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SEROLOGICAL AND BACTERIOLOGICAL INVESTIGATIONS ON BRUCELLA INFECTION IN ONE HUMPED CAMELS (*CAMELUS DROMEDARIUS*) IN EGYPT

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SUMMARY

Serodiagnosis supported by bacteriological examination is essential for the identification of brucellosis in camels. Out of 750 camel sera under investigation, 29 (3.9%) were positive brucella reactors to the buffer acidified plate antigen test (BAPA) and 37 (4.9%) were positive to Rose Bengal plate test (RBPT). The higher incidence of brucellosis were recorded among camels aged more than 5 years old and those between 4-5 years old. A total of 37 camel's serum samples positive to RBPT were subjected to both serum tube agglutination and complement fixation tests with reliability reaching 86.5% and 94.6% respectively. Correlation between positive RBPT and the rate of brucella isolation from 23 she-camels are described in details. Brucella organisms were isolated from 20 out of 23 serologically positive she-camels (87.0%). Morphological, cultural, biochemical and serological identification of the isolated brucella strains revealed the presence of Br. Abortus biovar "1" (55.0%), Br. Abortus biovar "7" (30.0%) and Br. melitensis biovar "3" (15.0%).

INTRODUCTION

The problem of brucellosis in camels seems to be related to the breeding and husbandry practices and high economic losses remains a matter of extreme importance (Abbas et al., 1988). Serological tests serve as important aids in the diagnosis of brucellosis, although they can not identify all infected animals in all stages of the disease including acute and chronic phases as well as latent infection (Huurne et al., 1993). There are no easy

formulae for the serodiagnosis of camel brucellosis due to large number of tests have been devised and each serological test has its advantages and limitations (Baumann and Zessin, 1992). By using the serological tests, the disease has been under control in many countries allover the world (Ayoub et al., 1978). The Rose Bengal plate test proved useful in diagnosis of camel brucellosis (Bassiony et al., 1996). The buffer acidified plate antigen test has been used increasingly in fixation and serum tube agglutination test aids in improving the accuracy and validity of serodiagnosis of camel brucellosis (Huurne et al., 1995).

Bacteriological isolation is the main access to a conclusive diagnosis of animal brucellosis but it is time, effort, money, proficiency-demanding task, besides, it is not usually possible to detect brucellae from live infected animals (Zowghi and Ebadi, 1988). The purpose of this study was to evaluate the different serological tests for detection of brucellosis from camel sera as well as identification and biotyping of the isolated brucella species.

MATERIAL AND METHODS

I- Collection and preparation of blood samples for serodiagnosis:

About 10 ml of the jugular vein blood were collected in Vacutainers (Becton, Dickinson), labelled then kept in an inclined position for about 2 hrs for complete clotting and the samples were kept overnight at 4°C, then centrifuged at 1000rom for 10 minutes to obtain amber clear serum. Sera were subjected first to serodiagnostic techniques included buffered acidified plate antigen test (Anon, 1984) and Rose Bengal plate test (Morgan et al., 1969) as screening techniques. All positive serum samples were subjected to serum agglutination test (Morgan et al., 1978) and complement fixation test (Weir et al., 1986).

II- Collection and preparation of samples for isolation of bracella from serologically positive she camels:

The specimens were taken from uterus, placenta, foetal membranes and aborted foeti if present from serologically positive she-camels. From the aborted foeti, specimens were taken from the stomach contents, liver, spleen and lungs. Smears were stained with Gram's and modified Zeihl-Neelson's method. Each specimen was streaked onto 4 serum brucella agar plates, 2 with antibiotics and 2 without antibiotics and onto liver infusion agar plates, (Alton et al., 1988). Inoculated plates were incubated in CO_2 incubator for 4-7 days. No culture was discarded as negative before the 10th day of incubation. Purified colonies of brucella isolates were subjected to: acriflavine test (Preston and Morrell, 1962); motility test, catalase test; nitrate reduction test; growth in presence of thionin and basic fuchsin; production of hydrogen sulphide and urease test according to Alton et al.,

(1988). Tube agglutination tests were set up in duplicate with A and M antisera for serotyping of the isolates. Male guinea pigs were inoculated S/C with 1ml of 10^4 viable organisms in duplicate and kept under observation for 6 weeks. Sera were obtained after 3 weeks for serum agglutination test and autopsy was carried out after 6 weeks and the affected lesions were examined and verified culturally and serologically.

