Part 1

GLANDERS EPIDEMIOLOGY

Geographical distribution,

disease & recent outbreaks

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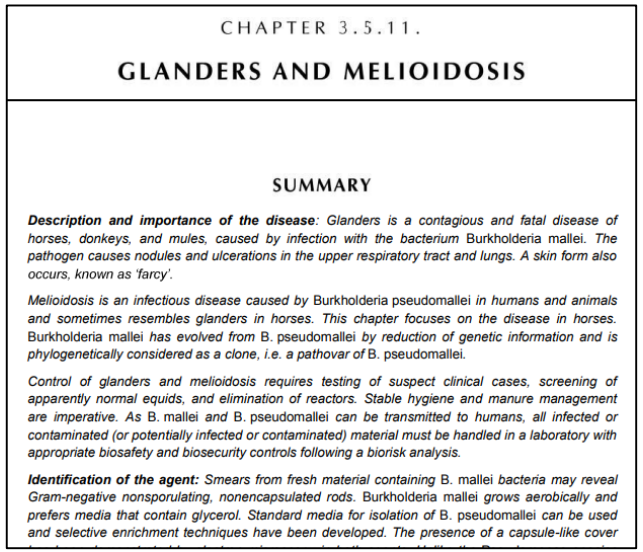
International Horse Movement in Asia and the Pacific
Webinar on Glanders, 30 August 2023

Glanders and Melioidosis: updated chapter



CHAPTER 3.5.11.

GLANDERS AND MELIOIDOSIS

SUMMARY

***Description and importance of the disease**: Glanders is a contagious and fatal disease of horses, donkeys, and mules, caused by infection with the bacterium* Burkholderia mallei. *The pathogen causes nodules and ulcerations in the upper respiratory tract and lungs. A skin form also occurs, known as 'farcy'.*

Melioidosis is an infectious disease caused by Burkholderia pseudomallei *in humans and animals and sometimes resembles glanders in horses. This chapter focuses on the disease in horses.* Burkholderia mallei *has evolved from* B. pseudomallei *by reduction of genetic information and is phylogenetically considered as a clone, i.e. a pathovar of* B. pseudomallei.

Control of glanders and melioidosis requires testing of suspect clinical cases, screening of apparently normal equids, and elimination of reactors. Stable hygiene and manure management are imperative. As B. mallei *and* B. pseudomallei *can be transmitted to humans, all infected or contaminated (or potentially infected or contaminated) material must be handled in a laboratory with appropriate biosafety and biosecurity controls following a biorisk analysis.*

***Identification of the agent:** Smears from fresh material containing* B. mallei *bacteria may reveal Gram-negative nonsporulating, nonencapsulated rods.* Burkholderia mallei *grows aerobically and prefers media that contain glycerol. Standard media for isolation of* B. pseudomallei *can be used and selective enrichment techniques have been developed. The presence of a capsule-like cover*

https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.06.11_GLANDERS.pdf

WOAH Reference Lab

Glanders

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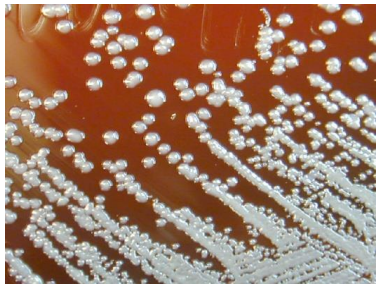
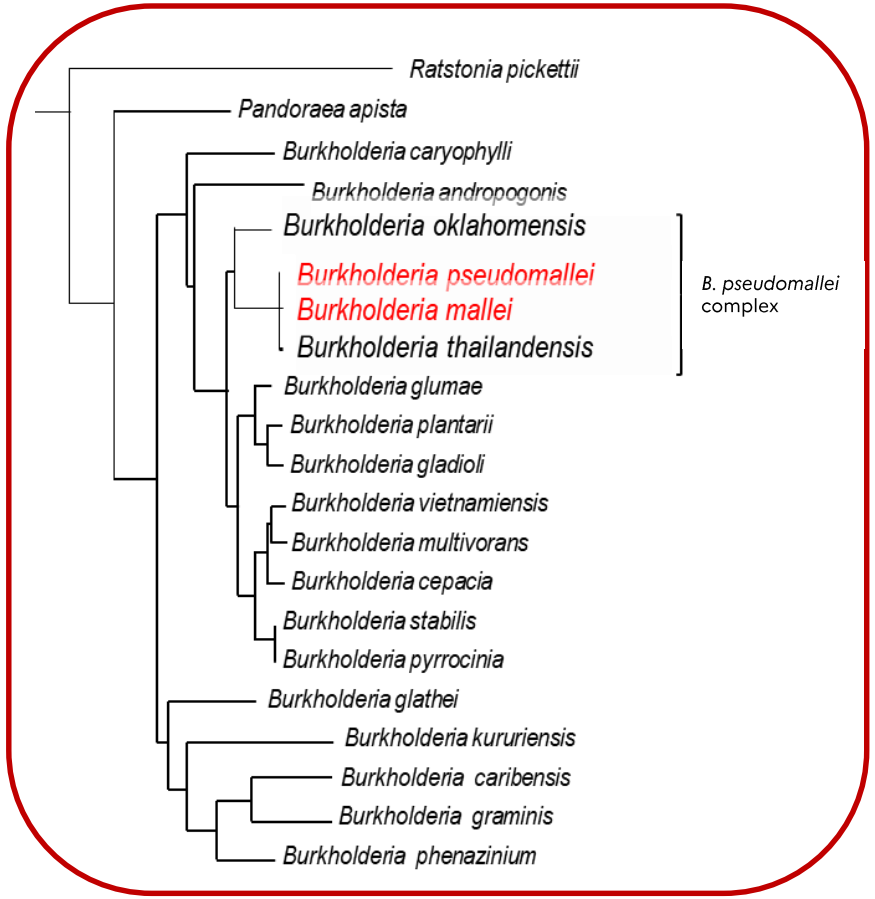
Burkholderia pseudomallei & Burkholderia mallei



Scientific classification	
Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Betaproteobacteria
Order:	Burkholderiales
Family:	Burkholderiaceae
Genus:	Burkholderia



Melioidosis

Burkholderia pseudomallei

- Present in water and damp soil
- Opportunistic pathogen
- Endemic in tropical countries

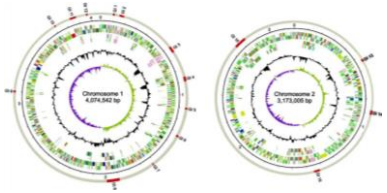
Loss of genetic material

Glanders

Burkholderia mallei

- Equid pathogen (+ humans)

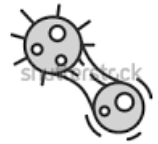
Burkholderia pseudomallei



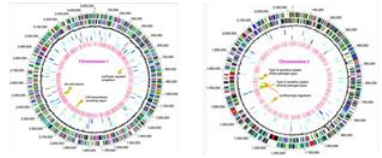
Strain K96243 7,25 Mb

Chromosome 1 4,07 Mb (3460 ORFs)

Chromosome 2 3,17 Mb (2395 ORFs)



Burkholderia mallei

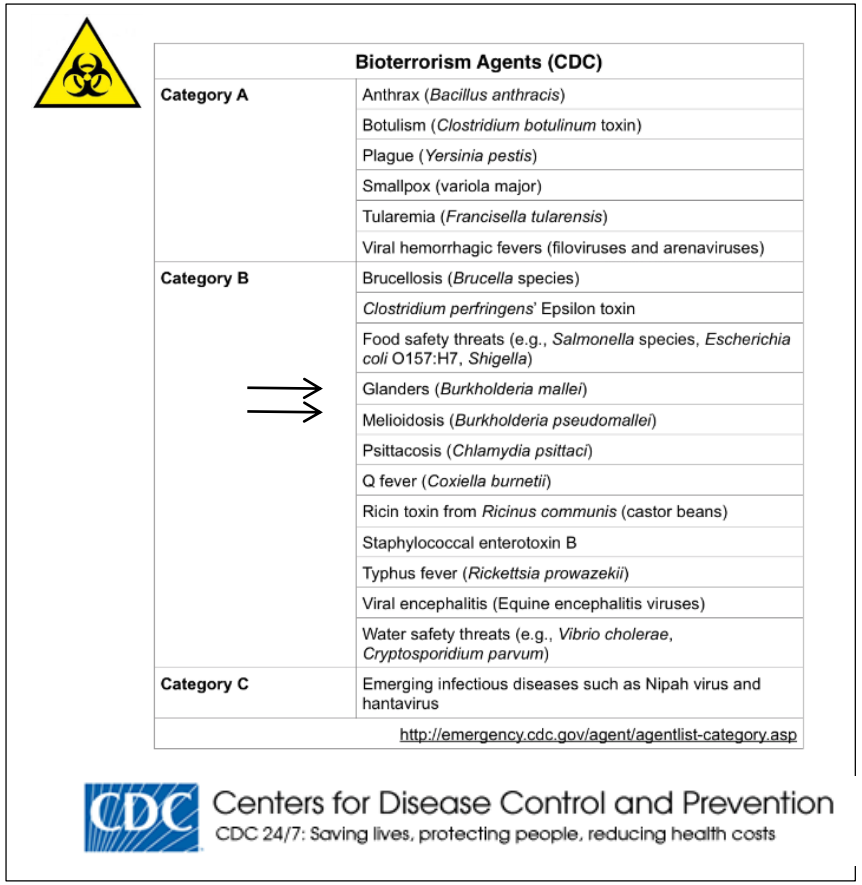


Strain ATCC23344 5,84 Mb

Chromosome 1 3,50 Mb (3344 ORFs)

Chromosome 2 2,30 Mb (2091 ORFs)

Potential of theses bacteria to be used in bioterrorism or other intentional acts to harm public health.



Bioterrorism Agents (CDC)	
Category A	Anthrax (*Bacillus anthracis*)
	Botulism (*Clostridium botulinum* toxin)
	Plague (*Yersinia pestis*)
	Smallpox (variola major)
	Tularemia (*Francisella tularensis*)
	Viral hemorrhagic fevers (filoviruses and arenaviruses)
Category B	Brucellosis (*Brucella* species)
	Clostridium perfringens' Epsilon toxin
	Food safety threats (e.g., *Salmonella* species, *Escherichia coli* O157:H7, *Shigella*)
	Glanders (*Burkholderia mallei*)
	Melioidosis (*Burkholderia pseudomallei*)
	Psittacosis (*Chlamydia psittaci*)
	Q fever (*Coxiella burnetii*)
	Ricin toxin from *Ricinus communis* (castor beans)
	Staphylococcal enterotoxin B
	Typhus fever (*Rickettsia prowazekii*)
	Viral encephalitis (Equine encephalitis viruses)
	Water safety threats (e.g., *Vibrio cholerae*, *Cryptosporidium parvum*)
Category C	Emerging infectious diseases such as Nipah virus and hantavirus

http://emergency.cdc.gov/agent/agentlist-category.asp

CDC Centers for Disease Control and Prevention
CDC 24/7: Saving lives, protecting people, reducing health costs

- **High Pathogenicity & Zoonotic potential**

Burkholderia mallei is responsible for causing glanders, a severe infectious disease primarily affecting horses, donkeys, and mules.
Burkholderia pseudomallei is the causative agent of melioidosis, a severe infectious disease with a wide range of clinical manifestations.

