebra, no Mule, no Donkey,

  NO WILD/ FERAL HORSES

漁農自然護理署
Agriculture, Fisheries and
Conservation Department

Distribution of Horses

Conghua Racecourse
(660 horses)

Riding School
(247 horses)

Riding School
(45 horses)

Conservation Farms
(5 horses)

Riding School
(29 horses)

Shatin Racecourse
(886 horses)

Riding School
(95 horses)

Riding School
(1 horse)

Riding School
(37 Horses)

Riding School
(36 Horses)

Happy Valley
Racecourse
(0 Horse)

As of 22/8/2024

漁農自然護理署
Agriculture, Fisheries and Conservation Department

Equine Animals in Hong Kong

Public Health (Animals and birds) Ordinance (Cap. 139)

- Quarantine and prevention of disease among animals and birds
- Importation and control of equines
- Issuance of licences for riding establishments

Pounds Ordinance (Cap. 168)

- Impound and control of stray animals

Wild Animals Protection Ordinance(Cap. 170)

- Conservation of wild animals

Rabies Ordinance (Cap. 421)

- Prevention and control of rabies

漁農自然護理署
Agriculture, Fisheries and
Conservation Department

Import & Export of Horses

- In 2023, a total of 478 horses were imported from overseas
- Bilateral import/export terms and veterinary health protocol with Schedule Countries
- Schedule countries: Argentina, Australia, Canada, Denmark, France, Germany, Italy, Japan, New Zealand, UAE, Great Britain and Northern Ireland and USA
- Testing requirements: EI, EIA, EVA and EP

2023 Horse Importing Countries

Argentina	Australia	France	England	Germany	Ireland	Italy	Japan	New Zealand	USA	**Total**
1	273	7	20	1	44	3	3	122	4	**478**

漁農自然護理署
Agriculture, Fisheries and Conservation Department

Establishment/ Full-time NCSC Staff and Responsibilities of Inspection and Quarantine Branch (596 staff)


(1AD, 1PVO, 1PSI, 1CCA*)

Technical Services Division

(1SVO, 1VO, 2SEOs, 1EOI, 1EOII, 2FOIs, 5EAs*, 2ACOs, 2PACOs*, 1CA/18 staff)

- Assist in formulation and review of animal health policy and veterinary legislation for IQ Branch
- Perform risk analysis and animal health related research
- Maintain close liaison on animal diseases and related issues with overseas and local authorities/ institutions
- Coordinate and provide administrative support to divisions of IQ Branch
- Provide administrative and professional support to the Veterinary Surgeons Board Secretariat

Animal Health Division

(1SVO, 4VOs, 1V*, 3SFOs, 10FOIs, 17FOIIs, 1AMI*, 9FAs, 1ART, 1WMI, 1WMII/49 staff)

- To monitor and control Avian Influenza (e.g. surveillance through collection of wild bird carcasses and poultry farm samples, handling bird nuisance cases, and combating illegal keeping of poultry, etc.)
- To monitor and provide advice for the usage of veterinary drugs and vaccine in local livestock farms
- To provide advice for local livestock farms on disease prevention and control (e.g. through enhancing farm hygiene conditions and biosecurity)
- To promote prudent and responsible antimicrobial use in food animals
- To conduct surveillance of antimicrobial resistance and antimicrobial use in food animals
- Responsible for coordinating culling operation (including allocation of manpower and necessary materials) in case of significant disease outbreak in local livestock farms such as Avian Influenza and Africa Swine Fever

Veterinary Laboratory Division

(1SVO, 1VP*, 7VOs, 2Vs*, 2SO(M)s, 1PSO(M)*, 1MO*, 1CRO*, 5VTs, 40AVTs, 2FOIs, 1SAHT*, 1AHT*, 1EA*, 1ESA*, 1PCO*, 1PACO*, 1SSA*, 2FAs, 7LAs, 1WMI/80 staff)

- Diagnostic and surveillance testing for livestock, poultry, bird, fish and other animals
- Laboratory testing for health certification of ornamental fish
- Diagnostic and surveillance testing for avian influenza
- Ante-mortem screening tests for chemical residues in food animals
- Antimicrobial resistance surveillance testing
- Post-mortem examination for animal cruelty and poisoning investigations

Import and Export Division

(1SVO, 2VOs, 3SFOs, 33FOIs, 54FOIIs, 13FAs, 2CFAs*, 2ARTs, 6WMIs, 1CWMI*, 1ACO, 1CA, 3CCAs*/122 staff)

- Prevention of the introduction of exotic animal disease into Hong Kong
- Enforce relevant regulations and policies related to the import and export of live animals, birds and animal products
- Facilitate and provide certification for export of live animals, aquatic animals and animal by-products

Equine Disease Division

(1SVO, 3VTs, 1CAVT*, 1EA*/6 staff)

- Laboratory testing for Equine Diseases to facilitate the movement of racehorses between Hong Kong and Conghua Equine Disease Free Zone in Guangdong

Plant and Pesticides Regulatory Division

(1SAO, 5AOs, 2PAOs*, 6SFOs, 1PSPO, 13FOIs, 23FOIIs, 1PFS*, 3PFOs*, 4CFOs*, 1FA, 4ACOs, 1CA, 1PCA*/66 staff)

- Plant import and phytosanitary control
- Plant varieties protection
- Pesticides control
- Prosecutorial services for AFCD

Animal Management (Development) Division

(1SVO, 2VOs, 3SFOs, 4FOIs, 12FOIIs, 1FS*, 1PFO*, 1ERO*, 1FA/26 staff)

- Draw up and implement publicity activities and public education programmes to promote animal welfare and Responsible Pet Ownership
- Combat illegal animal trading and boarding on the Internet and in pet grooming shops
- Review legislation, procedures and guidelines related to animal management and animal welfare
- Implement the 3-year "Trap-Neuter-Return" Trial Programme at designated places
- Issue Dog Breeder Licence (Category A) / One-off Permit / exemption documents under Cap. 139B, inspect licensed premises and discharge relevant duties
- Conduct public consultation and legislation amendment of Prevention of Cruelty to Animals Ordinance, Cap. 169

Animal Management (Operations) Division

(1SVO, 5VOs, 1V*, 6SFOs, 11FOIs, 47FOIIs, 4SFAs, 38FAs, 6PFAs*, 1CFA*, 10ARTs, 61WMIs, 8PWIs*, 20MDs, 1ACO, 3PACOs*, 1CA, 1PCA*/225 staff)

- Control of stray animals and enforce relevant regulations
- Issue dog licences and vaccinate dogs against rabies to prevent introduction of rabies
- Licensing and inspection of animal exhibitions, boarding facilities, riding facilities and animal traders
- Providing veterinary services to other disciplinary services
- Management of stray bovine animals
- Safeguard animal welfare

Remarks: AD-Assistant Director PVO-Principal Veterinary Officer SAO-Senior Agricultural Officer AO-Agricultural Officer SVO-Senior Veterinary Officer VO-Veterinary Officer SO(M)-Scientific Officer (Medical) SEO-Senior Executive Officer EOI-Executive Officer I EOII-Executive Officer II SFO-Senior Field Officer FOI-Field Officer I FOII-Field Officer II VT-Veterinary Technologist AVT-Associate Veterinary Technologist SFA-Senior Field Assistant FA-Field Assistant ART-Artisan LA-Laboratory Attendant WMI-Workman I WMII-Workman II PSI-Personal Secretary I ACO-Assistant Clerical Officer CA-Clerical Assistant MD-Motor Driver

Non Civil Servant Contract Staff: PAO-PRSC Agricultural Officer VP*-Veterinary Pathologist V*-Veterinarian PSO(M)*-PRSC Scientific Officer (Medical) MO*-Microbiological Officer CRO*-Chemical Residue Officer PSPO*-PRSC Senior Prosecution Officer CAVT*- Contract Associate Veterinary Technologist SAHT*-Senior Animal Health Technician AHT*-Animal Health Technician PFS*-PRSC Field Supervisor FS*-Field Supervisor PFO*-PRSC Field Officer CFO*-Contract Field Officer AMI*-Animal Management Inspector ERO*-Education Resources Officer EA*-Executive Assistant ESA*-Executive Service Assistant PCO*-PRSC Clerical Officer PACO*-PRSC Assistant Clerical Officer PCA*-PRSC Clerical Assistant CCA*-Contract Clerical Assistant SSA*-Support Service Assistant PFA*-PRSC Field Assistant CFA*-Contract Field Assistant PWI*-PRSC Workman I CWMI*-Contract Workman I

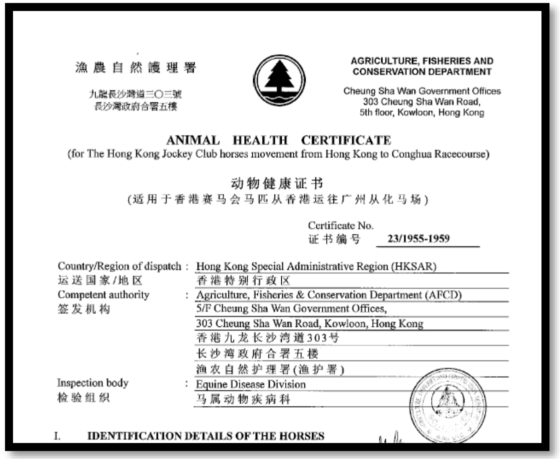
(Updated as at 01.07.2021)

漁農自然護理署
Agriculture, Fisheries and Conservation Department

Movement of Racehorses

- Since 2018, over 23 000 horses have traveled between Hong Kong and Conghua EDFZ
- More than 70 000 tests were conducted for:-

  Routine (fever) Cases, Biannual Surveillance and Ad-hoc Disease Testing
- Prior to travel, every horse is inspected by Certifying Veterinarian and accompanied by Animal Health Certificate



漁 農 自 然 護 理 署

九龍長沙灣道三〇三號
長沙灣政府合署五樓

AGRICULTURE, FISHERIES AND
CONSERVATION DEPARTMENT

Cheung Sha Wan Government Offices
303 Cheung Sha Wan Road,
5th floor, Kowloon, Hong Kong

ANIMAL HEALTH CERTIFICATE
(for The Hong Kong Jockey Club horses movement from Hong Kong to Conghua Racecourse)

动物健康证书
(适用于香港赛马会马匹从香港运往广州从化马场)

Certificate No.
证书编号 **23/1955-1959**

Country/Region of dispatch 运送国家/地区 : Hong Kong Special Administrative Region (HKSAR) 香港特别行政区

Competent authority 签发机构 : Agriculture, Fisheries & Conservation Department (AFCD)
5/F Cheung Sha Wan Government Offices,
303 Cheung Sha Wan Road, Kowloon, Hong Kong
香港九龙长沙湾道303号
长沙湾政府合署五楼
渔农自然护理署(渔护署)

Inspection body 检验组织 : Equine Disease Division
马属动物疾病科

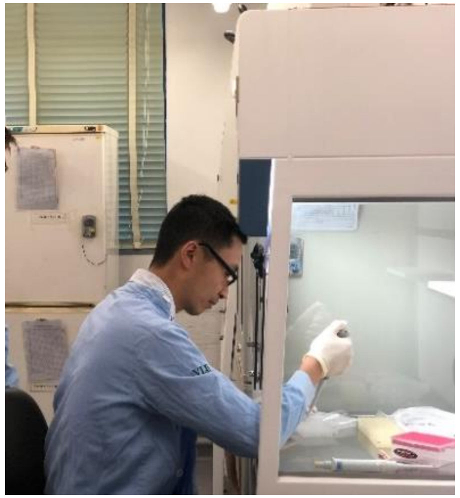
I. **IDENTIFICATION DETAILS OF THE HORSES**

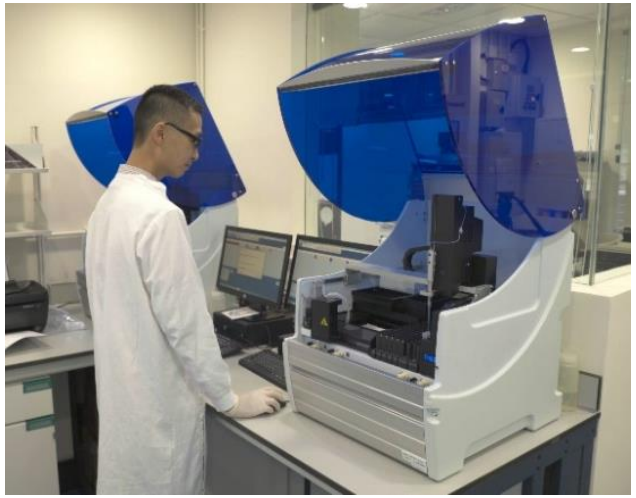
漁農自然護理署
Agriculture, Fisheries and
Conservation Department

Routine (fever) Cases

- Temperature of all racehorses taken twice daily
- Temperature > 38.5^{o}C (101.3^{o}F) must be reported and checked by an equine veterinarian
- Samples are submitted to AFCD Equine Disease Unit

  Equine Influenza, Strangles, Equine Viral Arteritis, African Horse Sickness





AGRICULTURE, FISHERIES & CONSERVATION DEPARTMENT, HKSAR.
香港特別行政區政府漁農自然護理署

Equine Disease Division, Tai Lung Veterinary Laboratory, Lin Tong Mei, Sheung Shui, HKSAR.