RESULTS AND DISCUSSION

Seroanalysis supported by bacteriological examination is primarily resorted for the identification of infected camels. The immunological responses are some times irregular and/or delayed according to the animal species and individual variations. Morearer brucella antibodies do not function equally well in all assays (Huurne et al., 1993).

From table (1), it is evident that out of 750 camel sera under investigation, 29 (3.9%) were positive brucella reactors to the PAPAT and 37 (4.9%) were positive by RBPT. A great deal of evidence about the behaviour of RBPT in various epidemiological situations has been recorded (FAO/WHO, 1986) and detects infection rapidly than does that ABPAT and its more efficient at identifying camels that are actively excreting brucella organisms. The high incidence of brucellosis by the RBPT was recorded from camels aged more than 5 years old and 4-5 years with an incidence of 5.5% and 5.3% respectively and lower in young camels aged 2-12 months old (2.7%). These results are nearly similar to that recorded by Wernery and Wernery (1990) who showed a high incidence of brucellosis among old camels more than 5 years old (4.2%) than that obtained from young aged ones. On the other hand, Yagoub et al., (1990) recorded that young infected she-camels had colostral agglutinins and they

Table 1: Results of the buffered acidified plate antigen and Rose Bengal test on camels sera.

Age	Total No. of blood samples	BAPAT		RBPT	
		No. positive	%	No. positive	%
2-12 M	110	2	1.8	3	2.7
1-4 Y	92	2	2.2	4	4.3
4-5 Y	133	5	3.8	7	5.3
More than 5 Y	415	20	4.8	23	5.5
Total	750	29	3.9	37	4.9

were resistant in young age and became susceptible a few months later.

Results of serological examination of 37 camel's serum samples positive to the RBPT were subjected to the standard tube agglutination test. Table (2) revealed that 3 serum samples (8.1%) showed agglutination titres, which did not exceed 1/20 and were considered as doubtful results. Meanwhile, 32 cases (86.5%) had markedly positive titres ranging from 1/40 up to 1/1280. On the contrary, the same test failed to identify 2 samples (5.4%) from the total 37 camels showed positive reactions to the RBPT. The reliability between the RBPT and SAT was reported by Sudibyo et al., (1990) who stated that RBPT is more reliable than SAT. In addition, Yagoub et al., (1990) suggested that RBPT may be the most satisfactory rapid technique for diagnosis and control of brucellosis. Meanwhile, Gameel et al., (1993) stated that SAT failed to eradicate brucellosis because many infected animals may gave negative reactions. Thus, the combined use of RBPT with the SAT is more reliable method in diagnosing bucellosis in camels. The positive results given by RBPT in a serum negative in the SAT or with a titre of 1 in 2 showed that the RBPT detected infected camels earlier than SAT. Moreover, sera positive to RBPT and negative or doubtful to SAT may mean that these camels were suffering from chronic brucellosis. It has been recognized that not every brucella infected camel will always shows a diagnostical titre, a finding that supports the limitation of the use of the SAT alone.

Table 2: Results of the serum tube agglutination test on brucella reactor camels positive to RBPT.

Age	No. of serum samples	positive		Doubtful at titre 1/20		Total No. of positive camels using SAT at titres of						Total positive	
		No.	%	No.	%	1/40	1/80	1/160	1/320	1/640	1/1280	No.	%
2-12 M	3	1	33.3	--	0.0	1	--	--	1	--	--	2	66.7
1-4 Y	4	--	0.0	1	25.0	1	1	1	--	--	--	3	75.0
4-5 Y	7	--	0.0	1	14.3	--	2	--	2	1	1	6	85.7
> 5 Y	23	1	4.3	1	4.3	5	6	4	4	2	--	21	91.3
Total	37	2	5.4	3	8.1	7	9	5	7	3	1	32	86.5

SAT = Serum Agglutination test.

