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EU Reference Laboratory
for equine diseases

GLANDERS
NEWS & RECENT ADVANCES IN SEROLOGY

KARINE LAROUCAU
BACTERIAL ZOONOSIS UNIT

14th Workshop of the EU RL for equine diseases – Maisons-Alfort - 16th of November 2021

Glanders: Update on the legislation & News

Commission européenne

Animal Health Law

EU: changes in the 'Animal Health Law'

COMMISSION DELEGATED REGULATION (EU) 2018/1629 of 25 July 2018 amending the list of diseases set out in Annex II to Regulation (EU) 2016/429 of the European Parliament and of the Council on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law').

NEW Glanders

efsa
European Food Safety Authority

working group
AHAW panel experts,
M. Elschner & K. Laroucau

EFSA received a mandate from the European Commission (EC) to assess the effectiveness of some of the control measures against diseases included in the Category A list according to Regulation (EU) 2016/429 on transmissible animal diseases ('Animal Health Law').

Assessment of the control measures of the category A diseases of Animal Health Law: *Burkholderia mallei* (Glanders)

To review the effectiveness of:
- the sampling procedures (in the event of suspicion or confirmation of Glanders/ for granting derogations for animal movements / for repopulation purposes)
- the monitoring period

Glanders: News

World Organisation for Animal Health

« Glanders and Melioidosis » updated chapter

☞ To be presented by U. Wernery

NEW OIE reference laboratory: Anses

Glanders

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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

anses

Glanders: Next challenges - Serology

Complement fixation test… harmonisation? (various antigens/protocols)

Presently, CFT is still the prescribed technique for trade purposes to certify individual animal freedom from disease. But CFT is difficult to standardise…

- **Detailed SOP available**
  - comments are welcomed
  - updated version for 2022

- **Need for a standard serum for CFT harmonisation**
  - positive sera have been collected (horses/donkeys sera from naturally infected animals / M. Saqib (Pakistan))
  - preliminary characterisation with FLI & CVRL

Interlaboratory ring trial to evaluate CFT proficiency of European laboratories for diagnosis of glanders in equines

K. Laroucau, C. Colaneri, M. Jaÿ, Y. Corde, A. Drapeau, B. Durand, S. Zientara, C. Beck, and European Union laboratories involved in glanders serodiagnosis

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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 and used successfully in the proficiency tests can be used for this assay.

1. TOPIC AND SCOPE

This document describes the method for the detection of antibodies specific to *Burkholderia mallei*, the agent of glanders, by the microtitre complement fixation test (CFT) according to the world organisation for animal health (OIE) international standard: OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals – Chapter 3.5.11, glanders and melioidosis (https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.05.11_GLANDERS.pdf).
It is applicable to the serological diagnosis of glanders from serum samples of any equid (horse, donkey, mule…).

A specific antigen is added to the serum to be tested. If specific antibodies against this antigen are present, immune complexes are formed. Heterologous complement is added. Once the specific antibody-antigen immune complexes are formed, the heterologous complement fixes to these complexes. Indigenous complement naturally present in the serum to be tested is prior destroyed by heat inactivation.

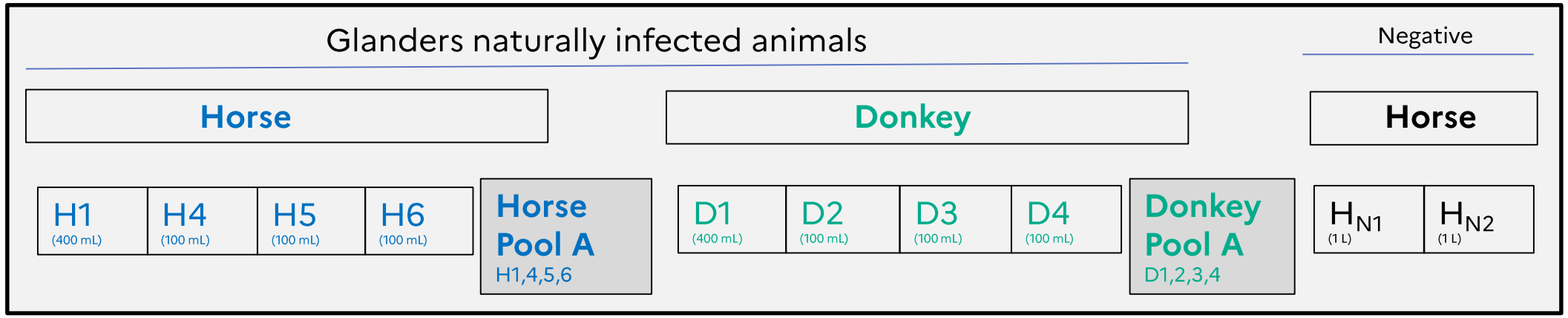
This reaction is revealed by adding a second immune system: erythrocytes-hemolysin (sensitised-Red Blood Cells (RBC)). The heterologous complement that was not fixed to the first complexes, will fix to the sensitised-RBC, thus causing the lysis of RBC to an extent that depends on the quantity of the complement that was not used on the first stage. The degree of hemolysis, observed through the colouring of the reaction medium (after centrifugation or sedimentation), is inversely proportional to the titre of specific antibodies originally present in the serum.

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).