LABORATORY REPORT 化驗報告

Final Report

Case Number 档案编号: ED23-0002
Sample Received Date 样本接收日期: 04 Jan 2023
Report Date 报告日期: 06 Jan 2023
Sample Type 样本类型: NPS

Note:
1. For information (except the results) related to the title of the table shown below are provided by the submitter
2. Results only apply to the samples as received
3. The report shall not be reproduced except in full, without written approval of the laboratory

To 致: Dr. Alexandra Davis
The Hong Kong Jockey Club,1st Floor Shatin Central Complex, Shatin Racecourse, Shatin N.T.

Sample ID 样本标识号	**Sampling date** 样本日期	**Horse ID** 马匹标识号	**Breed** 品种	**Equine Influenza** 马流行性感冒 **rRT-PCR (Matrix)**
ED23-0002-00001	31/12/2022	G401	Thoroughbred	NEGATIVE 阴性
ED23-0002-00002	02/01/2023	E445	Thoroughbred	NEGATIVE 阴性
ED23-0002-00003	02/01/2023	C466	Thoroughbred	NEGATIVE 阴性

Yours faithfully

Wai Kin-lung,Chris
for Dr. Lau Im-tung, Patrick
Senior Veterinary Officer / Equine Disease Division 高级兽医师 /马属动物疾病科

NATA Accredited Laboratory
Accredited for compliance with ISO/IEC 17025 - Testing
Number: 14756
NATA endorsed test report. This document shall not be reproduced, except in full.

VETERINARY LABORATORY DIVISION
NATA Accredited in the field of Veterinary Testing for the following: Bacteriology, Mycology, Other Microorganisms, Virology, Serology of infection, Biochemistry, Drugs and Drug metabolites, Anatomical Pathology, Histopathology, Necropsy.

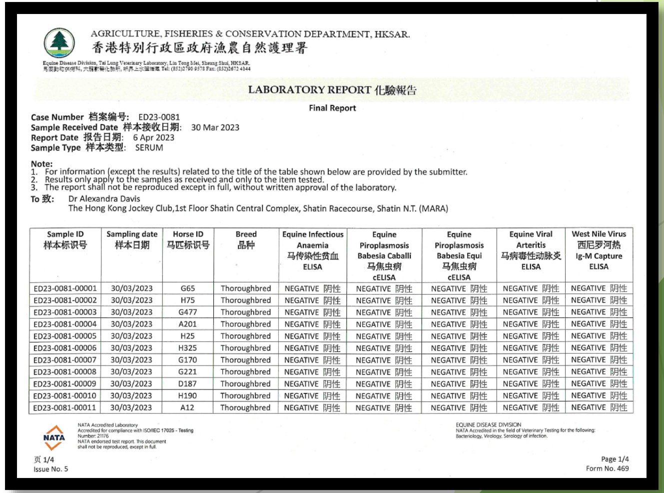
页 1/1 Page 1/1
Issue No. 4 Form No. 469

漁農自然護理署
Agriculture, Fisheries and Conservation Department

Biannual Surveillance

- Twice a year samples are collected randomly from racehorses in Hong Kong and Conghua EDFZ on the same day; usually in March and August
- Concerted effort between AFCD, MARA, GACC and HKJC
- Samples are submitted to AFCD Equine Disease Unit, HVRI and IQTC

EIA, EP, EVA, WNF, AHS, EI (Ab titre), JE (Ab titre)



AGRICULTURE, FISHERIES & CONSERVATION DEPARTMENT, HKSAR.
香港特別行政區政府漁農自然護理署
Equine Disease Division, Tai Lung Veterinary Laboratory, Lin Tong Mei, Sheung Shui, HKSAR.
馬類動物病務科, 大龍獸醫化驗所, 新界上水蓮塘尾 Tel: (852)2790 9578 Fax: (852)2672 4144

LABORATORY REPORT 化驗報告

Final Report

Case Number 档案编号: ED23-0081
Sample Received Date 样本接收日期: 30 Mar 2023
Report Date 报告日期: 6 Apr 2023
Sample Type 样本类型: SERUM

Note:
1. For information (except the results) related to the title of the table shown below are provided by the submitter.
2. Results only apply to the samples as received and only to the item tested.
3. The report shall not be reproduced except in full, without written approval of the laboratory.

To 致: Dr Alexandra Davis
The Hong Kong Jockey Club,1st Floor Shatin Central Complex, Shatin Racecourse, Shatin N.T. (MARA)

Sample ID 样本标识号	Sampling date 样本日期	Horse ID 马匹标识号	Breed 品种	Equine Infectious Anaemia 马传染性贫血 ELISA	Equine Piroplasmosis Babesia Caballi 马焦虫病 cELISA	Equine Piroplasmosis Babesia Equi 马焦虫病 cELISA	Equine Viral Arteritis 马病毒性动脉炎 ELISA	West Nile Virus 西尼罗河热 Ig-M Capture ELISA
ED23-0081-00001	30/03/2023	G65	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00002	30/03/2023	H75	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00003	30/03/2023	G477	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00004	30/03/2023	A201	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00005	30/03/2023	H25	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00006	30/03/2023	H325	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00007	30/03/2023	G170	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00008	30/03/2023	G221	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00009	30/03/2023	D187	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00010	30/03/2023	H190	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00011	30/03/2023	A12	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性

NATA Accredited Laboratory
Accredited for compliance with ISO/IEC 17025 - Testing
Number: 21176
NATA endorsed test report. This document shall not be reproduced, except in full.

EQUINE DISEASE DIVISION
NATA Accredited in the field of Veterinary Testing for the following:
Bacteriology, Virology, Serology of infection.

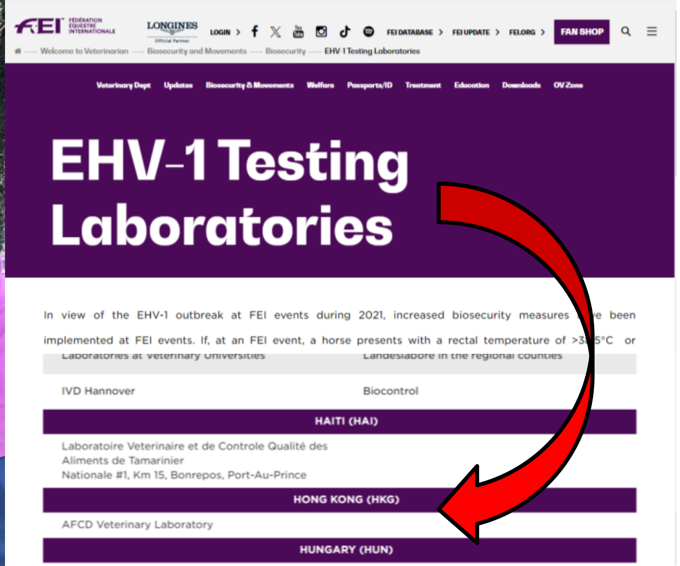
页 1/4
Issue No. 5

Page 1/4
Form No. 469

漁農自然護理署
Agriculture, Fisheries and
Conservation Department

Ad-hoc Disease Testing

- AHS outbreak in Thailand, testing peaked at an average of 60 tests per month
- EHV-1 outbreak in Europe, accredited laboratory for FEI
- Recently developed Taqman Array Card for screening of 18 equine infectious diseases



EHV-1 Testing Laboratories

In view of the EHV-1 outbreak at FEI events during 2021, increased biosecurity measures ve been implemented at FEI events. If, at an FEI event, a horse presents with a rectal temperature of >3 5°C or

Laboratories at Veterinary Universities	Landeslabore in the regional counties
IVD Hannover	Biocontrol

HAITI (HAI)

Laboratoire Veterinaire et de Controle Qualité des
Aliments de Tamarinier
Nationale #1, Km 15, Bonrepos, Port-Au-Prince

HONG KONG (HKG)

AFCD Veterinary Laboratory

HUNGARY (HUN)

Annual Surveillance Report

AGRICULTURE, FISHERIES & CONSERVATION DEPARTMENT, HKSAR.
香港特別行政區政府漁農自然護理署

Equine Disease Division, Tai Lung Veterinary Laboratory, Lin Tong Mei, Sheung Shui, HKSAR.
馬類動物疾病科，大龍獸醫化驗所，新界上水蓮塘尾 Tel: (852)2790 9578 Fax: (852)2672 4144

LABORATORY REPORT 化驗報告

Final Report

Case Number 档案编号: ED23-0081
Sample Received Date 样本接收日期: 30 Mar 2023
Report Date 报告日期: 6 Apr 2023
Sample Type 样本类型: SERUM

Note:
1. For information (except the results) related to the title of the table shown below are provided by the submitter.
2. Results only apply to the samples as received and only to the item tested.
3. The report shall not be reproduced except in full, without written approval of the laboratory.

To 致: Dr Alexandra Davis
The Hong Kong Jockey Club,1st Floor Shatin Central Complex, Shatin Racecourse, Shatin N.T. (MARA)

Sample ID 样本标识号	Sampling date 样本日期	Horse ID 马匹标识号	Breed 品种	Equine Infectious Anaemia 马传染性贫血 ELISA	Equine Piroplasmosis Babesia Caballi 马焦虫病 cELISA	Equine Piroplasmosis Babesia Equi 马焦虫病 cELISA	Equine Viral Arteritis 马病毒性动脉炎 ELISA	West Nile Virus 西尼罗河热 Ig-M Capture ELISA
ED23-0081-00001	30/03/2023	G65	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00002	30/03/2023	H75	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00003	30/03/2023	G477	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00004	30/03/2023	A201	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00005	30/03/2023	H25	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00006	30/03/2023	H325	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00007	30/03/2023	G170	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00008	30/03/2023	G221	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00009	30/03/2023	D187	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00010	30/03/2023	H190	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性
ED23-0081-00011	30/03/2023	A12	Thoroughbred	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性	NEGATIVE 阴性

NATA Accredited Laboratory
Accredited for compliance with ISO/IEC 17025 - Testing
Number: 21176
NATA endorsed test report. This document shall not be reproduced, except in full.

EQUINE DISEASE DIVISION
NATA Accredited in the field of Veterinary Testing for the following:
Bacteriology, Virology, Serology of infection.

