Veterinary Serum and Vaccine Research Institute,
Abbassia, Cairo.

IN VITRO ESTIMATION OF POTENCY OF SOME CLOSTRIDIAL TOXOIDS

(With 3 Tables)

By

M.M. FAYEZ; A.A. EL-MENISY and A.Z. HUSSEIN

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تحديد كفاءة توكسيدات بعض ميكروبات الكلوستريديا معمليا

محمود محمد فايز ، علاء عبد الفتاح المنيسي ، عبد السلام زكي حسين

في تلك الدراسة تم مقارنة اختبار الاتحاد السمي المثبط كاختبار معملي لقياس القوة المناعية للقـــاح الكلوستريديا الجامع باختبار السم المتعادل في الفئران. وكانت النتائج لكلا الاختبارين مـــتوازية ومتساوية ولذلك يمكن استخدام ذلك الاختبار بدلا من استخدام إختبار السم المتعادل توفيرا لاستخدام حيوانات التجارب.

SUMMARY

The efficacy of the toxin binding inhibition test (ToBi) as an in vitro testing of potency for multicomponent clostridial vaccine was compared with the currently used toxin neutralization test (TN). It was found that the antitoxin titers obtained with ToBi were highly correlated to TN. So this test could be used as simple, rapid and non-expensive alternative to TN test.

Key words: *Clostridial toxoids, antitoxin.*

INTRODUCTION

The potency of multicomponent clostridial vaccines is currently tested by their ability to stimulate an antibody response in rabbits, and it is measured in vivo by using toxin neutralization test (TN) in mice (British Veterinary Pharmacopoeia, 1993). While the test is known to be sensitive and reliable, however a high costing and the long time taken to perform the assay is regarded as inherent disadvantage. Moreover, there is a growing concern about the extensive use of non-protected animals in which clinical manifestation due to clostridial toxins especially tetanus may appear. For ethical, economical and practical reasons, several in vitro immunoassay techniques have been suggested by a number of

authors as an alternative to in vivo TN test such as haemagglutination test (Peel, 1980), and ELISA (EL-Idrissi and Ward, 1992; Ebert *et al.*, 1999). These immunological techniques are not preferred because non-neutralizing antitoxins are commonly detected. Moreover, the results of these in vitro techniques and those of the in vivo assay are not highly correlated especially when sera with low antibody titers are titrated (Simonsen *et al.*, 1987; Hagenaars *et al.*, 1984).

Recently an antigen competition ELISA has been described which showed a good correlation between in vivo and in vitro titers even in low level of antitoxin (Simonsen *et al.*, 1987; Marcjanna *et al.*, 1989). In the present study, the efficacy of the toxin binding inhibition test (ToBi) as an alternative approach to the currently used toxin neutralization test for estimating the potency of clostridial vaccines was evaluated.

MATERIALS and METHODS

Clostridial toxoids:

Clostridial toxins (*C. perfringens* types B; D, and *C. novyi* type B) were prepared according to (Gadalla *et al.*, 1974), while *C. tetani* toxin was prepared according to (Rijks, 1980). After estimation of the minimum lethal dose (MLD) of each toxin, 0.5% formalin was added for toxoiding all the prepared toxins. A polyvalent clostridial vaccine containing the above-prepared toxoids was formulated according to the regulation of (British Veterinary Pharmacopoeia, 1993).

Experimental Animals:

A group of ten Boscat rabbits (2.3-3Kg) and six sheep (8-12 month old) were used for evaluation of the potency of the prepared polyvalent clostridial vaccine. Both rabbits and sheep were injected S/C with two doses of 5 ml and 3 ml at 3 weeks interval. Rabbits were bled two weeks after boostering and sera were pooled. Sheep sera samples were collected two weeks post the second dose and then every 3 weeks until the end of the experiment.

Toxin neutralization test:

Antibody titers of rabbit and sheep sera were estimated in mice using the standard method described by (Frerichs and Gray, 1973).