- **Difficult Diagnosis and Limited Treatment Options**

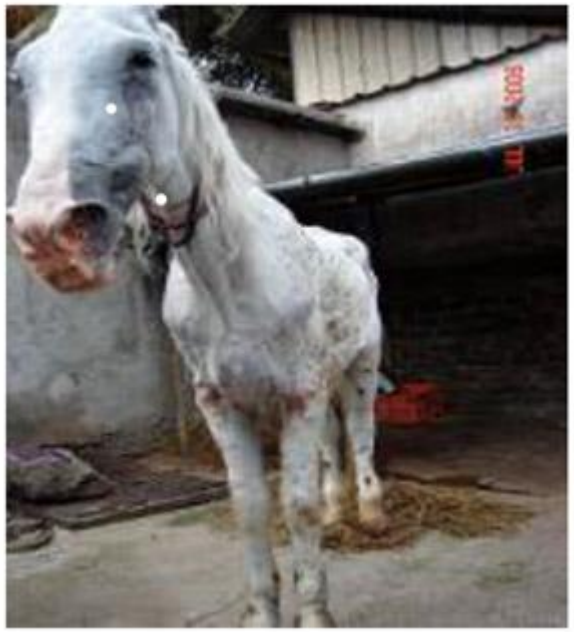
Non-specific symptoms and the need for specialized laboratory tests. Additionally, treatment options are limited, and antibiotic resistance has been reported in some cases, making effective management of the disease difficult.

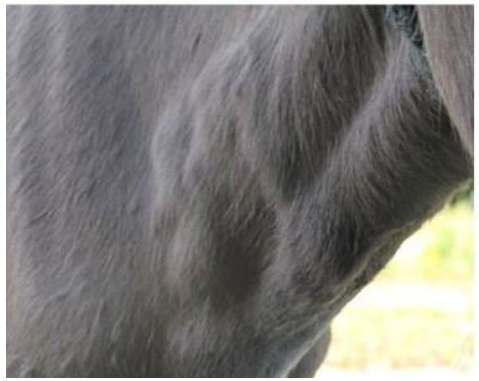
- **Environmental Resilience for *B. pseudomallei***

Burkholderia pseudomallei is naturally found in soil and water in specific regions, such as Southeast Asia and Northern Australia. It can persist in the environment for extended periods, making it challenging to control and eradicate in endemic areas.

- **Historical Use as a Biological Weapon for *B. mallei***

Glanders – Disease in equids





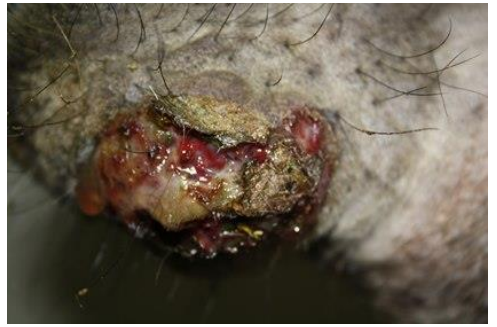


Photo: Ulrich Wernery

Glanders is a contagious, life threatening disease of equids which is generally fatal. It is also a very serious zoonotic disease, but rare in human.

Pictorial guide to the diagnosis of glanders

in horses, donkeys and camels

U. Wernery, D. Altemann, J. Kinne & R. Wernery

Central Veterinary Research Laboratory
PO Box 597, Dubai – United Arab Emirates

Organisation Mondiale de la Santé Animale • World Organisation for Animal Health • Organización Mundial de Sanidad Animal

Glanders – Disease in equids

- Horses, donkeys and mules are the only known natural reservoir of *B. mallei*
- Asymptomatic or carrier animals play a crucial role in the spreading of the agent in the healthy equine population.

Three recognised clinical presentations of glanders
- nasal
- pulmonary
- cutaneous

} according to the location of the initial infection

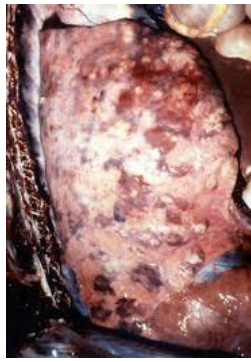
Three courses of disease: acute / chronic / latent

Forms of disease not clearly distinct:
- may occur simultaneously
- incubation period from 2-6 weeks to years

⇒ **Complexity & variability of glanders' clinical expression**

Nasal form
- Fever, cough, dyspnea, unilateral or bilateral nasal discharge, with highly infectious exudates, nodules in the nasal mucosa may produce ulcers.
- Lymph node and vessel involvement
- Death

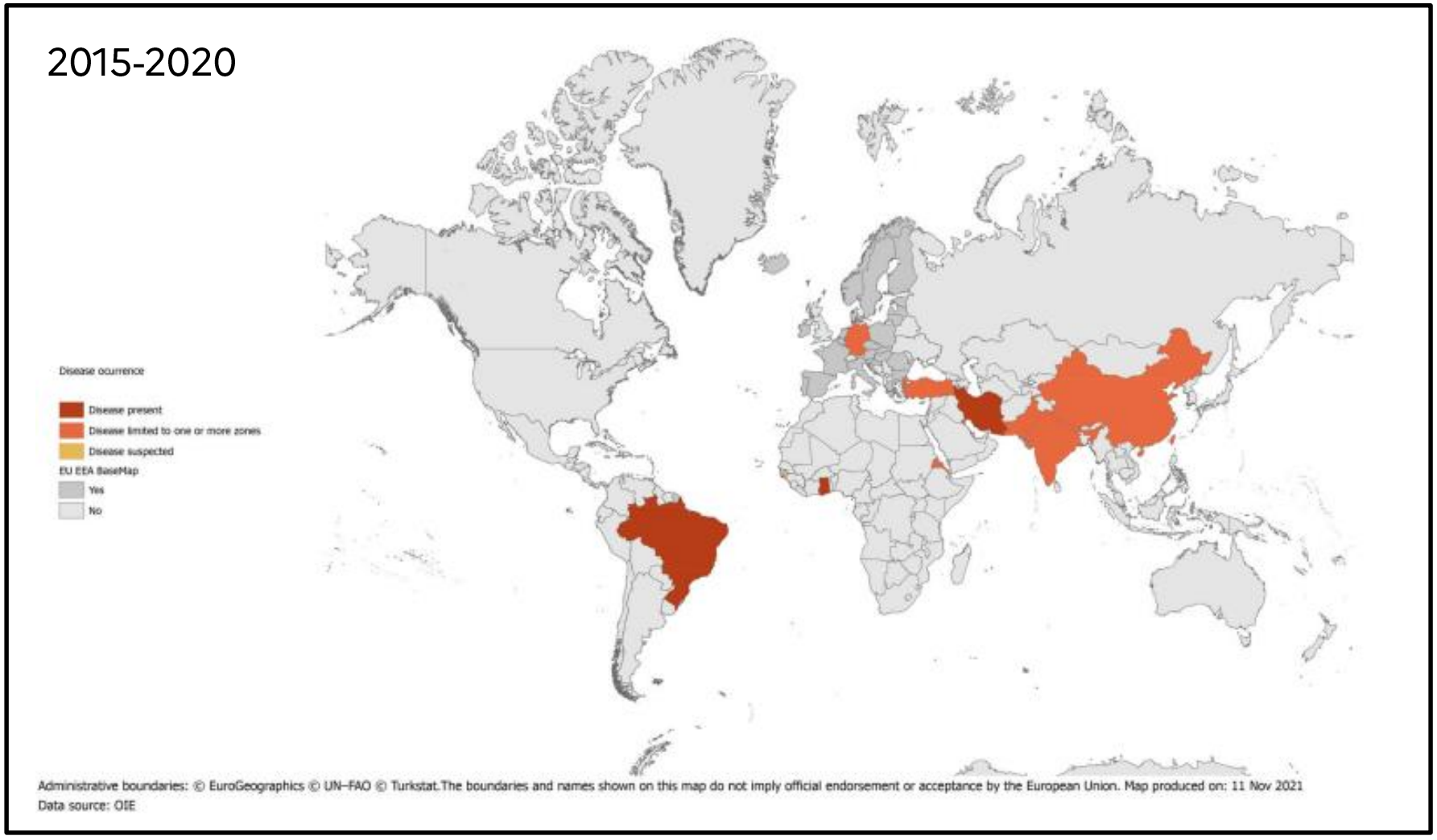
Pulmonary form
- Nodules and abscesses in lungs
- Dyspnea
- Cough
- Fever
- Progressive debilitation
- Requires several months to develop



Cutaneous form as "farcy"
- Nodules and ulcers on skin
- Lymphadenopathy
- Swollen joints and edema of legs
- Orchitis in males
- Develops insidiously over an extended period

Differential diagnosis : melioidosis, strangles (*Streptococcus equi*), ulcerative lymphangitis (*Corynebacterium pseudotuberculosis*), botryomycosis, sporotrichosis, guttural pouch empyema, pseudotuberculosis, tuberculosis…

Glanders – Geographical distribution

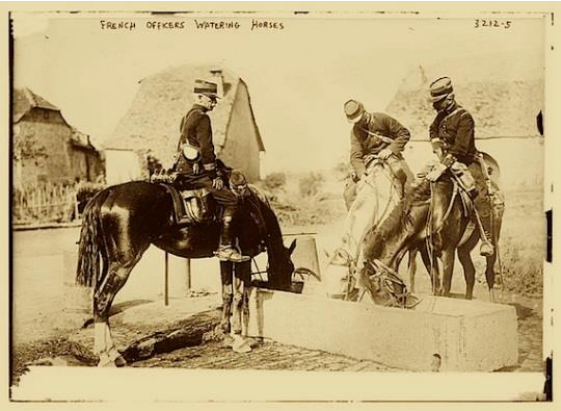


Historically, glanders was widespread worldwide, but over time, it has been eradicated or significantly reduced in many countries.

However, some regions in Asia, the Middle East, and parts of South America and Africa still report sporadic cases.

from EFSA (doi: 10.2903/j.efsa.2022.7069) - (Data sources: ADNS and WOAH)

Glanders – Transmission & Spreading



- *B. mallei* infection is usually introduced into horse populations by **diseased animals**.
- **Ingestion of contaminated food or water**
- **Contaminated aerosols** (produced by coughing and sneezing), and **contaminated fomites** brought to the animals via grooming equipment and tack
- Bacteria can also enter the body through **contact** with lesions or abrasions of the skin or through mucosa
- Poor husbandry and feeding conditions as well as animal transport
- Unsanitary conditions and over-crowded stables

Glanders – Recent outbreaks in animals

Over the last 10-20 years : increase of outbreak frequency

⇒ Glanders classified as **re-emerging disease**

2010 – Bahrain
First occurrence – Equids
Contact with infected animal(s) at grazing/watering points
2 †/15 cases/ 227 suspects/13 killed
Clinical signs
CFT/cELISA/Mallein skin test

2010 – Kuwait
Recurrence – Domestic horses
Unknown or inconclusive
0 †/6 cases/ 44 suspects/6 killed
No clinical signs
CFT

2011 - Afghanistan
First occurrence – Equids
Illegal movement of animals
Animals in transit
1 †/13 cases/ 1400 suspects/12 killed
Clinical signs
CFT/cELISA/Bacteriology

2011 – Lebanon
First occurrence – Equids
Illegal movement of animals
1 †/11 cases/ 82 suspects/30 killed
Clinical signs
CFT/WB

2015-2020 situation

2015 – Germany
Recurrence – Equids
Unknown or inconclusive source of contamination
0 †/1 case/ 31 suspects/ 1 killed
No clinical signs
CFT/WB/PCR

2017/2019 – Turkey
Recurrence – Equids
2017
Unknown or inconclusive
0 †/1 case/ 9 suspects/1 killed
No clinical signs
Mallein test
2019
Illegal movement of animals
0 †/92 case/ 1460 suspects/92 killed
No clinical signs
CFT

2023 – Russia
Recurrence – Equids
Unknown or inconclusive
2 †/6 cases/ 87 suspects/4 killed
Clinical signs
Bacteriology

2021 - Nepal
First occurrence – Equids
Illegal movement of animals
16 †/26 cases/ 87 suspects/0 killed
Clinical signs
PCR/sequencing

Brazil
Equids

Pakistan
Equids

India
Equids

2018 – China
First occurrence – Equids
Unknown or inconclusive
1 †/17 cases/ 17 suspects/16 killed
Clinical signs
PCR/sequencing

- Regular case notifications

› J Equine Vet Sci. 2023 Aug;127:104535. doi: 10.1016/j.jevs.2023.104535. Epub 2023 Apr 30.