漁農自然護理署
Agriculture, Fisheries and Conservation Department

Equine Infectious Disease Surveillance

- Public Private Partnership since horseracing began in 1846
- International horseracing since 1988, and partnership with overseas laboratories
- AFCD Equine Disease Unit – official laboratory testing established in 2017
- Equine Disease Free Zone established permanently in Guangzhou, Conghua

Equine disease free zones (EDFZ)

Facilitation of International Competition Horse Movement - HHP horses - Regional conferences

Principle

Complementary to the HHP concept, a **temporary establishment of a zone which is free of multiple specified equine diseases** ("Equine Disease Free Zone"- EDFZ) can be considered by countries that wish to host an international equine sport event but where the control and eradication of all equine diseases in their entire territory is not feasible or achievable.

The establishment of an EDFZ is an extension of the concept of zoning as defined in the *Code* (Chapter 4.3 Zoning and Compartmentalisation). Horses within an EDFZ are protected from diseases that may occur in other parts of a country. Separation of the subpopulation inside the EFDZ from the general population (i.e. equines and other species outside the EDFZ) is achieved by the implementation of sound biosecurity management, certification standards and procedures, contingency planning and the identification of all horses resident in the EFDZ and the capacity to trace their movement.

The combination of the HHP and the EDFZ concepts can allow countries not recognised as an approved country for the free movement of horses to host an international sport event.

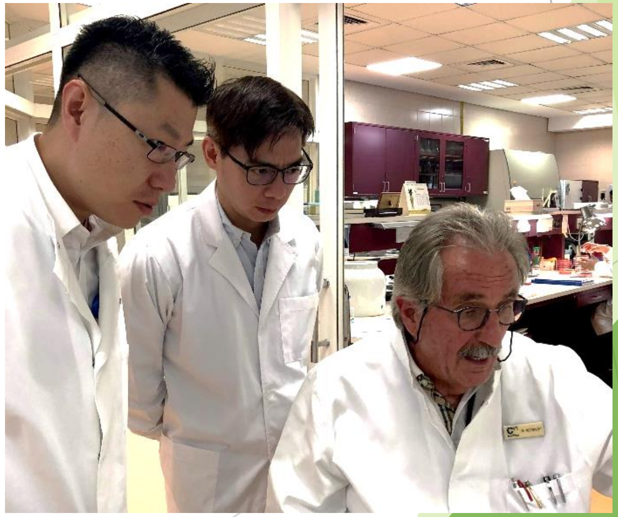
Implementation

EDFZ was successfully applied during the 16th Asian Games in Guangzhou (**China**) in 2010. Based on this successful experience, the Hong Kong veterinary services, Mainland China veterinary authorities and the Hong Kong Jockey Club have engaged in a Public Private Partnership to transform this venue into a permanent EDFZ in view of facilitating safe regular cross-border transportation of race horses between the Hong Kong Special Administrative region and the EDFZ in Guangzhou.

漁農自然護理署
Agriculture, Fisheries and
Conservation Department

Equine Infectious Disease Surveillance

- Partnership with WOAH Ref. Lab. (JP) NRCPD, (CN) HVRI, (GB) Pirbright, (IE) IEC, etc.
- Receiving samples from Australia and New Zealand authorities since 2022
- Inter-laboratory trials, comparisons and reviews with Mainland laboratories
- Equine disease laboratory network in the Greater Bay Area



漁農自然護理署
Agriculture, Fisheries and
Conservation Department

Equine Disease & Residues Control Division

- New building complex -
  Kai Tak Veterinary Testing Facility
- Establish and maintain a network with other institutions designated for the international movement of racehorses
- Identify, cultivate and maintain consultant(s) in this field of expertise
- Organise, coordinate, present and harmonise the international standard for the movement of racehorses

漁農自然護理署
Agriculture, Fisheries and
Conservation Department

Thank You

patrick_it_lau@afcd.gov.hk

漁農自然護理署
Agriculture, Fisheries and
Conservation Department

EDFZ Hangzhou Asian Games 2023 in People's Republic of China

Kenneth Lam BSc(Hons), BVetMed, PhD, CertVA, MRCVS
Executive Manager, International Veterinary Liaison & Epidemiology
Hong Kong Jockey Club
WOAH IHSC

Publication of self-declarations on horse related diseases including EDFZ at the WOAH website

2010

16TH ASIAN GAMES
Guangzhou 2010

2014

17th Asian Games
INCHEON 2014

2016

Rio2016

Rio2016
PARALYMPIC GAMES

2017

Asian Indoor & Martial Arts Games
TURKMENISTAN
Ashgabat 2017

2018

18th ASIAN GAMES
Jakarta
Palembang
2018

2021

SYRIAN EQUESTRIAN FEDERATION
الاتحاد العربي السوري للفروسية

2021

TOKYO 2020

TOKYO 2020
PARALYMPIC GAMES

2022

19th Asian Games
Hangzhou 2022

2022

INTERNATIONAL SPORTS CONFEDERATION
IHSC

World
Organisation
for Animal
Health

Organización
Mundial
de Sanidad
Animal

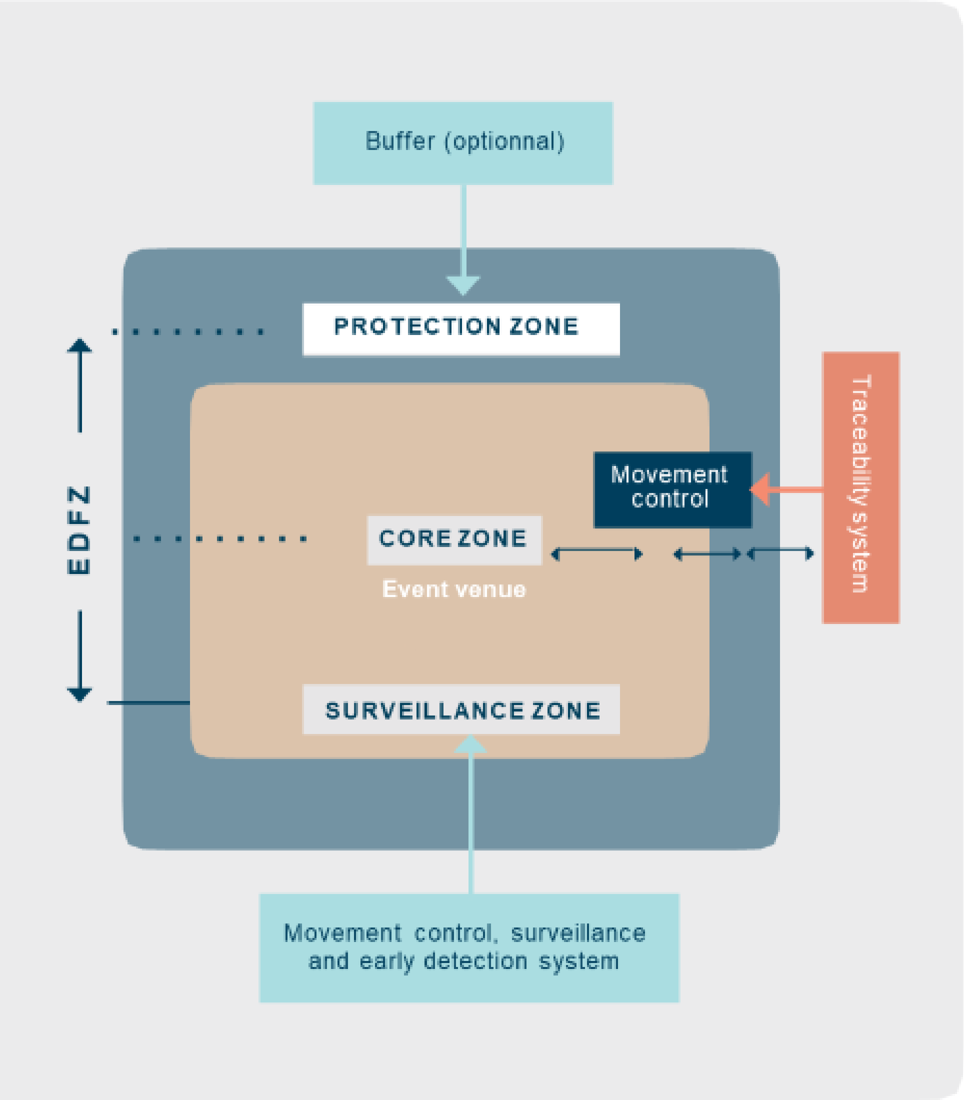
Adoption of a risk-based approach

When international equestrian sport competitions are planned in countries where the control or eradication of specific equine diseases is not achievable throughout the whole territory, it is possible to define specific zone(s) in which equine disease risks are mitigated.

Equine Disease Free Zones (EDFZ) allow the separation of imported horses from the local equid population. They aim to protect both imported horses from diseases that may occur in other parts of the territory and the equid population of the host country from imported diseases.

What are the criteria for an EDFZ?

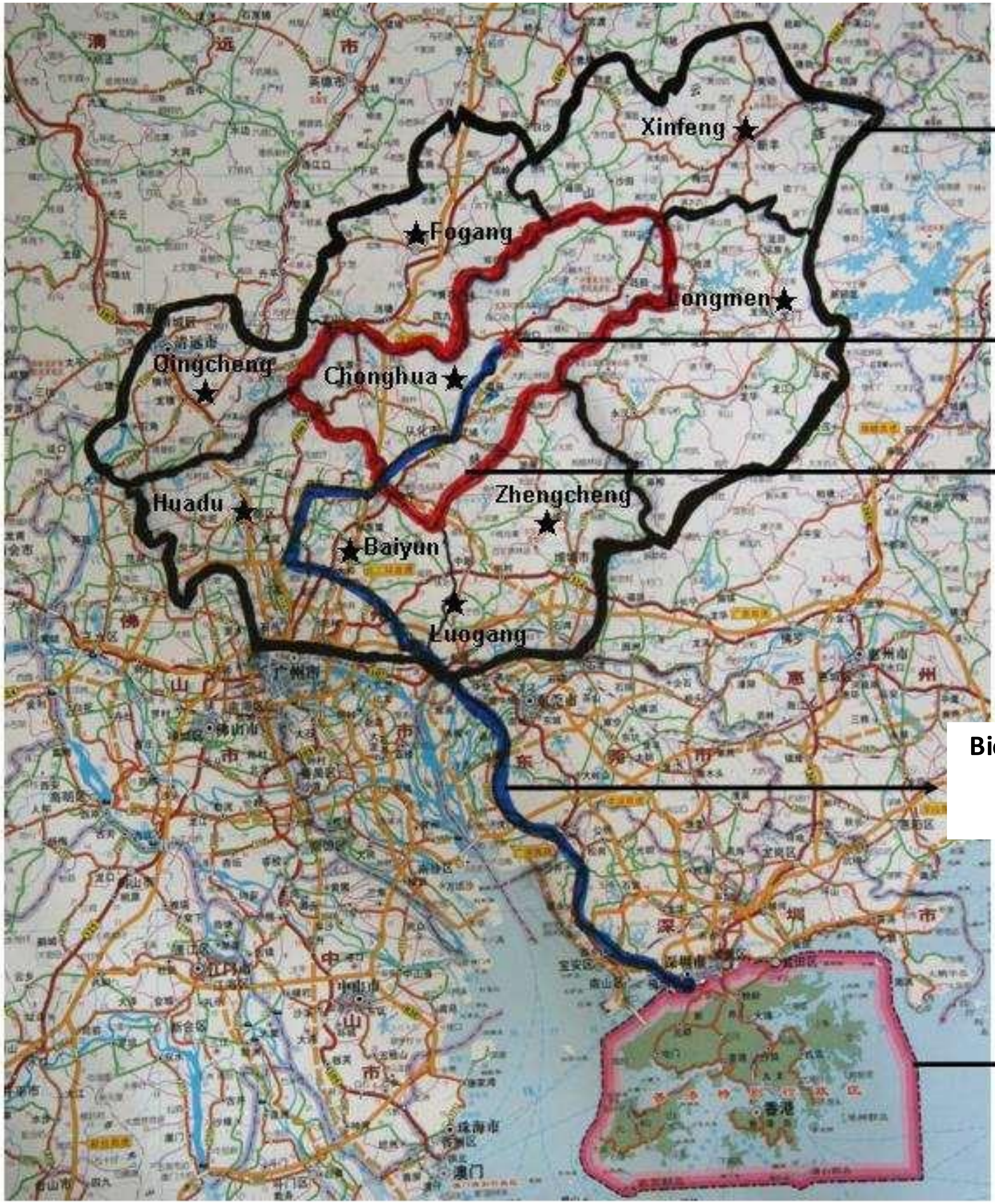
- Establishment of a defined area under the control of the Veterinary Authorities.
- Identification of a limited equine population with a known health status.
- Implementation of specific biosecurity measures.
- Traceability and movement control: certification procedures for all horses moving into and out of the zone.
- Existence of a contingency plan for effective risk management.



