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STUDIES ON CAMEL BRUCELLOSIS WITH PRELIMINARY REPORT ON SOME IMMUNOGENETIC MARKERS

(With 6 Tables and 2 Figures)

By

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دراسات على مرض البروسيلا في الجمال مع التركيز على بعض دلالات المناعة الوراثية

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استخدمت هذه الدراسة عدد (١٢٣) من الجمال الموجودة بسوق برقاش بالجبزة وذلك لإجراء الدراسات السيرولوجية لمرض البروسيلا، وقد تم استخدام (٣٠) من الحالات الإبجابية لعمل دراسات على صورة الدم ، بروتينات المصل (طريقة التهجير الكهربائي) وكذلك دراسات مناعية ورائيــة. وقــد كــانت نســبة الإصابــة بالبرومـــيلا (٤,٣٩٪) ، (٢٩,٦٩٪) ، (٢١,١٣) ، (٢٣,٥٧) ، الستخدام الحتب الرات الروز بنجال والتارن الانبوبي الحساسية (٢٣,٢٥) ، بينما كان اختبار التلزن الانبوبي الافضل من حب ث نسبة الدقة (٨١,٣٠)، وقد اثبتت الدراسات الهيماتولوجية عن زيادة معنوية في معدل الترسيب وكريات الدم البيضاء مصحوبة بارتفاع في الكريات الليمفاوية وانخفاض في الكريات متعادلة الاصباغ ووحيدة النواة وكريات الايوسين ، كما وجدت زيادة معنوية في الالبيومين مصحوبة بنقص معنوي في جلوبيولينات المصل الكلي (جلوبيولينات الفا وبيتا) ، بينما ارتفع معدل جلوبيولين جاما وذلك في الحيوانات المصابة بالبروسيلا. اما فيما يتعلق بالخصائص المناعية الوراثية فقد اظهرت النتائج أن بروتينات المصل في الجمال تتميز بالتتوع الكبير حيث المتماثلة "Homozygotic" ما عدا بروتين الترانسفيرين الذي سادت فيه التراكيب الوراثيسة غير المتماثلة "Heterozygotic" وهي DE, AD, AE. وقد تميزت أيضا الحيوانسات السليمة بسيادة الجينات الوراثية التالية ! CATB, Tf⁰, Fa₂B مما قد يرجح إلى حد كبير مسئولية هذه الجينات عن المقاومة الطبيعية ضد مرض البروسيلا في الجمال. أما بالنسبة للحيوانات المصابة فقد انخفضت فيها حزم البروتين إلى ١٠ حزم فأقَل ، وقد تأثرت بشــــــدة

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مناطق ما قبل الالبيومين والالبيومين وما بعد الالبيومين نتيجة للاصابة بالبروســـيلا ، وقـــد تميزت هذه الحيوانات المصابة بسيادة الحياات الوراثية Tf^, PrB, Pal مما يرجح إلى حد كبير مسئولية هذه الجينات عن قابلية الجمال للاصابة بمرض البروسيلا.

SUMMARY

A total number of 123 camels, collected from Berkash Village Market at Giza, were used to perform scrological brucella surveillance; of which, 30 brucella positive sero-reactors were used for haematological, serum proteins electrophoresis and immunogenetical studies. The incidence was 30 (24.39%), 23 (18.69%), 26 (21.13%) and 29 (23.57%) using RBPT, TAT, MET and Rivanol tests, respectively. The estimated sensitivity cleared that RBPT (32.25%) was the highest sensitive test, meanwhile, TAT (81.30%) was the highest specific test for detection of brucellosis in camels. The haematological study revealed significant increase in ESR and total leucocytic count accompanied with lymphocytosis, neutrocytopenia, monocytopenia and eosinocytopenia in brucella positive sero-reactors. Serum proteins electrophoresis revealed significant increase in serum albumin accompanied with marked decrease in the total serum globulins (α -globulins and α -globulins) in brucella positive sero-reactors. However, significant increase of α globulins was recorded. Concerning the immunogenetic characteristics, it was evident that serum of camel includes large number of bands ranged from 10-17 protein bands in healthy cases with predominance of homozygotic genotypes in most genetic loci except transferrin in which heterozygotic genotypes AE, AD and DE are predominant. These healthy negative sero-reactors are characterized by high frequency of $F\alpha$ αf^D and CAT^B genes, and the condition may be due to the responsibility of these genes to natural resistance of animals against brucella. In the brucella positive sero-reactors, we noticed that protein bands did not exceed 10 bands as a result to infection and the most affected area is albumin, pre-albumin (AA) and post-albumin (BB). Pal^A , Pr^B and Tl^A were the highest frequent genes in brucella positive sero-reactors. The high frequency of these genes in brucella positive cases gives evidence to correlation between these genes and susceptibility to infection by brucella.

Keywards: Brucellosis – Camel – Epidemiological – Haematological – Immunogenetic.