Serological and Bacteriological Surveillance of Glanders Among Horses in Central Region of Iran

Peyman Dehghan Rahimabadi [1], Alireza Nazari [2], Mostafa Kamyabi [3], Nader Mosavari [4]

Glanders – Occupational risk & Zoonotic potential

Clinical repercussions of Glanders (*Burkholderia mallei* infection) in a Brazilian child: a case report

Eusébio Lino dos Santos Júnior[1], Juliane de Carvalho Rocha Moura[1], Bruna Karoline Pinheiro França Protásio[1], Vanise Aragão Santos Parente[2] and Maria Helena Neves Dorea Veiga[2]

Brazil, 2020
21-year-old boy
Chest pain, dyspnea
Close contact with families who owned horses
Culture

- Glanders can be transmitted from infected animals to humans, although human cases are relatively rare
- People who work closely with horses, especially those involved in veterinary care, horse racing, or the military, are at higher risk
- Once infected, humans can develop localized or disseminated forms of the disease

Iran, 2023
22-year-old man
headache, fever, chills, diarrhea, and vomiting of blood
In contact with a dead horse
Serology

Case report

Glanders (*Burkholderia mallei* infection) in an Iranian man: A case report

Maryam Nasiri [a,*], Amirali Zarrin [b], Sina RoshankarRudsari [c], Javad Khodadadi [d]

Case Report

A Case Report of *Burkholderia mallei* Infection Leading to Pneumonia

Author(s): Guanfeng He, Yu Zeng, Qizhong He, Tuxuan Liu, Nanhong Li, Hui Lin, Muhong Zeng, Yonglong Li, Min Peng*, Junfen Cheng*, Wang Liu* and Weimin Yao*

Volume 26, Issue 1, 2023

China, 2023
60-year-old man
history of diabetes
cough, expectoration, and fever
PCR identification

Glanders – Control measures

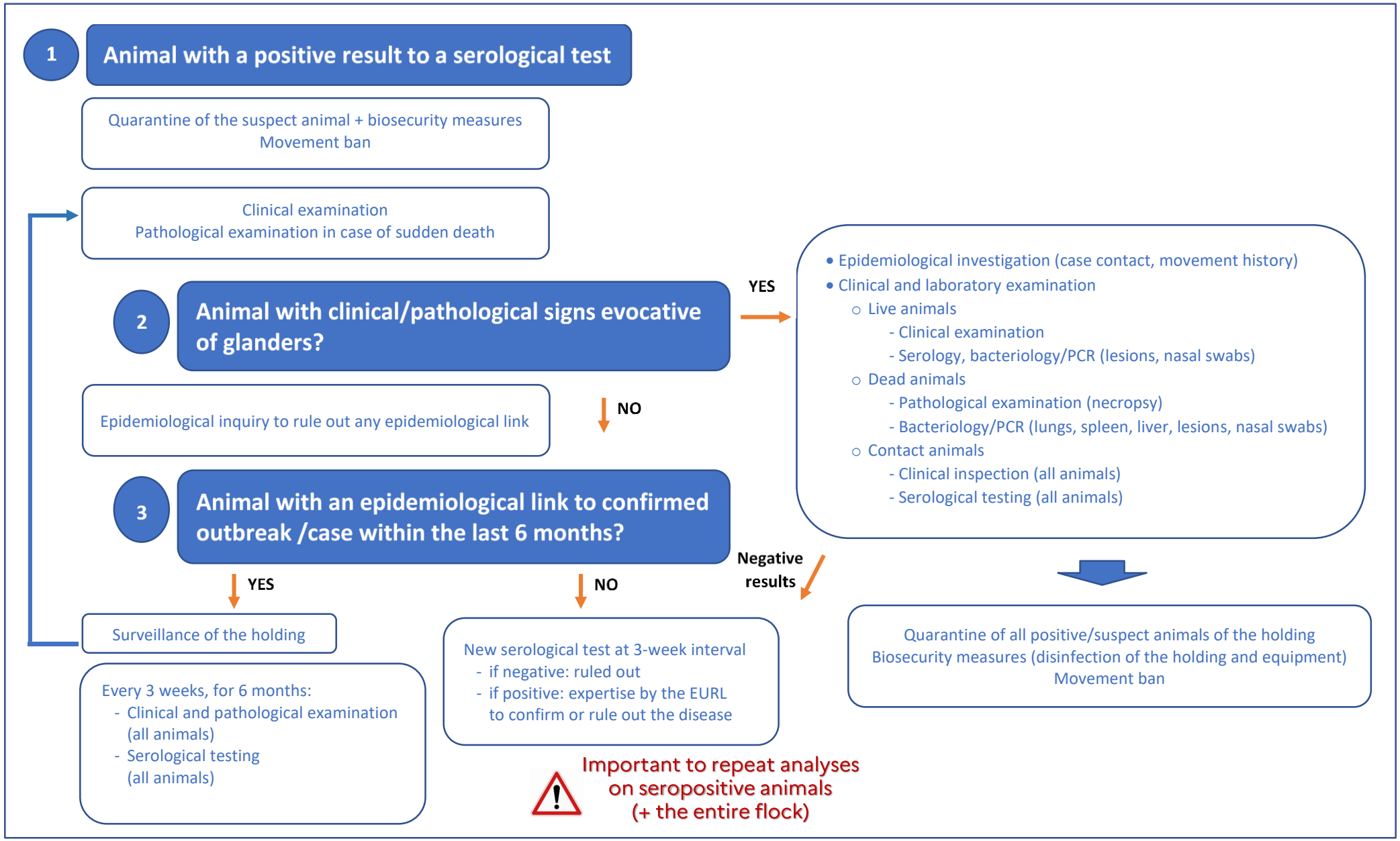
- No vaccine available
- Measures include:
  - quarantine and isolation of infected animals,
  - testing and culling of infected animals,
  - strict biosecurity protocols, and
  - limiting animal movement in affected regions

Limitations
Animal owners and stakeholder compliance/International coordination/Cost, resources and government compensation

Many governments recommend a test-and-slaughter policy for all equids infected with *B. mallei*

Using antibiotics in infected animals is not recommended due to the organism's high resistance to antibiotics (varying sensitivity of *B. mallei* to sulfadiazine, tetracycline, neomycin, and erythromycin [Gregory and Wagg, 2007]). Literature reports [Khan et al, 2012] indicate treatment protocols that resolve clinical signs without confirming complete eradication of the organism, potentially leading to asymptomatic carriers.

Glanders – Decision tree in case of glanders suspicion (e.g. Europe)



- Europe: glanders free zone
- Decision tree to be adapted to each country situation

from EFSA
(doi: 10.2903/j.efsa.2022.7069)

Glanders – Susceptibility of other animals

- Susceptibility to glanders has also been demonstrated in camels, felines living in the wild, bears, wolves and dogs
- Carnivores may become infected by eating infected meat
- Guinea pigs and hamsters are highly susceptible
- Cattle and pigs are resistant

Natural *Burkholderia mallei* Infection in Dromedary, Bahrain

Ulrich Wernery, Renate Wernery, Marina Joseph, Fajer Al-Salloom, Bobby Johnson, Joerg Kinne, Shanti Jose, Sherry Jose, Britta Tappendorf, Heidie Hornstra, and Holger C. Scholz

2010

Linked to an outbreak in horses in the United Arab Emirates in 2004 – the strain might be endemic in this region.

Glanders outbreak at Tehran Zoo, Iran 2012

Khaki P[1], Mosavari N[1*], Khajeh Nasiri S[2], Emam M[2], Ahouran M[2], Hashemi S[2], Mohammad Taheri M[1], Jahanpeyma D[3], Nikkhah S[3]

1 tiger (+ seropositive lions)

Prepared solipeds for feeding canivores ?

Part 2

DIAGNOSTIC TESTS FOR GLANDERS & ROLE OF WOAH REFERENCE LABORATORIES

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Dr **Karine Laroucau**, Anses, France

Glanders – Diagnosis

WOAH Terrestrial manual: available methods for glanders diagnosis and their purposes

B. DIAGNOSTIC TECHNIQUES

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

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification						
PCR	–	–	–	+	–	n/a
Culture	–	–	–	+	–	n/a
Detection of immune response						
Complement fixation	++	++[1]	+++	+	+++	n/a
ELISA	+	+	++	+	++	n/a
Mallein skin test	+	+	+	+	+	n/a
Western blotting	+	+	++	+	++	n/a

Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation;
+ = may be used in some situations, but cost, reliability, or other factors severely limits its application;
– = not appropriate for this purpose; n/a = purpose not applicable.
PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay.

WOAH 2018
chapter 3.5.11
Glanders and Melioidosis

Detection of immune response

To detect Antibodies

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
Complement fixation	++	++[1]	+++	+	+++	n/a

B. DIAGNOSTIC TECHNIQUES

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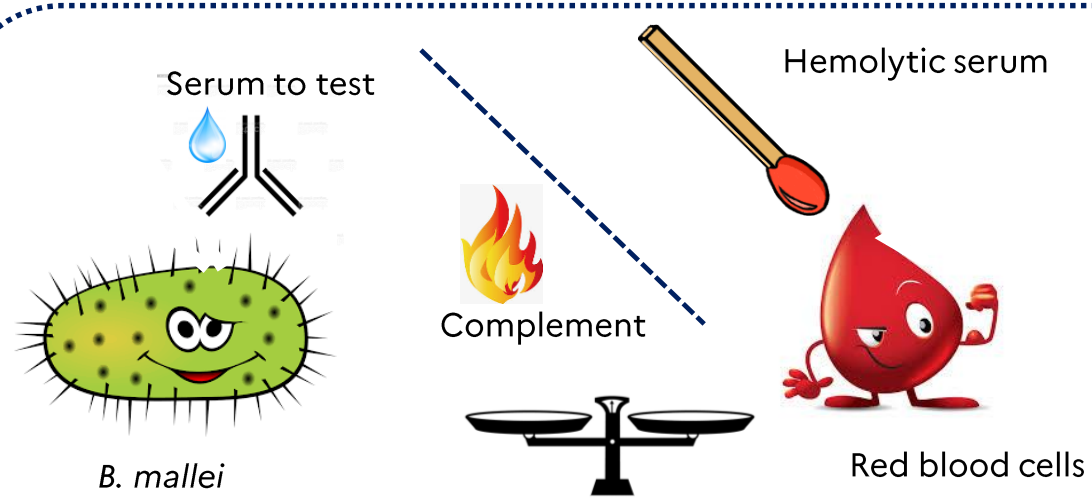
Agent identification						
PCR	–	–	–	+	–	n/a
Culture	–	–	–	+	–	n/a
Detection of immune response						
Complement fixation	++	++[1]	+++	+	+++	n/a
ELISA	+	+	++	+	++	n/a
Mallein skin test	+	+	+	+	+	n/a
Western blotting	+	+	++	+	++	n/a

Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose; n/a = purpose not applicable.
PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay.