Case study on 1st EDFZ in 2010 (Grandfather)

- **16th Asian Games, Conghua, Guangzhou, China – November, 2010.**
- **Based on experiences e.g. Sydney & China Equestrian Olympics and Paralympics (Hong Kong)**

Application of OIE Zoning principles



Protection Zone

Core Zone
(Equestrian Site)

Surveillance Zone

Biosecurity Highway Passage

Hongkong

★ Locations of animal health supervision institution of relevant areas

Panorama 2019-2

Français | **English** | Español

World Organisation for Animal Health
Founded as OIE

bulletin

SUBSCRIBE / LOG IN

THE OFFICIAL | PANORAMA | OIE NEWS | ARCHIVES

OIE WORLD ORGANISATION FOR ANIMAL HEALTH
Protecting animals, preserving our future

PANORAMA > AROUND THE WORLD > 04-3-2 LONG-TERM, EQUINE-DISEASE-FREE ZONE (EDFZ) IN GUANGZHOU, THE PEOPLE'S REPUBLIC OF CHINA

AROUND THE WORLD

SUCCESS STORIES

04-3-2 Long-term, Equine-Disease-Free Zone (EDFZ) in Guangzhou, the People's Republic of China

https://bulletin.woah.org/?panorama=04-3-2-edfz-conghua-en

AUTHORS

Kenneth Lam(1)* & Brian Stewart(1)

(1) Hong Kong Jockey Club, Hong Kong Special

Font size - AAA +

SUMMARY

A long-term, Equine-Disease-Free Zone (EDFZ) has been in operation in Guangzhou, the People's Republic of China,

PRC Hangzhou Asian Game 2023

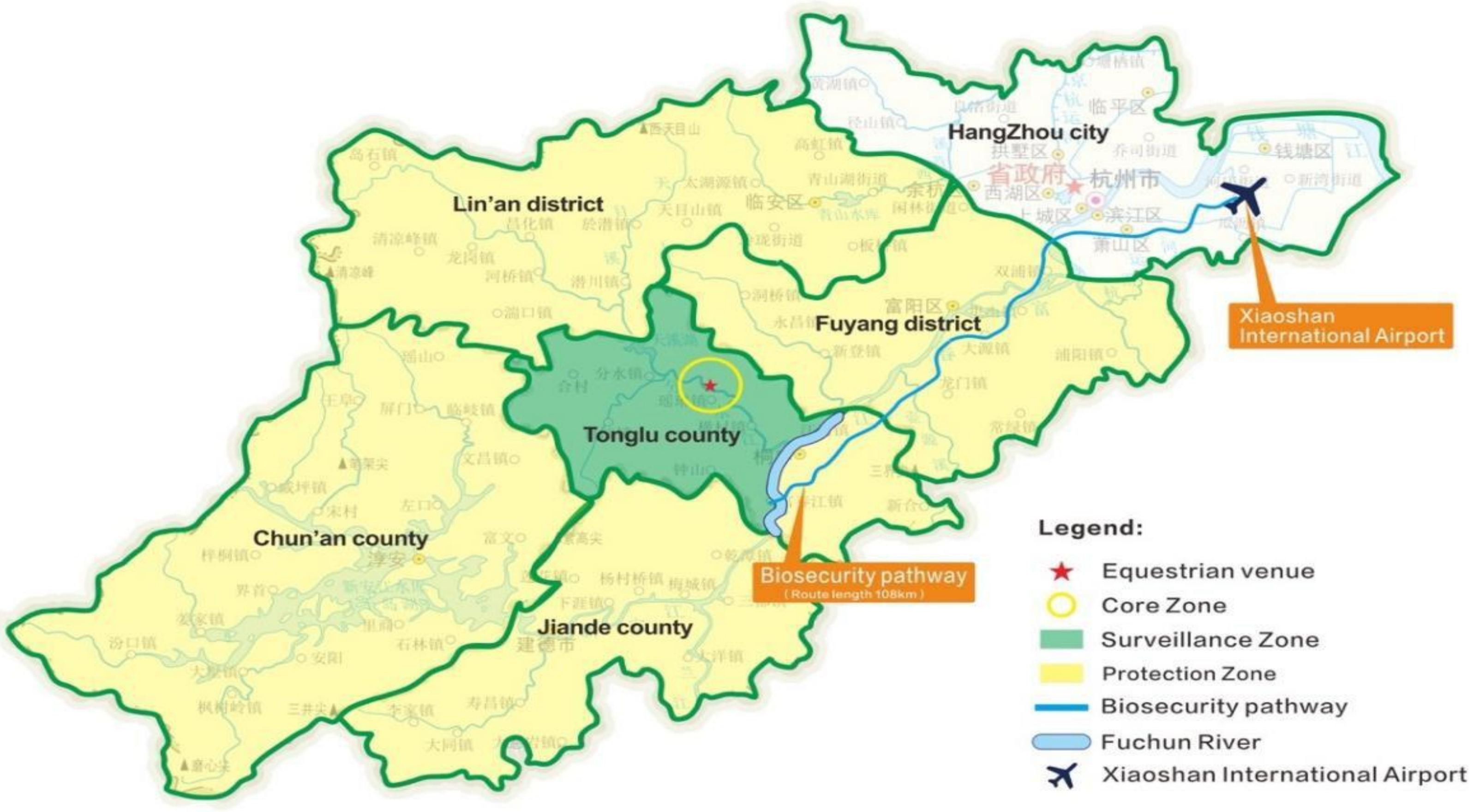
Self-declaration of an Equine Disease-Free Zone in Tonglu, Hangzhou, China for the purpose of facilitating the Equestrian competitions for the 19th Asian Games Hangzhou 2022, Equine Disease-Free Zone (EDFZ) is established in Tonglu, Hangzhou, Zhejiang province in China.

Self-declaration submitted to the World Organisation for Animal Health on 8 February 2022 by Dr Baoxu Huang, Delegate of China to the OIE.

A. Executive Summary

1.Acronyms
2.Veterinary Services
3.Structure and barrier of the EDFZ
4.Equine disease situation
5.Maintenance of EDFZ
6.Conclusions

https://www.woah.org/app/uploads/2022/03/2022-03-chinapeop-s-rep-ofedfz-selfd.pdf



1.1 CZ

The CZ includes the venue and surrounding areas with a radius of 5 km (Figure 2). The construction of the CZ fully considers the requirements of biosecurity, ease of management, and reasonable spatial layout. No equine animals and other susceptible animals to horse-related diseases (such as pigs, cattle, sheep) are kept in the area. Biosecurity measures are taken against vectors and susceptible wild animals.

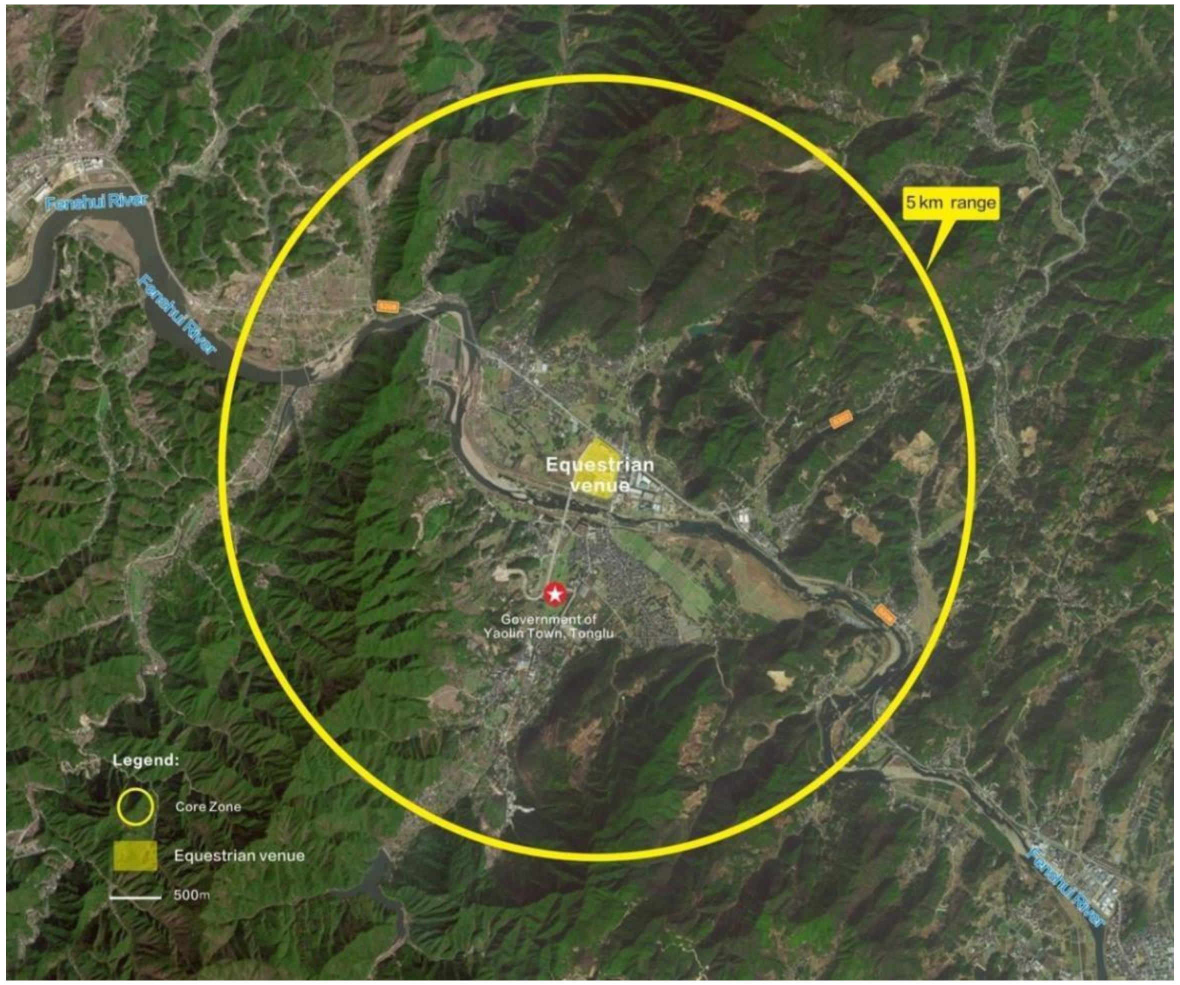


Figure 2: Schematic diagram of equestrian venue and CZ

Highlights of Hangzhou EDFZ (1)

- Similar approach to establishment of Conghua EDFZ, Guangzhou

- *Clear layout of EDFZ including The Core Zone (5km radius) with no resident horses in the venue and The Survelliance Zone (about 55km long from east to west, 46 km wide from north to south, and covers an area of about 1,220 km^2). There are no equids in the CS & SZ of EDFZ.*

- *The Protection Zone is set up around the EDFZ (with an area of about 12300 km^2)*

- *Airport connected to venue via highway biosafety passage*

- The declaration covers **19 diseases**

- Additionally, **China has been officially recognized as historically free from AHS by the WOAH and maintained the AHS-free status since 2014**.

Highlights of Hangzhou EDFZ – Veterinary Services

- PRC Ministry of Agriculture & Rural Affairs (MARA) is responsible for the national animal husbandry and veterinary work in China.