Nowadays, the CFT is widely used as aid to proper serodiagnosis in chronic stages of brucellosis. Table (3) illustrates the results of CFT on the tested 37 camels sera proved to be positive for RBPT. The CFT proved to be more efficient than SAT. This is especially so in those cases where the results of the SAT are either negative or inconclusive as may happen in the incubative stage or in the late chronic stages of the disease. The reliability of reactors to the CFT was 94.6% than that in SAT 86.5%. These findings nearly coincide with results obtained by Al-Khalaf and El-Khaladi (1989) in Kuwait who stated that the CFT is superior to other serological techniques in diagnosing brucellosis in camels. This Observation is in accord with the facts that all sera of camels must be subjected to CFT irrespective of the SAT results (Baumann and Zessin, 1992). The results of the present study support the necessity for the use of more than one test for the diagnosis of brucellosis in camels. These findings along with those of other investigators indicate that the RBPT is highly suitable as a rapid screen test and the standard SAT and CFT are quantitative ones (Walfgang et al., 1992).

Table 3: Results of complement fixation test on brucella reactors camels positive to RBPT

Age	No. of serum samples tested	No. of positivereactors at titres of							Total	
		1/4	1/8	1/16	1/32	1/64	1/128	1/256	No.	%
2-12 M	3	--	1	1	--	--	--	--	2	66.7
1-4 Y	4	--	--	--	1	2	--	--	3	75.0
4-5 Y	7	--	--	1	2	3	1	--	7	100.0
More than 5 Y	23	1	1	6	5	4	3	3	23	100.0
Total	37	1	2	8	8	9	4	3	35	94.6

Table4: Numbers and biotypes of the brucella strains isolated from positive reactor shecamels for RBPT

No. of serologically positive she-camels examined.	Bacteriological findings for brucella				Strains and biotypes of the isoalted brucella					
	positive		Negative		Br. abortus biovar 1		Br. abortus biovar 7		Br. melitensis biovar 3	
	No.	%	No.	%	No.	%	No.	%	No.	%
23	20	87.0	3	13.0	11	55.0	6	30.0	3	15.0

The correlation between positive RBPT and the rate of brucella isolation from 23 she-camels are shown in table (4). Although the isolation of brucella organisms needs laborious efforts, yet it offers an essential base for diagnosis (Abbas et al., 1988) especially in cases of false negative serodiagnosis. Brucella organisms were isolated from 20 out of 23 serologically positive she-camels (87.0%) as bacteriological examination alone is not absolutely accurate and there is a varying degrees of sensitivity. Seroanalysis supported by bacteriological investigation is primarily resorted to for the identification of infected camels. As shown in table (4), morphological, cultural, biochemical and serological identification of the isolated brucella strains revealed the presence of Br. abortus bivar "1" in an incidence of 55.0%, followed by Br. Abortus biovar "7" (30.0%) and then Br. melitensis biovar ì3î (15.0%) as incriminators to camel brucellosis. These results are in accordance with those reported by Ayoub et al., (1978); Sudibyo et al., (1990) and Zowghi and Ebadi (1990) who stated that camels are highly susceptible to infection with Br. abortus bivar "1" more than other biotypes.

The present data revealed that Br. melitensis biovar "3" was isolated from camels in an incidence of 15.0% . The occurrence of this biotype in camels may suggest the possibility of this biotype being acquired by contact to man or sheep and goats. Concerning Br. Abortus biotype "1" and "7" and Br. melitensis biovar "3" recorded in the present work seems to be a reflection to the vice of farmers who rear such animals and this magnitudes the chances of human being to catch brucella infection from their camels. The present data indicates the approximate prevalence of brucellosis in camels in Egypt, which undoubtedly points to the problem of considerable magnitude not only due to its impact on economical point of view but also from the public health significance of brucellosis. This implies the necessity of different efforts to determine rapid and accurate diagnosis with valid records to fulfill the necessary measures of control.

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