Complement fixation test

the **prescribed technique** for **trade purposes** to certify individual animal freedom from disease



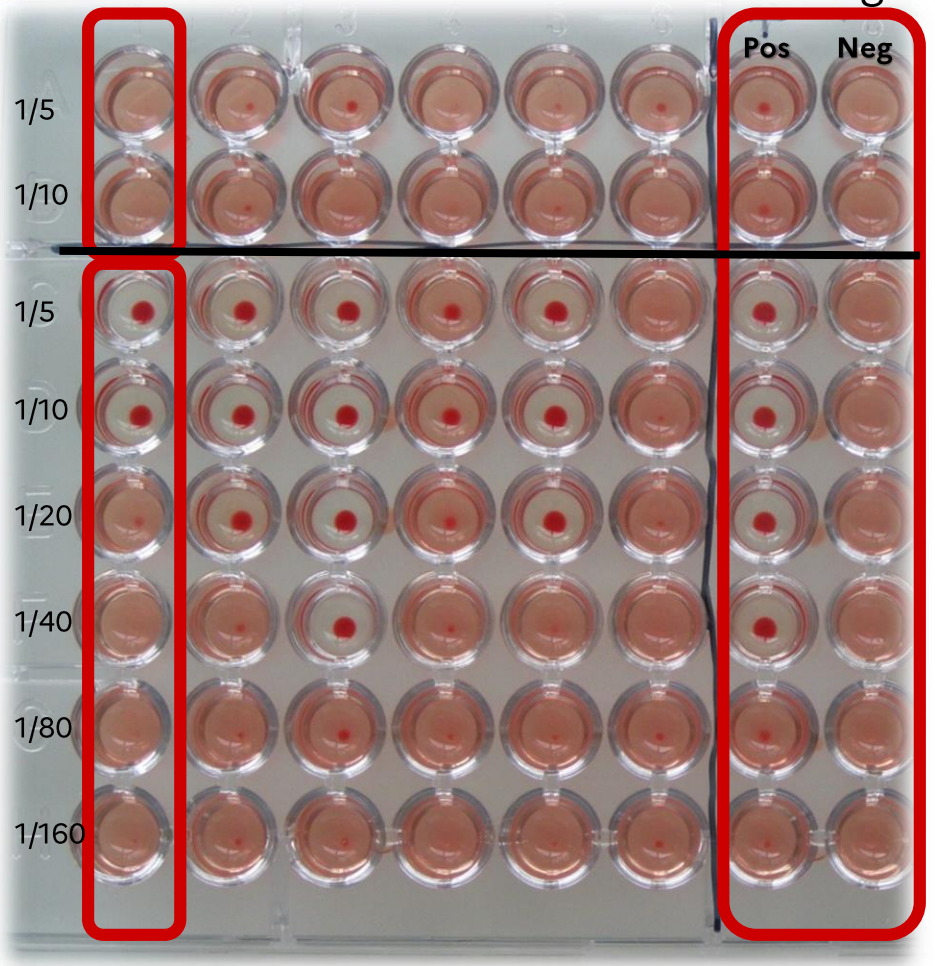
If no Ab, the complement is not fixed by the Ag/Ab complex
⇒ the complement is free and induces the hemolysis of RBC

- Complement fixation reaction (or complement bypass): detection of antibodies in de-complemented sera ....
- Complement fixation on the antigen-antibody complex inhibits lysis of the hemolytic complex

Detection of immune response

Complement fixation test

Sample A



3- Control n°3
(Anticomplementarity)

4- Reading of samples
(supernatant color)

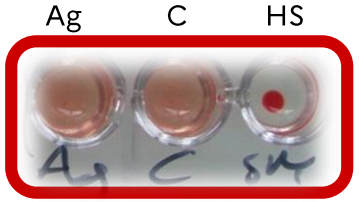
Positive if 100 % hemolysis at 1/5

Controls

2- Control n°2
Reading of Pos and Neg controls

With no Ag added

With Ag added



1- Control n°1
(Reagent controls)

Detection of immune response

Complement fixation test (prescribed technique for trade purposes)

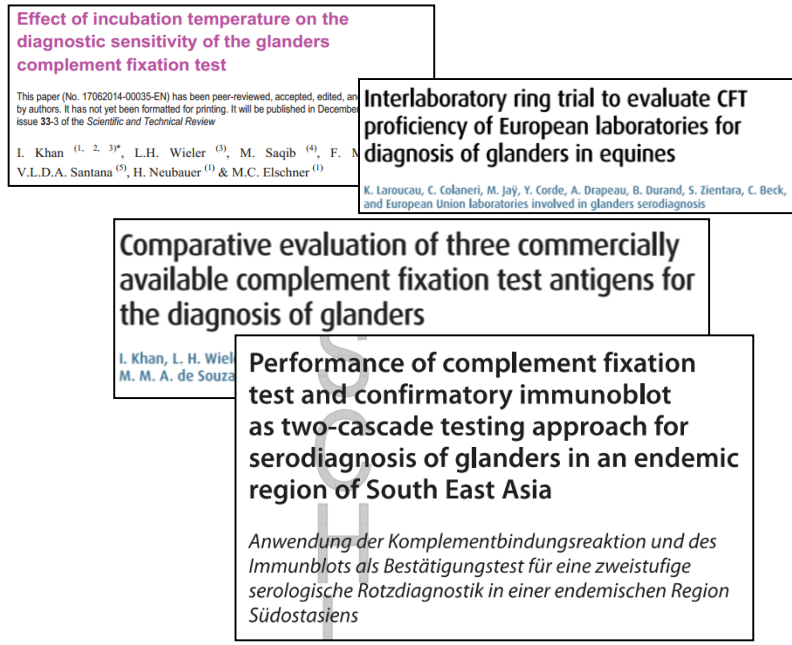
DSp testing on 400 true-negative samples	False Pos	True Neg	DSp %	CI 95%
	12	388	97,0	94,8 - 98,4
DSe testing on 370 true-negative samples	**False Neg**	**True Pos**	**DSe %**	**CI 95%**
	13	357	96,5	94,1 - 98,1

Performances in reference/trained laboratories

[Elschner *et al.* 2021]

But CFT has limitations:

- needs technical expertise
- difficult to standardise (influence on DSe & DSp)
  - various antigens
  - various protocols (warm *vs* cold incubation)
  - no standardised reagents
- sera from old, pregnant and emaciated animals can give false negative results
- limitation to test anticomplementary sera (donkeys)
- false positive results to due cross reactions (crude whole cell preparations)



⇒ advised to combine **CFT with a <u>more specific</u> and <u>complementary/independent</u> test**

Detection of immune response: available complementary tests

- Commercial ELISA tests

Name	Company, Country	Caracteristics	References
Mormo ELISAi	Biovetech, Brazil	Semi-purified fraction (proteins and LPS) [Sp 98,2%, Se 100%] (171 neg/129 pos)	Teles et al. 2012
ELISA-BKM16 (Panaftosa, Brazil)	Panaftosa, Brazil	TssB recombinant protein [Sp 100%, Se 100%] (22 neg/34 pos)	Report-Performance-ELISA-BKM16-eng.PDF (paho.org)
ID Screen® Glanders Double Antigen Multi-species	IDvet, France	Recombinant protein [Sp 99,8%, Se 98,1%] (400 neg/370 pos)	Elschner et al. 2021
Glanders – Ab Rapid Detection Test kit	Genomix, India	Hcp1 recombinant protein [Sp 99,6%, Se 95,3%] (2959 neg, 254 pos)	Elschner et al. 2019
AsurDx™ Burkholderia Mallei Antibody Test Kit	Biostone, USA	*B. mallei* antigen – competitive ELISA No data available	/

Tests can vary in terms of their
- purpose,
- accuracy,
- reliability,
- sensitivity,
- specificity....

Detection of immune response: available complementary tests

- Tests only available in WOAH reference laboratories

Method	WOAH ref lab performing the test	Caracteristics	References
WB	only in WOAH lab (FLI, Germany)	LPS [Sp 99,2%, Se 97,3%] (400 neg/370 pos)	Elschner et al. 2021
ELISA	only in WOAH lab (CVRL, UAE)	LPS (competitive ELISA) [Sp 100%, Se 97,2%] (41 (+136) neg/31 pos)	Wernery et al., 2021
Luminex	only in WOAH lab (Anses, France)	Recombinant proteins (set of proteins) Hcp1 [Sp 99,5%, Se 100%] (198 neg/99 pos) GroEL [Sp 99,5%, Se 97,0%] (198 neg/99 pos)	Laroucau et al., 2020

Detection of immune response

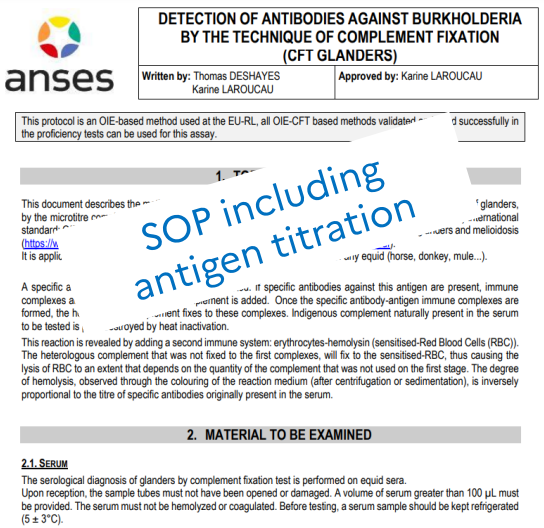
In summary

Methods	Sample
CFT (reference test) ELISA tests WB Luminex } Confirmatory tests	serum

Serology: How WOAH Reference Lab can help?

World Organisation for Animal Health
Founded as OIE

- **detailed SOP available for CFT**
- **standard serum for CFT harmonisation** (antigen titration)
- **Training sessions** (CFT, ELISA, including ISO 17025 quality management principles)
- **Proficiency tests**
- **Confirmatory testing of suspect samples** (CFT, ELISA and in-house methods)


anses

DETECTION OF ANTIBODIES AGAINST BURKHOLDERIA BY THE TECHNIQUE OF COMPLEMENT FIXATION (CFT GLANDERS)

Written by: Thomas DESHAYES, Karine LAROUCAU
Approved by: Karine LAROUCAU

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

SOP including antigen titration

2. MATERIAL TO BE EXAMINED

2.1. SERUM

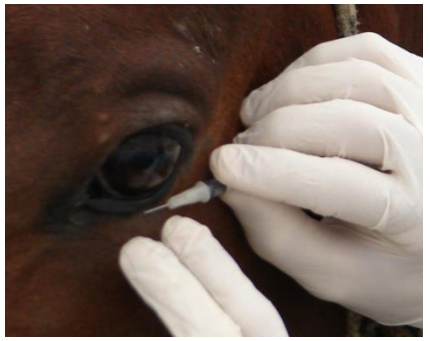
The serological diagnosis of glanders by complement fixation test is performed on equid sera.
Upon reception, the sample tubes must not have been opened or damaged. A volume of serum greater than 100 µL must be provided. The serum must not be hemolyzed or coagulated. Before testing, a serum sample should be kept refrigerated (5 ± 3°C).

Lyophilised positive control for antigen calibration and control

Mallein test

To detect a cellular response

- Based on a protein fraction of *B. mallei* (purified protein derivative (PPD) - solution of watersoluble protein fractions of heat-treated *B. mallei*)
- Injected intradermo palpebrally, subcutaneously or given by eye drop
- Resulting fever, swelling or efflux of pus from the eye in positive animals



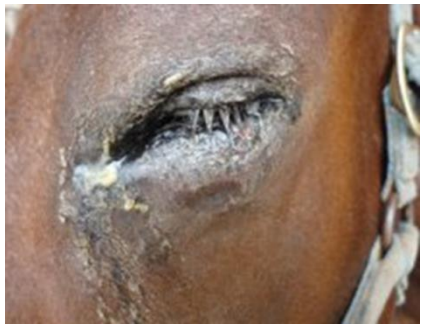
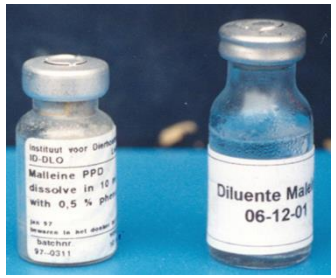


Figure 1: Inoculation sous-cutanée de malléine à des fins diagnostiques. AXE Prof. J. Wortley, M.R.C.V.S., Ex-President of the Royal College of Veterinary Surgeons [...]. *The Horse, its treatment in health and disease, with a complete guide to breeding, training and management*, London, The Gresham Publishing Co, 1906, 9 vol., t. 4, p. 38.