- The Animal Husbandry and Veterinary Bureau of MARA is responsible for organizing and implementing animal disease prevention and the supervision and management activities in the People's Republic of China.

- The General Administration of Customs of the People's Republic of China is responsible for the quarantine, supervision and administration of entry and exit of animals and animal products.

Highlights of Hangzhou EDFZ (3)

- The local people's governments of Zhejiang Province, Hangzhou City and the counties (cities and districts) involved in EDFZ have established a comprehensive veterinary work system, including veterinary administration, technical support and supervision and enforcement systems, which can effectively carry out animal disease **prevention and management, disease monitoring, supervision and inspection**.

Animal Population in EDFZ, PZ & Biosafety Passage

- As of 30 December 2021, there are no equids kept in the CZ & SZ of EDFZ.

- The livestock in the Surveillance Zone of EDFZ were pigs 53,251, cattle 572, sheep 2,482 and dogs 14,911.

- The 166 equids that have been registered in the PZ, including 23 working horses and 39 mules, as well as 104 ornamental horses, donkeys and zebras, are all kept in Fuyang Zoo, Hangzhou.

- The livestock in the PZ were pigs 446,398, cattle, 5,155, sheep 62,767 and dogs 77,306.

- There are no equids and susceptible animals to horse-related diseases (pig, cattle, sheep) kept along the biosecurity pathway.

Equine Disease Situation

- In accordance with the Animal Disease Prevention Law of the People's Republic of China, MARA has established an animal disease reporting system, published a list of reportable animal diseases, and regularly reported the reportable equine diseases to WOAH as required. The People's Government of Hangzhou, Zhejiang Province formulated the Emergency Plan for Tonglu Equine Disease-Free Zone, Hangzhou, and **listed 19 equine diseases**

19 Equine Diseases under surveillance

No.	Notifiable equine disease in China	Diseases status (last case)	Surveillance
1	AHS	Never occurred, China is officially recognised as free from AHS by the OIE	General and Targeted surveillance
2	Vesicular stomatitis	Disease never reported	General surveillance
3	West Nile fever	Disease never reported	General surveillance
4	Nipah virus disease	Disease never reported	General surveillance
5	Hendra disease	Disease never reported	General surveillance
6	Equine encephalomyelitis (including Eastern and Western)	Disease never reported	General surveillance
7	Venezuelan equine Encephalomyelitis	Disease never reported	General surveillance
8	Contagious equine metritis	Disease never reported	General surveillance
9	Equine infectious anemia	(01/2010, in Guangdong, equine)	General and Targeted surveillance
10	Glanders	(12/2019, in Chongqing, equine)	General and Targeted surveillance

19 Equine Diseases under surveillance

11	Equine viral arteritis	Disease never reported	General surveillance
12	Equine piroplasmosis	Disease never reported	General surveillance
13	Equine influenza	(12/2021, in Yunnan, equine)	General surveillance
14	Japanese encephalitis	(06/2021, in Henan, Hunan, Jiangxi, Shaanxi, pig)	General surveillance
15	*Trypanosoma evansi* (surra)	(12/2021, in Shaanxi, equine)	General surveillance
16	Dourine	(01/2021, in Inner Mongolia, equine)	General surveillance
17	Anthrax	(09/2021, in Inner Mongolia, cattle and other animals; in Tibet, sheep)	General surveillance
18	Rabies	(09/2021, in Inner Mongolia, other animals; in Henan, dog)	General surveillance
19	Equine rhinopneumonitis (EHV-1)	(06/2021, in Tibet, equine)	General surveillance

Veterinary Laboratory Capabilities

- The veterinary laboratory system consists of a national laboratory and local veterinary laboratories, which effectively support the surveillance, diagnosis and epidemiological investigation of the EDFZ equine diseases.
- At the national level, there are national veterinary laboratories such as **National Center for Diagnosis of Exotic Animal Diseases (NCDEAD) in China Animal Health and Epidemiology Center**,
- **OIE Reference Laboratory for equine infectious anaemia** and the National Reference laboratory for **glanders** in **Harbin Veterinary Research Institute (HVRI) of Chinese Academy of Agricultural Sciences.**
- At the local level, the Local Veterinary Laboratories (LVL) in Zhejiang Provincial Animal Disease Control Center, Hangzhou, EDFZ and PZ are in charge of implementing the equine disease testing.

Equine Disease Surveillance

- In order to control the situation of the aforementioned 19 diseases in the EDFZ including Surveillance Zone and Protection Zone, routine epidemiological investigation and surveillance plans, regular epidemiological investigations on susceptible animals are conducted in the region and carry out active and passive monitoring.

- Vectors and susceptible wild animals are sampled for investigation and active monitoring. The results of laboratory serology and pathogen surveillance conducted showed no positive cases to the 19 equine diseases in the EDFZ in the past **12 months**.

Surveillance in the Protection Zone (PZ)

- There is no equids in the Surveillance Zone.
- In the PZ, a total of 1,316 equine samples (including 446 serum, 437 nasal swabs and 433 blood samples) were collected, for the surveillance of 19 diseases.
- Results of testing showed that all tests for the detection of the pathogenic agent were negative, and all tests for the detection of immune response were negative except for equine influenza and Japanese encephalitis
- Vector investigation and surveillance in the EDFZ and the PZ
- Investigation and surveillance in susceptible wildlife

Surveillance results of 19 equine disease in PZ

Disease	Surveillance time	No. equids	Category of equid*			No. sample	Method	Lab	Result	
			Working horse	Working mule	Ornamental horses/ donkeys/ zebras, etc.				pos	neg
Equine infectious anemia	Aug.2020	278	61	83	134	156	AGID	VDC-ZAFU	0	156
	Nov.2020	230	42	64	124	118	AGID	VDC-ZAFU	0	118
	May.2021	207	33	48	126	93	cELISA	LVL	0	93
	Oct.2021	173	24	43	106	79	cELISA	LVL	0	79
Glanders	Aug.2020	278	61	83	134	156	CFT	HVRI	0	156
	Nov.2020	230	42	64	124	118	CFT	HVRI	0	118
	May.2021	207	33	48	126	93	CFT	HVRI	0	93
	Oct.2021	173	24	43	106	79	CFT	HVRI	0	79
Equine viral arteritis	Aug.2020	278	61	83	134	156	ELISA	VDC-ZAFU	0	156
	Nov.2020	230	42	64	124	118	ELISA	VDC-ZAFU	0	118
	May.2021	207	33	48	126	93	ELISA	LVL	0	93
	Oct.2021	173	24	43	106	79	ELISA	LVL	0	79
Equine piroplasmosis	Aug.2020	278	61	83	134	156	ELISA	VDC-ZAFU	0	156
	Nov.2020	230	42	64	124	118	ELISA	VDC-ZAFU	0	118
	May.2021	207	33	48	126	93	ELISA	LVL	0	93
	Oct.2021	173	24	43	106	79	ELISA	LVL	0	79
Trypanosoma evansi (surra)	Aug.2020	278	61	83	134	156	CATT	VDC-ZAFU	0	156
	Nov.2020	230	42	64	124	118	CATT	VDC-ZAFU	0	118
	May.2021	207	33	48	126	93	ELISA	LVL	0	93
	Oct.2021	173	24	43	106	79	ELISA	LVL	0	79
Dourine	Aug.2020	278	61	83	134	156	ELISA	HVRI	0	156
	Nov.2020	230	42	64	124	118	ELISA	VDC-ZAFU	0	118
	May.2021	207	33	48	126	93	ELISA	LVL	0	93
	Oct.2021	173	24	43	106	79	ELISA	LVL	0	79
Equine influenza	Aug.2020	278	61	83	134	151	RT-PCR	VDC-ZAFU	0	151
	Nov.2020	230	42	64	124	114	RT-PCR	VDC-ZAFU	0	114
	May.2021	207	33	48	126	93	RT-PCR	LVL	0	93
	Oct..2021	173	24	43	106	79	RT-PCR	LVL	0	79
	Aug.2020	278	61	83	134	147	RT-PCR	HVRI	0	147

Japanese encephalitis	Nov.2020	230	42	64	124	114	RT-PCR	HVRI	0	114
	May.2021	207	33	48	126	93	RT-PCR	LVL	0	93
	Oct.2021	173	24	43	106	79	RT-PCR	LVL	0	79
Equine rhinopneumonitis (EHV-1)	Aug.2020	278	61	83	134	156	ELISA	HVRI	0	156
	Nov.2020	230	42	64	124	118	ELISA	HVRI	0	118
	May.2021	207	33	48	126	93	ELISA	HVRI	0	93
	Oct.2021	173	24	43	106	79	ELISA	HVRI	0	79
Africa horse sickness	Aug.2020	278	61	83	134	31	RT-PCR	HVRI	0	31
	Nov.2020	230	42	64	124	24	RT-PCR	HVRI	0	24
	May.2021	207	33	48	126	93	ELISA	NCDEAD	0	93
	Oct.2021	173	24	43	106	79	ELISA	NCDEAD	0	79
Equine encephalomyelitis (including Eastern and Western)	Aug.2020	278	61	83	134	31	RT-PCR	HVRI	0	31
	Nov.2020	230	42	64	124	24	RT-PCR	HVRI	0	24
	May.2021	207	33	48	126	93	RT-PCR	NCDEAD	0	93
	Oct.2021	173	24	43	106	79	RT-PCR	NCDEAD	0	79
Vesicular stomatitis	Aug.2020	278	61	83	134	31	RT-PCR	HVRI	0	31
	Nov.2020	230	42	64	124	24	RT-PCR	HVRI	0	24
	May.2021	207	33	48	126	93	RT-PCR	NCDEAD	0	93
	Oct.2021	173	24	43	106	79	RT-PCR	NCDEAD	0	79
Nipha virus disease	Aug.2020	278	61	83	134	31	RT-PCR	HVRI	0	31
	Nov.2020	230	42	64	124	24	RT-PCR	HVRI	0	24
	May.2021	207	33	48	126	93	ELISA	NCDEAD	0	93
	Oct.2021	173	24	43	106	79	ELISA	NCDEAD	0	79
West Nile fever	Aug.2020	278	61	83	134	31	RT-PCR	HVRI	0	31
	Nov.2020	230	42	64	124	24	RT-PCR	HVRI	0	24
	May.2021	207	33	48	126	93	ELISA	NCDEAD	0	93
	Oct.2021	173	24	43	106	79	ELISA	NCDEAD	0	79
Hendra disease	Aug.2020	278	61	83	134	31	RT-PCR	HVRI	0	31
	Nov.2020	230	42	64	124	24	RT-PCR	HVRI	0	24
	May.2021	207	33	48	126	93	ELISA	NCDEAD	0	93
	Oct.2021	173	24	43	106	79	ELISA	NCDEAD	0	79
Venezuelan equine encephalomyelitis	Aug.2020	278	61	83	134	31	RT-PCR	HVRI	0	31
	Nov.2020	230	42	64	124	24	RT-PCR	HVRI	0	24
	May.2021	207	33	48	126	93	RT-PCR	NCDEAD	0	93
	Oct.2021	173	24	43	106	79	RT-PCR	NCDEAD	0	79
Contagious equine metritis	Aug.2020	278	61	83	134	31	RT-PCR	HVRI	0	31
	Nov.2020	230	42	64	124	24	RT-PCR	HVRI	0	24
	May.2021	207	33	48	126	93	RT-PCR	HVRI	0	93
	Oct.2021	173	24	43	106	79	RT-PCR	HVRI	0	79
Rabies	Aug.2020	278	61	83	134	15	ELISA	HVRI	0	15
	Nov.2020	230	42	64	124	13	ELISA	HVRI	0	13
	Oct.2021	173	24	43	106	10	ELISA	HVRI	0	10
Anthrax	Aug.2020	278	61	83	134	15	PR	HVRI	0	15
	Nov.2020	230	42	64	124	13	PR	HVRI	0	13
	May.2021	207	33	48	126	10	PR	HVRI	0	10
	Oct.2021	173	24	43	106	10	PR	HVRI	0	10