❶ Selection of sera [20 animals, 75-450 mL] (Heat inactivation (Pakistan) + Filtration 0.22µm)



❷ Preliminary testing

	FLI (Ccpro)		Anses (Bioveta)		CVRL (Ccpro)	CVRL (in house)
Sample	warm	cold	warm	cold	warm	warm
Horse 1 ●	44442	44441	444441	444441	4444	4444
Horse 4 ●	444442	44441	444444	444444	*(AC 1+)* 4444	*(AC +1)* 4442
Horse 5 ●	44442	4443	444441	4443	*(AC 1+)* 4442	*(AC +1)* 44421
Horse 6	4444	*(AC 4+)* 44442	44444	*(AC 4+)* 44444	*(AC 4+)* 444	*(AC 4+)* 442
Horse Pool A (H1+H4+H5+H6)	44443	*(AC 3+)* 44442	44431	444441	*(AC 4+)* 4444	*(AC 4+)* 4442
Donkey 1 ●	444	4443	4441	4443	*(AC 4+)* 44	*(AC 4+)* 42
Donkey 2 ●	444	44443	4441	4443	*(AC 4+)* 44	*(AC 4+)* 42
Donkey 3 ●	444	4443	4441	4443	*(AC 4+)* 44	*(AC 4+)* 42
Donkey 4	444	4443	4441	4443	*(AC 4+)* 44	*(AC 4+)* 42
Donkey Pool A (D1+D2+D3+D4)	44444	4443	442	443	*(AC 4+)* 44	*(AC 4+)* 42

- AC results for almost all sera for CVRL
- Horse 6 gave AC results with the cold method for Anses and FLI

❸ Selection of sera New pools

Horse Pool B: Horse 1, Horse 4 & Horse 5
Donkey Pool B: Donkey 1, Donkey 2 & Donkey 3

Horse Pool B: Horse 1, Horse 4 & Horse 5
Donkey Pool B: Donkey 1, Donkey 2 & Donkey 3

❹ Selection for lyophilisation

	Anses (Bioveta)			
Sample	cold	cold	warm	warm
Horse Pool B - undiluted	4442	44431	44443	44441
Horse Pool B - 1/2	442	443	4444	4441
Horse Pool B - 1/5	3	31	431	441
Horse Pool B - 1/10	0	1	3	41
Donkey Pool B - undiluted	4431	441	431	431
Donkey Pool B - 1/2	43	31	2	31
Donkey Pool B - 1/5	1	0	0	0
Donkey Pool B - 1/10	0	0	0	0

4 First panels lyophilised:

- Horse Pool B – undiluted
- Horse Pool B – 1/5
- Donkey Pool B – undiluted
- Donkey Pool B – 1/2

❺ Lyophilisation…

❺ … and preliminary testings

Stability (+4°C)

	21/07/2021	22/07/2021	26/07/2021	09/08/2021	09/08/2021
	cold	cold	cold	cold	warm
Horse Pool B - undiluted	44442	444431	44442	44431	444441
	44442	444431	44442	44431	444441
	44442	44431	44442	44431	444441
Horse Pool B - 1/5	441	442	441	42	443
	441	442	441	42	443
	441	442	441	42	443
Donkey Pool B - undiluted	4442	4442	4442	442	443
	4442	4442	4442	442	443
	4442	4442	4442	442	443
Donkey Pool B - 1/2	441	441	441	42	42
	441	441	441	42	42
	441	441	441	42	42

Limit of detection (triplicates)

			1/5	1/10	1/20	1/40	1/80	1/160	1/320	1/640
Horse Pool B - undiluted	09/08/2021	Cold	4	4	4	3	1	0	0	0
Horse Pool B - undiluted	16/08/2021	Cold	4	4	4	4	2	0	0	0
Horse Pool B - undiluted	09/08/2021	Warm	4	4	4	4	4	1	0	0
Horse Pool B - undiluted	16/08/2021	Warm	4	4	4	4	4	0	0	0

To do: Comparative tests – to be tested in triplicate & in dilution

❻ ELISA? (ID Screen Glanders Double antigen (IDVet))

	DO_{450}	% pos	Result
Negative control	0,046	0	n
Negative control	0,046	0	n
Positive control	1,738	99	P
Positive control	1,758	101	P
Horse Pool B - undiluted_1/50	6,000	350	P
Horse Pool B - undiluted_1/100	4,874	284	P
Horse Pool B - undiluted_1/250	6,000	350	P
Horse Pool B - undiluted_1/500	4,682	272	P
Horse Pool B - undiluted_1/1000	4,179	243	P
Horse Pool B - undiluted_1/2000	2,654	153	P
Horse Pool B - undiluted_1/3000	1,826	122	P
Horse Pool B - undiluted_1/4000	1,558	104	P
Horse Pool B - undiluted_1/5000	1,137	75	P
Horse Pool B - 1/5_1/500	2,045	137	P
Horse Pool B - 1/5_1/1000	1,028	67	n
Horse Pool B - 1/5_1/2000	0,531	33	n
Horse Pool B - 1/5_1/4000	0,273	15	n
Donkey Pool B - undiluted_1/50	1,900	109	P
Donkey Pool B - undiluted_1/100	0,752	41	n
Donkey Pool B - undiluted_1/250	0,320	16	n
Donkey Pool B - undiluted_1/500	0,205	9	n
Donkey Pool B - undiluted_1/1000	0,128	5	n
Donkey Pool B - undiluted_1/2000	0,189	8	n
Donkey Pool B - 1/2_1/50	0,860	56	n

Horse Pool B - undiluted

Horse Pool B – 1/5

Donkey Pool B - undiluted

Donkey Pool B – 1/2

ID.vet

› ID Screen® Glanders Double Antigen Multi-species

Result	Status
S/P % < 70 %	NEGATIVE
S/P % ≥ 70 %	POSITIVE

- Ok for ELISA, dilutions to determine

❼ And now?