Advanced clinical cases in horses and acute cases in donkeys and mules may give inconclusive results

- Still used in some countries
- Require consideration for its replacement (a 2nd visit required for reading the reaction in the animal (at least 48h), and concerns for animal welfare)

Agent Identification

B. DIAGNOSTIC TECHNIQUES

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

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification						
PCR	–	–	–	+	–	n/a
Culture	–	–	–	+	–	n/a
Detection of immune response						
Complement fixation	++	++[1]	+++	+	+++	n/a
ELISA	+	+	++	+	++	n/a
Mallein skin test	+	+	+	+	+	n/a
Western blotting	+	+	++	+	++	n/a

Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose; n/a = purpose not applicable.
PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay.

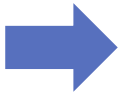


Table 1. *Test methods available for the diagnosis of glanders and their purpose*

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification						
PCR	–	–	–	+	–	n/a
Culture	–	–	–	+	–	n/a

Agent Identification – Culture

To isolate the strain

B. DIAGNOSTIC TECHNIQUES

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

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification						
PCR	–	–	–	+	–	n/a
Culture	–	–	–	+	–	n/a
Detection of immune response						
Complement fixation	++	++[1]	+++	+	+++	n/a
ELISA	+	+	++	+	++	n/a
Mallein skin test	+	+	+	+	+	n/a
Western blotting	+	+	++	+	++	n/a

Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose; n/a = purpose not applicable.
PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay.

- Growth on **routine culture media** (eg. nutrient, blood and Mc Conkey agar)
- Viscid, smooth and creamy colonies can be obtained after 48h at 37° C
- *B. mallei* can be grown in pure culture from fresh glanderous lesions or tissues during active infection, but can be **overgrown** by the normal flora because of its slow growing nature
- **Glycerol enrichment** enhances growth – New media recently described

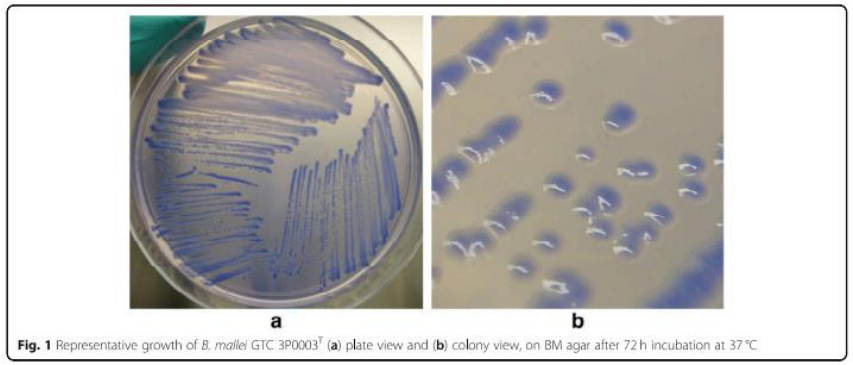


Fig. 1 Representative growth of *B. mallei* GTC 3P0003[T] (**a**) plate view and (**b**) colony view, on BM agar after 72 h incubation at 37 °C

NEW

RESEARCH ARTICLE Open Access

A novel selective medium for the isolation of *Burkholderia mallei* from equine specimens

Yuta Kinoshita[1,2*], Ashley K. Cloutier[3], David A. Rozak[3], Md. S. R. Khan[2], Hidekazu Niwa[1], Eri Uchida-Fujii[1], Yoshinari Katayama[1] and Apichai Tuanyok[2]

Circular, purple, smooth, >1 mm colonies, 48-72h

Bpm, Bt, Bcc grow also

- Isolation from equine blood culture has also been described (but rare)

Agent Identification - Culture

In summary

Methods	Samples
Culture	Tissues, Lesions, Swabs

Pus, open ulcers, lungs, organ abcesses, nasal mucosa...

Tissues/samples

- should be **sent under secure conditions**
- should be **kept cool** and shipped on ice as soon as possible

Agent Identification: Molecular detection

To detect DNA

B. DIAGNOSTIC TECHNIQUES

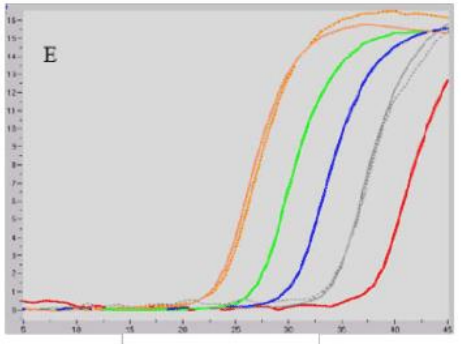
Table 1. Test methods available for the diagnosis of glanders and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification						
PCR	–	–	–	+	–	n/a
Culture	–	–	–	+	–	n/a
Detection of immune response						
Complement fixation	++	++[1]	+++	+	+++	n/a
ELISA	+	+	++	+	++	n/a
Mallein skin test	+	+	+	+	+	n/a
Western blotting	+	+	++	+	++	n/a

Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose; n/a = purpose not applicable.
PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay.

In summary

Methods	Samples
PCR	Tissues, Lesions, Swabs Isolates



PCR can offer better sensitivity compared to culture
(success of isolation is influenced by sample preservation, commensal overgrowth, and generally low bacterial load in tissues)

General information

- *B. mallei* (around 5,8 Mbp) is a clonal descendant of *B. pseudomallei* (around 7,2 Mbp) that has undergone genome decay and lost the capacity for environmental survival.

- Nearly all *B. mallei* genes have orthologs in *B. pseudomallei.*
- *B. mallei* genome continues to evolve through random IS-mediated recombination events

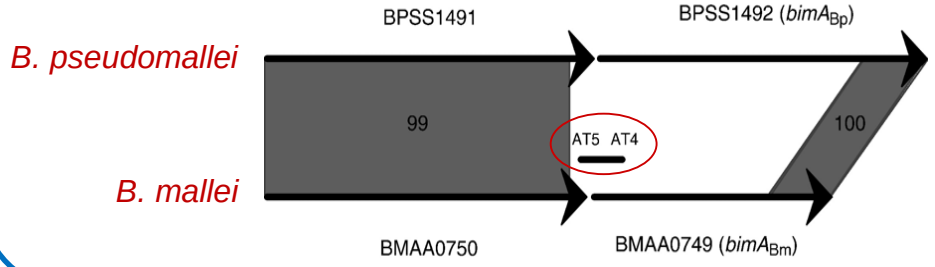
⇒ **Specific *B. mallei* PCR systems are available**

Agent Identification: *B. mallei* specific PCR systems

Several PCR systems are available, targeting distinct and unique *B. mallei* sequences, including:

bimA (*Burkholderia* intracellular motility A)

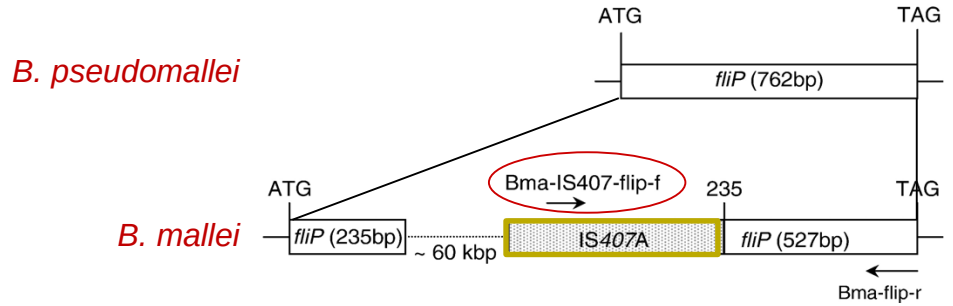
= a polarly localized surface protein that binds actin and promotes its polymerization. The N terminal nucleotide sequence of *bim*A contains a unique Bm region not present in Bpm and Bt *bim*A genes



fliP (flagellin gene)

= IS407A insertion inside the *fli*P locus of Bm

A frequently used PCR system overlapping *fli*P and IS407A for the species-specific identification of *B. mallei*



[Tomaso et al, 2006; Scholz et al, 2006]

Insertion sequence: genetic element with the ability to move within a genome

⇒ Genetic evolutions of *B. mallei* may lead to the emergence of new variants that can no longer be detected by the current PCR systems

Strains evolving continuously….

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

Karine Laroucau[1] | Rachid Aaziz[1] | Fabien Vorimore[1] | Koshy Varghese[2] | Thomas Deshayes[1] | Claire Bertin[1] | Sabine Delannoy[3] | Attia M. Sami[2] | Maha Al Batel[2] | Mamdouh El Shorbagy[2] | Khaled A. W. Almutawaa[2] | Saad J. Alanezi[2] | Yousef S. N. Alazemi[2] | Vanina Guernier-Cambert[4] | Ulrich Wernery[5]

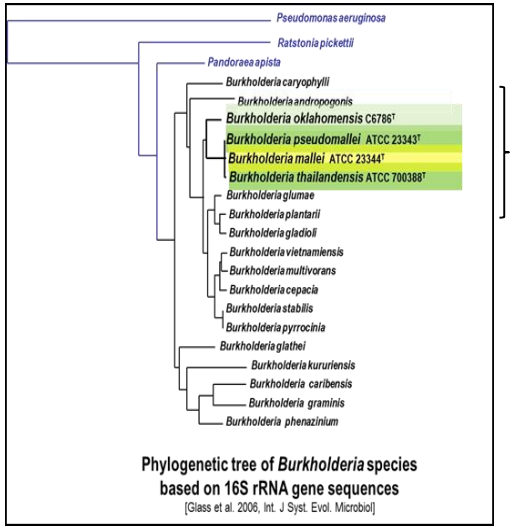
Kuwait « atypical » case

Serology

Horse Id	CFT		iELISA		Luminex®			
	Result	Titer	Result	% pos	BimA	Hcp1	GroEL	TssB
3	P	444443	P	73	P	P	P	P
4	P	4442	P	107	P	P	P	P
6	P	444441	P	119	P	P	P	P
37	P	4441	P	96	P	P	P	P
38	P	333	n	47	P	P	P	P
39	D	1	n	29	P	P	P	P

Positive animals

P: positive, D: Doubtful, n: negative



Phylogenetic tree of *Burkholderia* species based on 16S rRNA gene sequences
[Glass et al. 2006, Int. J Syst. Evol. Microbiol]

B. pseudomallei complex
- *B. mallei*
- *B. pseudomallei*
- *B. thailandensis*
- *B. oklahomensis*

multi-targets PCR scheme

Molecular detection
1st analysis

Horse Id	Description	IPC	real-time PCR: *B. mallei* *fliP*	*B. mallei* 16,5 kDa	*B. pseudomallei* *orf11*	*B. thailandensis* 70 kDa	*B. pseudomallei* complex *aroA*	*fliC*	*bpscU2*
3	Skin nodule 1	30,7	-	39,7	-	-	-	-	/
	Skin nodule 2	30,1	-	34,7	-	-	35,0	33,1	35,2
	Nasal turbinate	30,4	-	-	-	-	38,0	*42,4* *	/
	Nasal swab	30,6	-	*40,3* *	-	-	38,0	-	/
6	Retropharyngeal lymph noc	31	-	39,6	-	-	37,8	37,1	*40,4* *
	Nasal turbinate	30,3	-	-	-	-	-	-	/
	Trachea	29,6	-	-	-	-	-	-	/
	Lung	30,5	-	-	-	-	-	-	/

B. mallei *B. mallei* *B. pseudomallei* *B. thailandensis* Complexe Bpm

IPC: Internal control with exogenous DNA, (1)after pre-amplification, - (negative), / (not done)
*Cq over 40 were not considered

False negative results with *fliP* PCR
- Mutations on the primers/probe binding sites?
- Loss of the IS407A?
- Recombination in this region of the genome?