Surveillance results of susceptible animals (pigs, cattle and sheep) other than equids in EDFZ

Sample size
Assuming
1% Disease
Prevalence at 95%
confidence interval

Disease	Surveillance time	Category	No. samples	Method	Lab	Results		Complementary testing or investigation results
						pos	neg	
Trypanosoma evansi (surra)	Aug.2020	pig, cattle, sheep	898	CATT	TLVL	0	898	
	Nov.2020	pig, cattle, sheep	986	CATT	TLVL	0	986	
	May.2021	pig, cattle, sheep	1080	CATT	TLVL	0	1080	
	Oct.2021	pig, cattle, sheep	1497	CATT	TLVL	0	1497	
Japanese encephalitis (antibody detection)	Aug.2020	pig	745	ELISA	TLVL	30	715	Positive results due to vaccination, based on clinical examinations and pathogenic negative tests
	Nov.2020	pig	831	ELISA	TLVL	0	831	
	May.2021	pig	870	ELISA	TLVL	364	506	All pigs in the EDFZ have been vaccinated since March 2021, thus, the positive results are due to vaccination
	Oct.2021	pig	1267	ELISA	TLVL	1059	208	
Japanese encephalitis (antigen detection)	Aug.2020	pig	12	RT-PCR	TLVL	0	12	
	Nov.2020	Pig	135	RT -PCR	VDC-ZAFU	0	135	Among them, 117 samples were from antibody-positive pig farms
	May.2021	Pig	1110	RT -PCR	TLVL	0	1110	
	Oct.2021	Pig	1604	RT -PCR	TLVL	0	1604	
Vesicular stomatitis (antibody)	Aug.2020	pig, cattle, sheep	898	ELISA	TLVL	0	898	
	Nov.2020	pig, cattle, sheep	986	ELISA	TLVL	0	986	
Vesicular stomatitis (antigen detection)	May.2021	pig, cattle, sheep	1200	RT-PCR	TLVL	0	1200	
	Oct.2021	pig, cattle, sheep	1639	RT-PCR	TLVL	0	1639	
Nipah virus disease	Aug.2020	Pig	745	ELISA	TLVL	0	745	
	Nov.2020	Pig	831	ELISA	TLVL	0	831	
	May.2021	Pig	870	ELISA	TLVL	0	870	
	Oct.2021	Pig	1267	ELISA	TLVL	0	1267	
Rabies	Aug.2020	pig, cattle, sheep	91	ELISA	TLVL	0	91	
	Nov.2020	pig, cattle, sheep	96	ELISA	TLVL	0	96	
	May.2021	pig, cattle, sheep	109	ELISA	TLVL	0	109	
	Oct.2021	pig, cattle, sheep	158	ELISA	TLVL	0	158	
Anthrax	Aug.2020	pig, cattle, sheep	91	PR	TLVL	0	91	
	Nov.2020	pig, cattle, sheep	96	PR	TLVL	0	96	
	May.2021	pig, cattle, sheep	109	PR	TLVL	0	109	
	Oct.2021	pig, cattle, sheep	158	PR	TLVL	0	158	

Sample size
Assuming
1% Disease
Prevalence at 99%
confidence interval

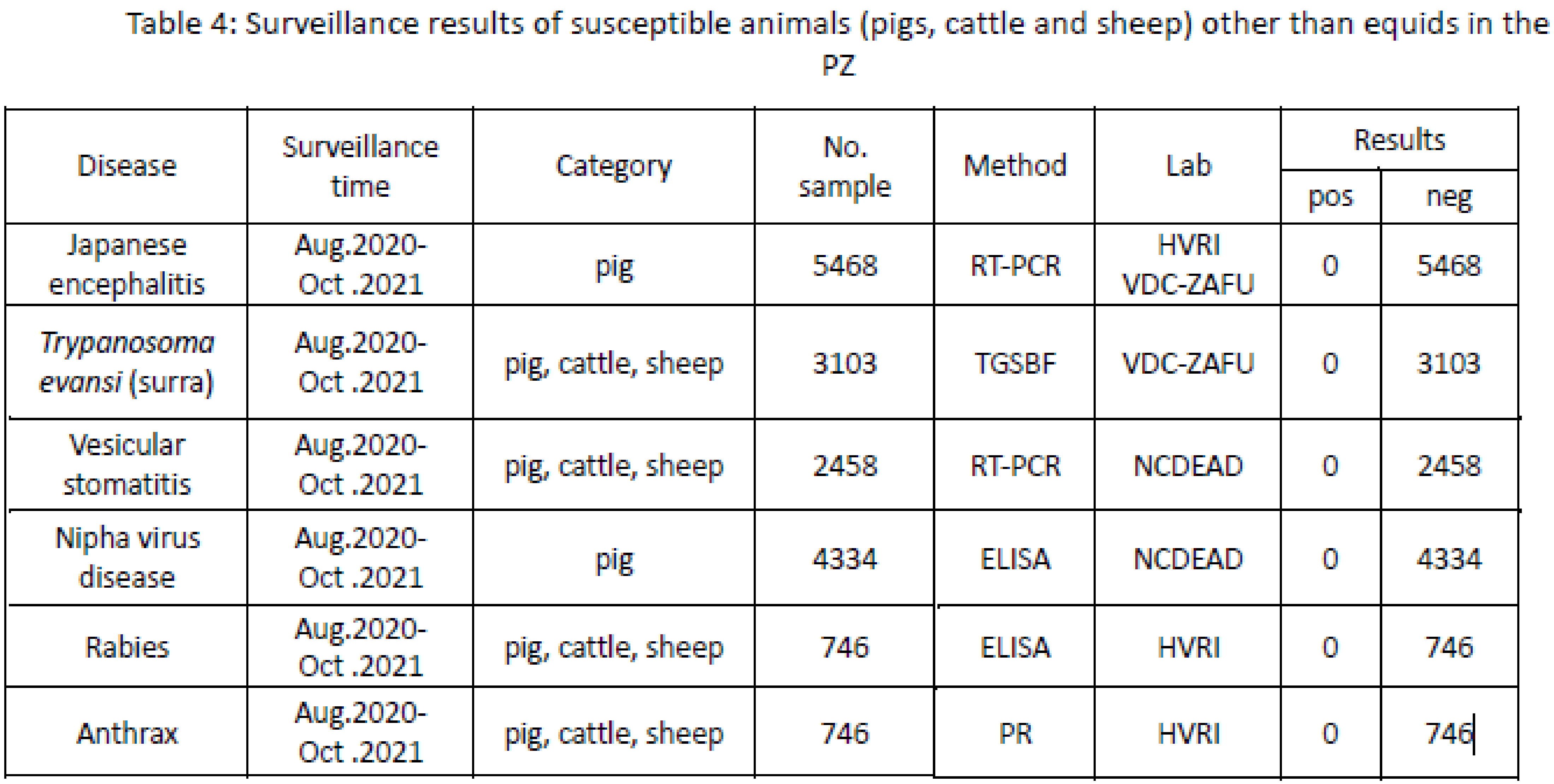


Table 4: Surveillance results of susceptible animals (pigs, cattle and sheep) other than equids in the PZ

Disease	Surveillance time	Category	No. sample	Method	Lab	Results	
						pos	neg
Japanese encephalitis	Aug.2020-Oct .2021	pig	5468	RT-PCR	HVRI VDC-ZAFU	0	5468
Trypanosoma evansi (surra)	Aug.2020-Oct .2021	pig, cattle, sheep	3103	TGSBF	VDC-ZAFU	0	3103
Vesicular stomatitis	Aug.2020-Oct .2021	pig, cattle, sheep	2458	RT-PCR	NCDEAD	0	2458
Nipha virus disease	Aug.2020-Oct .2021	pig	4334	ELISA	NCDEAD	0	4334
Rabies	Aug.2020-Oct .2021	pig, cattle, sheep	746	ELISA	HVRI	0	746
Anthrax	Aug.2020-Oct .2021	pig, cattle, sheep	746	PR	HVRI	0	746

Equine disease surveillance and early warning system

- MARA has established a comprehensive animal disease reporting system, and veterinary institutions at the provincial, city and county levels report the occurrence of animal diseases to MARA according to reporting requirements.

- In order to ensure early warning, horse breeders in the PZ carry out body temperature measurement and clinical observation of the horses in stock every day. Livestock owners are required to immediately report to the local veterinary institution if there is any finding of any clinical abnormality.

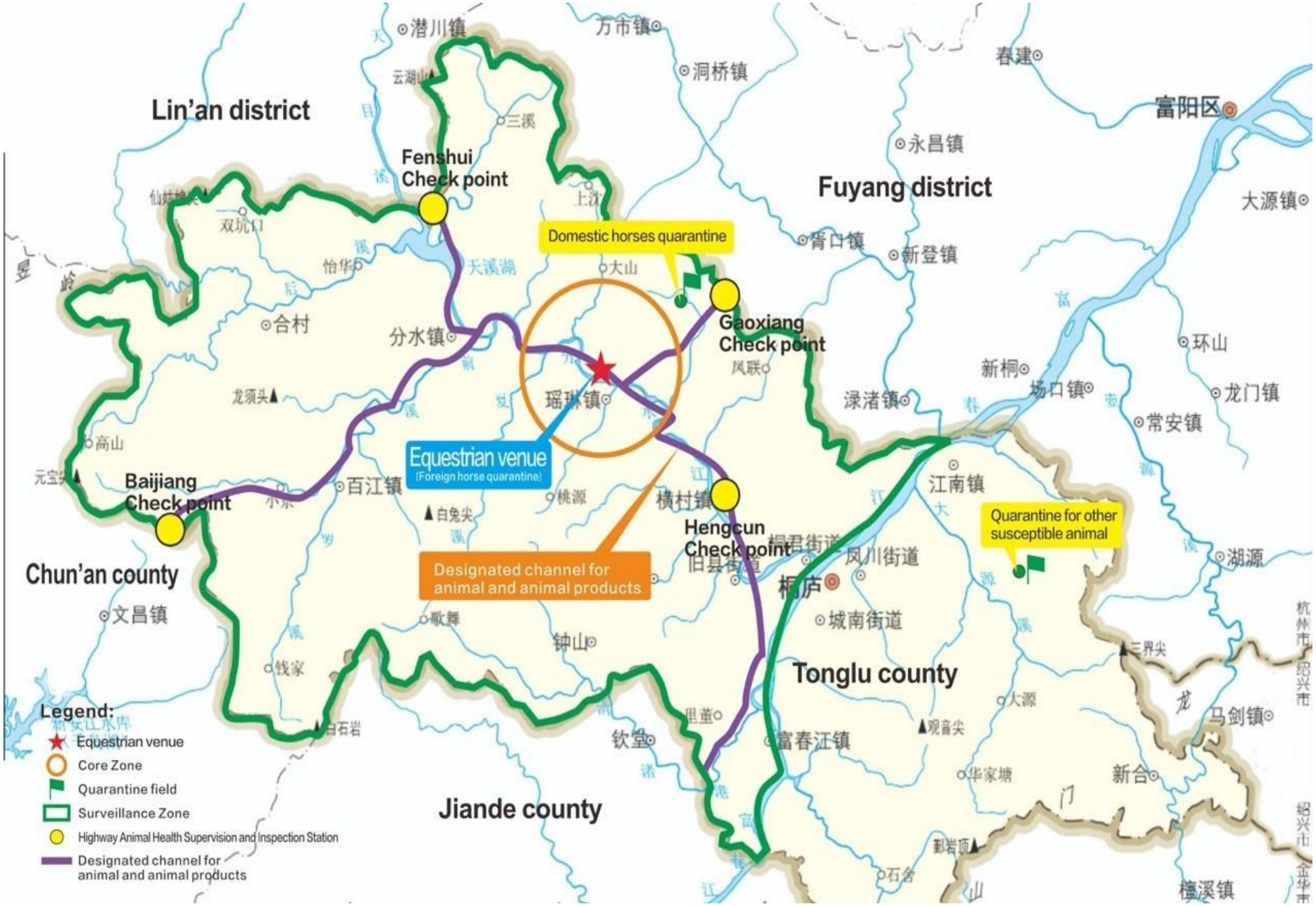
Exporting Country of competition horses- centralised hubs for import into EDFZ