INTRODUCTION

Brucellosis is among the important infectious diseases that attack farm animals, particularly the genital organs causing great economic losses. It is a serious zoonotic disease, which is considered as one of the great public health problems all over the world (Rhadostits et al., 1994). Through intensive health control measures, many countries have succeeded in eradicating brucellosis. However, in developing countries, the disease remains widespread among both domesticated and wild animal populations (Wernery and Kaaden, 1995). Control of brucellosis depends primarily on the elimination of animal reservoirs. The most effective plan for elimination of the disease is the detection of infected animals by periodic examination of blood for presence of specific antibodies and elimination of positive reactors (Nielsen and Duncan, 1990). Camels play an important role in the epidemiology of brucellosis and act as important source of infections to other domestic animals and human (El-Sawalhy et al., 1996). Although camels appear to be very susceptible to infections with brucella, isolation of brucella from animal samples proved less successful (Wernery and Kaaden, 1995). Therefore, diagnosis of camel brucellosis is based on both serological and bacteriological examination (Nada and Ahmed, 1993). Brucellosis may cause some pathological alterations in blood picture and serum biochemical profile (El-Sawalhy et al., 1996).

The immunogenetic studies still very limited in camels (Rendel, 1967). Borozden and Kleeberg (1990) used genetic polymorphism to evaluate the resistance of animals against infectious diseases, while Penedo et al. (1998) used microstellite markers to evaluate polymorphism and estimation of gene frequencies in South America camels.

Therefore, this study was planned to perform brucella surveillance using different serological tests for evaluation of serological diagnosis of brucellosis in camels. The immunogenetic analysis was also undertaken to characterize the genetic markers, which correlated to and responsible for the natural resistance to brucellosis and those which related to susceptibility to infection with brucellosis. In addition, changes in haematological parameters and serum protein electrophoretic profile were included.

MATERIAL and METHODS

1- Animals:

A total number of 123 carnels (Camelus dromedarius), aged 3-5 years, collected from Berkash Village Market - Giza, at which camels come from many locations in the Upper Egypt after long transport, were used to perform serological surveillance for brucellosis. These camels either used for fattening or breeding purposes in camels farms or immediately send for slaughter. All these animals were clinically examined for any diseases or abnormalities, and they were apparently healthy. Of these camels, 30 brucella positive sero-reactors were used to perform haematological, serum proteins electrophoresis and immunogenetic studies, and 30 brucella negative sero-reactors were used as control group.

2- Samples:

- * Anticoagulated blood samples: used for haematological studies.
- * Blood smears: used for differential leucocytic count.
- * Serum samples: used for serum protein electrophoresis, serological and immunogenetic studies.

3- Serological examination for brucellosis:

Rose Bengal plate test (RBPT), Tube agglutination test (TAT), Mercaptocthanol test (MET) and Rivanol test were done as described by Alton et al. (1988). All these antigens were supplied by Vet. Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. A titre of 1/40 (80 IU/ml) in TAT, 1/10 in MET and 1/25 in Rivanol test or higher is considered as brucella positive reactor (Pinsent and Fuller, 1997).

4- Haematological examination:

Total erythrocytic count (RBCs), total leucocytic count (WBCs), differential leucocytic count (DLC), haemoglobin content (IIb), packed cell volume (PCV) and erythrocytic sedimentation rate (ESR) were done as described by Schalm et al. (1986) and Cheesbrough and McArthur (1980).

- 5- Serum proteins electrophoresis and immunogenetic studies:
 1- The total serum proteins (TSP) were fractionated polyacrylamide gel on one dimensional electrophoretically electrophoresis (PAGE) according to Lacmmeli (1970) and Carlstrom and Johnson (1983). Determination of the total serum proteins was performed by Biuret method described by Weichslbaum (1946).
- 2- Genotyping of blood protein loci and determination of gene frequencies were done according to Mercoreva (1977) as follows:

- i- For 2 allelic loci:
 - $NP^2 AA + N.2 PqAB + Nq^2BB = 1$
- ii- For 3 allelic loci:
 - $P^2 + 2Pz + q^2 + 2qz + z^2 + 2Pq = 1$

RESULTS

Concerning with the incidence of brucellosis among camels examined with different serological tests, the incidence was 30 (24.39%), 23(18.69%), 26(21.13%) and 29(23.57%) with RBPT, TAT, MET and Rivanol tests, respectively (Table 1). The results of RBPT, TAT, MET and Rivanol tests (Table 2) were estimated and evaluated by comparing their absolute sensitivity and specificity as described by Ruppanner et al. (1980). The estimated sensitivity and specificity cleared that RBPT (32.25%) was the highest sensitive test followed by Rivanol test (30.85%), MET (26.80%) and finally TAT (23.00%). Meanwhile, TAT (81.30%) was the highest specific test for detection of brucellosis, followed by MET (78.86%), then Rivanol (76.42%) and finally RBPT (75.60%).