Agent Identification

to use a multi-targets PCR scheme
(even if rare event)

Example of a multi-targets PCR scheme

- for *B. mallei* *(FliP [Tomaso et al., 2006])*
- for *B. pseudomallei* *(orf11 [Thibault et al., 2004])*
- for *B. thailandensis* *(70 kDa [Lowe et al., 2016])*
- for complex *B. pseudomallei* *(in house)*

After obtaining an isolate or detecting a positive PCR signal from a tissue sample, what are the molecular traits of the circulating isolate(s)?

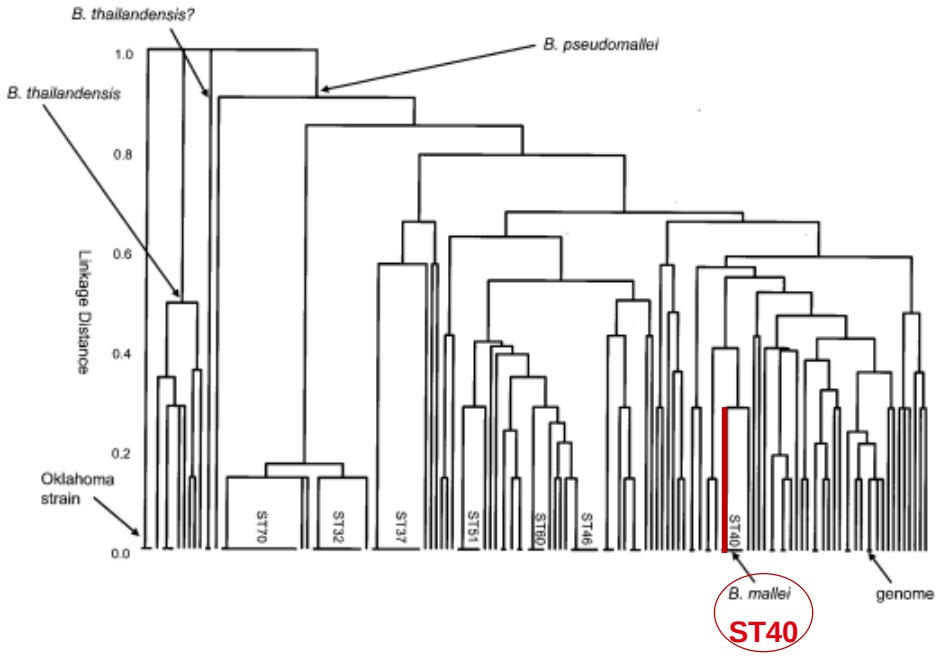
Agent Identification: Genotyping

Molecular typing of bacterial strains is a crucial method in epidemiology, enabling the differentiation and linkage of strains at the molecular level to :

- track disease spread
- understand transmission pathways
- develop prevention strategies

Genotyping methods developed for *B. pseudomallei* and applied to *B. mallei*

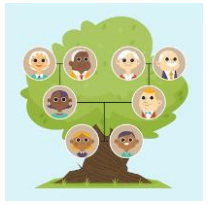
MLST (Multi-locus sequencing typing)
7 housekeeping genes [Godoy et al, 2003]



MLST, a method that enables discrimination for *B. pseudomallei* but not for *B. mallei* (single ST40)

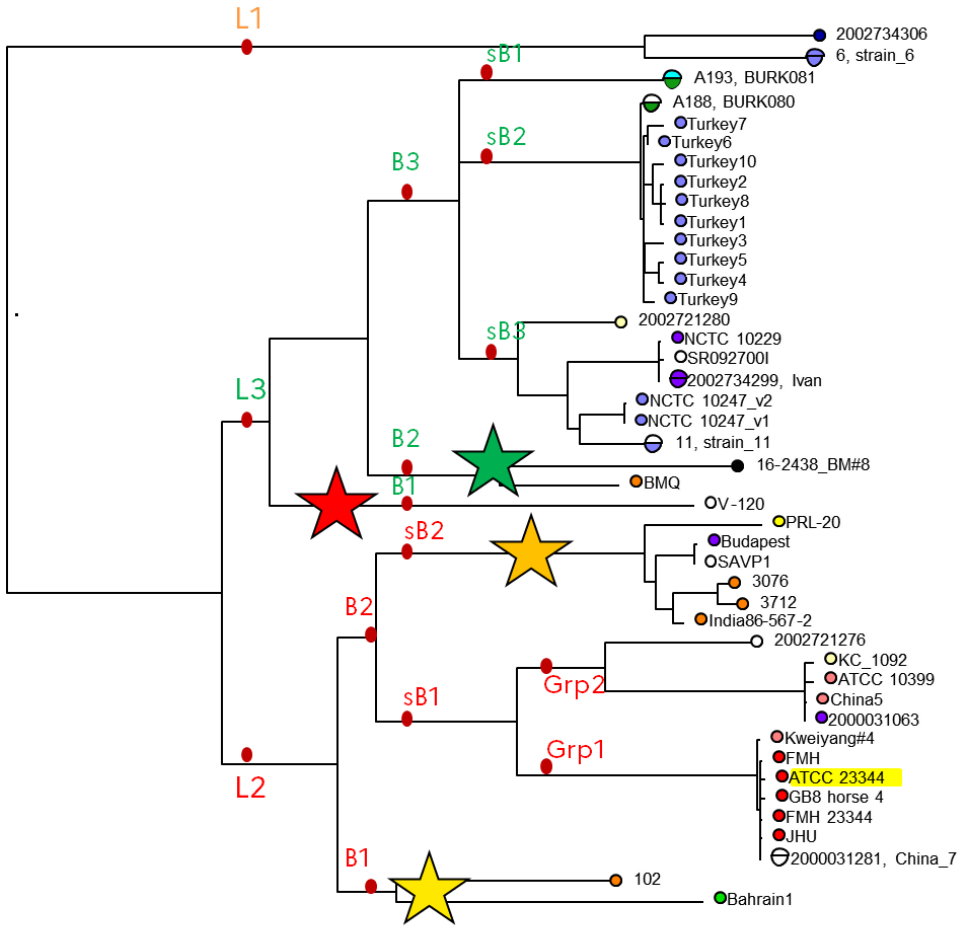
Agent Identification: Genotyping

The phylogenetic analysis of bacterial genomes, similar to the construction of a family tree, reveals evolutionary patterns and relationships between different strains.



B. mallei strains are grouped according to their respective geographical origin

PCR-HRM typing method (SNP-based) [strain or sample DNA]

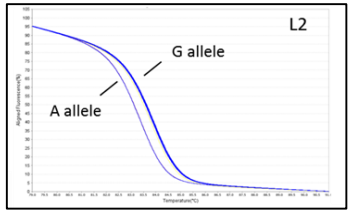


Brasil

East Asia

India/Pakistan

Middle East



anses

- Initial panel of 15 markers
- Synthetic DNA as positive control for each alleles
- Extra-markers available for geographical areas (Middle East, Brasil, India/Pakistan)

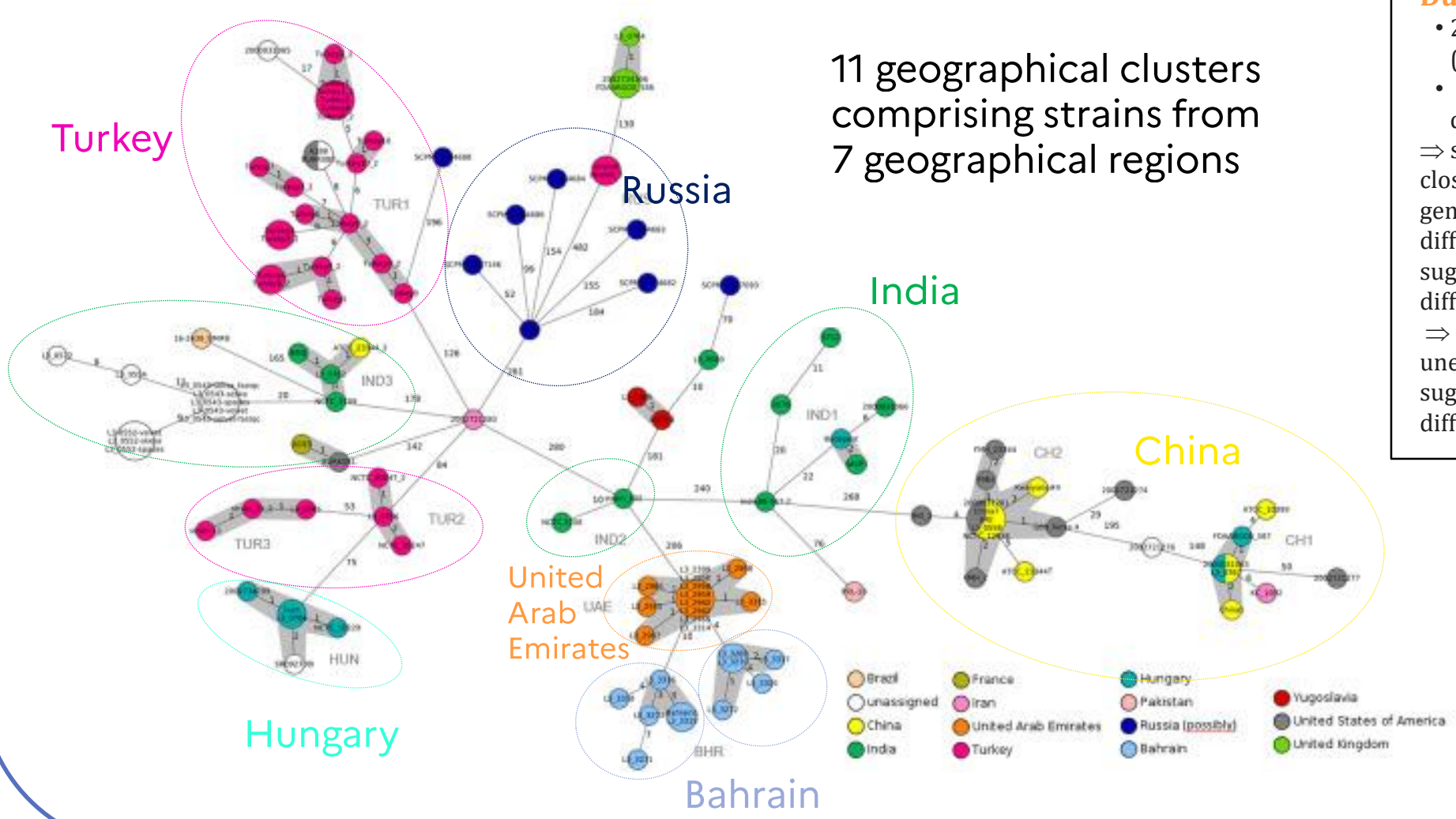
High-resolution melting PCR analysis for rapid genotyping of *Burkholderia mallei*

Girault G.[a], Wattiau P.[b], Saqib M.[c], Martin B.[a], Vorimore F.[a], Singha H.[d], Engelsma M.[e], Roest H.J.[e], Spicic S.[f], Grunow R.[g], Vicari N.[h], De Keersmaecker S.C.J.[i], Roosens N.H.C.[i], Fabbi M.[h], Tripathi B.N.[d], Zientara S.[a], Madani N.[a], Laroucau K.[a,*]

Agent Identification: Genotyping

Core MLST based method through WGS data [Strain + quality WGS data]

(3328 genes – 55,2% of the total seed genome)



11 geographical clusters comprising strains from 7 geographical regions

Dubai/Bahrain outbreaks

- 2004 – Dubai (UAE) - 6 horse strains (during quarantine)
- 2010/2011 – Bahrain 8 horse strains + 2 dromedary strains (large area in the north)

⇒ strains from Dubai (UAE) and Bahrain were closely related, but strains from Bahrain were genetically more diverse and formed two different clusters called BH-1 and BH-2, suggesting that the outbreak was caused by different strains.

⇒ the two strains of the same dromedary unexpectedly differed in 7 alleles, which suggests a simultaneous infection with two different *B. mallei* strains.