Toxin binding inhibition test: (ToBi)

The ToBi was carried out as described by (Hendriksen *et al.*, 1988) with some modification, keeping in consideration a fixed concentration of each toxin and antitoxin as illustrated in Table (1). In brief, a flat-bottomed polystyrene micro titer plates were coated with

250µl/well phosphate buffered saline (PBS), pH 7.2 containing 0.5% bovine serum albumin (BSA); 0.05% Tween 80. After incubating at 37°C for 2 hours, two fold dilution of each serum sample starting with 1:4 dilution were made in PBS in 100 µl volumes. Each serum dilution was mixed with 100µl of the toxin in PBS (A reference serum with known antitoxin concentration (one I.U. /ml) was titrated for comparison. The antitoxin concentration for this reference serum has been previously determined in vivo). The plates were gently shaked and incubated overnight at 37°C in humid atmosphere. Next day, 100µl of the serum toxin mixture was transferred from the micro titer plates to the corresponding wells of immunoassay micro titer plates coated with (one I.U./ml) antitoxin. All plates were incubated for 1.5 hours at 37°C, and then washed with tape water containing 0.05% tween 80. After washing, (one I.U./ml) antitoxins were added again each well in 100 µl quantities, and incubated at 37°C for 1.5 hours. Thereafter a diluted peroxidase labeled antispecies was added in 100 µl/well quantities, incubated at 37°C for 1.5 hours, followed by washing with tape water containing 0.05% tween 80. Finally, 100 µl/well of the substrate (34mg OPD dissolved in 100 ml phosphate citrate buffer, pH 5, and 15µl of 30% H_2O_2) was added to each well. After ten minutes the reaction was stopped by addition of 100 µl of 2M H_2SO_4 to each well. The absorbance was measured at 490nm.

Table 1: Concentration of toxins and antitoxins of different clostridial strains.

Toxins	Concentration	Antitoxin	Concentration
Beta toxin of C. perfringens type B	1L+/10	Beta antitoxin of C. perfringens type B	One I.U./ml
Epsilon toxin of C. perfringens type D	1L+/100	Epsilon antitoxin of C. perfringens type D	
Alpha toxin of C. novyi type B	1L+/10	Alpha antitoxin of C. novyi type B	
Tetanus toxin	0.1 Lf/ml	Tetanus antitoxin	

Reproducibility of ToBi:

The reproducibility of ToBi was determined by repeated testing of 10 serum samples for several weeks. The inter and intra assay coefficient of variance was determined as described by (Dawson-Sounders and Trapp, 1990).

RESULTS and DISCUSSION

Despite the serum neutralization assay in mice for measuring clostridial antitoxin is laborious and expensive and uses large number of laboratory animals, it is still the in vivo method of choice for demonstrating the protective (neutralizing) antitoxin. The need for simple in vitro test, which makes possible rapid titration of sera for potency testing of clostridial vaccines has been previously recognized (Peel, 1980). To discriminate neutralizing from non-neutralizing antitoxins is an essential prerequisite for many techniques that replacing the TN. In the present study, the ToBi test is based on the detection of free toxin in toxin-antitoxin mixture by an ELISA with preoxidase-labeled antitoxin and the only difference between the ToBi and in vivo TN test being the way in which free toxin is detected (Hendriksen *et al.*, 1991).

During this work, pooled sera collected from rabbits vaccinated with the prepared polyvalent clostridial vaccine was titrated using both TN and ToBi tests. The results illustrated in Table (2) revealed that the antitoxin values measured by TN test were 10,5,5 and 20 I.U. for Beta, Epsilon, Alpha, and Tetanus antitoxin respectively. On the other hand, the corresponding antitoxin values determined by ToBi were 11,5,5 and 21 respectively. The obtained antitoxin titers satisfy the requirements of British Veterinary Standard for all vaccine components.

By inspecting the data shown in Table (2), it is clear that there is a significant correlation coefficient r= 0.89 between both TN and ToBi test in the estimation of antitoxin level, as also documented by (Hendriksen *et al.*, 1991).

