

## Detection of Glanders in horses of eight Iraqi provinces by ELISA

Zaid Salah Hussein

College of Veterinary Medicine/ University of Baghdad

### Abstract

The present study was carried out to determine the prevalence of Glanders in horses of Iraq. For this reason, a serological surveillance was conducted during the period extended between November 2015 and March 2016. Four hundred and four samples of serum were collected from horses lived in eight different Iraqi provinces that were Al-Muthanna, Baghdad, Al-Najaf, Babylon, Al-Qadysia, Diyala, Karbala and Wasit. Sera transported to the lab of clinical pathology/ department of internal and preventive veterinary medicine at Baghdad University, where they were analyzed by cELISA technique to determine the seropositivity against *Burkholderia mallei*. The results of sera analysis showed that 2.97 % (12 horses) were positive toward the applied test. The positive cases were recorded in Baghdad and Muthanna governorate (11 and 1, respectively). Statistical analysis revealed no significant variations ( $P < 0.05$ ) in the seropositivity against Glanders between different genders or ages.

Keywords: Glanders, *Burkholderia mallei*, Horse, ELISA, Epidemiology, Iraq

e-mail: zaid.salah.hussein@gmail.com

### التحري عن مرض الرعام في الخيول في ثمان محافظات عراقية باستخدام الاليزا

زيد صلاح حسين

كلية الطب البيطري/ جامعة بغداد

### الخلاصة

هدفت هذه الدراسة لمعرفة مدى انتشار مرض الرعام في الخيول العراقية وذلك عن طريق اجراء مسح مصلي خلال الفترة الممتدة من تشرين الثاني 2015 الى آذار 2016 جمعت خلالها 404 عينة مصلية من خيول متواجدة في ثمانية محافظات عراقية وهي المثنى وبغداد والنجف وبابل والقادسية وديالى وكربلاء وواسط تم نقل هذه الامصال بعد جمعها الى مختبر التشخيصات في فرع الطب الباطني والوقائي البيطري التابع لكلية الطب البيطري في جامعة بغداد حيث اخضت هناك لفحص الاليزا التنافسي (cELISA) للتحري عن ايجابية المصول لجرثومة الـ *Burkholderia mallei*. أظهرت النتائج ان 2.07% من امصال الخيول (12 مصل) كانت موجبة للفحص جميع هذه الحالات سجلت في محافظتي بغداد والمثنى (1 و 11 على التوالي) كما اظهر التحليل الاحصائي للنتائج عدم وجود أي فروق معنوية ( $P < 0.05$ ) بين الاعمار المختلفة وعامل الجنس بالنسبة لإيجابية المصول لمرض الرعام.

الكلمات المفتاحية: مرض الرعام، *Burkholderia mallei*، الخيول، الاليزا، العراق.

## Introduction

Glanders is one of the most ancient recorded equine diseases (1). The effect of infection may involve respiratory tract or/and skin and lymphatics (2). Since many names were known for the disease including Malleus, Equinia, Farcy and Farcy Buds but Glanders is the common choice in term of use (3). Despite the fact that the disease affect primarily equidae, horses usually exhibit the chronic form of the disease whereas, mules as well as donkeys develops the acute form only (4, 5). However carnivores may be infected particularly when consumed meat from infected horses (6). Human infection may also rarely encountered (7, 8), yet one case was reported in 2009 (9). The disease is caused by *Burkholderia mallei* bacterium which is non-motile, non-spore forming, Gram-negative and aerobic rods (10). The transmission of the disease mostly occurs by ingestion of contaminated food and water utensils (11). Nevertheless, the contaminated grooming instruments by the secretion of infected skin or flies may play a significant role in the transmission and spreading of this disease (1, 11). Animals infected by Glanders manifests clinically variable signs that are ranged from asymptomatic carrier to pneumonia and nasal involvement which may develops to skin form (sometime this form occurs primarily due to the presence of skin wounds and subsequent contamination). Both the nasal and skin forms gives characteristic pathognomonic lesions such as nodules that may rupture leaving on the cartilaginous nasal septum usually accompanied by bloody mucopurulent nasal discharge (3) or the chains of cutaneous nodules ruptures giving rise ulcers with thick yellowish sticky purulent material (12). However the ambiguous pulmonary form is the most common one with characteristic granulomatous lesions and main signs of bronchopneumonia (13). The strict regimen of surveillance and culling have been managed previously to eradicate the disease from major horse population worldwide (14). Nevertheless, many evidences of potential presence of the disease was encountered in many parts of Asia (15, 16, 17). In Iraq, despite that numerous records confirming the endemicity nature of this disease, yet, paucity of works and studies conducted in the last two decades imposed the repetition of Glanders surveillance especially when taking in consideration the local dramatic changes that occur in the both of equine population and the government efforts to eradicate this dangerous disease. Accordingly, the current investigation aimed to updated the records concerning the existence and distribution of the Glanders in Iraqi horses by Applying serological surveillance test using a commercial cELISA kit.