Genetic diversity and spatial distribution of *Burkholderia mallei* by core genome-based multilocus sequence typing analysis

Sandra Appelt[1], Anna-Maria Rohleder[1], Daniela Jacob[1], Heiner von Buttlar[2], Enrico Georgi[2], Katharina Mueller[2], Ulrich Wernery[3], Joerg Kinne[3], Marina Joseph[3], Shantymol V. Jose[3], Holger C. Scholz[1,2]*

Direct detection: How WOAH Reference Lab can help?

- **detailed SOP**
- **Training sessions**
- **Proficiency tests**
- **Confirmatory testing of suspect samples / glanders & melioidosis**
- **Typing and WGS approaches**

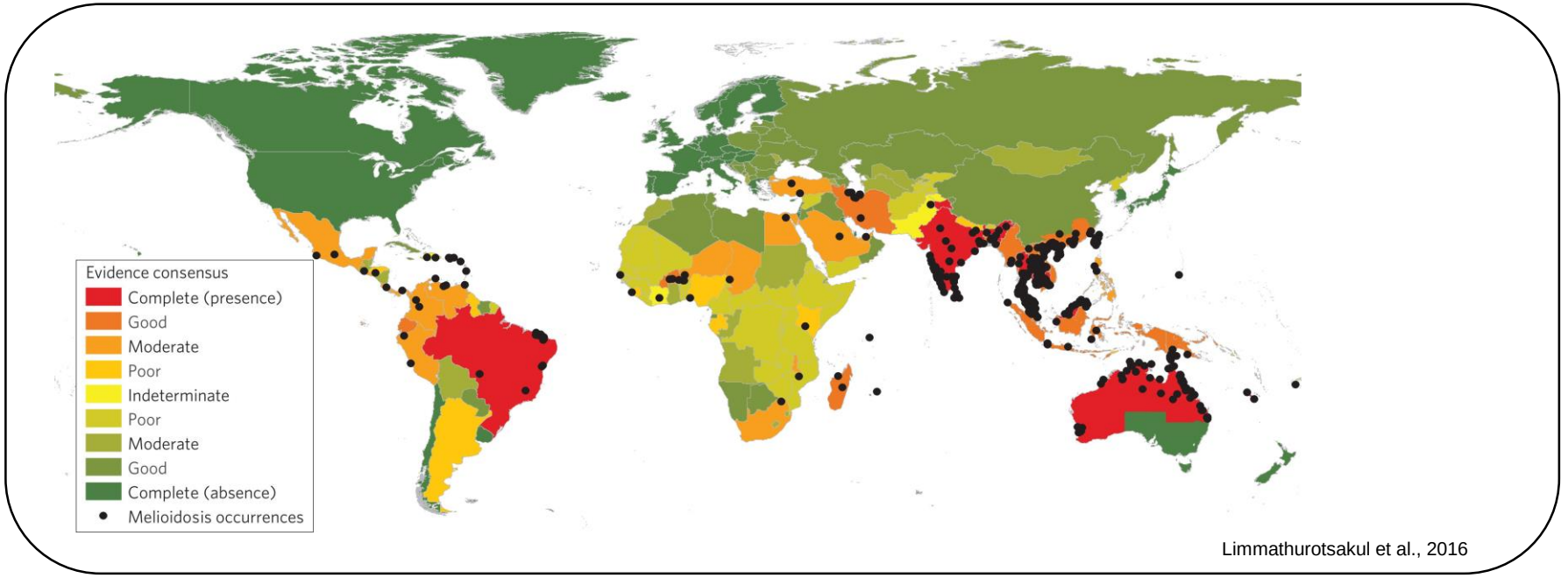
World Organisation for Animal Health
Founded as OIE

Part 3

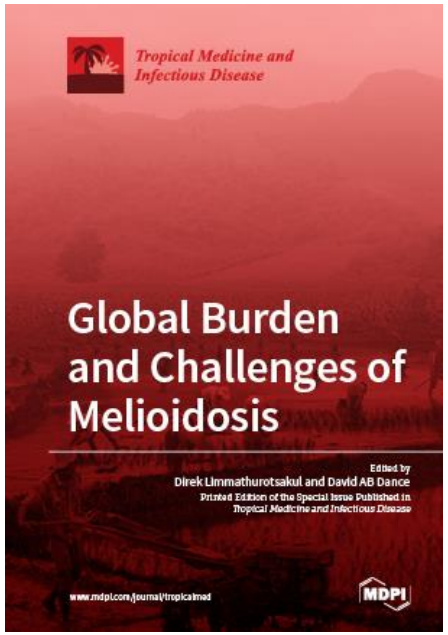
MELIOIDOSIS – A DISEASE TO CONSIDER

Priv. Doz. Dr. Dr. habil. **Ulrich Wernery**, CVRL, UAE
Dr **Karine Laroucau**, Anses, France

Melioidosis is so neglected that it is missing from all the lists of neglected tropical diseases…



Limmathurotsakul et al., 2016



Human melioidosis

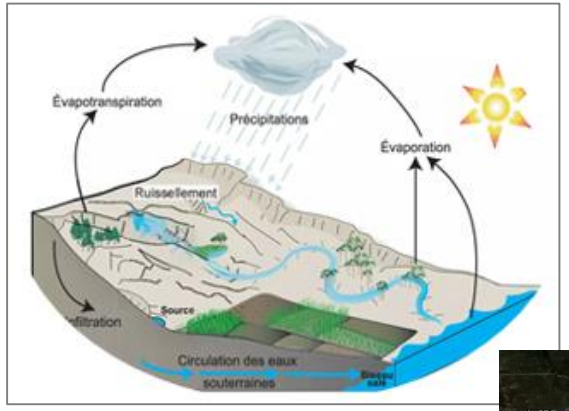
- Underestimated infectious disease
- Endemic in **Southeast Asia** and **Northern Australia**, sporadic in Africa and the Americas
- Estimates: 165,000 human cases/year, 89,000 deaths/year
- Difficult to diagnose and treat
- Many risk factors
- Environmental bacteria (*B. pseudomallei*)

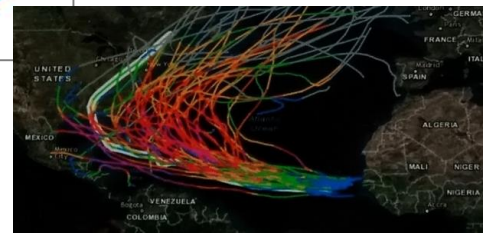
Melioidosis: a perfect illustration of the One Health concept

Burkholderia pseudomallei

Present in the environment (soil/water)

- High prevalence at soil depths > 30 cm
- Resistant to extreme conditions
- Seasonal effect - Dissemination through cyclones, floods





Many hosts (human/animal)

- Contamination through inhalation/ingestion/cutaneous route

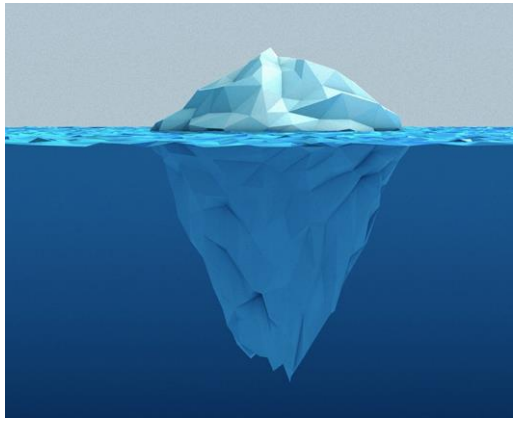
Wide range of clinical signs that can be mistaken for tuberculosis: Acute pulmonary infection (the most common), Focal infection, Septicemia, Neurological (rare)

Main risk factors: **diabetes**, liver disease, kidney disease, anemia, cancer or other immune disorders, chronic lung disease

Animal melioidosis: less data…

B. pseudomallei can infect a wide range of animals:
mammals, **birds**, **reptiles**, **fish**...

Sheep	Fever, severe cough, respiratory distress, nasal and ocular discharge, lameness, neurological signs.
Goats	Less severe respiratory infection than in sheep. Frequent mastitis with palpable abscesses. Emaciation, lameness, weakness of hind limbs, and abortion.
Pigs	Few significant clinical signs in adults, but progressive emaciation, neurological signs including lack of coordination, skin ulcers, and diarrhea. Juveniles can develop acute disease with fever, anorexia, cough, and nasal and ocular discharge.
Camelids	Respiratory infection with cough, nasal discharge, and breathing difficulties. Weakness of hind limbs or lack of coordination and emaciation. Acute infection leading to sudden death in camels and alpacas.
Bovine	Rarely reported infection. Fever, respiratory difficulties, and neurological signs.
Equids	Clinical signs similar to those of glanders: weakness, emaciation, limb swelling, mild colic, diarrhea, cough, and nasal discharge. Cases of acute disease with high fever, limb swelling, diarrhea, and death.
Birds	Relatively resistant to melioidosis, but cases of lethargy, anorexia, and diarrhea progressing to death have been reported.
Primates	Similar to humans

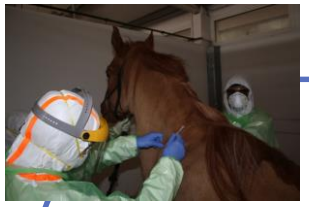


Melioidosis reported cases in equids

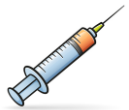
	Country	Total analysed	Cases		References		
			serology	bacteriology			
Horses/Ponies/Mules	Malaysia	1	0	1	Stanton 1927	imported horse from Australia / clinical signs	
	Malaysia	3	0	3	Davie & Wells 1952	imported race-horses / clinical signs	
	Egypt	1	1	0	McLennan 1952	polo pony /clinical signs	
	Malaysia	1	0	1	Omar 1963	horse / clinical signs	
	Australia	1	0	1	Laws & Hall 1963	horse / clinical signs	
	Iran	3	0	3	Baharsefat & Amjadi 1970	horses + mule / clinical signs	
	France	2	1	1	Nouvel 1977		Jardin des Plantes
	France	44	11	1	Bourrier 1978		
	France	300	79	33	Desbrosse 1978		
	Australia	8	0	1	Thomas 1981/Ladds 1981	horse / clinical signs	
	Malaysia	157	0	1	Ouadah 2006	horse / identification post-mortem	
	Thailand	1	0	1	Limmathurostsakul 2012	horse / identification post-mortem	
Zebras	Thailand	1	0	1	Limmathurostsakul 2012	in captivity / identification post-mortem	
	Thailand	1	0	1	Kasantikul 2015	in captivity / identification post-mortem	

Experimental infection of horses with *B. pseudomallei*

Research Article
Clinical and Pathological Changes of 6 Horses Infected with *Burkholderia pseudomallei*



Inoculum: 6.6×10^7 CFU/mL



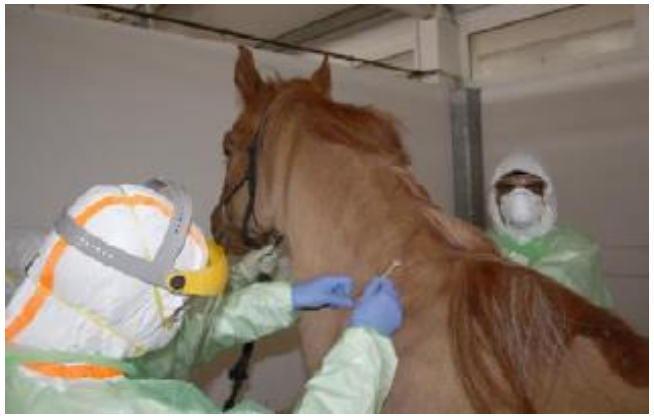
2 mL of inoculum subcuta -neously

piece of bread with 5 mL of inoculum

Horse ID	Route of infection	Clinical signs	Loss of weight	Euthanazed days pi	Pathology	Positive cultures
1	Sub-cutaneous	Fever (40ºC), nasal and ocular discharge	Yes	13	Pulmonary and renal abscesses; acute hemorrhagic cystitis. No lesions in nasal septum/ conchae	EDTA blood Urine swab Right and left lung Kidney Urinary bladder Injection site Left prescapular lymph node
2	Sub-cutaneous	No fever, mucopurulent nasal discharge	No	31	Pulmonary hemorrhages; subacute pyogranulative cystitis. No lesions in nasal septum/ conchae	Urine Urine swab Urinary bladder Injection site
4	Sub-cutaneous	Slight fever (38.6ºC), weight loss, stopped eating, no ocular or nasal discharge	Yes	44	Granulative cystitis. No pulmonary and renal lesions. No lesions in nasal septum/conchae.	Urinary bladder
3	Oral	No fever, lameness of both front legs swelling at chest, no nasal or ocular discharge	Yes	37	Granulative cystitis. No pulmonary and renal lesions. No lesions in nasal septum/conchae.	Tonsil
5	Oral	None	No	51	No pathological lesions.	/
6	Oral	None	No	58	No pathological lesions.	/