In vaccinated sheep Table (3), statistical analysis revealed that there is a good correlation between TN and ToBi for their sera (for Beta antitoxin r= 0.999, Epsilon antitoxin r= 0.994, *C. novyi* type B antitoxin r= 0.986, tetanus antitoxin r= 0.999) $p<0.05$. Such results almost agree with these of (Hendriksen *et al.*, 1989; Marcjanna *et al.*, 1989; Hendriksen *et al.*, 1991) as they used ToBi instead of TN and in vitro neutralization test in vero cells for titration of tetanus and diphtheria antitoxin.

The reproducibility of the test for the 10 sera samples that tested for 5 times proved that the ToBi test was reliable. Statistical analysis of the obtained results showed that the intra assay coefficient of variance was (1-4%) Where the inter assay coefficient of variance was (0-36%)

which are very satisfactory and within the normal range (Dawson-Sounders and Trapp, 1990).

In conclusion, the ToBi was found to be a practically simple; reproducible, and quick to perform and it is correlated well with TN test, it could be also act as non expensive, alternative to TN test by saving for the laboratory animals needed for testing the potency of multicomponent clostridial vaccines.

Table 2: Antitoxic values of pooled rabbit sera vaccinated with polyvalent clostridial vaccine as estimated by serum neutralization test and toxin binding inhibition test.

Test	Antitoxin titer (IU/ml)			
	C. perfringens type B	*C. perfringens* type D	*C. novyi* type B	*C. tetani*
Serum neutralization test	10	5	5	20
Toxin binding inhibition test	11	5	5	21

Table 3: Immune response of sheep to polyvalent clostridial vaccine as measured by TN and ToBi test.

Time after vaccination	Antitoxin titer (I.U./ml)							
	C. perfringens Type B Beta antitoxin		*C. perfringens* Type D Epsilon antitoxin		*C. novyi* Type B Alpha antitoxin		Tetanus antitoxin	
	TN	ToBi	TN	ToBi	TN	ToBi	TN	ToBi
Prevaccination	0	0	0	0	0	0	0	0
4 weeks	5	5	3	3	2	2	5	5
7 weeks	20	21	10	10	5	6	20	21
10 weeks	15	15	8	7	5	5	20	20
13 weeks	10	10	5	5	4	4	15	15

I.U.= International Unit.

REFERENCES

British veterinary pharmacopoeia, (1993): HMSO, London, pp. 118.

Dawson-Sounders, B., and Trapp, R.G. (1990): Basic and clinical biostatistics Appleton and lange, Norwalk CT.

Ebert, E.; Oppling, V.; Werner, E., and Cussler, K. (1999): Development and prevalidation of two different ELISA systems for potency testing of *C. perfringens* type B and epsilon toxoid containing veterinary vaccines. FEMS Immunol. Med. Microbiol. 24: 299-311.

EL-Idrissi, A.H., and Ward, G.E. (1992): Development of double sandwiches ELISA for Clostridium perfringens beta and epsilon toxins. Vet. Microbiol. 31: 89-99.

Frerichs, G.N., and Gray, A.k. (1973): Immune response of rabbits to multicomponent clostridial vaccine. J. Biol. Stand. 1:33-36.

Gadalla, M.S.; Farrag, I., and Sharaf, D. (1974): Effect of growth requirement on the improvement of clostridial vaccines. J. Egypt. Vet. Med. Ass. 34 (112):19-28.

Hagenaars, A.M.; Van Delft, R.W., and Nagel, J. (1984): Comparison of ELISA and toxin neutralization for detection of tetanus antibodies. J. Immunoassay, 5: 1-11.

Hendriksen, C.F.M.; Gun, V.D.J.; Nagel, J., and Kreeftenberg, J.G. (1988): The toxin binding inhibition test as a reliable in vitro alternative to toxin neutralization test in mice for the estimation of tetanus antitoxin in human sera. J. Biol. Stand. 16: 287-297.

Hendriksen, C.F.M.; Gun, V.D.J., and Kreeftenberg, J.G. (1989): Combined estimation of tetanus and diphtheria antitoxin in human sera by in vitro toxin binding inhibation (ToBi) test. J. Biol. Stand. 17:181-190.

Hendriksen, C.F.M.; Gun, V.D.J.; Marsman, F.R., and Kreeftenberg, J.G. (1991): The use of the in vitro toxin binding inhibition (ToBi) test for the estimation of the potency of tetanus toxoid. J. Biol. Stand. 19: 23-29.

