

SEROPREVALENCE STUDY ON THE CURRENT SITUATION OF BRUCELLOSIS IN HORSES AND DONKEYS IN ASSIUT GOVERNORATE

S.M. EL BERBAWY

Animal Health Research Institute (AHRI), Assiut Lab.

ABSTRACT

Received at: 12/6/2012

Accepted: 18/7/2012

Seroprevalence study was carried out on 423 donkeys and 48 horses of different ages and both sex for detection of the incidence of brucella. Seroreactive cases among donkeys at different locations in Assiut Governorate were 2.13%, 1.65% and 1.42% by using BAPAT, RBPT and TAT respectively, while by using Rivanol test (which detected the true positive only) it was 0.24%. Quantitatively, 6 serum samples were seroreactive using TAT; 5(1.18%) of them revealed titer 1/10 and last one (0.24%) had a titer 1/40 and also gave a titer 1/50 by using Rivanol test with a percent 0.24% (the only true positive case all over the study). Two cases only showed clinical signs with a titer 1/10, the first had a history of abortion at the 7 month of pregnancy and the other had a sore back lesion. The incidence of infection among young donkeys (less than 5 years old) was less than that of old ones (more than 5 years old) and in females it was higher than in males. There are no any seroreactive cases among the 48 examined horses using the above tests. It can be concluded that the disease was not widely prevalent among horses and donkeys reared in Assiut Governorate, however even this low percent among donkeys can threaten human and other animals. BAPAT was the most sensitive tests for the serological detection of brucellosis among donkeys, and horses but TAT & Rivanol tests were the most accurate.

Key word: *Brucellosis, Donkeys, Horses, Seroprevalence.*

Abbreviation: (TAT) = Tube Agglutination Test- (RBPT) = Rose Bengal plate Test. (BAPAT) = Buffered Acidified plate Antigen Test.

INTRODUCTION

Historically, equine brucellosis was recognized early when *B. abortus* was isolated from an aborted fetus of a mare (McNutt and Murray, 1924), where the disease is important not only as a clinical existence but also as a potential source of infection for man and other animals (Tel *et al.*, 2011). Equines can contract infection through either contact with infected cattle or other species, ingestion of contaminated food or water and via penetration of skin or mucous membrane (Megid *et al.*, 2010).

Equines showed a wide range of brucellosis occurrence, from 0.24% up to 37.5% (Gul and Khan, 2007). Worldwide brucellosis among donkeys were 5.5% (Dragonas *et al.*, 1967), 3.61% (Yadav *et al.*, 1991), 3.6% (Musa, 2004), 7.4% (Abo-Shehada, 2009), 2.12% (Abdalla *et al.*, 2010) and 0.25% (Tel *et al.*, 2011), in Greece, India, Sudan-Darfur, Jordan, Eastern Sudan and Southeast Turkey, respectively. While through Egyptian previously research works, it was recorded as 46% (Fahmy & Salem 1974), 27.05% (El-Bohy, 1979), 20.61% (Abdel-Kader *et al.*, 1995), 19.42 in male donkeys, and 21.15% in she- donkeys (Hamoda & Montaser, 1998) in Assiut, Elsharkia, Assiut and Elkaliobia Egyptian Governorates, respectively.

Brucellosis among horses was widely investigated abroad and recorded as 0.8% (Dragonas *et al.*, 1967),

16.28% (Ali *et al.*, 1985), 42.40% (Izgur *et al.*, 1988), 4.40% in male horses, 12.29% in mares (Yadav *et al.*, 1991), 5.78% (Ahmed & Munir, 1995), no positive reactors (Omer *et al.*, 2000), 60.59 (Solmaz *et al.*, 2004), 0.24% (Acosta- González *et al.*, 2006), 9.5% (Göz *et al.*, 2007), 2.5 (Namavari *et al.*, 2008), 20.7% (Wadood *et al.*, 2009), 2.5% (Tahamtan *et al.*, 2010), 14.7% (Ehizibolo *et al.*, 2011) and 13.68% (Tel *et al.*, 2011) in India, Netherlands, Greece, Iraq, Turkey-Ankara, India, Lahore-Pakistan, Eriteria, Van province of Turkey, Mexico, Hakkari- Turkey, Mashhad-Iran, Faisalabad- Pakistan, Northeast of Iran, North Nigeria, and Southeast Turkey respectively.