- Comparative tests – to be tested in triplicate & in dilution (panels ready)
- Horse Pool B – undiluted has been included in the proficiency test

Proficiency test 2021
- Horse 4 - 1/5
- Horse 6 - 1/5
- Horse Pool B - undiluted

2022: Lyophilisation of a large batch

- CFT Ag titration / CFT validation
- ELISA validation

	FLI (Ccpro)		Anses (Bioveta)		CVRL (Ccpro)	CVRL (in house)
Sample	warm	cold	warm	cold	warm	warm
Horse 1	44442	44441	444441	444441	4444	4444
Horse 4	444442	44441	444444	444444	(AC 1+) 4444	(AC +1) 4442
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Horse 6	4444	(AC 4+) 44442	44444	(AC 4+) 44444	(AC 4+) 444	(AC 4+) 442
Horse Pool A (H1+H4+H5+H6)	44443	(AC 3+) 44442	44431	444441	(AC 4+) 4444	(AC 4+) 4442
Donkey 1	444	4443	4441	4443	(AC 4+) 44	(AC 4+) 42
Donkey 2	444	44443	4441	4443	(AC 4+) 44	(AC 4+) 42
Donkey 3	444	4443	4441	4443	(AC 4+) 44	(AC 4+) 42
Donkey 4	444	4443	4441	4443	(AC 4+) 44	(AC 4+) 42
Donkey Pool A (D1+D2+D3+D4)	44444	4443	442	443	(AC 4+) 44	(AC 4+) 42

Glanders: Next challenges - Serology

CFT is still the prescribed technique

- **Alternatives / Complement tests to CFT** (false positive cases, anti-complementarity)?

2015-2017 - Large international consortium
(OIE funded project, led by FLI)

Project Application

OIE- INTERNATIONAL CALL FOR TENDER- Tender ref.: AD/SR/2015/1885

Applicant: OIE Reference Laboratory for Glanders - Friedrich Loeffler Institute, Federal Research Institute for Animal Health, Institute of Bacterial Infections and Zoonoses, Jena Germany, Head of OIERL: Prof. Heinrich Neubauer

Title of the project

Validation study of a western blot (WB) technique and ELISAs for serological diagnosis of glanders in equids for the purpose of certifying freedom from infection in individual animals for trade or movement.

RESEARCH ARTICLE

Evaluation of the comparative accuracy of the complement fixation test, Western blot and five enzyme-linked immunosorbent assays for serodiagnosis of glanders

Mandy Carolina Elschner[1]*, Karine Laroucau[2], Harisankar Singha[3], Bhupendra Nath Tripathi[3], Muhammad Saqib[4], Ian Gardner[5], Sheetal Saini[3], Subodh Kumar[6], Hosny El-Adawy[1,7], Falk Melzer[1], Iahtasham Khan[8], Praveen Malik[9], Carola Sauter-Louis[10], Heinrich Neubauer[1]

3000 negative sera
254 positive sera

- WB [LPS]
- 4 ELISA (BimA, Hcp1, TssA, TssB) [recombinant proteins]
- IDVet-ELISA [crude antigen] (prototype)

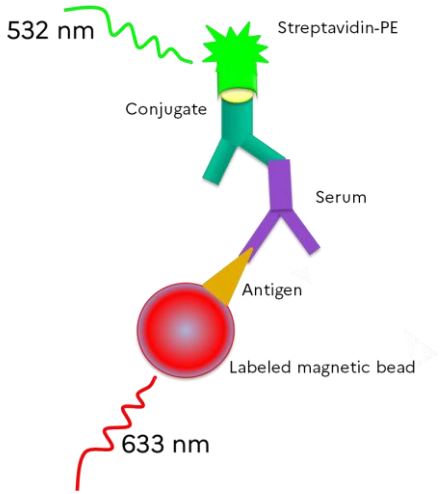
⇒ Comparable sensitivities and specificities for CFT (98.0%, 96.4%), WB (96.8%, 99.4%), Hcp1-ELISA (95.3%, 99.6%) and IDVet-ELISA (92.5%, 99.5%)
⇒ Promising results, but tests must be available (commercial format)

Glanders: Next challenges - Serology

- **Alternatives / Complement tests to CFT** (false positive cases. anti-complementarity)?

2020 – Luminex® approach

Recombinant proteins?



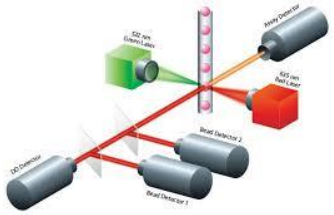
The microsphere-based immunoassay is a method that allows the analysis of antigens individually coated on labeled magnetic beads, within a micro array format requiring only a small volume of sample to test.

