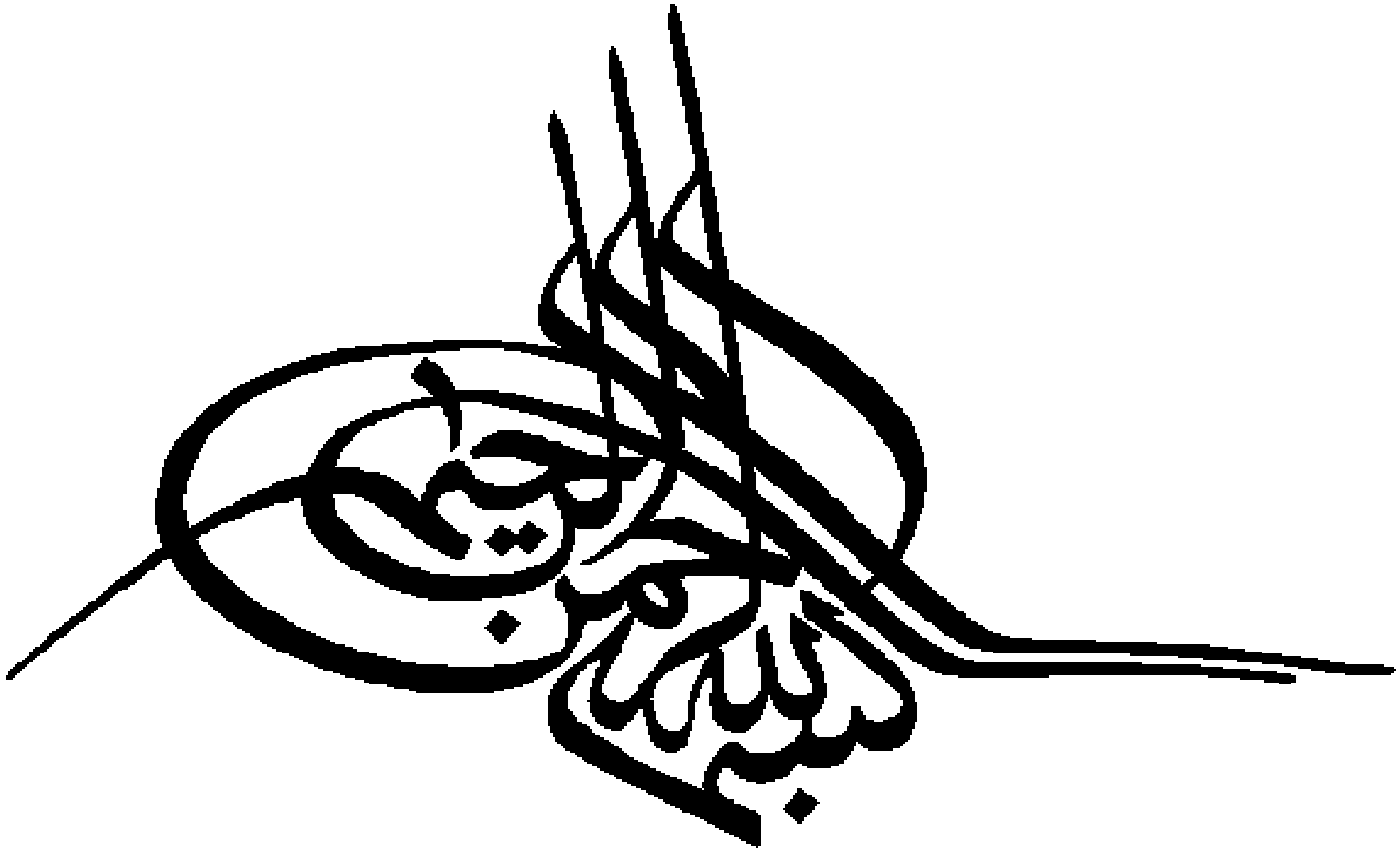

Occurrence of Glanders in Fars and Alborz provinces in Iran and isolating *Burkholderia mallei*

Nader Mosavari

Head of Tuberculosis and Glanders Department

Basic Facts, History and Indigenous Potential

- Aristotle was first report of Glanders, 330 B.C.
- Foundation of Lyon veterinary school, mid 1700s
- Systematical studies, delayed until 19th century
- The first published human case, 1821
- Isolation of the agent, 1881
- Development of mallein test, 1892
- Glanders outbreak at Tehran zoo about 5 years ago
- Unfortunately Glanders in Iran has increased thrice mainly due to legal and illegal increase in trafficking of equids from the borders of the country

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Glanders outbreak at Tehran Zoo, Iran

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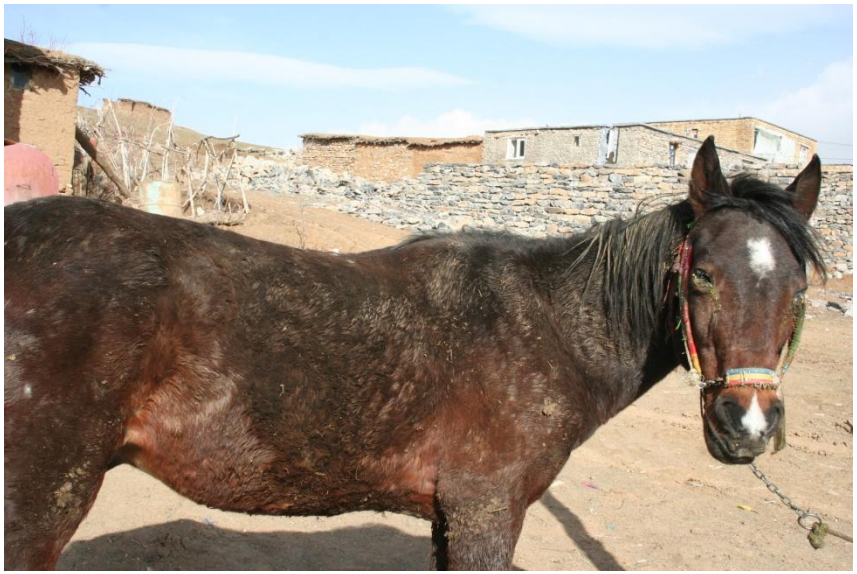
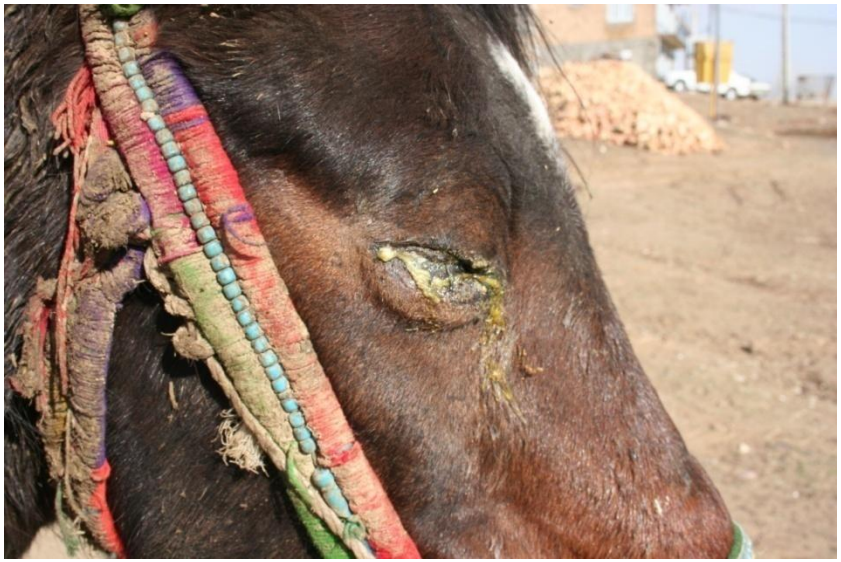
Isolation & identification of Burkholderia mallei in IRAN after Mallein, CF & ELISA Test

Shiraz mini-epidemics (2013)
Kordestan (2013)