Few Egyptian studies recorded it as: 37% in Assiut (Fahmy & Salem 1974), no positive reactors (Nashed, 1977) and 5.88% (Abdel-Kader *et al.*, 1995) and 8 % in Elsharkia (Esmat, 1996).

Clinically equine brucellosis is generally asymptomatic since many serological surveys have indicated that horses may be exposed to brucella infection without developing clinical signs of the disease (Cyetic *et al.*, 2005; Acosta-González *et al.*, 2006; Göz *et al.*, 2007; Namavari *et al.*, 2008 and Ehizibolo *et al.*, 2011). However some clinical signs were reported in horses due to *B. abortus* infection as fluctuating temperature, arthritis, intermittent lameness, lethargy, painful swellings were found in

different sites of the body mainly on supraspinous bursa (Fistulous withers) and atlantal bursa (Poll evil) (Carrigan *et al.*, 1987; Esmat 1996 and Ocholi *et al.*, 2004), or reproductive impairments as infertility in stallion and abortion in mare (Garg and Manchanda 1986).

Studies in relation to brucellosis in Egypt have focused mainly on domesticated ruminants (cattle, sheep and goats) neglecting the other contact farm animals which may threaten the spread of infection, so this work aimed to determine the prevalence of brucellosis among horses and donkeys in Assiut Governorate using 4 different serological tests (RBPT, BAPAT, TAT and Rivanol test) and in turn to evaluate the efficiency of these tests.

MATERIALS and METHODS

I- Animals:

A- Donkeys: 423 (150 ♂ & 273 ♀) animals were involved in the serosurvey for detection of brucella Infection, 303 old animals (over 5 years old) while the rest 120 were young (less than 5 years). 350 of them were apparently healthy and the rest 73 were suffering from injuries, lameness or bursitis at different parts of the body. They were gathered from four different management sources as follows:-

1-) 285 personal domestic animal holding (PDAH) (rural domestic animal holding) were collected from different localities belonging to Assiut Governorate.

- 2-) 36 animals from village markets.
- 3-) 85 animals working in private farms.
- 4-) 17 animals working in governmental farms

B- Horses: Forty eight animals (12 ♂ & 36 ♀), where 41 of them were old (over 5 years old) and the rest 7 animals were young (less than 5 years) and 39 of them apparently healthy, while the rest 9 were suffering from injuries, lameness and bursitis at different parts of the body. Managementally, 35 were PDAH, while the rest 12 were belonging to farms (11 to private and one to governmental farms).

II- Antigens

- 1- Buffered Acidified Plate Antigen 0.5 % phenol was obtained from SAS-Scientific San Antonios, Texas, USA, and was carried out as described by Angus and Barton (1984).
- 2- Rose Bengal Antigen was supplied from Merieux Institute – France and was performed as described by Alton *et al.* (1988).
- 3- Standard *B.abortus* Agglutination Antigen for TAT was obtained from Veterinary Sera and Vaccine Research Institute, Abbasia, Egypt. The test was performed according to that described by Alton *et al.* (1988).
- 4- Rivanol Antigens were supplied by Veterinary Sera and Vaccine Research Institute, Abbasia, Egypt and the test was carried out according to Alton *et al.* (1988).

III- Test agreement % with Rivanol results was calculated according to Saylor (1995) as follows:-

$$\text{Agreement Percent between two serological tests} = \frac{\text{No of Samples gave +Ve with both tests} + \text{No of samples gave -Ve with both tests}}{\text{Total examined cases}} \times 100$$

RESULTS

The obtained results were recorded in Tables (1-3).

Table 1: Seroreactive animals using BAPAT, RBPT, TAT and Rivanol serological tests.