## Materials and Methods

Four hundred and four samples were collected randomly from horses (work and sport horses) lived in different eight Iraqi provinces, during the period extended between November 2015 and March 2016. Samples of blood were collected directly from the jugular vein of these selected horses using plain vacutainer tubes to obtain subsequently serum according to the method previously described (18) These collected blood samples were transported immediately in cooled sterile conditions to the Department of Internal Veterinary Medicine/ Baghdad university where they were subjected to cELISA test (*Lilli Test Glanders cElisa Test Kit*, Lillidaie Diagnostics, UK) to find out antibodies against *Burkholderia mallei* if present following the instructions of the applied commercial kit provided by the manufacturer.

Data of the present investigation was analyzed using the SAS statistical analysis system (Ver.9.2, 2002, SAS institute Inc.Cory.NC, USA), the estimated proportions were compared using Chi-square and  $P < 0.05$  was considered significant level.

## Results and Discussion

Despite the fact that horses considered precious animal especially in the Arabian Peninsula and many Europe countries as well as USA, breeding of these animals is diminished in Iraq with estimated total number of approximately 6000. This dropping is takes place due to a series of military conflicts which prevented any international activates, reduced the transport and outboard trading of horses. The largest horse population which is bred together located mainly in Baghdad province where they engaged in local races or some Olympic activities whereas in most other parts are reared individually and used for traction. This situation caused the majority of the samples (260 out of the total 404) were collected from horses lived in Baghdad. In Iraq, previous study conducted on 125 clinically diseased horses by Al-Ani *et al.* (19) establish that ELISA was a highly sensitive diagnostic technique for Glanders compared to other available serological tests such as Complement fixation test (CFT), agar gel immunodiffusion (AGID) or haemagglutination assay (HA), so that current study selected such test for the current surveillance. Out of the total 404 samples, only 12 horses (2.97%) were seropositive to Glanders, only one of these seropositive samples was recorded in Al-Muthanna while all the remain samples (11 serum) were obtained from horses located in Baghdad province (Table1). The current results confirmed many previous findings concerning the endemic nature of the Glanders as a disease in horses in Iraq (20, 21, 22). The presence of *Burkholderia mallei* infection in horses was predicted because of the lack or cessation of scientific efforts to eradicate the disease by veterinary authorities. Although the current legislation measures obligate owners of horses to cull their horses suffered Glanders and subsequent disposal their carcasses. However, the majority of the owners evade Glanders surveillance or submission of their horses to any field or laboratory tests by veterinary services since the insignificant compensation or bad responses of the government to compensate their losses besides the unworthy sell value of the horses confirmed their infection with this dangerous disease. Eleven seropositive horses were recorded in Baghdad province and only one case in Al-Muthanna while all the other examined horses in the rest areas were negative to the applied test. The current study can't affirm the absence of Glanders in those areas due to the low number of examined horses. The current study couldn't avoid such shortage since the small the number of horse's population in these governorates which breed individually and in separated localities may be the main reason of low infection rate in them. Conversely, horses are comparatively numerous and gathered in a large groups in specified areas namely equestrian clubs (Al-Froseha and Al-Jadriya Equestrian club) or Arabian horse location at Al-Zawraa Zoo increase the chance of Glanders spreading since *Burkholderia mallei* infection is often takes place via contaminated food and water (11). The lack of references concerned genders or age as a potential risk factor for Glanders infections, current serological surveillance included these parameters to find out whether they have an impact on the epidemiology of the disease in Iraqi horses. Statistical analysis revealed no significant differences ( $P < 0.05$ ) of both factors between seropositive horses (Tables 2, 3) which suggest to rule out them in any upcoming future surveillances.