Marcjanna, G.S.; Victor, J.W.; Cloristopher, J.T., and Peter, L.P. (1989): The detection of *Clostridium perfringens* epsilon antitoxin in rabbit serum by monoclonal antibody based competition ELISA. J. Biol. Stand. 17: 117-124.

Peel, M.M. (1980): Measurement of tetanus antitoxin 1-Indirect haemagglutination. J. Biol. Stand. 8:177-189.

Rijks, I. (1980): Instructions for the preparation of vaccines. Rijiks Institute Voor Volksgezondheid Bilthoven, The Netherland.

Simonson, O.; Kjeldsen, K.; Bentzon, M.W. and Heron, J. (1987): Susceptibility to diphtheria in populations vaccinated before and after elimination of indigenous diphtheria in Denmark. Acta. Pathol. Microbiol. Immunol. Scand. 13: 229-234.

Animal Health Research Institute,
Assiut Laboratory.

SEROLOGICAL STUDY OF BRUCELLOSIS ON CAMELS IN ASSIUT AND THE NEW VALLEY GOVERNORATES

(With One Table)

By

M.M. ALI; N.H. MAKAR and S.R. SEDDEK

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دراسة سيرولوجية تشخيصية عن البروسيلا فى الجمال فى محافظتى أسيوط والوادى الجديد

محمد مصطفى على ، نبيل حبيب مقار ، صديق رشوان صديق

أجريت هذه الدراسة على عدد ٣٠٠ عينة من دم الجمال التى تم تجميعها من محافظتى أسيوط والوادى الجديد لتحديد نسبة الإصابة بالبروسيلا بين الجمال. وقد تم فحص جميع العينات سيرولوجيا بواسطة اختبارى الانتيجن الشريحى المحمض المخمد والروز بنجال والعينات الإيجابية أجرى لها اختبارى التلازن الأنبوبى والريفانول. وأسفرت النتائج عن ٧ (٢,٣٣%) حالات إيجابية للبروسيلا منها ٢ ذكور (٠,٦٦%) و ٥ إناث (١,٦٦%) وكانت نسبة الإصابة فى محافظة أسيوط (٣,٠٤%) فى حين لم تسجل محافظة الوادى الجديد أى حالات إيجابية.

SUMMARY

A serological study was performed on 300 camels sera collected from Assiut and New Valley Governorates to estimate the incidence of brucella infection among camels. All samples were examined serologicaly by Buffered acidified plate antigen test (BAPAT) and Rose Bengal plate test (RBPT) and positive reactors were confirmed by tube agglutination test (TAT) and Rivanol tests (R.T.). Of the 300 camel sera tested, 7 positive reactors (2.33) were detected, 2 males (0.66%) and 5 females (1.66%). The incidence in Assiut was 3.04% while no positive reactors were detected in the New Valley.

Key words: *Brucella, Camel, Rose Bengal, New valley.*

INTRODUCTION

Brucellosis is still one of the important zoonotic diseases of a serious public health and economy problem in many countries. It was

being eradicated among domestic animals in some countries, is still prevalent in some others where it poses a potential threat to the consumers of milk and cheese and those working with animals and meat of slaughtered animals (Almer, 1985). In Egypt camels are still important animals for meat production and help in transportation of agricultural crops. The infection is caused by different biotypes of Brucella abortus and Brucella melitensis. There is no clear policy in any of the camel-keeping countries regarding the control of brucellosis in camels (Abbas and Agab, 2002).

The incidence of positive results were recorded by Ayoub *et al.* (1978), they found that the percentage of camel brucellosis was 24-25% in females and 14-28% in males when 216 camels sera were examined by (SAT) and (RBPT). Okoh (1979) in a survey on 232 camels serum samples found that 1.5% was positive. Damir *et al.* (1984) said that from 740 camels sera, brucella antibodies were 5.6% in males and 4.5% in females.