Species	Locality	No.of Animals	Seroreactors							
			BAPAT		RBPT		TAT		Rivanol test	
			No	%	No	%	No	%	No	%
Donkeys	PDAH*	285	6	2.1	5	1.75	4	1.40	1	0.35
	Village markets	36	2	5.56	1	2.78	1	2.78	-	-
	Private farms	85	1	1.18	1	1.18	1	1.18	-	-
	Government farms	17	-	-	-	-	-	-	-	-
Total		423	9	2.13	7	1.66	6	1.42	1	0.24
Agreement % with Rivanol test			98.1		98.6		98.8			
Horses	PDAH*	35	-	-	-	-	-	-	-	-
	Private farms	12	-	-	-	-	-	-	-	-
	Government farms	1	-	-	-	-	-	-	-	-
Total		48	-	-	-	-	-	-	-	-

*PDAH = Personal Domestic Animal Holding.

Table 2: Different titres of seroreactive donkeys for TAT and Rivanol Tests.

Locality	No of donkeys	Tube agglutination test						Rivanol test				
		1/10	1/20	1/40	1/80	Total reactors		1/25	1/50	1/100	Total reactors	
						No	%				No	%
PDAH	285	3	-	1	-	4	1.40	-	1	-	1	0.35
Village markets	36	1	-	-	-	1	2.78	-	-	-	-	-
Private farms	85	1	-	-	-	1	1.18	-	-	-	-	-
Government farms	17	-	-	-	-	-	-	-	-	-	-	-
Total	423	5 (1.18%)	-	1 (0.24%)	-	6	1.42	-	1	-	1	0.24

Table 3: Seroprevalence of brucellosis in donkeys in relation to sex and age.

Parameter	Total no	Positive seroreactors								
		BAPAT		RBPT		TAT		Rivanol test		
		NO	%	NO	%	NO	%	NO	%	
Sex	Male donkeys	150	1	0.67	1	0.67	1*	0.67	-	-
	She donkeys	273	8	2.93	6	2.20	5	1.83	1	0.37
Age	<5 years	120	1	0.83	1	0.83	1*	0.83	-	-
	>5 years	303	8	2.64	6	1.98	5	1.65	1	0.33

* a male donkey < 5 years had a titer of 1/10

DISCUSSION

Donkeys have a multipurpose use (especially in developing countries) for agricultural practices as transport and cart dragging where they are grazed, watered and kept in close contact with cattle and other farm animal species. Thus, donkeys may be exposed to contract (Ehizibolo *et al.*, 2011) or transmit brucella infection since, they are possibly shedders of brucella organisms (Ocholi *et al.*, 2004).

In the present work, brucella seroprevalence among donkeys in Assiut Governorate using BAPAT, RBPT, TAT and Rivanol tests on 423 donkey blood sera revealed that 9 (2.13%), 7 (1.66%), 6 (1.42%) and 1 (0.24%) were seroreactors respectively (Table 1). Nearly similar results among donkeys were obtained in Sudan (Musa, 2004; Abdalla *et al.*, 2010), India (Yadav *et al.*, 1991) and Turkey (Tel *et al.*, 2011), while higher incidences were recorded in Greece (Dragonas *et al.*, 1967), Jordan (Abo-Shehada, 2009) and through all available Egyptian studies (Fahmy & Salem 1974; El-Bohy 1979; Hamoda & Montaser 1998 and Abdel-Kader *et al.*, 1995). The higher incidence of brucella seroprevalence might be due to variation of samples collection, practical management and diagnostic techniques.

From Table (1), the seroreactive prevalences in-between categories among donkeys showed that the higher BAPAT ratio was in village markets (5.56%) then those of PDAH (2.1%) and finally private farms (1.18%) which indicate the high risk of disease transmission through animal movement in-between different locations.

Since Rivanol test acts with the true positive samples only as it detects IgG precipitating other immunoglobulins (Sayour 1995), it was the reference test in the present study. The only seropositive case all over this study (Rivanol positive) necessitated to study the agreement % of other serological tests with Rivanol which revealed that BAPAT, RBPT, TAT agree with Rivanol test by 98.1%, 98.6% and 98.8% respectively. TAT was the most serological test agreed with Rivanol test and it was the recommended test by many authors (EL-Bohy 1979; Abdel-Kader *et al.*, 1995) in diagnosis of equine brucellosis as the superior test.