Alborz outbreak (2014)
Razi farm





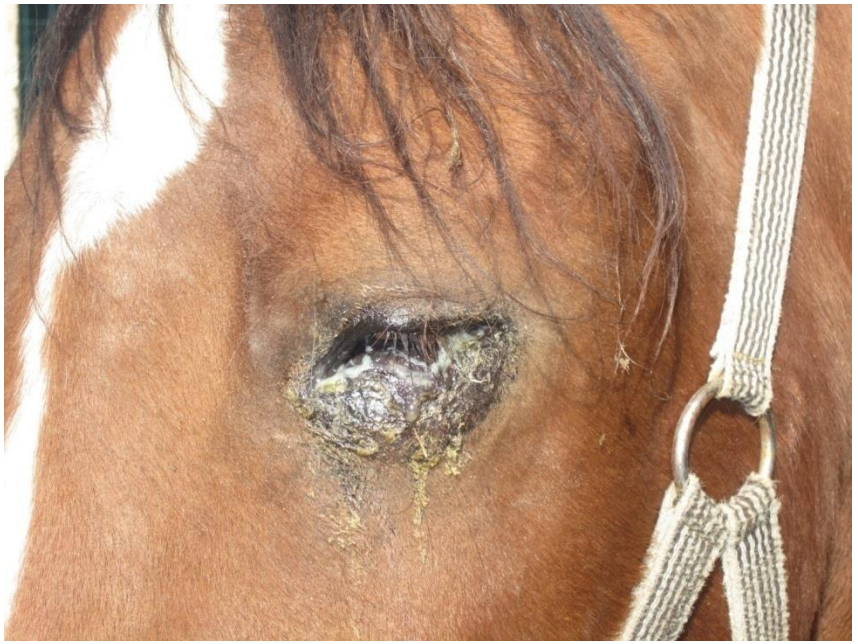
B. DIAGNOSTIC TECHNIQUES

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

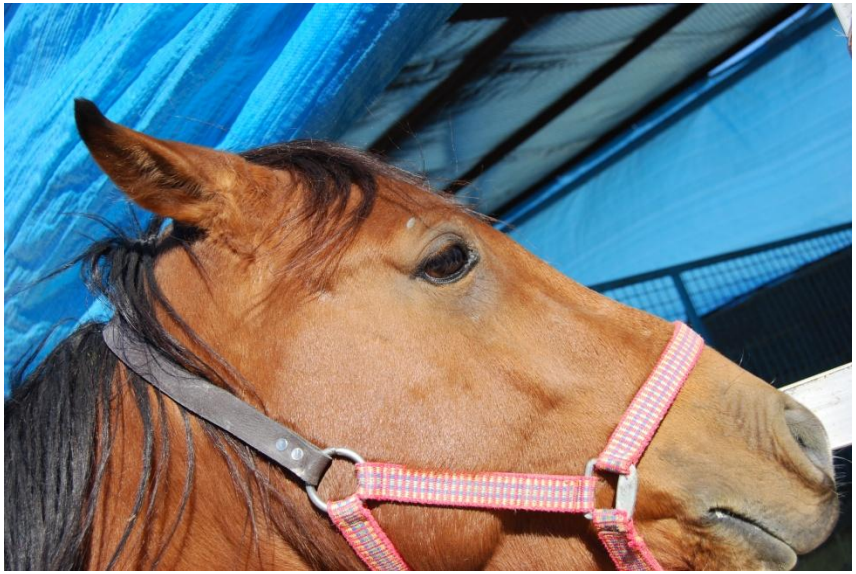
Method	Purpose				
	Population freedom from infection	Individual animal freedom from infection	Efficiency of eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance
Complement fixation	+	+	+++	+	+++
Western blotting	+	+	++	+	++
ELISA	+	+	++	+	++
Malleinisation	+	+	+	+	+
PCR	–	–	–	+	–
Animal inoculation	–	–	–	+	–
Culture	–	–	–	+	–

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose.
Although not all of the tests listed as category +++ or ++ have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.
ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction.

Scenario 1: Shiraz Glanders mini-epidemics (2013)