Zaghloul and Kamel (1985) recorded that the incidence of brucellosis in camels in Assiut province were 8.11% by (RBPT) and (STAT), also Lotfi *et al.* (1987) found that the percentage was 7.9%. Yaqoub *et al.* (1990) in a five years investigation of brucella antibody prevalence in camel, the incidence of positive results was 6.95±1.55%. Among adult one-humped camels the rate was 4.94±2.51% in males and 13.76±4.41% in females. Antibodies against brucella abortus were prevalent in one-humped camel sera throughout the five years of the survey with incidence rate of 6.54, 5.79, 9.32, 5.03 and 8.06% respectively from 1985-1989. Baumann and Zessin (1992) determined the prevalence for burcellosis as 1.9% by (SAT) and 0.3% by (CFT). At the same time. Radwan *et al.* (1992) said that the overall seroprevalence of brucellosis in tested camels was 8%. Omer *et al.* (2000) documents the first serological evidence of brucella species in camels (3.1%) in Eritrea. El-Ansary *et al.* (2001) found that of 64 camel sera tested with (SAT) and (TAT) non were positive, while Teshome *et al.* (2003) indicated that sera collected from 1442 accessible camels were screened with (RBPT), 82 (5.7%) of them reacted and the results of complement fixation test (CFT) on those reactors indicated 4.2% prevalence of brucellosis.

The present study attempts to investigate the incidence of brucellosis among camels in Assiut and New Valley Governorates by the different serological tests.

MATERIALS and METHODS

(A) Samples collection:

A total of 300 blood samples were aseptically collected from clinically healthy camels, each of 10 cc collected in clean and dry screw capped tubes. These samples were left at room temperature or at 37°C for 1/2 hours in inclined position, then placed in the refrigerator for 18 hours. Centrifugation for each sample at 3500 R.P.M. for 15 minutes, then the serum were kept at 4°C in the refrigerator till use for serological examination. The tested sera must be inactivated in water bath at 56°C for 1/2 hour to destruct the non specific antibodies before being tested (Amerault *et al.*, 1961).

(B) Serological examination:

All the sera were subjected to four serological tests: Rose Bengal Plate test (RBPT), Buffered Acidified Plate test (BAPT), Tube Agglutination test (TAT) and Rivanol test (R.T).

The four used antigens were supplied by serum and vaccine Research Institute, Abbassia, Cairo, Egypt. The techniques of RBPT, BAPT and TAT were carried out according to Anon (1992), while that of Rivanol test was performed according to Anon (1984).

RESULTS

The results were tabulated in table No. (1).

Table 1: Seroprevalence of brucellosis among camels in Assiut and New Valley Governorates by different serological tests.

Locality	No. of Examined animals	Sex	Serological tests									
			RBPT		BAPT		TAT			Rivanol		
			+	%	+	%	1/160	1/320	%	1/200	1/400	%
Assiut	230	160♂	6	3.7	7	4.3	1	1	1.3	1	-	0.6
		70♀	2	2.8	3	4.3	1	4	7.1	-	6	8.6
			8	3.5	10	4.3	2	5	3.04	1	6	3.04
New Valley	70	50♂	-ve	-	-ve	-	-ve	-ve	-	-ve	-ve	-
		20♀	-ve	-	-ve	-	-ve	-ve	-	-ve	-ve	-
			-ve	-	-ve	-	-ve	-ve	-	-ve	-ve	-
Total	300		8	2.7	10	3.3	2	5	2.3	1	6	2.3

DISCUSSION

Brucellosis is still a serious problem in most countries of the world due to its zoonotic and economic importance. So the early detection of brucella infection in a herd or flock is a pre-request for the successful control and elimination of one of the major problems considered to be a predisposing factor leading to infertility and sterility along with the possible transmission of infection to human (FAO/WHO) 1986. Control of the disease in animals depends mainly upon the use of efficient diagnostic procedures that insure the lowest possible incidence of false negative reaction (specific test) and false positives (sensitive test). In this study four different serological testes were used for diagnosis of brucellosis in camels, (BAPT and RBPT) were used as screening tests which revealed an incidence of (3.3%) and (2.7%) respectively and (TAT and Rivanol test) were used as confirmatory tests with an incidence of (2.3%). From the obtained results, it is evident that screening tests BAPT (3.3%) & (RBPT (2.7%) showed the highest percentage of positive reactors if compared with TAT and Rivanol test (2.3%). These results agrees with that obtained by Teshome *et al.* (2003) and Abdel Rasheed (2004). This may be claimed to the higher sensitivity of these testes as reported by El-Bauomy (1989). Also the acidic pH (3.6 in RBPT and 4.0 in BAPT) of the used antigen inhibit to a certain extent the activity of non specific immunoglobulins. Stemshorn *et al.* (1985) reported that BAPT detected higher incidence of positive reactors than RBPT, this may be ascribed to the fact that the test is more sensitive in detecting IgM and IgG, it could also depend on the amount of serum used in this test in which is more than the amount of serum used in RBPT. This results agree with our obtained results that recorded BPPT (3.3%) and RBPT (2.7%).