Development of a microsphere-based immunoassay for the serological detection of glanders in equids

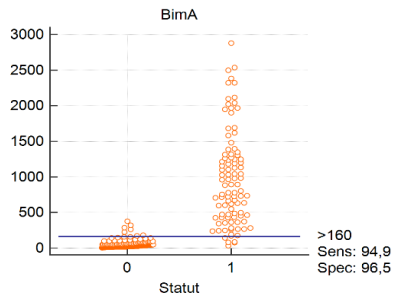
K. Laroucau[a,*], M. Saqib[b], B. Martin[a], T. Deshayes[a], C. Bertin[a], U. Wernery[c], S. Joseph[c], H. Singha[d], B.N. Tripathi[d], C. Beck[e]

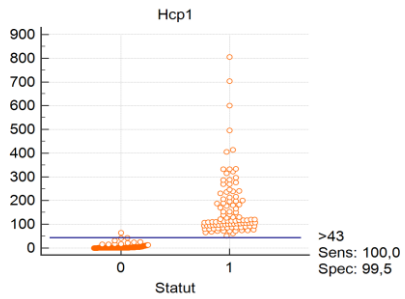
- BimA (intracellular motility A protein)
- TssB (secreted protein of the TT6S)
- GroEL (heat shock protein)
- Hcp1 (hemolysin-coregulated protein)

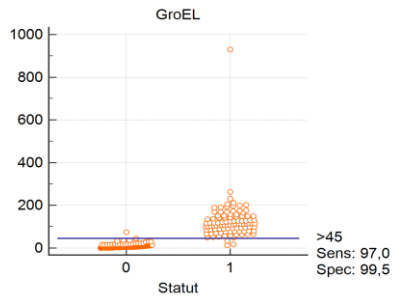
- P1 – infection free (n = 198) (EU or North Africa)
- P2 – glanders positive (n=99) (Pakistan & India)
- P3 – sera with Dbt/Pos CFT results
  - 17 from EU or North Africa
  - 14 from 4 French horses with Pos or Dbt CFT results

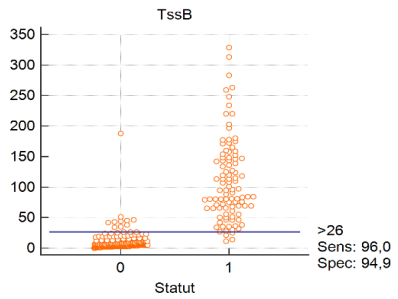


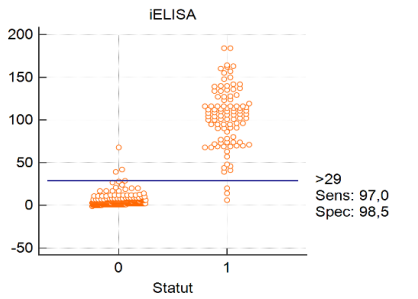
	Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
BimA	> 160	**94.9**	88.6-98.3	**96.5**	92.9-98.6	26.9	13.0-55.7	0.05	0.02-0.10
Hcp1	> 43	**100**	96.3-100	**99.5**	97.2-100	198.0	28.1-1398.7	0.00	
GroEL	> 45	**97.0**	91.4-99.4	**99.5**	97.2-100	192.0	27.2-1356.8	0.03	0.01-0.09
TssB	> 26	**96.0**	90.0-98.9	**94.9**	90.9-97.6	19.0	10.4-34.8	0.04	0.02-0.10
iELISA	> 29	**97.0**	91.4-99.4	**98.5**	95-6-99.7	64.0	20.8-196.8	0.03	0.01-0.09