In the present study quantitatively, among the 6 seroreactors through TAT, their only 2 she-donkeys had clinical signs (showing titre 1/10 using TAT) from the 9 seroreactive donkeys (using the most sensitive BAPAT). The first one had a previous history of abortion at the 7 month of pregnancy where

the second one had sore back lesions. The rest of seroreactive donkey show no clinical signs even that case which had a titer of 1/40 with TAT and a titre of 1/50 with Rivanol test (Table2). As the latent or dormant infection is the most probable form of brucellosis in equine (Acosta–González *et al.*, 2006; Göz *et al.*, 2007; Namavari *et al.*, 2008 and Ehizibolo *et al.*, 2011) even on experimental infection with *B. abortus* of a group of horses did not result in any clinical signs, but serological response of the organism was both marked and prolonged (MacMillan *et al.*, 1982; MacMillan & Cockrem 1986).

In the present study, Table (3) show the seroprevalence of brucellosis was found to be much less in male donkeys than that in she- donkeys which are in accordance to Hamoda & Montaser (1998) as the females experience comparatively greater physiological stress during pregnancy and lactation due to which they are more susceptible to infection.

Moreover aged donkeys showed higher incidence than younger ones since the increase of antibodies positive reactions in older donkeys could possibly due to increase chance for exposure to infection (Ahmed & Munir, 1995; Agab, 1997 and Kazi *et al.*, 2005). Low prevalence in young may be explained on the basis that the animal may harbor the organism without expressing any detectable antibodies until their first parturition or abortion Wadood *et al.* (2009).

At Assiut, as owning horses as a kind of priding, the animals were kept in separate special pens away from other domestic giving no chance of infection transmission so no positive results were obtained either through the present study and Nashed (1977) while Abdel – Kadder *et al.* (1995) detected 5.88%.

CONCLUSION

The results indicated that brucellosis is not widely distributed among horses and donkeys raised in Assiut province. However low percent donkeys reacted serologically to brucella infection without clinical evidence should not be neglected. The present findings proved that BAPAT was the most sensitive tests for the serological detection of brucellosis among donkeys, and horses but TAT was the most accurate serological test that agreed with Rivanol test.