TAT was included in these serological tests as it detects mainly IgM and IgG classes of antibodies (Barton 1994). Also, Rivanol test is a useful and reliable test in detecting brucellosis without serious number of false positive, it is a highly specific, dependable and an official diagnostic test as it detects mainly the presence of the specific IgG through the precipitation of IgM (Hamdy, 1992).

The obtained results in table (1) revealed that the incidence of brucellosis in camels recorded higher detection by screening tests BAPT 3.3%, RBPT 2.7% and 2.3% by TAT and Rivanol test as a confirmatory tests. A higher incidence were recorded by many authors, Ayoub *et al.*

(1978) recorded an incidence of 24-25% in females and 14-28% in males by using SAT and RBPT. In Assiut, Zaghloul and Kamel (1985) recorded an incidence of 8.11% while Lotfi *et al.* (1987) recorded an incidence of 7.9% with STAT and RBPT. Barsoum *et al.* (1995) recorded an incidence of 8% in Sharkia, 4% in Kaliobia and 6% in Dakahlia. Also, Abdel Rasheed (2004) recorded an incidence at Behira province of 8.74%, 9.53%, 9.92%, 8.09%, 8.87%, and 9.26% of camel brucellosis with RBPT, BAPT, TAT, Mercaptoethanol test, Rivanol test and Elisa respectively.

As regards this high incidence that recorded by many authors, it may be explained as they don't use the heat inactivation technique to the serum samples before being used serologically to inactivate the non specific brucella agglutinins (Amerault *et al.* 1961) so, false positive samples were included in these incidence. Another factor may be attributed to the wet weather (muddy and rainy) of most of this regions, that represent a predisposing factor for spread of brucella infection.

The negative results of brucella infection obtained in the present study in New Valley Governorate may be attributed to the deserty dry hot weather of this locality beside being isolated districts that limit the spreading of brucella micro-organisms where it can not survive for long period and consequently limit the spread of infection (Nashed, 1977 and Gadalla, 1991). Also most of the samples collected from restricted newly free non infected areas.

Epidemiologically, brucellosis in camels represents a serious public health risk and high-risk human other than occupational contactors through consumption of milk, milk products and meat products of seropositive animals (Almer, 1985). So camels must be included in the national program for control and eradication of brucellosis in Egypt.

REFERENCES

Abbas, B. and Agab, H. (2002): A review of camel brucellosis. Prev. Vet. Med. Sept., 10; 55 (1): 47-56.

Abdel Rasheed, F. Abdel Moghney (2004): A preliminary study on brucellosis on camels at Behira province. Assiut Univ. Bull. Environ. Res. Vol. 7 No. (1), March 2004.

Almer, L.O. (1985): A case of brucellosis complicated by endocarditis and desseminated intravascular coagulation. Acta Med Scand.; 217 (1): 139-140.

Amerault, T.E.; Manthel, C.A.: Goode, E.R. and Lambert, G. (1961): A heat-inactivation test for differentiating specific and non specific agglutination reactions for bovine brucellosis. Am. J. Vet. Res., May. Pag. 564-569.

Anon, (1984): Brucellosis in Britain. Br. Med. J. 289. 29 Sep.; pp. 817.

Anon, (1992): Office international Dis Epizooties Manual of Standards and vaccines. A. Diagnostic Techniques. Paris-France.