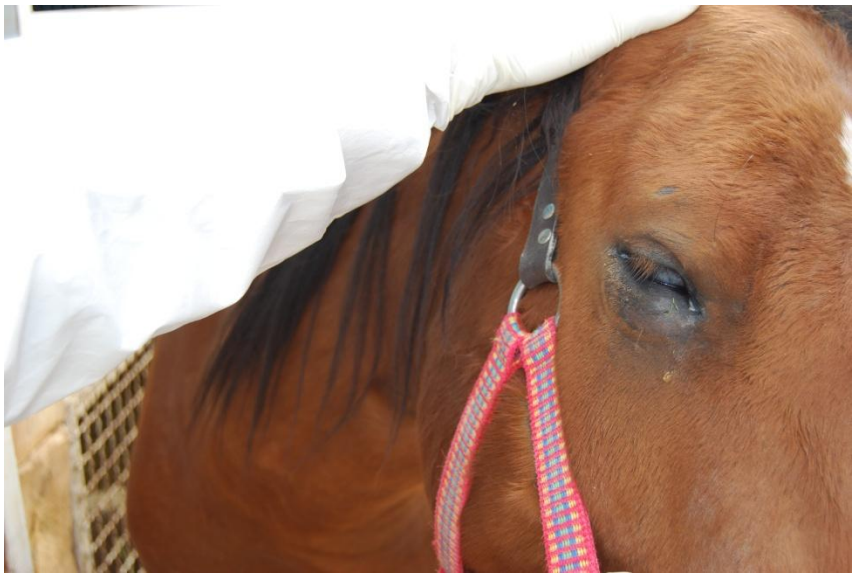


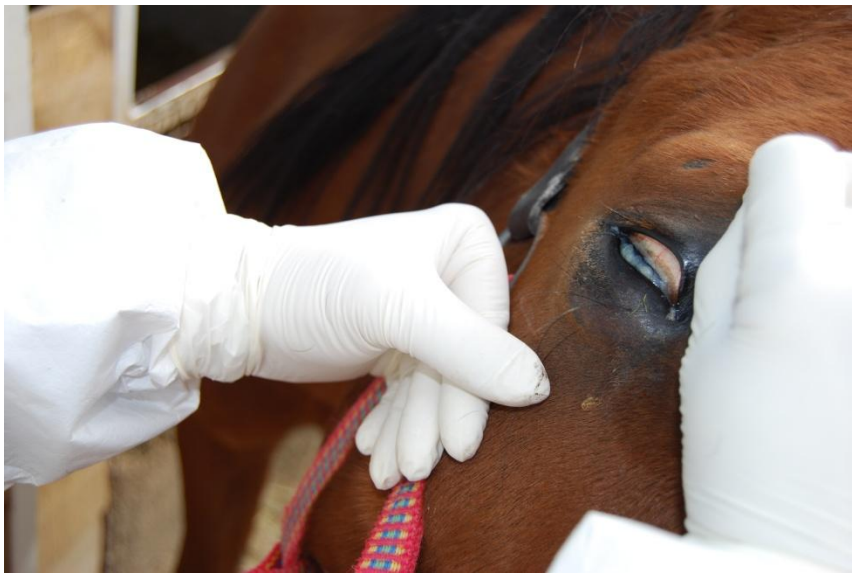
Suspect horse before Malleinisation



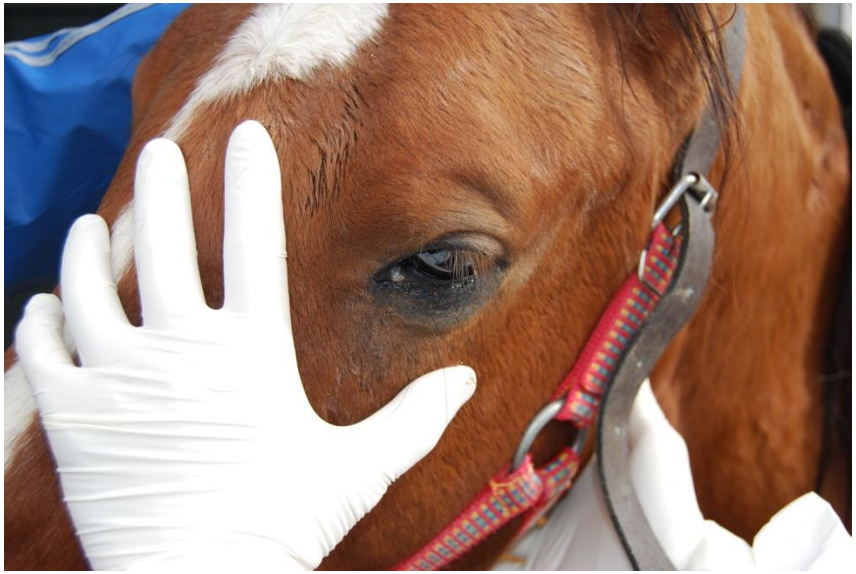
PPD Mallein

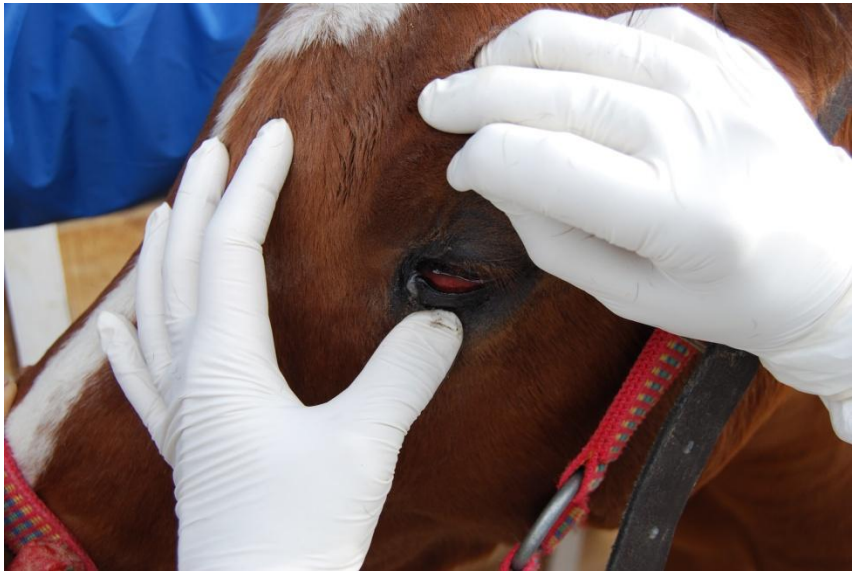
Malleinisation by Brute Mallein



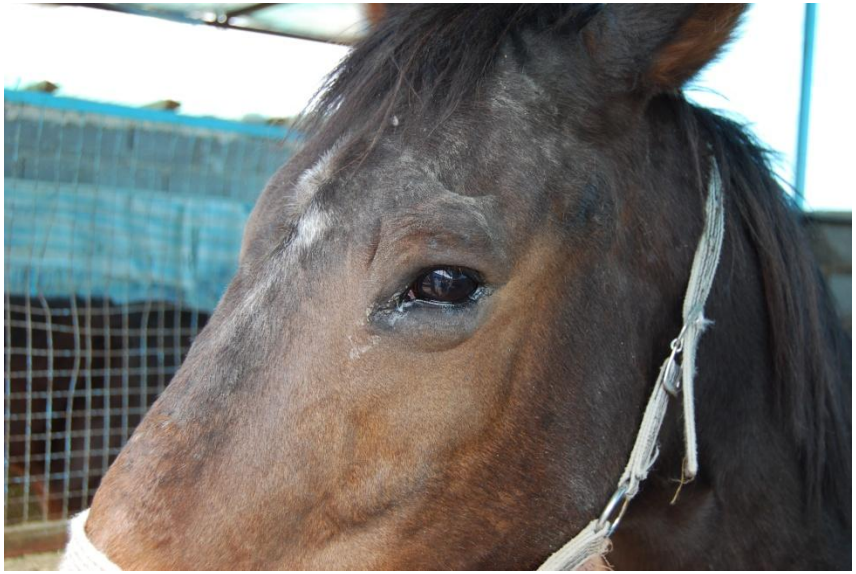


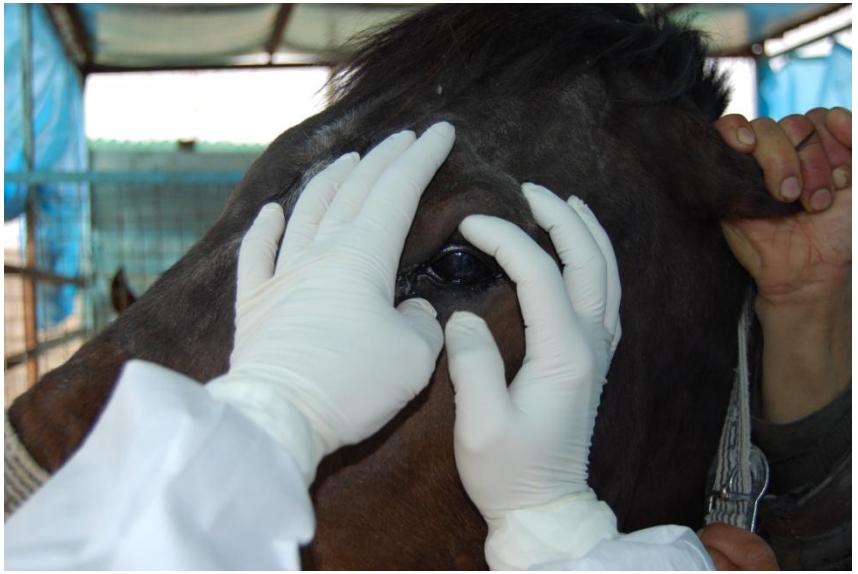
Malleinisation by PPD Mallein



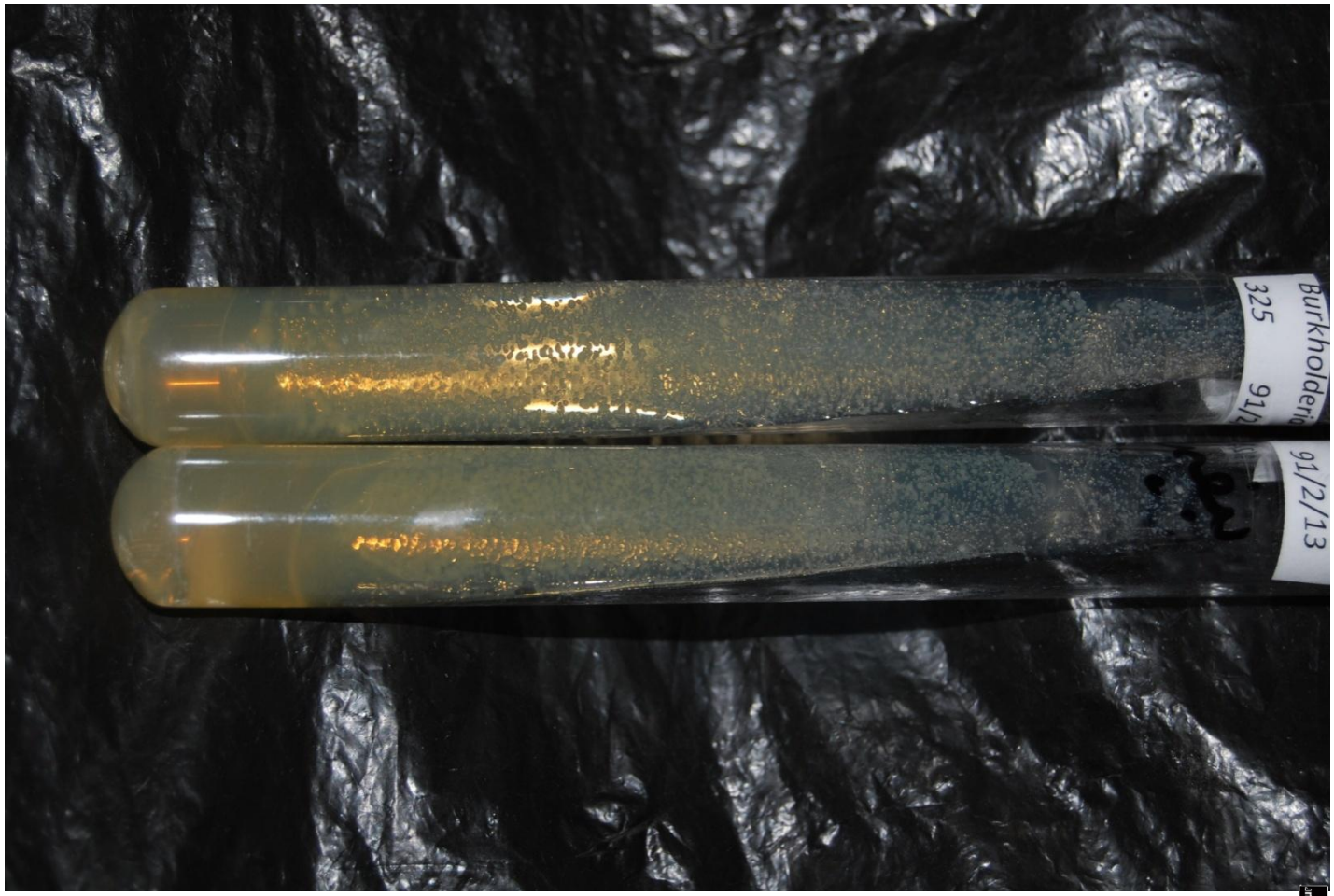


Suspect horse near to positive horse





Culture



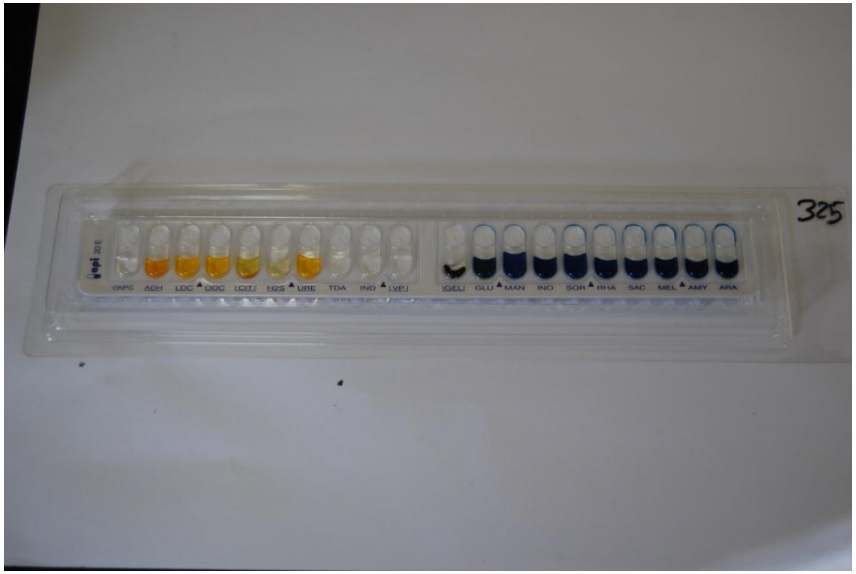
Identification