REFERENCES

- Abdalla, M.A.E.; Abdalla, S.H. and Elzaki, R. (2010):* Prevalence of brucella abortus Gaderef State of Eastern Sudan. Tropentage, September 14- 16, Zurich "Word Food System – A contribution from Eurpe".
- Acosta–González, R.I.; González–Reyes, I. and Flores–Gutiérrez, G.H. (2006):* Prevalence of brucella abortus antibodies in equines of a tropical region of Mexico. Can. J. Vet. Res., 70 (4): 302 -304.
- Abdel- Kader, H.A.; Abdel- Hafeez, M.M. and Bastawrows, A.F. (1995):* Seroprevalence of brucellosis in Equines in Assiut Governorate. Assiut Vet. Med. J., 33(66): 84- 90.
- Abo-Shehada, M.N. (2009):* Seroprevalence of brucella species in equids in Jordan. Vet. Rec., 165: 267- 268.
- Agab, H. (1997):* Clinical signs of animal brucellosis in Eastern Sudan Rev. Elev. Med. Vet. Trop., 50(2): 97-98.
- Ahmed, R. and Munir, M.A. (1995):* Sero-prevalence of brucellosis in Horses, Dogs, Cats and Poultry. Pakistan Vet. J. 15 (2): 85 – 88.
- Ali, A.H.; Zaidan, W.A. and Sharma, V.K. (1985):* Seroprevalence of brucellosis in horses in Iraq. Indian Vet. J., 62: 917–921.
- Alton, G.G.; Jones, L.M.; Angus, R.D. and Verger, J.M. (1988):* Techniques for brucellosis: laboratory. Paris: Institut National de la Recherche Agronomique, pp 63-129.
- Angus, R.D. and Barton, C.E. (1984):* The production and evaluation of a buffered plate antigen for use in a Presumptive test for brucellosis (cattle and swine). Develop. boill. Standard, 56: 349-351.
- Carrigan, M.J.; Cockram, F.A. and Nash, G.V. (1987):* Brucella abortus biotype 1 arthritis in a horse. Aust. Vet. J., 64: 190. Cited by Tel *et al.* (2011).
- Cyetic, Z.; Spicic, S. and Curic, S. (2005):* Isolation of *B. suis* biovar 3 from horses in Croatia. Vet. Rec., 156: 584–585.
- Dragonas, P.N.; Stotores, E.N. and Eftathiou, LE. (1967):* Bull. Soc. Vet. Hell. 18: 20 -23. After Abdel – Kadder *et al.* (1995).
- Ehizibolo, D.O.; Gusi, A.H.; Ehizibolo, P.O.; Mbuk, E.U. and Ocholi, R.A. (2011):* Serologic prevalence of brucellosis in horse stable in two North state of Nigeria. J. Equine Sci., 22 (1): 17- 19.
- El–Bohy, Z.M.A. (1979):* Some studies on brucellosis in donkey. M Sc. Thesis, Fac. Vet. Med., Zagazig Univ.
- Esmat, M. (1996):* Some studies on brucellosis in horses 7th Sci. Cong. 17-19 Nov. Fac. Vet. Med. Assiut, Egypt, pp. 637- 643.
- Fahmy, I.S. and Salem, A.A. (1974):* Brucella as a cause of bursitis in domestic Animals. Assiut Vet. Med. J., 1(1 & 2): 191 – 198.
- Garge, D.N. and Manchanda, V.P (1986):* Prevalence and etiology of equine abortion. Ind. J. Anim. Sci., 56 (7): 730-735.
- Göz, Y.; Babür, C.; Aydin, A. and Kilic, S. (2007):* Seroprevalence of toxoplasmosis, brucellosis and listeriosis in horses in Hakkari, eastern region of Turkey, Revue. Med. Vet., 158 (11): 534-539.
- Gul, S.T. and Khan, A. (2007):* Epidemiology and epizootology of brucellosis: a review. Pakistan Vet. J., 27: 145-151.
- Hamoda, F.K. and Montaser, A.M. (1998):* Clinico-epizootiological study on brucellosis in donkeys. Beni-Suef. Vet. Med. J., 8: 105-118.
- Izgun, M.; Akay, O.; Candas, A.; Inan, A.; Ayhan, H. and Esendal, O. (1988):* Study on the

- prevalence of equine brucellosis in Ankara. Etlik Veteriner Mikrobiyoloji – Enstitüsü – Dergisi, 6: 117–126.
- Kazi, M.; Amin, R.; Bahman, M.B.; Rahman, M.S.; Han, J.; Park, J. and Chae, J., (2005): Prevalence of antibodies in sera of cows in Bangladesh. J. Vet. Sci. 6: 223-226.
- MacMillan, A.P. and Cockrem, D.S. (1986): Observation of the long term effects of B. abortus infection in the horse, including effects during pregnancy and lactation. Equine Vet. J., 18: 388-390.
- MacMillan, A.P.; Baskerville, A.; Hambleton, P. and Corbel, M.J. (1982): Experimental Brucella abortus infection in the horse; observation during the three months following inoculation. Research in Veterinary Science 33: 351-259.
- McNutt, S.H. and Murray, C. (1924): Bacterium abortum (Bang) isolated from the fetus of an aborting mare, J. Am. Vet. M.A., 65: 215.
- Megid, J.; Mathias, L.A. and Robles, C.A. (2010): Clinical manifestations of brucellosis in domestic animals and humans. The Open Veterinary Science Journal, 4: 119- 126.
- Musa, M.T. (2004): A serological study on Equine brucellosis in Darfur, Western Sudan. The Sudan J. Vet. Res., 19: 7- 11.
- Namavari, M.M.; Mohammadi, G.H.R.; Tahamtan, M.R. and Tahamtan, Y. (2008): Prevalence of brucellosis in equines of Mashhad – Iran. International Journal Of Infectious Diseases. Vol 12, page 134.
- Nashed, S.M. (1977): Brucellosis in Upper Egypt (Assiut – governorate) in cattle, buffaloes, sheep, goats and its relation to public health Ph. D. Thesis, Faculty of Vet. Med. Cairo University.
- Ocholi, R.A.; Kawaga, J.K.P.; Ajogi, I. and Bale, J.O.O. (2004): Phenotypic characterization of Brucella strains isolated from livestock in Nigeria. Vet. Microbiology, 103: 47-53.
- Omer, M.K.; Skjerve, E.; Holstad, G.; Woldehiwet, Z. and MacMillan, A.P. (2000): Prevalence of antibodies to Brucella spp in cattle, sheep, goats, horses and camels in the State of Eritrea; influence of husbandry system. Epidemiol Infect., 125: 447–453.
- Sayour, A.E.M. (1995): An approach towards the use of some unconventional serological tests for the diagnosis of brucellosis. M Sc Vet. Thesis, Fac. of Vet. Med. Cairo, University.
- Solmaz, H.; Tutuncu, M.; Akhan, H.A.; Aksakal, A.; Gulhan, T. and Boynukara, B. (2004): Brucellosis in horses around Van, Turkey. Indian. Vet. J., 81: 748–749.
- Tahamtan, Y.; Namavari, M.M.; Mohammadi, G. and Jula, G.M. (2010): Prevalence of brucellosis in horse North – East of Iran. J. Equine Vet. Sci., 30 (7): 376-378.
- Tel, O.Y.; Neva, B.A. and Oktay, K. (2011): Seroprevalence of Equine brucellosis in Southeast Turkey. YYU Veteriner Fakultesi Dergisi .22(3): 181-183.
- Wadood, F.; Ahmed, M.; Khan, A.; Gul, S.T. and Rehman, N. (2009): Seroprevalence of brucellosis in horses in and around Faisalabad. Pakistan Vet. J., 29 (4): 196-198.
- Yadav, M.P.; Singh, B.K. and Uppal, P.K. (1991): Sero-prevalence of brucellosis in equines in some of the states in India. Indian. J. Anim. Sci., 62 (1): 41-42.