- Mainland China - 2 horses (180 days under official supervision at place of origin + 30 days quarantine in EDFZ prior to entry to competition venue)

- Europe- 100 horses

- Japan- 5 horses

- South Korea- 7 horses



Layout of EDFZ highway animal health supervision check points, designated transportation pathways and quarantine fields

WOAH Public Private Partnership for Hangzhou Asian Games 2023

- **WOAH – International Horse Sports Confederation (Liaison Role)**

- PRC Ministry of Agriculture & Rural Affairs (MARA)

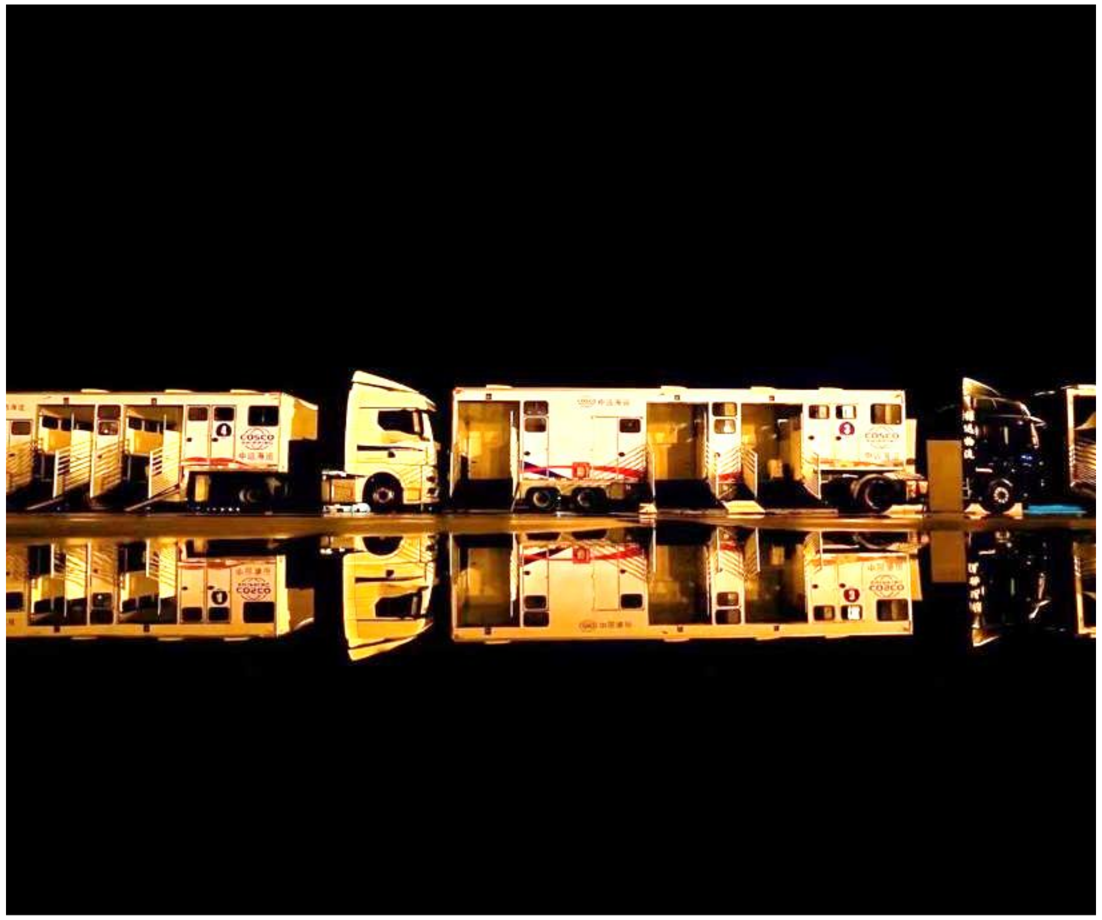
- China Animal Health and Epidemiology Centre

- PRC General Administration of China Customs

- Hong Kong Agriculture, Fisheries & Conservation Department (Expert group for China Customs with veterinary laboratory diagnostic support)

- Hong Kong Jockey Club (Technical Support for Biosecurity, Stable and Event Management)


QATAR
CARGO
القطرية


COSCO


eart to Heart, @Future 心心相融,@未来
19th Asian Games
Hangzhou
中国
China M
19th Asian Ga
Hangzh

THANK YOU


19th Asian Games
Hangzhou
杭州亚运会马术项目管理指挥中心
Hang zhou Asian Games Equ… an Ev… Management and Operations Centre

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

Updated Laboratory survey results (2024)

Susanne Münstermann, WOAH consultant
s.munstermann@outlook.de

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

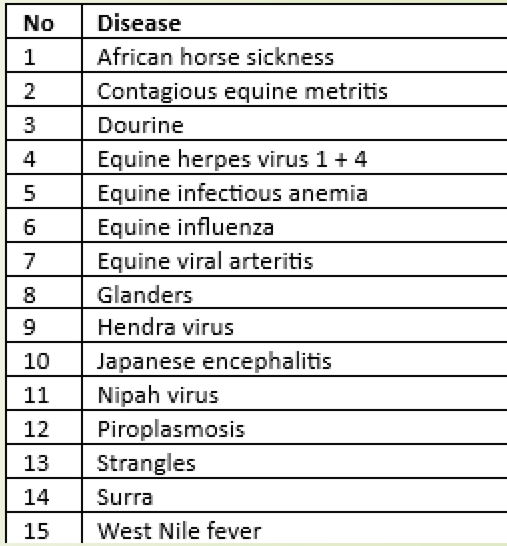
Importance of laboratory capacity

- Adequate capacity to diagnose equine diseases is key to
  - Early recognition of diseases
  - Differential diagnosis
  - Surveillance and control programmes
  - Certification of country / zone / individual disease freedom
  - Being trustworthy to trade partners
- Few WOAH Reference laboratories in the region
- Different level of laboratory capacity
  - To understand this better, a survey was carried out during 2023
  - The survey was updated in 2024 in view of presenting a proposal for a Phase II of our project, focusing on laboratory capacity development

Laboratory capacity survey

Methodology:

- Questionnaire with 11 questions and referenced to 15 listed diseases of importance for international horse movement
- Sent to project liaison person and WOAH laboratory Focal points
    - Capacity for diagnosis of equine diseases
    - Participation in regional and international PTs
    - Collaboration with regional and international laboratories
    - Interest in WOAH laboratory twinning programme
    - National experts
    - Topics of interest for online training
    - Research or routine work on AMR in equines



No	Disease
1	African horse sickness
2	Contagious equine metritis
3	Dourine
4	Equine herpes virus 1 + 4
5	Equine infectious anemia
6	Equine influenza
7	Equine viral arteritis
8	Glanders
9	Hendra virus
10	Japanese encephalitis
11	Nipah virus
12	Piroplasmosis
13	Strangles
14	Surra
15	West Nile fever

General results

- **16** / 15 of the 21 countries replied
  - 11 countries included ALL state Veterinary laboratories
  - 5 countries gave different answers from individual labs or one document for selected labs
- Information was captured in a database and analysed
- A full report is available and was updated to reflect the situation in 2024
- For privacy reasons in this presentation no countries are named individually

Results

Capacity to diagnose the 15 equine diseases in the region

Disease	**Number of countries capable to diagnose disease**
EIA, EI	13
AHS, EHV	11/12
EVA, glanders,	10
piroplasmosis	10/11
Strangles	9/10
WNF	8/9
Surra, JE, CEM	7/8
Hendra	4/5
Dourine	3/5
Nipah	1 (1)

Results: Proficiency testing

2. Internal national Proficieny tests

6 / 16 countries carry out internal PTs with their decentralised or partner laboratories, including University laboratories

Diseases covered by these PTs: EI, EVA, piro, surra, EIA, glanders, AHS, Hendra, EHV, CEM (10)

3. International Proficiency Tests

10 / 16 countries engaged with WOAH Ref labs on international PTs in the last 5 years.

Diseases covered were the same as above plus strangles and rabies (12)

4. Interest in participating in international Proficiency tests

Interest was expressed by 12 /13 to participate in PTs if organised by WOAH Ref labs

Rank	Disease desired for international PT	Disease already tested in international PTs
1	AHS	AHS
2	Glanders, EIA	EIA, piro
3	Surra	EI, EVA

Results: improvement of capacity

5. Desire to establish additional capacity

12 / 16 want to increase their capacity to diagnose equine diseases

Rank	Disease
1	EHV, dourine
2	EIA, Nipah, Hendra, WNF
3	Strangles, AHS, JE

6. Interest in WOAH twinning program

4 / 6 expressed interest for a wide range of diseases

7. Interest in receiving training

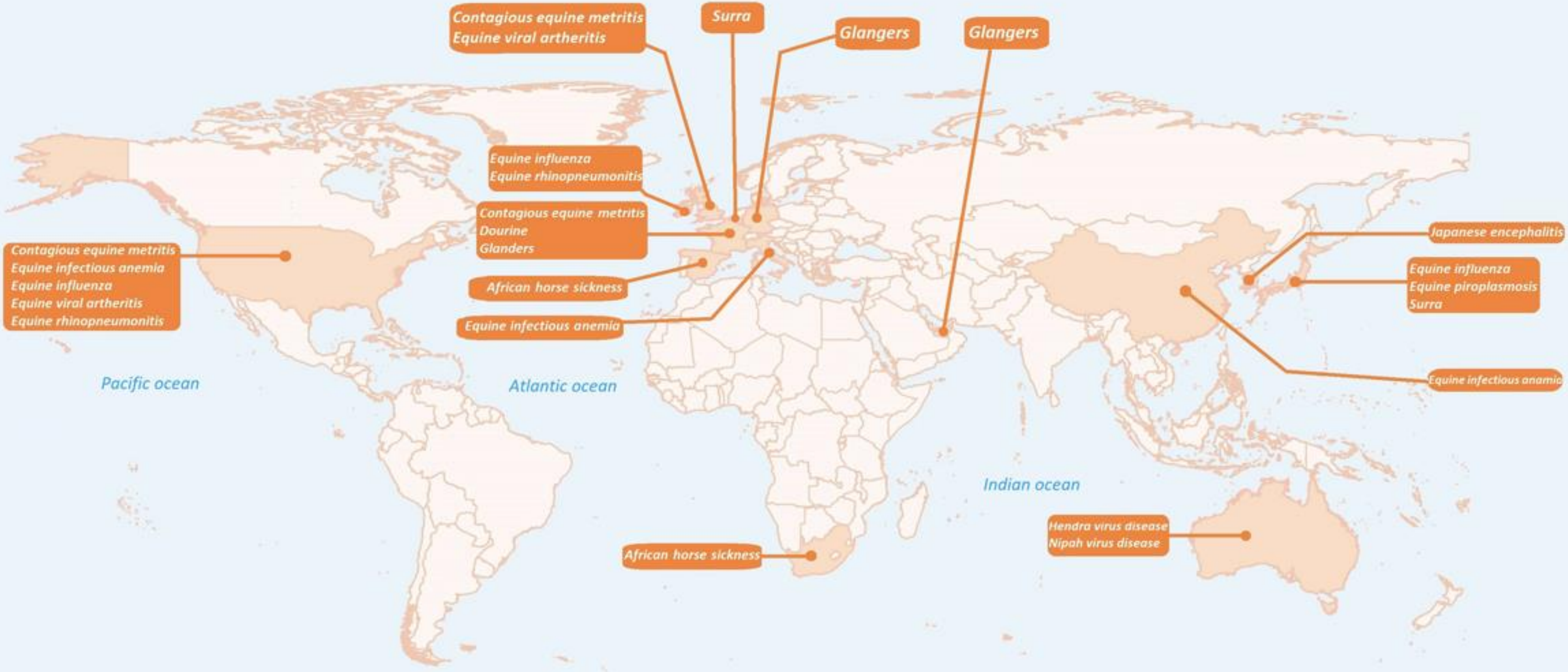
12 / 16 want to receive training in different diseases and tests (prefered type of training: in person!)