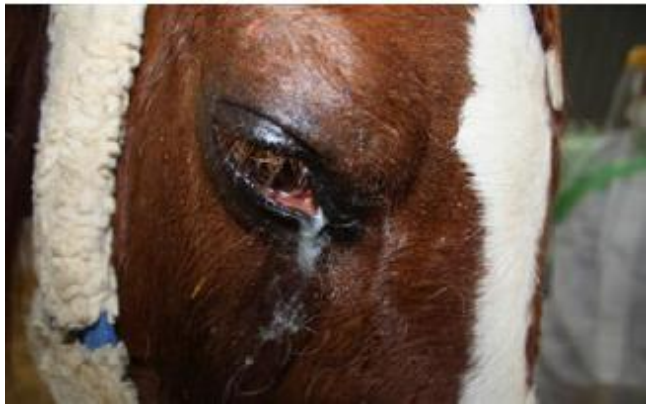
CVR LABORATORY

Clinical and Pathological Changes of 6 Horses Infected with *Burkholderia pseudomallei*

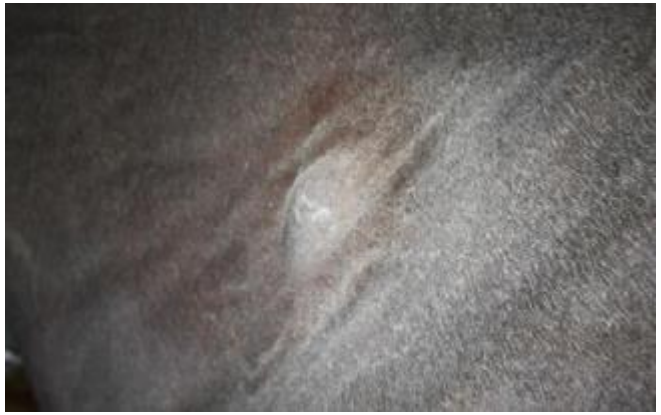


Subcutaneous injection

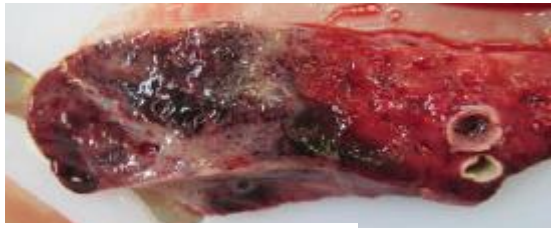
Occular discharge (8 dpi)



Abscessation of the injection site (7 dpi)



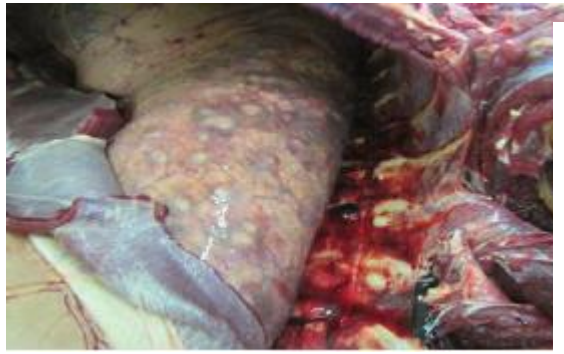
Mucopurulent nasal discharge (8 dpi)

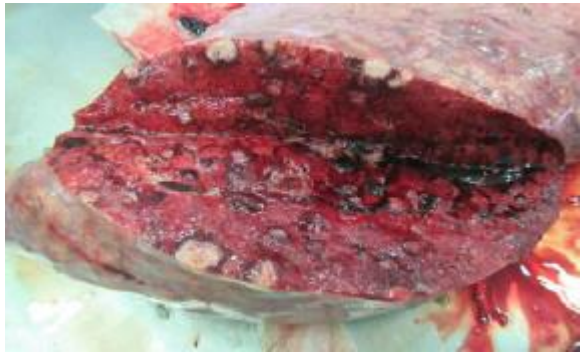


Lung cut surface with hemorrhagic oedema

Lungs

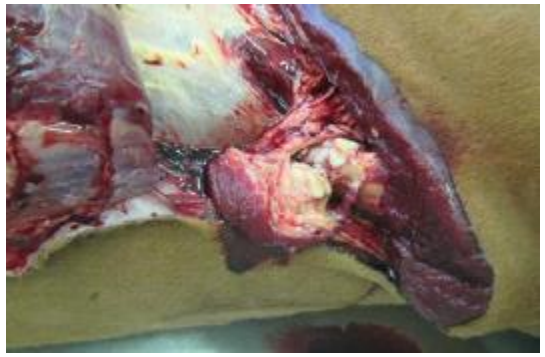
Subpleural lung abscesses of different size



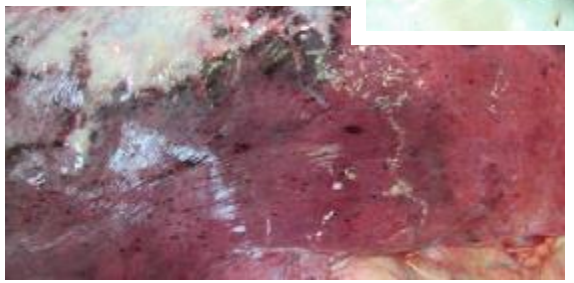


Lung cut surface showing whitish necrotic granulomas

Neck

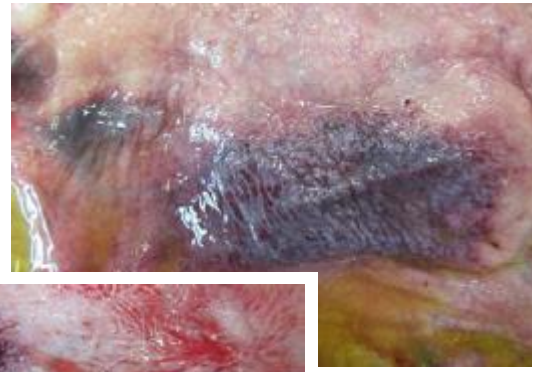


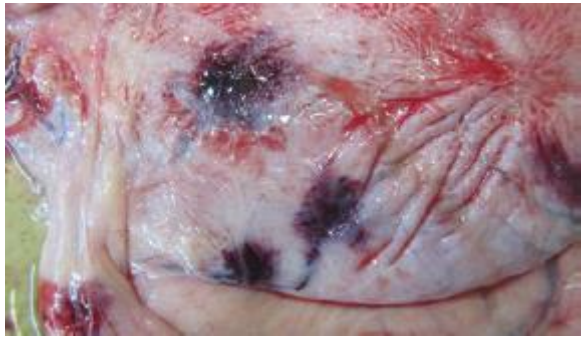
Left prescapular lymphnode abscess



Dorsal lung surface with greyish plaque and hemorrhages

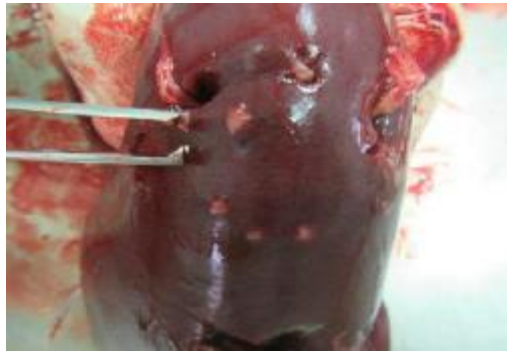
Oedematous urinary bladder wall with mucosal hemorrhages





Bladder

Thickened oedematous urinary bladder wall with massive ecchymosis



Pea size whitish abscesses in the renal cortex

Kidney

Diagnosis tools for Melioidosis in horses

Agent identification

Bacteriology: Sheep blood, Ashdown and Sabouraud dextrose agars…

PCR analysis: species-specific molecular tools (*B. mallei*/*B. pseudomallei*)

Example of a multi-targets PCR scheme

- for *B. mallei* (*FliP [Tomaso et al., 2006]*)
- for *B. pseudomallei* (*orf11 [Thibault et al., 2004]*)
- for *B. thailandensis* (*70 kDa [Lowe et al., 2016]*)
- for complex *B. pseudomallei* (*in house*)

Detection of immune response

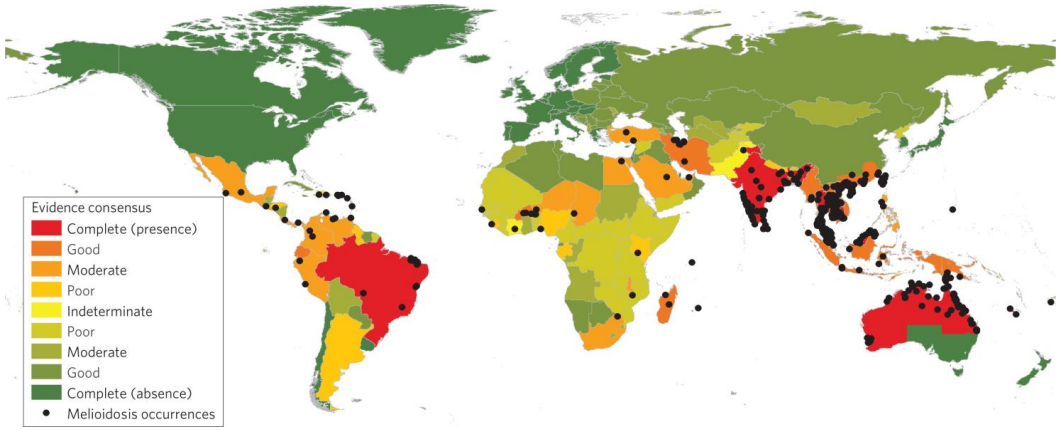
Current diagnostic tests developed for *B. mallei* are suitable for detecting an infection caused by *B. pseudomallei* (no distinction possible using serological tools): CFT, ELISA…

Evaluation of serological responses in horses challenged with* Burkholderia pseudomallei *using current diagnostic tests for glanders

Ulrich Wernery[1*] Marina Rodriguez Caveney[1], Renate Wernery[1], Rekha Raghavan[1], Karine Laroucau[2], Ginu Syriac[1], Shruti Miriam Thomas[1], Jeeba John[1], Marina Joseph[1], Shantymol Jose[1], Sunitha Joseph[1] and Patrick Woo[3]

In areas where *B. pseudomallei* is endemic, essential consider the possibility of melioidosis

- Differential diagnosis with glanders
- Potential impact on serology (exposure to *B. pseudomallei* in the environment)



Melioidosis: How WOAH Reference Lab can help?

- **Confirmatory testing of suspect samples / glanders & melioidosis**
- **Typing and WGS approaches**

World Organisation for Animal Health
Founded as OIE

Glanders and melioidosis are of Public Health importance, therefore:

- Probably underdiagnosed diseases
- Surveillance for both diseases is very important
- Capacity for rapid diagnosis and capacity to differentiate between the two diseases is essential
- Control options should be agreed with stakeholders
- Political support for control programs is essential