Ayoub, M.M.; Shawkat, M.A. and Fayed, A.A. (1978): Serological investigation on brucellosis in camels in Egypt. Assiut Vet. Med. J.

Barsoum, S.A.; El-Sayed, M.M. and El-Fayoumy (1995): Sero-Epidimiological study on camel brucellosis. Beni-Suef. Vet. Med. Res. Vol. V. No. (2): 119-126.

Barton, C.E. (1994): Immunology and Serology of bovine brucellosis. Brucellosis Seminar, Cairo, Egypt, 25-30 June 1994.

Baumann, M.P. and Zessin, K.H. (1992): Productivity and health of camels (Camelus dromedarius) in Somalia: association with trypanosomosis and brucellosis. Trop. Anim. Health & Prod., Aug, 24 (3): 145-156.

Damir, H.A.; Kenyon, S.J.; Khalaf Alla, A.E. and Idris, O.F. (1984): Brucella antibodies in sudanese camels. Trop. Anim. Health & Prod. Nov., 16 (4): 209-212.

El-Ansary, E.H.; Mohammed, B.A.; Hamad, A.R. and Karom, A.G. (2001): Brucellosis among animals and human contacts in eastern sudan. Saudi Med. J., Jul.; 22 (7): 577-579.

El-Bauomy, E.E. (1989): Some studies on brucellosis in sheep and goats. M.V. Sc. Thesis, Fac. of Vet. Med., Cairo Univ.

FAO/WHO (1986): Joint FAO/WHO expert committe on brucellosis 6th report world health organtech. Rep. No. 740, WHO, Geneva, Switzerland.

Gadalla, Z.b. (1991): Epidemiological studies of brucellosis in upper Egypt with special reference of its prevalence among different species of animals. M.V. Sc. Thesis, Fac. of Vet. Med. Assiut University.

Hamdy, M.E. (1992): Epidemiological studies on Br. melitensis in dairy animals and man. Ph.D.V. Ps. Thesis, Faculty of Vet. Med., Cairo University.

Lotfi, A.; Youssef, J.; El-Khatibe, T.; Saddik, I.; El-Timaway, A.; El-Gibaly, S.M. and Gafar, M. (1987): Brucellosis in slaughtered carcasses in upper Egypt. Assiut Vet. Med. J. Vol. 19, No. (37): 102-105.

Nashed, S.M. (1977): Brucellosis in upper Egypt (Assiut Governorate) in cattle, buffaloes, sheep, goats and its relation to public health. Ph.D. Thesis, Fact. of Vet. Med., Cairo University.

Okoh, A.E. (1979): A survey of brucellosis in camels in Kano, Nigeria. Trop. Anim. Health & Prod. Nov.; 11 (4): 213-214.

Omer, M.K.; Skierve, E.; Holstand, 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 systems. Epidemiol. Infect., Oct.; 125 (2); 447-453.

Radwan, A.I.; Bekairi, S.I. and Prasad, P.V. (1992): Serological and bacteriological study of brucellosis in camels in central Saudi Arabia. Rev. Sci. Tech., Sept., 11 (3): 837-844.

Stemshorn, P.W.; Forbes, L.B.; Fagiesome, M.D.; Nielsen, K.H.; Robertson, F.J. and Sammagh, B.S. (1985): A comparison of standard serological tests for diagnosis of bovine brucellosis in Canada. Can. J. Comp. Med. 49 (4): 391-394.

Teshome, H.; Molla, B. and Tibbo, M. (2003): A seroprevalence study of camel brucellosis in three camel-rearing regions of Ethiopia. Trop. Anim. Health & Prod., Oct.; 35 (5): 381-390.

Yaqoub, I.A.; Mohamed, A.A. and Salim, M.O. (1990): Serological survey of brucella abortus antibody prevalence in the one-humped camel (Camelus dromedarius) from eastern sudan. Rev. Elev. Med. Vet. Pays Trop., 43 (2): 167-171.

Zaghloul, A.H. and Kamel, Y.Y. (1985): Incidence of brucellosis among farm animals in Assiut Governorate. Assiut Vet. Med. J. Vol. 14, No. (28): 117-122.