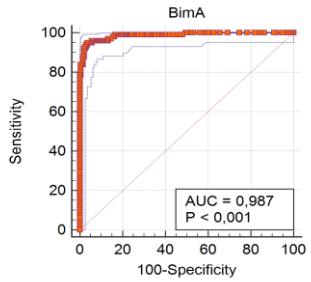


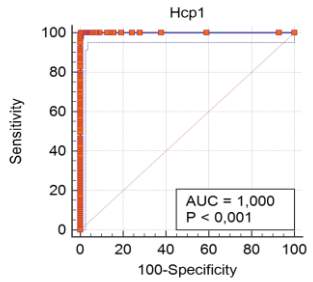


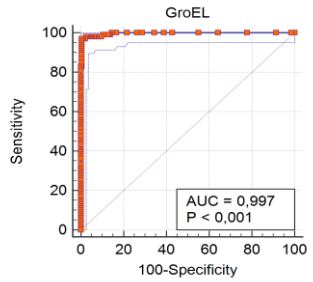


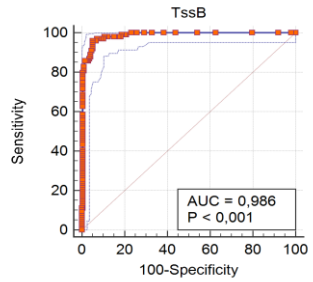


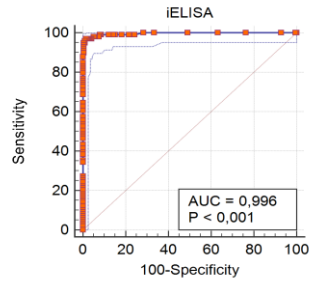






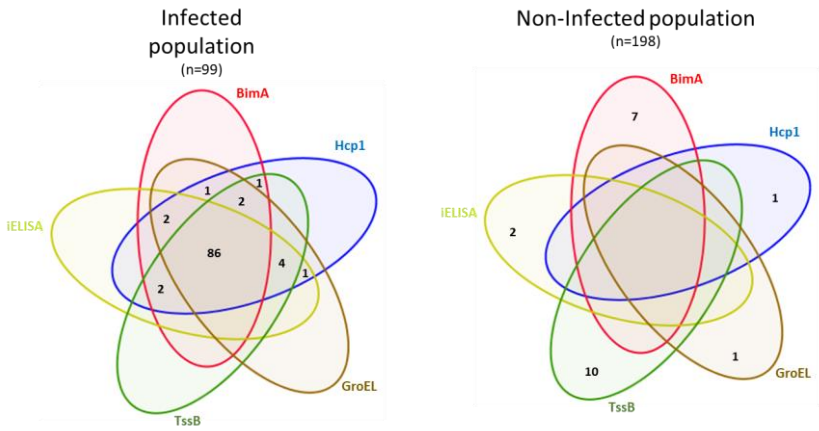






Id	Country	Sampling time	CFT (titer at 1/5)	iELISA (% pos) cut off >40	BimA (MFI) cut off >160	Hcp1 (MFI) cut off >43	GroEL (MFI) cut off >45	TssB (MFI) cut off >26
Single analysis								
18-4469/6911	France		D (1)	n (14%)	n (67)	n (3)	n (4)	n (4)
17-3597/7496	France		D (2)	n (4%)	n (39)	n (4)	n (14)	n (21)
17-3597/7495	France		D (1)	n (3%)	n (41)	n (3)	n (14)	n (20)
17-3376	Tunisia		D (1)	n (14%)	n (131)	n (0)	n (4)	n (12)
17-2173	France		D (2)	n (12%)	n (121)	n (6)	n (3)	n (5)
16-4070_10-363	UK		D (2)	n (25%)	n (28)	n (38)	n (3)	n (2)
15-965	France		D (1)	n (3%)	n (28)	n (4)	n (6)	n (26)
15-3119	France		D (2)	n (3%)	n (14)	n (3)	n (2)	n (3)
15-2480	Italy		D (2)	n (4%)	n (17)	n (4)	n (5)	n (8)
13-2741	France		D (1)	n (4)	P (240)	n (2)	n (3)	n (7)
13-2636	France		D (2)	n (9%)	n (97)	n (1)	n (26)	n (5)
13-1676/2877	France		D (3)	n (4%)	n (98)	n (3)	n (4)	P (70)
13-165/0308	Algeria		D (1)	n (5%)	n (44)	n (2)	n (22)	n (5)
13-165/0307	Algeria		D (1)	n (6%)	n (104)	n (1)	n (8)	n (1)
13-115/0251	Algeria		D (1)	n (16%)	n (34)	n (1)	n (13)	n (4)
13-082	France		D (2)	n (1%)	n (35)	n (1)	n (5)	n (3)
12-4065_8066	Ireland		D (2)	n (4%)	n (27)	n (1)	n (1)	n (1)
Follow-up								
Horse n°1	France	27/12/12	P (4)	n (18%)	n (83)	n (3)	n (2)	n (4)
		08/01/13	P (4)	n (8%)	n (58)	n (3)	n (2)	n (3)
Horse n°2	France	17/05/13	P (4)	n (3%)	n (72)	n (11)	n (2)	n (1)
		28/05/13	D (3)	n (2%)	n (91)	n (10)	n (2)	n (1)
Horse n°3	France	26/11/15	D (1)	n (6%)	n (15)	n (27)	n (4)	n (12)
		03/12/15	n	n (1%)	n (17)	n (17)	n (4)	n (11)
		21/12/15	n	n (1%)	n (27)	n (24)	n (3)	n (9)
Horse n°4	France	19/05/16	P (4)	n (3%)	n (79)	n (28)	n (29)	n (12)
		10/06/16	P (4)	n (4%)	n (56)	n (28)	n (20)	n (14)
		04/07/16	P (4)	n (4%)	n (52)	n (20)	n (17)	n (11)
		25/07/16	P (4)	n (5%)	n (46)	n (28)	n (13)	n (13)
		20/10/16	P (4)	n (4%)	n (29)	n (33)	n (6)	n (27)
		06/12/16	D (2)	n (6%)	n (22)	n (22)	n (8)	n (20)
		01/02/17	D (3)	n (27%)	n (29)	n (19)	n (15)	n (17)

- None of the EU or North Africa CFT positive/doubtful samples gave a positive response with ELISA (IDVet) or with the recombinant proteins
- Except 2 samples, with 1 reaction with 1 recombinant protein



Glanders: Next challenges - Serology

- **Alternatives / Complement tests to CFT** (false positive cases. anti-complementarity)?

2021 – Evaluation of a new commercial kit by FLI

- ELISA double antigen, based on a recombinant *B. mallei* antigen

Validation of a Commercial Glanders ELISA as an Alternative to the CFT in International Trade of Equidae

Mandy Carolina Elschner[1], Falk Melzer[1], Harisankar Singha[2], Saqib Muhammad[3], Ian Gardner[4] and Heinrich Neubauer[1]*

☞To be presented by M. Elschner

Glanders: Next challenges - Serology

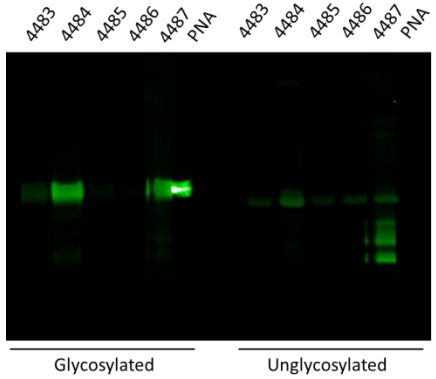
- **Alternatives / Complement tests to CFT** (false positive cases. anti-complementarity)?