**Table (1) Results of cELISA test of sample from different provinces**

Governorates	No. of tested horses	No. of seropositive samples	Seropositivity	X <sup>2</sup> Value	P
Al-Muthanna	22	1	4.54	5.35	0.61
Baghdad	260	11	4.23		
Al-Najaf	26	0	0		
Babylon	13	0	0		
Al-Qadsyia	30	0	0		
Diyala	20	0	0		
Karbala	18	0	0		
Wasit	15	0	0		
<b>Total</b>	404	12	2.97		

X<sup>2</sup>: Chi-square value, P: probability

**Table (2) Results of ELISA test showing effect of the gender on the incidence of Glanders**

Genders	No. of tested horses	No. of seropositive samples	Seropositivity	X <sup>2</sup> Value	P
Females	195	7	3.59	0.5	0.47
Males	209	5	4.99		
<b>Total</b>	404	12	2.97		

X<sup>2</sup>: Chi-square value, P: probability

**Table (3) Results of ELISA test showing effect of the age on the incidence of Glanders**

Ages by years	No. of tested horses	No. of seropositive samples	Seropositivity	X <sup>2</sup> Value	P
<10	102	2	1.96	1.29	0.52
11-15	204	8	3.92		
>15	98	2	2.04		
<b>Total</b>	404	12	2.97		

X<sup>2</sup>: Chi-square value, P: probability

**Conclusions,** the current study provides a new evidence of *Burkholderia mallei* infection in horses in Iraq, particularly in Baghdad province. This obliges the veterinarians to take Glanders into consideration during examination and treatment of chronic and non-responsive ambiguous pneumonia in horses since the potential risks of human fetal infection on the other hand, the current result should urge the veterinary authorities to re-evaluate the existing legislation and determine the means of effective implementation.

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## References

1. Henning, M. W. (1956). Animal diseases in South Africa. 3<sup>rd</sup> ed., Central News Agency. Pretoria, South Africa. PP. 159-181.
2. Julini, M. (1990). The turin vandelic glanders commission of 1847-1848. Obiettivi. Documenti. Vet., 11: 44-45.
3. Jubb, K. V.; Kennedy, P. C. & Palmer, N. (1985). Glanders. Pathology of Domestic Animals, 3<sup>rd</sup> ed., Academic Press. New York. PP. 423-425.
4. Mahadevan, S.; Dravidamani, S. & Dare, B. J. (1987). Glanders in horses. Centaur., 3: 135- 138.
5. Theves, G. (1993). Glanders in Luxembourg and elsewhere from the 13<sup>th</sup> Century to the end of the 19<sup>th</sup> Century or the arduous advancement of ideas on an animal and