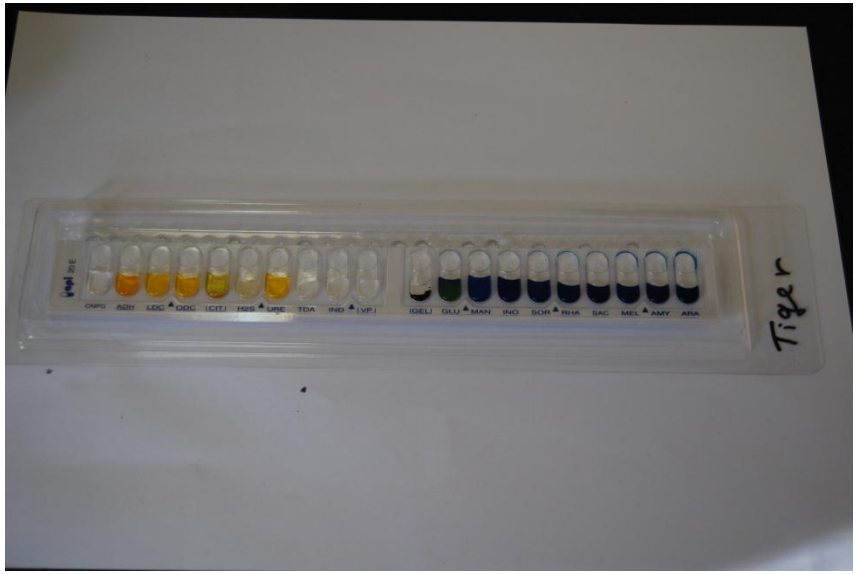
Table 1. Identification of *B. mallei* and *B. pseudomallei* (2).

Tests	Oxidase	Growth at 42°C	Nitrate Reduction	Gelatin Liquified	Motility	Oxidizes Glucose	Oxidizes Lactose	Oxidizes Mannitol	Arginine dihydrolase	Citrate	TSI	Indol
B. pseudomallei	+	+	+	v	+	+	=	=	+	+	Alk/NC1 Gas-, H2S -	–
B. mallei	v	=	+	=	-	=	=	=	+	v	Alk/NC1 Gas-, H2S -	–

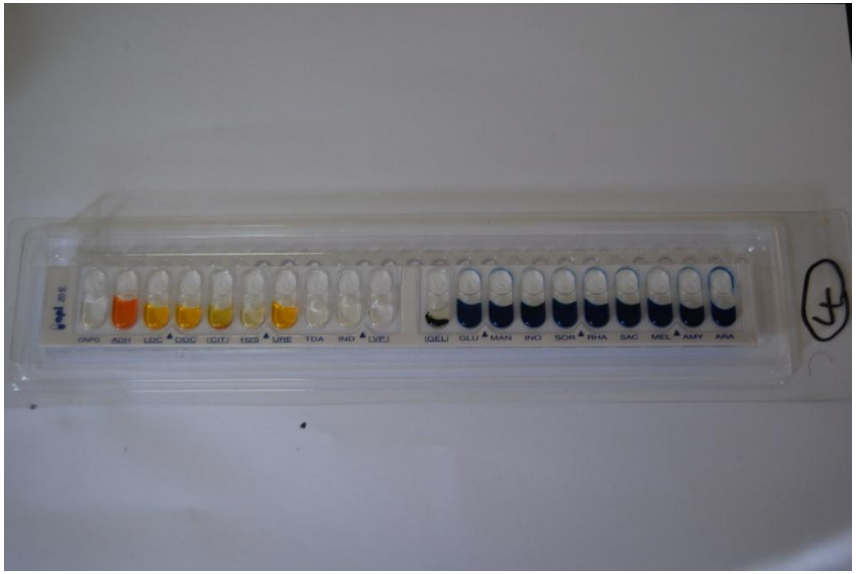
V = variable; +, > 90% of strain are positive; =, > 90% of strain are negative, NC1 = No Change.