دراسة مصلية استيبائية علي الموقف الحالي للإجهاض المعدي (البروسيلة) في الخيول والحمير في محافظة أسيوط

سعد محروس البرياوي

أجريت الدراسة علي عدد ٤٢٣ حمار ، ٤٨ حصان من مناطق مختلفة بمحافظة أسيوط من كلا الجنسين ومن مختلف الأعمار وذلك بغرض استبيان نسبة تواجد الإجهاض المعدي (البروسيلة) بها. أظهرت النتائج أن نسبة الحالات الإيجابية في الحمير كانت ١٣،٢% ، ١،٦٥% ، ١،٤٢% باستخدام اختبارات المحمض المخمد الشريحي والروزبنجال والتلازن الأنبوي البطئ علي التوالي. وباستخدام اختبار الريفانول والذي يعتبر هو الاختبار الوحيد بين الاختبارات المستخدمة الذي يحدد الإيجابي الحقيقي كانت النسبة ٠،٢٤% معيارياً أظهرت ٥ عينات مصل دم حمير نتائج إيجابية بعبارية ١٠/١ (منها حالتين أظهرتا أعراض إكلينيكية الأولى لها تاريخ إجهاض في الشهر السابع من الحمل والأخري بها تقرحات في الظهر ولم تظهر اي أعراض إكلينيكية اخري في باقي الدراسة)، وعينة واحدة بعبارية ٤٠/١ باختبار التلازن الأنبوي البطئ ونفس العينة أعطت عيارية ٥٠/١ باختبار الريفانول وتعتبر هذه العينة الوحيدة الإيجابية الحقيقية على مدار الدراسة. كانت نسبة الإصابة في الحمير التي اعمارها اصغر من ٥ سنوات أقل من الحمير الأكثر من ٥ سنوات وفي الإناث كانت أعلى من الذكور. لم تسجل الدراسة أي نتائج إيجابية في الخيول وذلك بنفس الاختبارات السابقة. ويمكن الخلاصة بأن نسبة المرض في الخيول والحمير ضئيلة بمحافظة أسيوط ولكن لا يمكن إهمال هذه النسبة في الحمير وما يمكن أن تشكله من خطورة علي الإنسان والحيوان. وأن اختبار المحمض الشريحي أكثر الاختبارات حساسية في تشخيص البروسيلة في الخيول والحمير بينما اختباري الريفانول والتلازن الأنبوي البطئ أكثرها دقة.