- human disease. *Annales Med. Vet.*, 137: 469-481.
6. Alibasoglu, M.; Yesildere, T.; Calislar, T.; Inal, T. & Calsikan, U. (1986). Glanders outbreak in lions in the Istanbul zoological garden. *Berl. Münch. Tierärztl. Wschr.*, 99: 57-63.
  7. Miller, W. R.; Pannell, L.; Cravitz, L.; Tanner, W. A. & Ingalls, M. S. (1948). Studies on certain biological characteristics of *Malleomyces mallei* and *Malleomyces pseudomallei*. I: Morphology, cultivation, viability and isolation from contaminated specimens. *J. Bacteriol.*, 55(1): 115-126.
  8. Gangulee, P. C.; Sen, G. P. & Sharma, G. L. (1966). Serological diagnosis of glanders by haemagglutination test. *Indian Vet. J.*, 43(5): 386-391.
  9. Srinivasan, A.; Kraus, C. N.; DeShazer, D.; Becker, P. M.; Dick, J. D.; Spacek, L.; Bartlett, J. G.; Byrne, W. R. & Thomas, D. L. (2001). Glanders in a military research microbiologist. *N. Engl. J. Med.*, 345(4): 256-258.
  10. Quinn, P. J.; Markey, B. K. & Maguirem, D. (2003). Concise review of veterinary microbiology, Blackwell publishing, P. 41.
  11. Gilbert, R. O. (2007). Glanders. In: Foreign animals diseases. The Gray book. Richmond; Va: United states Animals Health Association, 1998. Available At: [www.vet.uga.edu/vpp/gray-book](http://www.vet.uga.edu/vpp/gray-book) O 2/fad/gla.ph.p.
  12. Al-Kafawi, A. A.; Al-Ani, F. K.; Al-Bassam, L. S. & Youkob, A. Y. (1977). Hematological changes in Arabian horses infected with glanders. *Vet. Rec.*, 101(21): 427.
  13. Zubaidy, A. J. & Al-Ani, F. K. (1978). Pathology of glanders in horses in Iraq. *Vet. Pathol.*, 15(4): 566-568.
  14. Wiser, V.; Mark, L.; Graham, H.; Robertson, D. V. & M. M. Jacobs. (1986). One hundred years of animal health 1884 to 1984. *J. National Agri. Libr. Assoc.*, 11: 1-4.
  15. Krishna, L.; Gupta, V. K. & Masand, M. R. (1992). Pathomorphological study of possible glanders in solipeds in Himachal pradesh. *Indian Vet. J.*, 69: 211-214.
  16. Yehya, G. (1994). Problems associated with international trade and the movement of horses in the Middle East. Comprehensive Reports on Technical Items Presented to the International Committee or to Regional Commissions, Ministry of Agriculture, rue Riad solh, Beirut, Lebanon. PP. 165-181.
  17. Nagal, K. B.; Joshi, V. B.; Katoch, R. C.; Sharma, M. & Kumar, R. K. (1995). Glanders outbreak in Himachal pradesh. *Centaur.*, 12: 30-35.
  18. Barger, A. M. & MacNeil, A. L. (2015). Clinical pathology and laboratory techniques for veterinary technicians. Willey Blackwell, UK.
  19. Al-Ani, F. K.; Al-Omran, A. H. & Al-Zubaidy F. S. (1993). A micro-enzyme- linked immunosorbent assay (ELISA) for detection of antibody to *Pseudomonas mallei* infection in horses. *Pak. Vet. J.*, 13: 70-73.
  20. Al-Ani, F. K.; Al-Delaimi, A. K. & Ali, A. H. (1987). Glanders in horses: clinical and epidemiological studies in Iraq. *Pak. Vet. J.*, 7: 126-129.
  21. Al-Izzi, S. A. & Al-Bassam, L. S. (1989). In vitro susceptibility of *Pseudomonas mallei* to antimicrobial agent. *Comp. Immunol. Microbiol. Infect. Dis.*, 12: 5-8.
  22. Al-Ani, F. K.; Al-Rawashdeh, O. F.; Ali, H. A. & Hassan, F. K. (1998). Glanders in horses: clinical, biochemical and serological studies in Iraq. *Vet. Arhiv*, 68(5): 155-162.