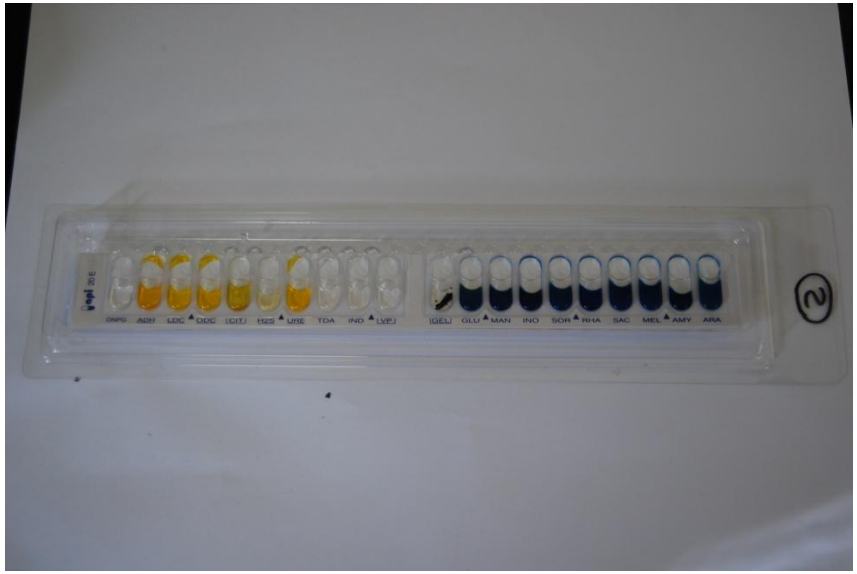
API





API





PCRs

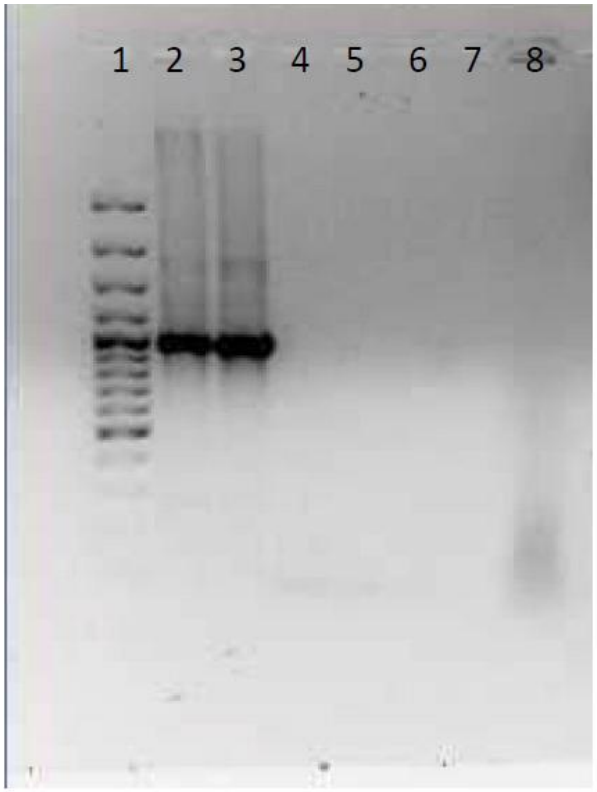


Fig. 2. Lane 1: 100 bp Size Marker, Lane 2: Tiger Isolates, Lane 3: Positive control (Standard strain from Razi Institute), Lane 4: *E. coli*, Lane 5:Empty, Lane 6: Negative control, Lane 7: *Salmonella enteritidis*, Lane 8: *Pseudomonas aeruginosa.*

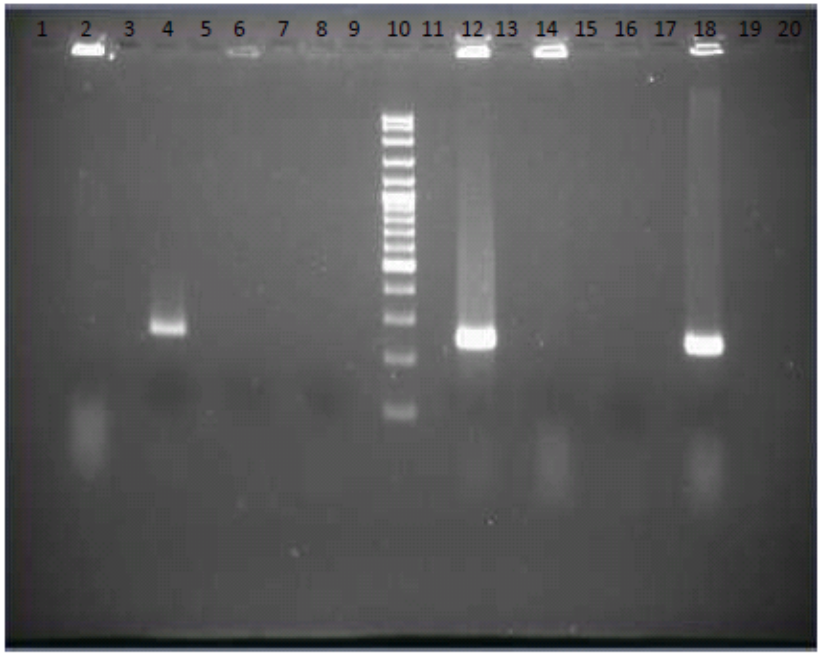
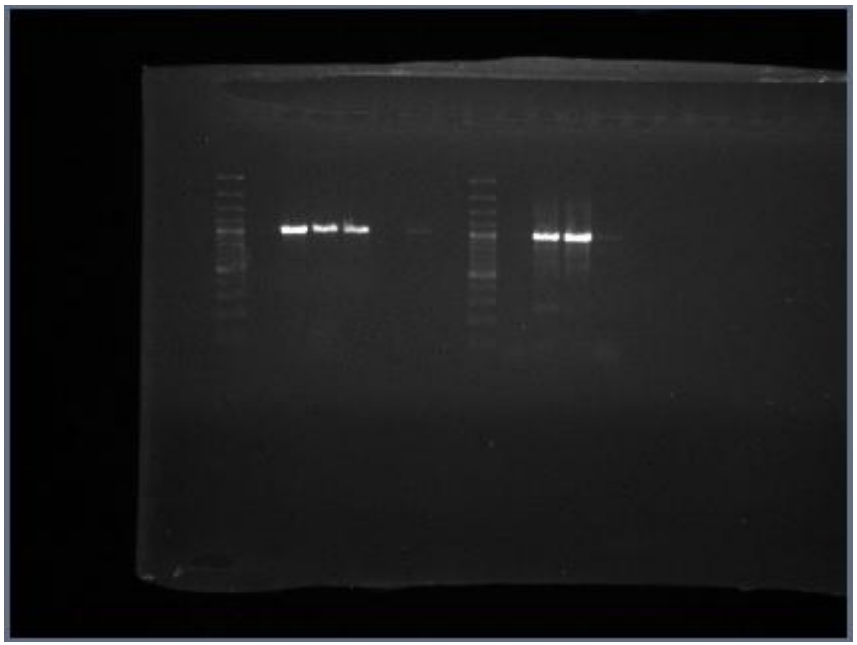


Fig. 1. Lanes 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 20 are empty, Lane 2: *E. coli*, Lane 4: DNA of Tiger isolates submitted from Pasteure laboratory, Lane 6: *Salmonella enteritidis*, Lane 8: Negative control, Lane 10: 100 bp Size marker, Lane 12: Positive control (Standard strain from Razi Institute), Lane 14: *Pseudomonas aeruginosa*, Lane 16: Negative control, Lane 18: Tiger isolates.

Molecular Identification

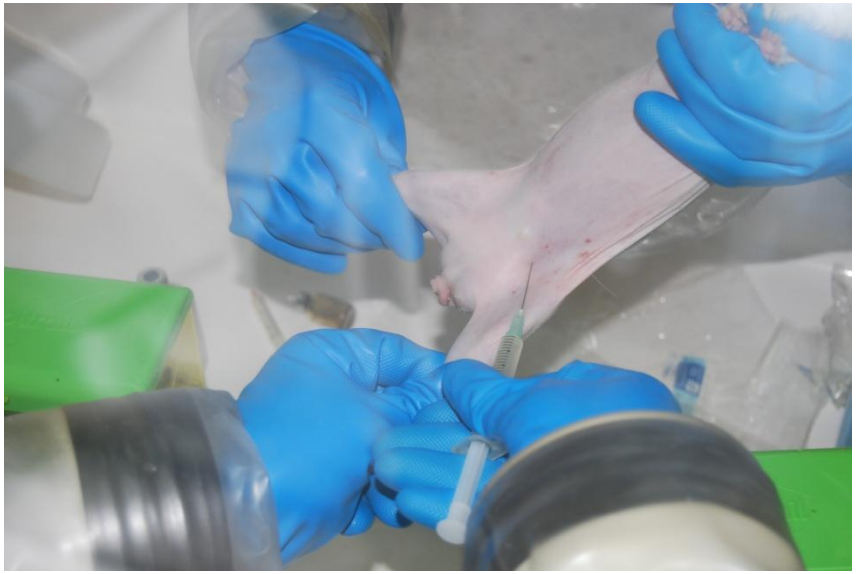
Molecular



Primers

- 5’-TCA-GGT-TTG-TAT-GTC-GCT-CGG-3’
- 5’-CTA-GGT-GAA-GCT-CTG-CGC-GAG-3’