Rank	Disease
1	AHS
2	CEM, dourine, glanders, Hendra, Nipah, piro, strangles
3	JE, EIA, EHV

Indicated need for the establishment of additional capacity to test for certain diseases either to identify the pathogen or for antibodies

Lab /Disease /country	AUS	Bhutan	China	Chinese Taipei	India	Indonesia	Mongolia	Myanmar	Singapore	Sri Lanka	Thailand
	Glanders	EIA	Harbin	Nipah	Hendra	AHS	AHS	EI	Dourine	AGS	dourine
	CEM	EI	dourine	Hendra	Nipah	JE		Strangles	JE	EHV	
		Glanders	TC of Quindao	EIA		Glanders		Glanders	Nipah	EEE	
		Strangles	Surra	EVA		Hendra		piro	WNF	WNF	
		Dourine		CEM		Dourine				EIA	
		EHV		EHV		strangles				Hendra	
		EVA		Glanders						Glanders	
		WEE, EEE		Strangles						Strangles	
				piro						Surra	
				dourine						dourine	

World distribution of WOAH Reference laboratories for equine diseases



Dr Gregorie Hermann Bazimo (WOAH World Animal Health Information and Analysis Department)

WOAH experts for equine diseases

Species and Prof Yokoyama	**Disease Name**	**Asia and the Pacific**		**Other regions**	
		Nations	**Experts**	**Nations**	**Experts**
Multiple Species	Japanese encephalitis	Korea(RO)	Dr. Dong. Kun. Yang	-	-
	West Nile fever	-	-	Italy	Dr. Federica. Monaco
		-	-	USA	To be Decided
	Surra (*Trypanosoma evansi*)	Japan	Prof. Noboru. Inoue	Belgium	Dr. Nick Van Reet
Equidae	Infectious with African horse sickness virus			South Africa	Dr. Baratang. Alison. Lubisi
		-	-	Spain	Dr. Montserrat. Agüero. Garcia
		-	-	Spain	Dr. José. Manuel. Sanchez-Vizcaino
		-	-	UK	Dr. Carrie Batten
	Contagious equine metritis	-	-	UK	Dr. Ian. Mawhinney
		-	-	USA France	Dr. Kristina Lantz Dr Sandrine Petry
	Dourine	-	-	France	Dr. Laurent. Hébert
	Equine encephalomyelitis	-	-	~~USA~~	Not anymore a WOAH Reference Lab
	Equine infectious anaemia	China(PRO)	Dr. Xiaojun. Wang	USA	Currently under study
				Italy	Dr. Maria. Teresa. Scicluna
	Infection with equine influenza virus	**Japan (JRA)**	Dr Manabu Nemoto	Ireland	Prof. Ann. Cullinane
		-	-	USA	Dr. Thomas. M. Chambers
	Equine piroplasmosis	Japan	Prof. Naoaki. Yokoyama		
	Equine rhinopneumonitis (Infection with equid herpesvirus-1)	-	-	Ireland	Prof. Ann. Cullinane
		-	-	USA	Dr Lutz Goehring
	HENDRA, NIPAH	**AUSTRALIA**	DR KIM HALPIN	-	-
	Infection with equine arteritis virus	-	-	UK	Prof. Falko. Steinbach
		-	-	USA	To be decided
	Glanders	-	-	Germany	Dr. Heinrich. Neubauer
		-	-	UAE	Prof. Ulrich Wernery
		-	-	France	Dr Karine Laroucau
	Venezuelan equine encephalomyelitis	-	-	~~USA~~	Not anymore a WOAH Reference Lab

Inquiry on work on AMR in equines (2024)

- During the update of the survey, the following additonal questions were asked:

1. **Has your laboratory been involved in testing for AMR in equines?**
2. **Are you aware of any studies on AMR in equines in your country**

- Six countries indicated that they are involved either in research on AMR in equines or have included it into regular surveillance programmes, testing any reports of confirmed bacterial diseases for AMR.
- Most of these countries indicated their increasing interest in research on AMR in equines

Conclusion and recommendations

- There is a lot of capacity in the region, including WOAH Ref labs, but also at national level and the update of the survey indicates an increase
- Countries are encouraged to utilise regional capacity when national capacity is not sufficient
  - E.g. only 4 countries have sent samples for confirmatory diagnosis to other countries in the region in the last 5 years!
- The need for a „regional Collaborating Centre for diagnostics in equine diseases“ should be discussed
- The need for a WOAH Reference Lab for glanders in key regions like Asia (but also in the Americas)
- The use and recognition of private laboratories to carry out diagnosis for certification should be considered
  - Only 3 countries indicate that they use private laboratories
- The region is home to a large number of experts which could be called upon for training activities
  - 8 countries provided names of their experts

Thank you for your attention

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

Concept for intra-regional training programme

Susanne Münstermann, WOAH consultant

s.munstermann@outlook.de

World Organisation for Animal Health
Founded as OIE

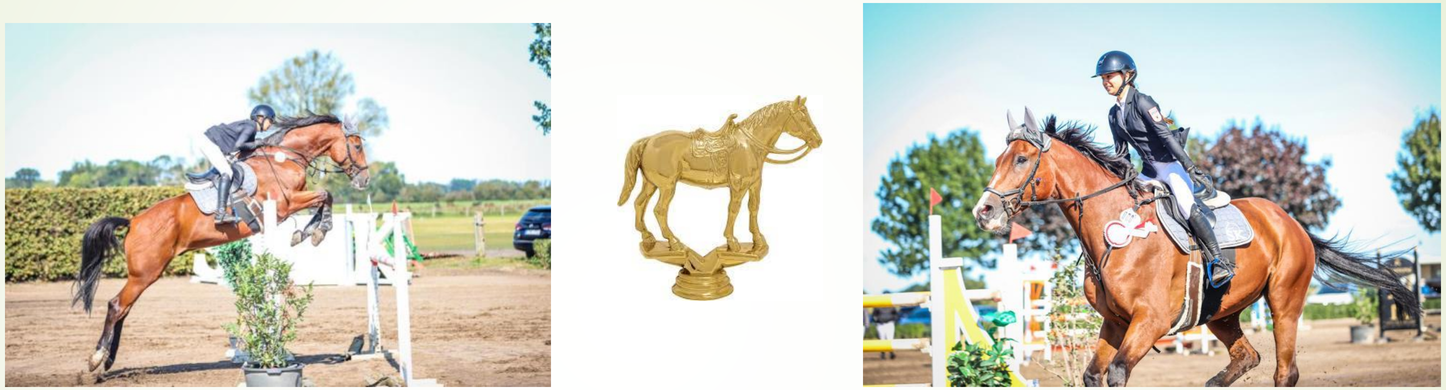
Organisation mondiale de la santé animale
Fondée en tant qu'OIE

Organización Mundial de Sanidad Animal
Fundada como OIE

Regional Workshop, Tokyo, 17 – 18 September 2024

Introduction & justification

- Equestrian sports are on the increase in the Region – competitive sport and leisure sport
- Economic importance - employment, national and international events



- Against this background, the number of intra-regional events are still small, competition horses often based in Europe
  - Difficult and different import conditions
  - Laboratory diagnostic capacity for equine diseases
  - Evidence provided in the laboratory survey and update
  - Very few Reference Laboratories in the Region
- Clear need for capacity building program in the Region

Objective of the proposal

- To increase capacity in countries with an identified gap
- Strengthen regional collaboration and networking
- Strengthen links between laboratories in the Region and WOAH Ref labs outside the region and other networks (e.g. EU network)

Methodology

Step 1

- Identify up to 3 experts from laboratories in the Region with high standards and capacity, e.g.
  - Equine Research Institute, Japan
  - National Institute for Animal Health, Thailand
  - National Research Centre for Equines, India
  - Veterinary Laboratory Division of AFCD, Hongkong
  - Harbin, China

Methodology *cont*.

Step 2

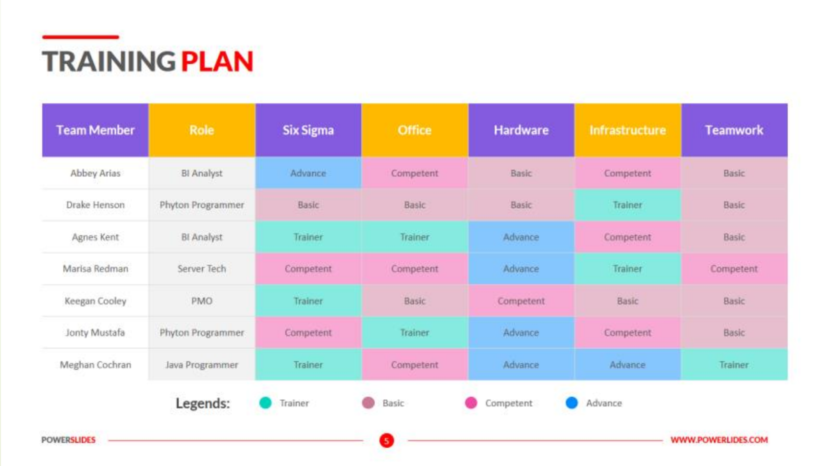
- Confirm identified needs with the countries (based on Table 8 in the survey report) during online meeting

Lab /Disease /country	AUS	Bhutan	China	Chinese Taipei	India	Indonesia	Mongolia	Myanmar	Singapore	Sri Lanka	Thailand
	Glanders	EIA	Harbin	Nipah	Hendra	AHS	AHS	EI	Dourine	AGS	dourine
	CEM	EI	dourine	Hendra	Nipah	JE		Strangles	JE	EHV	
		Glanders	TC of Quindao	EIA		Glanders		Glanders	Nipah	EEE	
		Strangles	Surra	EVA		Hendra		piro	WNF	WNF	
		Dourine		CEM		Dourine				EIA	
		EHV		EHV		strangles				Hendra	
		EVA		Glanders						Glanders	
		WEE, EEE		Strangles						Strangles	
				piro						Surra	
				dourine						dourine	

Methodology cont.

Step 3

- Identify the diagnostic methods needed per disease (serology, agent identification, others)
- Match with suitable experts
- Develop a training plan per expert



TRAINING PLAN

Team Member	Role	Six Sigma	Office	Hardware	Infrastructure	Teamwork
Abbey Arias	BI Analyst	Advance	Competent	Basic	Competent	Basic
Drake Henson	Phyton Programmer	Basic	Basic	Basic	Trainer	Basic
Agnes Kent	BI Analyst	Trainer	Trainer	Advance	Competent	Basic
Marisa Redman	Server Tech	Competent	Competent	Advance	Trainer	Competent
Keegan Cooley	PMO	Trainer	Basic	Competent	Basic	Basic
Jonty Mustafa	Phyton Programmer	Competent	Trainer	Advance	Competent	Basic
Meghan Cochran	Java Programmer	Trainer	Competent	Advance	Advance	Trainer

Legends: ● Trainer ● Basic ● Competent ● Advance

POWERSLIDES 5 WWW.POWERLIDES.COM

Methodology cont.

Step 4:

- Contract experts according to their capacity to implement the training plan

Proposal:

- Country visits of 1 week each
- Each expert max 3 countries during 1 year
- Project to provide DAS, travel and accommodation - NO honorarium

Expected results

- Training under local conditions with local equipment and reagents, experiencing local difficulties and challenges
- Approach increases networking between lab experts in the region and the establishment of long lasting links between laboratories
- Ultimately laboratories can diagnose diseases correctly, and support the certification of horses for export / participation in competitions / international horse movement

Thank you for your attention