2021 – ELISA based on a glycoengineered antigen

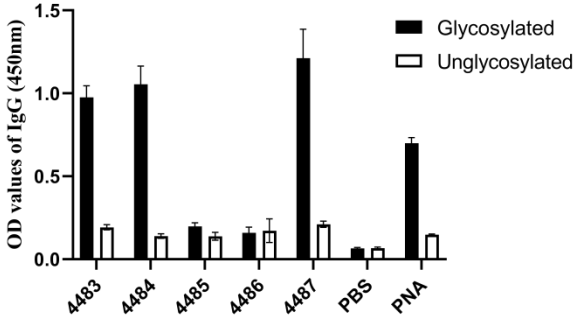
A glycoengineered antigen exploiting a conserved protein O-glycosylation pathway in the Burkholderia genus for detection of glanders infections

Guanbo Wang [a], Lena Glaser [a], Nichollas E. Scott [b], Yasmine Fathy Mohamed [a,c], Rebecca Ingram [a], Karine Laroucau [d], and Miguel A. Valvano [a]

- Evidence of glycosylated proteins in *Burkholderia*
- *Burkholderia*-specific glycan (β-Gal-(1,3)–α-GalNAc-(1,3)–β-GalNAc trisaccharide)
- O-glycan–specific antibodies in patients infected by *B. cenocepacia*, *B. multivorans*, *B. pseudomallei*
- Glanders??



Constructions to produce glycosylated and unglycolysated proteins (glycosylation-deficient mutants)



Sample	Origin	CFT[a]	iELISA[b]-semi-purified *B. mallei* (S/P%)	iELISA[c] CtxB-BCAL2737a	Western blot CtxB-BCAL2737a
18–117/302	France	-	- (3%)	-	-
18–1575/2905	France	± (1)	- (4%)	-	-
13–3290/7936	Ireland	-	- (3%)	-	-
13–3290/7937	Ireland	-	- (1%)	-	-
13–3290/7938	Ireland	-	- (0%)	-	-
13–3290/7939	Ireland	-	- (12%)	-	-
13–3290/7940	Ireland	-	- (1%)	-	-
18–103/214	Tunisia	-	- (7%)	-	-
18–103/215	Tunisia	-	- (3%)	-	-
18–103/216	Tunisia	-	- (4%)	-	-
18–103/218	Tunisia	-	- (7%)	-	-
14–566_1831	South America	+ (421)	+ (149%)	+	+
14–566_1850	South America	+ (44,431)	+ (128%)	+	+
14–566_1861	South America	+ (42)	+ (180%)	+	+
14–566_1899	South America	+ (4)	+ (134%)	+	+
14–566_1917	South America	+ (432)	+ (173%)	+	+
16–2439_81	South America	+ (44,442)	+ (63%)	+	+
16–2439_85	South America	+ (4442)	+ (146%)	+	+
19–5577_3	Middle East	+ (444,443)	+ (73%)	+	-
19–5577_4	Middle East	+ (4442)	+ (107%)	+	+
19–5577_6	Middle East	+ (444,441)	+ (119%)	+	+
19–5577_37	Middle East	+ (4441)	+ (96%)	+	+
19–5577_38	Middle East	+ (333)	+ (47%)	+	-
19–5577_39	Middle East	± (1)	- (29%)	-	-
Horse_1 (Océane)	$D_{08/02}$ immunization trial[d]	-	-	-	-
Horse_2 (Poupée)	$D_{08/02}$ immunization trial[d]	-	-	-	-
Horse_3 (Princese)	$D_{08/02}$ immunization trial[d]	-	-	-	-
Horse_4 (Quirina)	$D_{08/02}$ immunization trial[d]	-	-	-	-
Horse_1 (Océane)	$D_{19/04}$ immunization trial[d]	+ (32)	+ (47%)	-	-
Horse_2 (Poupée)	$D_{19/04}$ immunization trial[d]	+ (444)	+ (60%)	-	+
Horse_3 (Princese)	$D_{26/04}$ immunization trial[d]	+ (443)	+ (119%)	-	+
MRI#1	Lyophilized serum from a naturally infected horse	-	+ (111%)	-	-

Glanders (horses)

⇒ Alternative confirmatory test (not specific to *B. mallei*)

Glanders: Next challenges - Serology

Conclusions & Perspectives

- First panel of reference sera to be evaluated by the EU network for CFT standardisation

- New serological tests developed (partially validated, commercially available or only in research lab)

> "According to OIE (2018), supporting evidence of infection may be provided by a positive result in, e.g. CFT, which should be confirmed by a second test with equal or higher sensitivity and higher specificity"

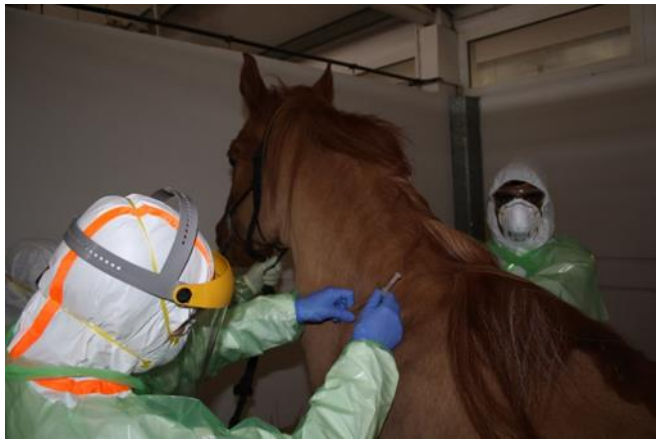
ELISAs are currently considered to be the most accurate and reliable assays...