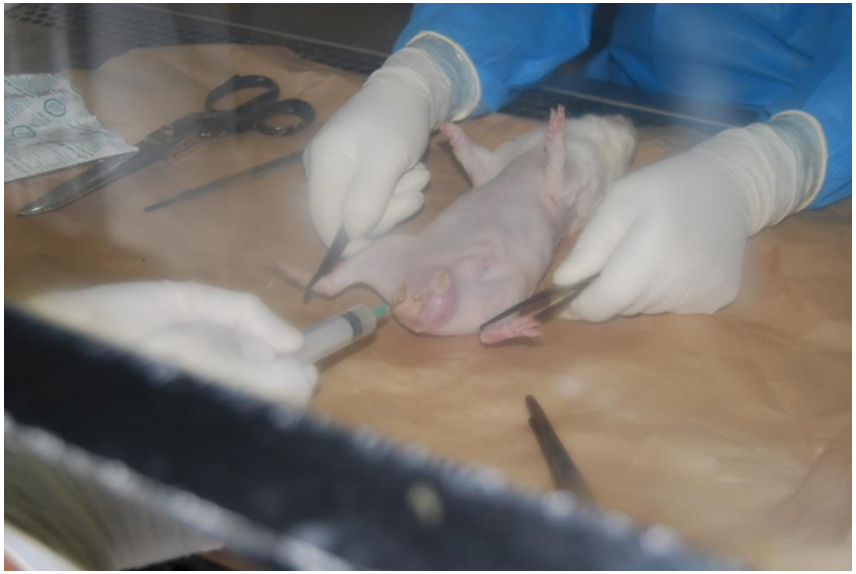
Injection



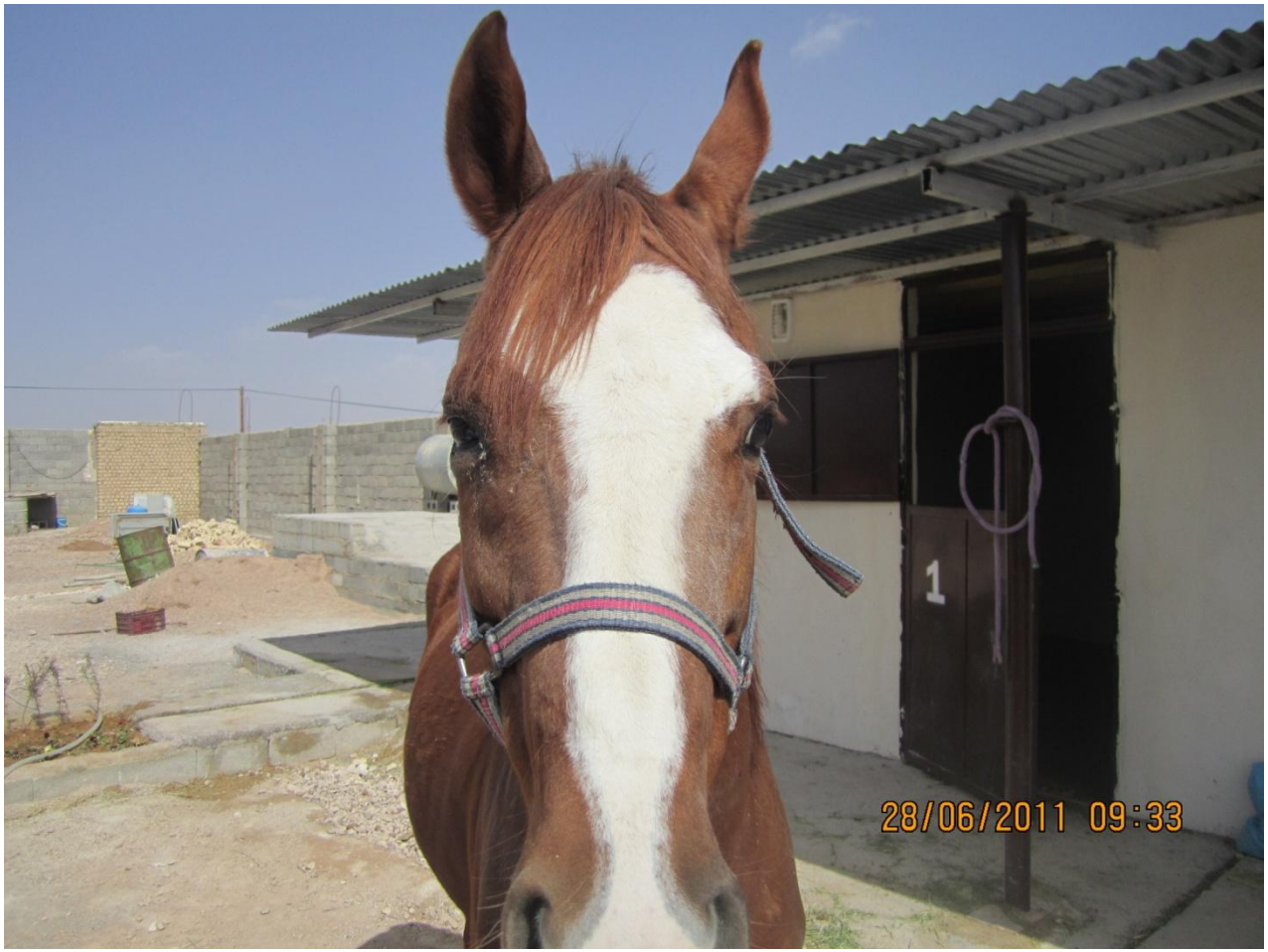
Isolation

Fig. 3. Inoculated guinea pigs by *B. mallei* tiger (Red Mark-Right) and standard (Blue Mark-Left) isolates.

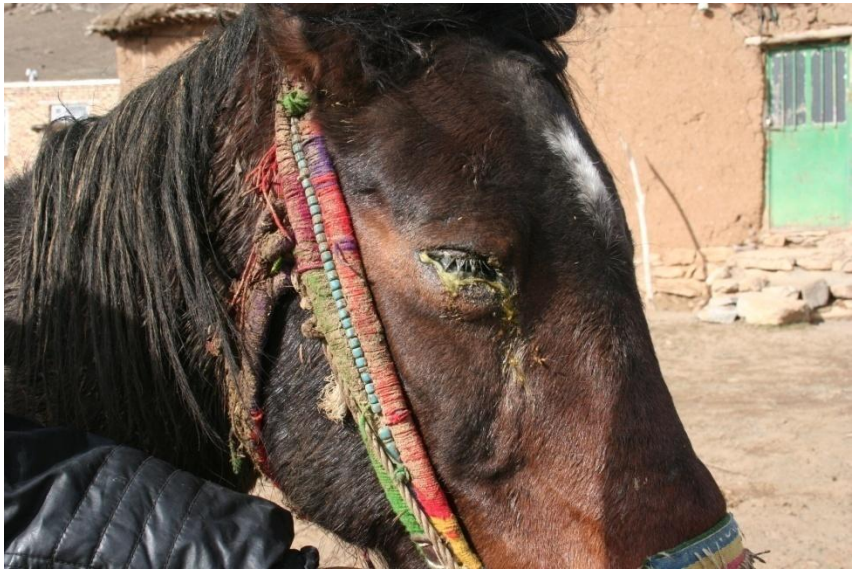
Isolation



Farm to farm transmission of Glanders



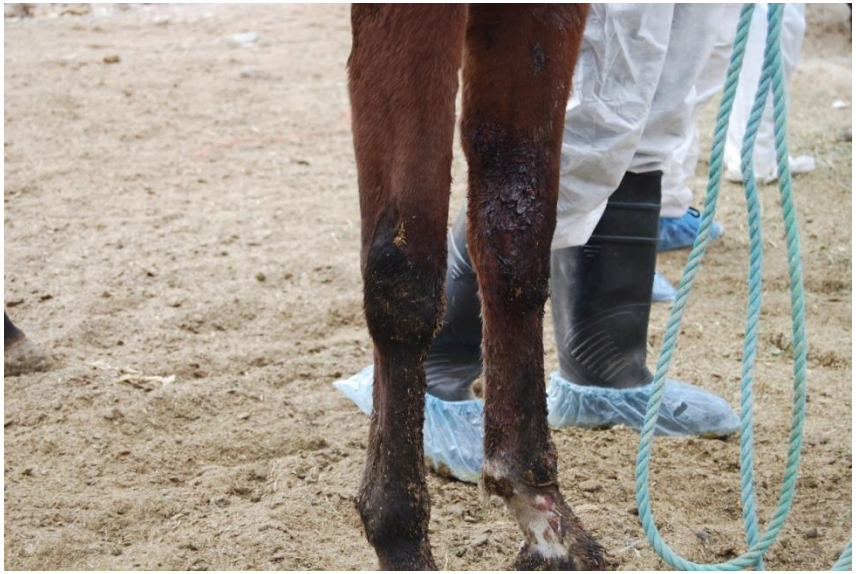
Scenario 2: Just one positive case out of 4 suspect horse