... but efforts are still needed for their full validation

What about melioidosis?

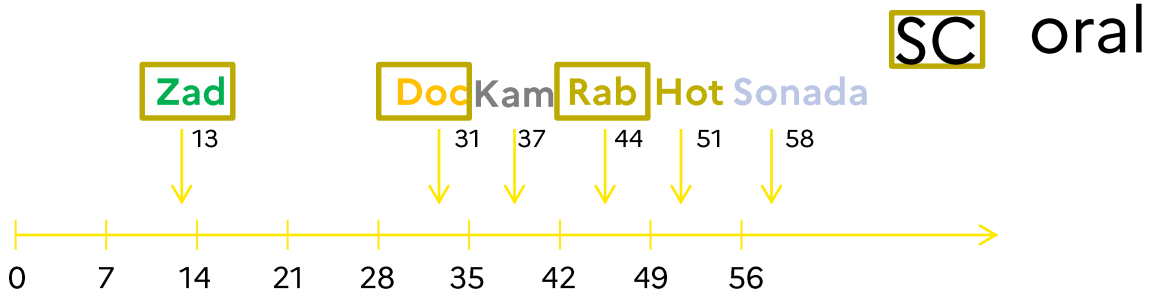
Evaluation of serological responses in horses challenged with* Burkholderia pseudomallei *using current diagnostic tests for glanders

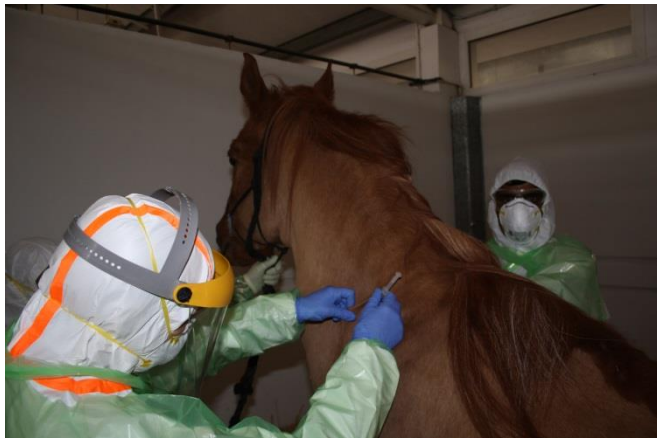
Ulrich Wernery[1*] Marina Rodriguez Caveney[1], Renate Wernery[1], Rekha Raghavan[1], Karine Laroucau[2], Ginu Syriac[1], Shruti Miriam Thomas[1], Jeeba John[1], Marina Joseph[1], Shantymol Jose[1], Sunitha Joseph[1] and Patrick Woo[3]



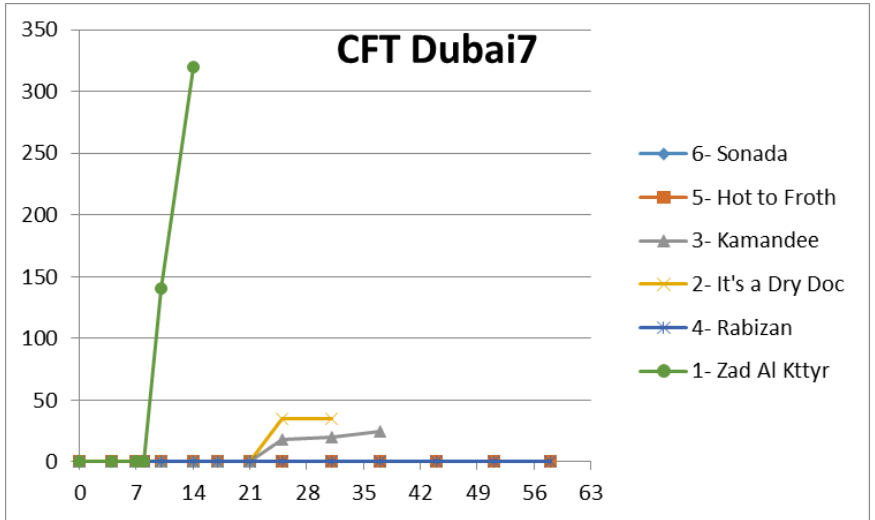
Thank you for your attention

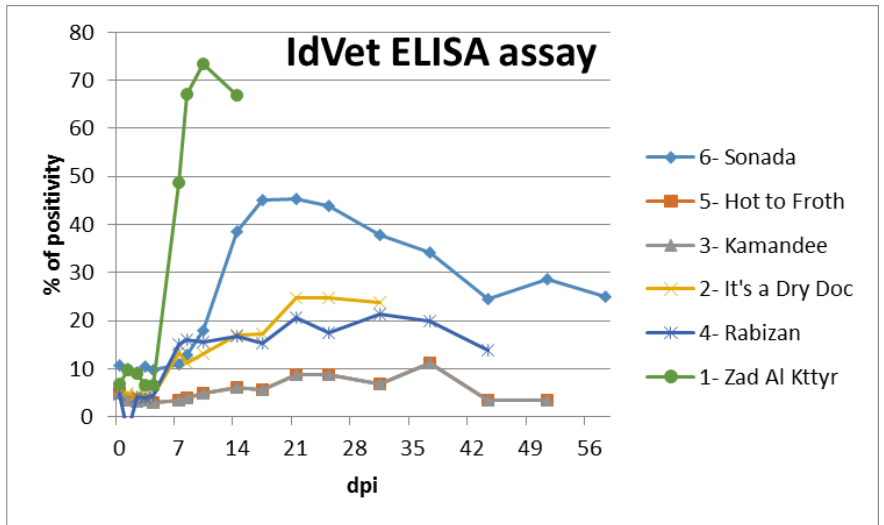
Cinétique d'infection avec *B. pseudomallei*

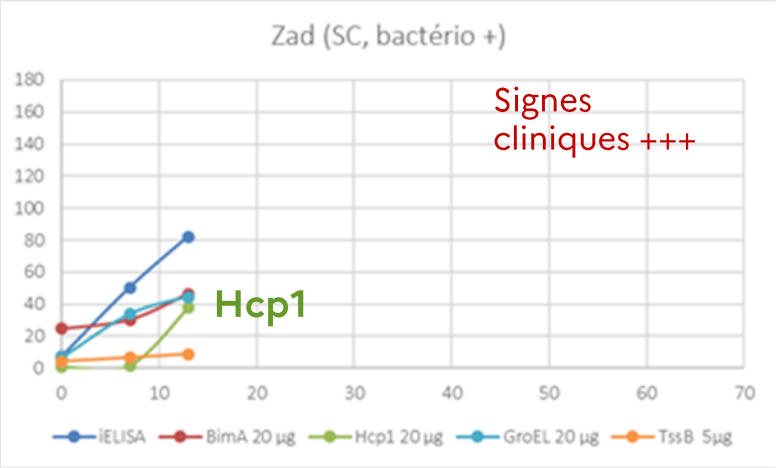


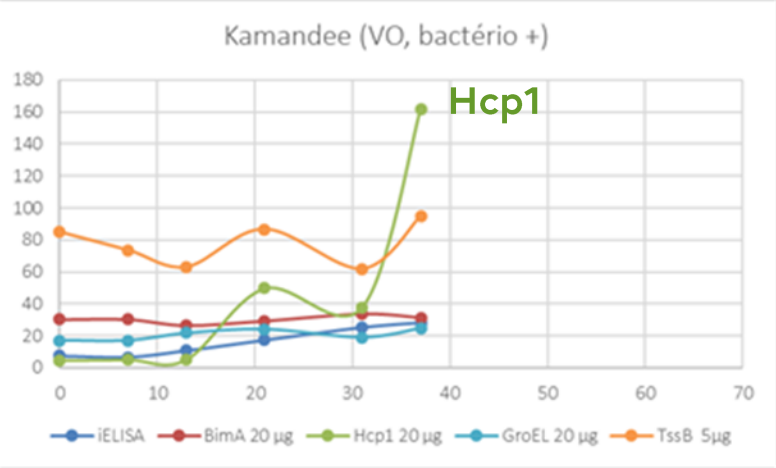


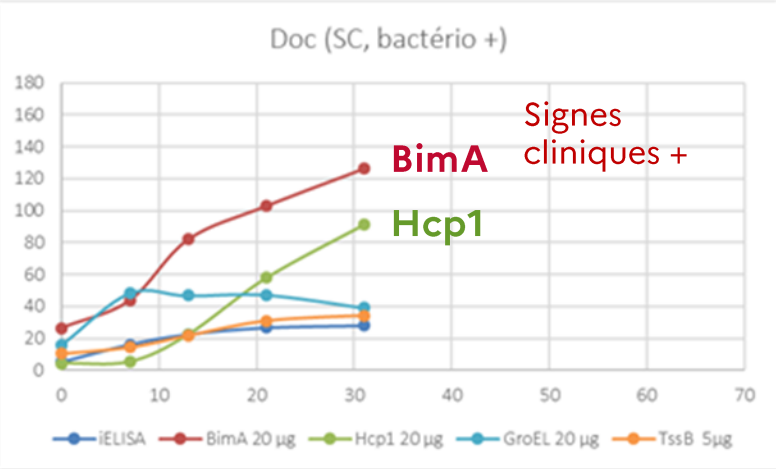
Collecte : sérums. plasma. tissus
(bactério. PCR. Histo)

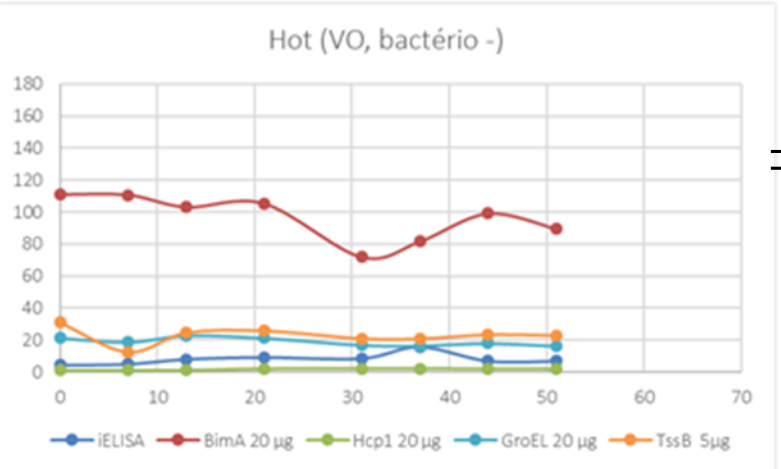












⇒Hcp1 principal ement