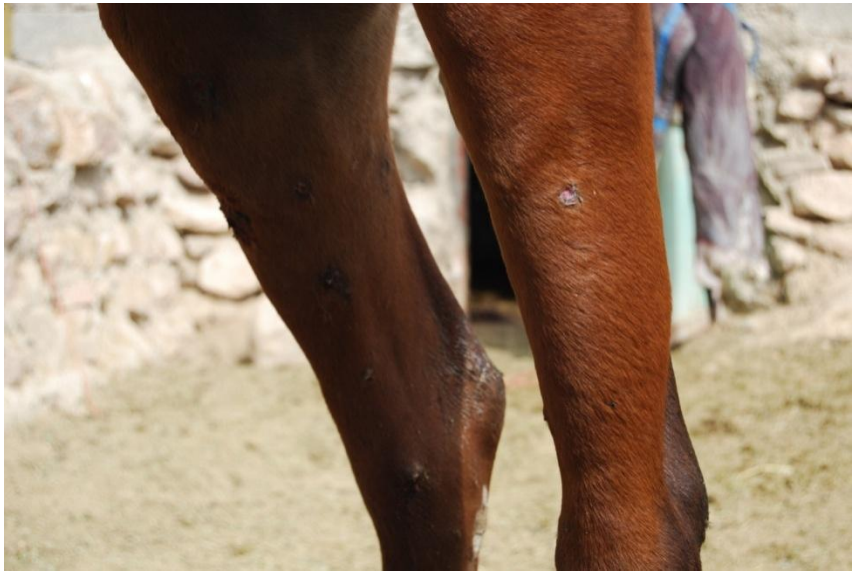


Scenario 3: Isolation & Identification of *Burkholderia mallei* from a mare & a young foal

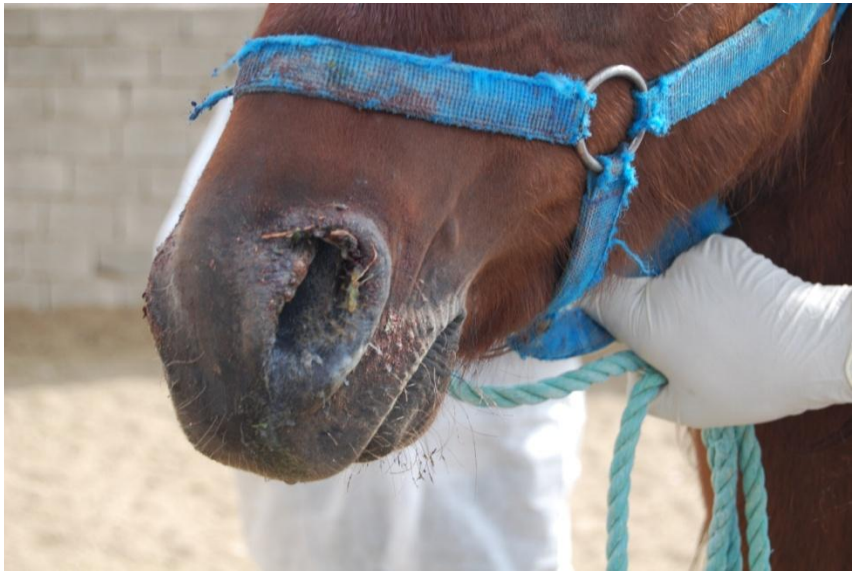
Alborz, Kordan (2013)

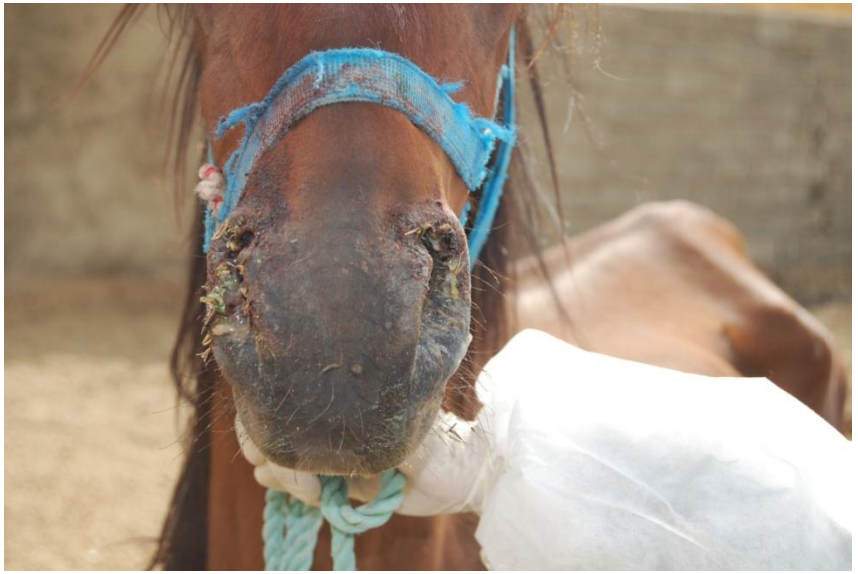
Foot injuries



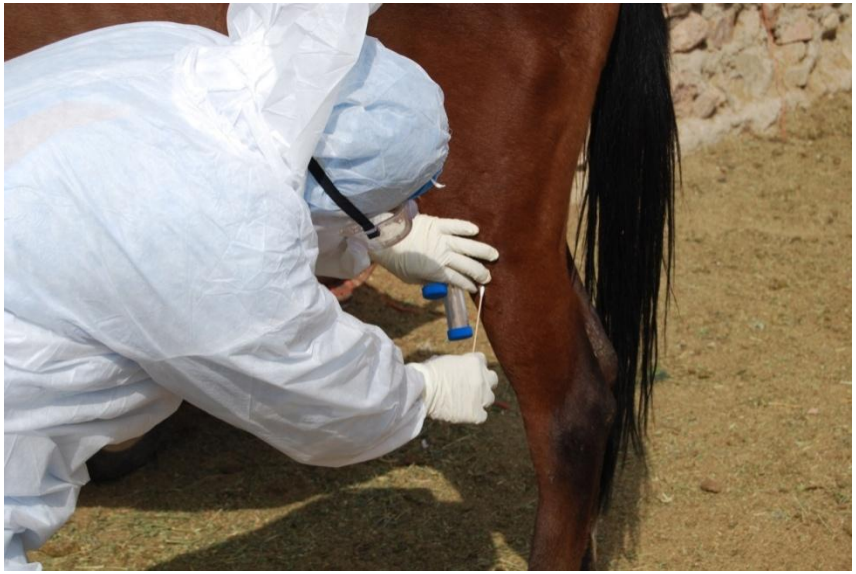


Nostril lesions



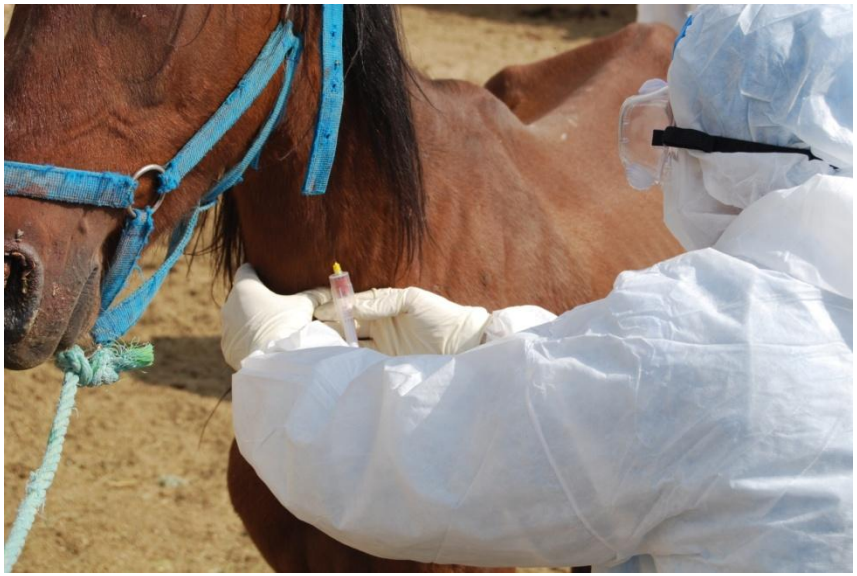


Specimen collection (swab)



Specimen collection

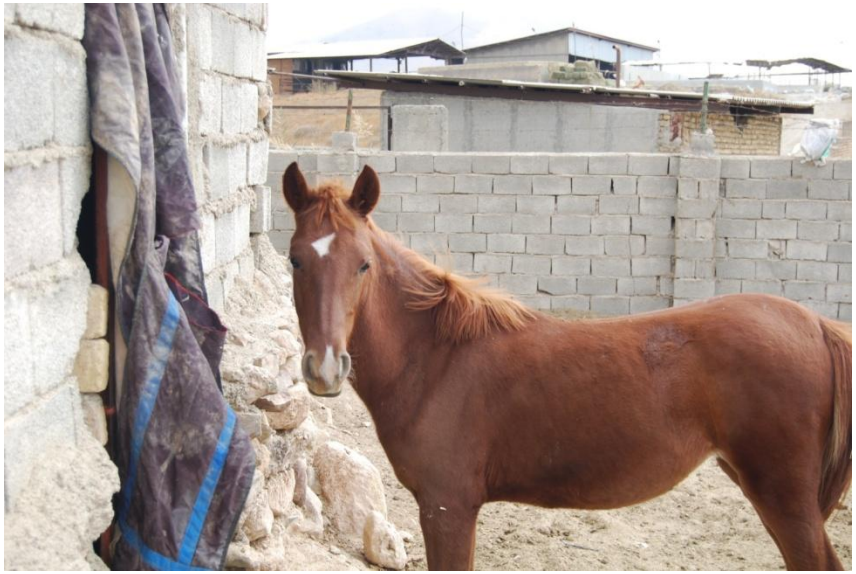
Blood

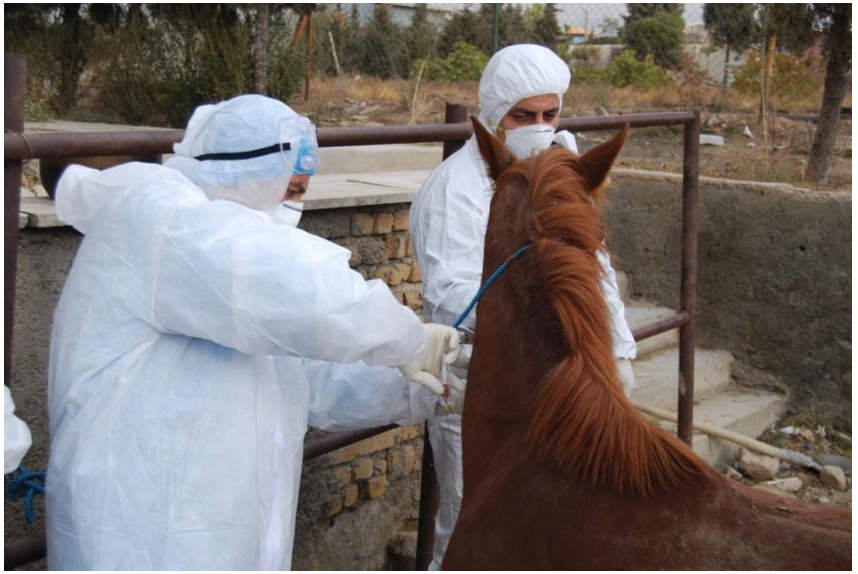


Pus

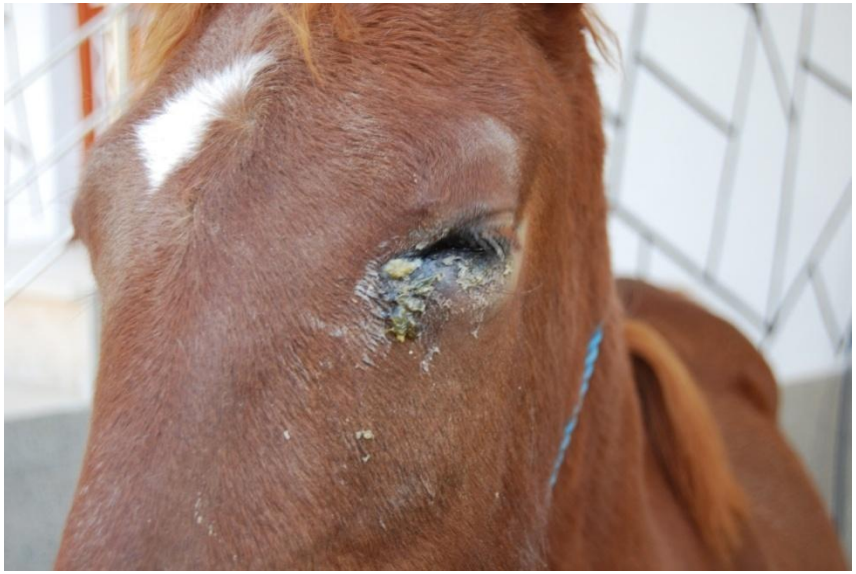
Puppies straying in the farm

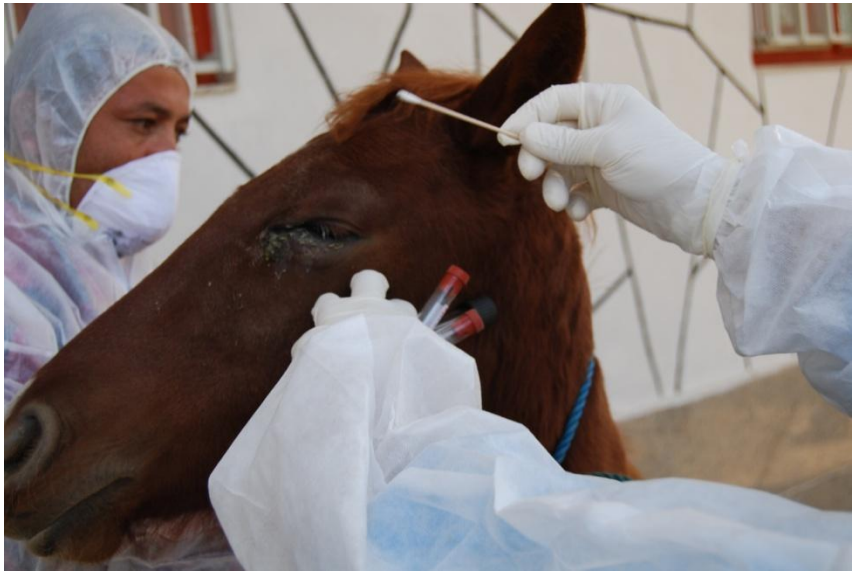
Foal of the suspected mare





Intradermopalpebral test (Malleinisation)





Scenario 4: Razi farm

Results

- Isolation & identification of *Burkholderia mallei* from Shiraz and Alborz provinces
- Introdermopalpebral test can diagnose faster than CF & ELISA tests
- CF & ELISA test can diagnose better than introdermopalpebral test in weak horses

Discussion

- We have to use of all mentioned tests to control and eradicate program of Glanders in IRAN
- It’s better we use of CF & ELISA tests to finding positive cases
- Then we perform Malleination test in infected mentioned farms
- We are trying to set up new techniques like agglutinations and indirect immunoflourescent so as to diagnose the infectious agent at faster speed and more accuracy.

Acknowledgements

Thanks for your attention

90 Years of Honour 1924 - 2014

Razi Vaccine & Serum Research Institute