Concerning the haematological study on the brucella positive reactor camels (Table 3), there was a significant increase in ESR and total leucocytic count attributed to marked lymphocytosis. Moreover, there was neutrocytopenia, monocytopenia and eosinocytopenia.

Results of scrum protein electrophoresis in brucella positive sero-reactor camels table 4 revealed significant increase in serum albumin associated with significant decrease in total scrum globulins (α -globulins and α -globulins). Meanwhile, there was significant increase in α -globulins.

It was evident that serum of camel includes large numbers of bands ranged from 10–17 protein bands in healthy cases with predominance of homozygotic genotypes in most genetic loci except transferrin in which heterozygotic genotypes AF, AD and DE are predominant. These immunogenetic characteristics are shown in Tables 5 & 6 and Figs. 1 & 2.

On the other hand, these healthy negative scro-reactor camels are characterized by high frequency of $F\alpha^B$, Tf^D and CAT^B genes, and the condition may be due to the responsibility of these genes to natural resistance of animals against brucella (Table 5 and Fig. 1).

Concerning the brucella positive sero-reactor cases, we noticed that protein bands not exceed 10 bands as a result to infection and the

most affected area is albumin, pre-albumin (AA) and post-albumin (BB). Results were illustrated in Table (6) and Fig. (2), which demonstrate that Pal^A, Pr^B and Tf^A were the highest frequent genes in brucella positive scro-reactor cases. The high frequency of these genes in brucella positive cases gives evidence to correlation between these genes and susceptibility to infection by brucella.

DISCUSSION

Brucellosis is a serious reproductive disease threatening the animal wealth and is of public health importance. Camel is one of those domestic animal species which were known to be infected with brucellosis (Ghazi, 1996). Inspite of that camel may be infected with brucella: it is rare to show any clinical signs or abortion (Higgins, 1986).

Concerning results of different serological tests among the examined camels (Table, 1), higher percent of positive reactors was recorded using RBPT (24.39%) and lower percent was reported by Rivanol test (23.57%), MET (21.13%) and TAT (18.69%). Evaluation of the different serological tests was estimated on the basis of absolute sensitivity and specificity (Ruppanner et al., 1980). The results of absolute sensitivity were 32.25% (RBPT), 30.85% (Rivanol test), 26.80% (MET) and 23.00% (TAT). Meanwhile, the absolute specificity was 81.30% (TAT), 78.86% (MET), 76.42% (Rivanol test) and 75.60% (RBPT). These results revealed highest sensitivity of RBPT followed by Rivanol, MET and finally TAT. On the contrary, the specificity was highest using TAT and the MET, Rivanol and finally RBPT (Table, 2). The higher sensitivity of RBPT and Rivanol as compared with other serological test indicates their ability to detect higher numbers of brucella positive reactors in camels. Similar results obtained by El-Sawalhy et al. (1996); Ghazi (1996) and Hegazy et al. (1998), who reported that RBPT was the most sensitive and easily applied test for serological examination of brucellosis. On the other hand, the lack of sensitivity leads to false negative results and the lack of specificity leads to false positive results (Martin, 1977). However, the present results disagree with that reported by Nada (1990), who reported that TAT was the most sensitive scrological test for examination of brucellosis.

RBPT is still considered by several authors as more efficient, inexpensive and easily performed method in the detection of both early and chronic brucella infection. RBPT detects IgM antibodies more

efficiently than IgG_1 or IgG_2 antibodies (Allan et al., 1976) and it was suggested that its acidic buffer inhibits immunologically the non-specific agglutinins (Davies, 1971).

Haematological study on the brucella positive sero-reactor camels (Table 3), revealed a significant increase in ESR and total leucocytic count attributed to marked lymphocytosis. Moreover, there was neutrocytopenia, monocytopenia and eosinocytopenia. Higher ESR may be due to hyper-fibrinogenemia associated with most chronic disorders (Schalm et al., 1986). Lymphocytosis is known to be a body defense mechanism (Benjamin, 1978). It is a feature mainly related to activation of the lymphoreticular system for production and transportation of antibodies in a trial to combat infection (Ahmed and Nada, 1992). The significant lymphocytosis and neutrocytopenia in brucella positive camels may be due to aggregation of neutrophils in the affected tissue and their withdrawal from the peripheral circulation (Cabana et al., 1990). However, our results agree with Ahmed et al. (1994), who reported significant decrease in neutrophils percentage while, no changes in basophils, RBCs count, PCV (%) and Hb content, while, disagree with the same authors, who found no significant change in WBCs count together with the percentage of lymphocytes, monocytes, eosinophils and ESR in working she-camels with high incidence of brucellosis (25%) compared with non-working she-camels with low incidence of brucellosis (8%). Our findings agree also with those mentioned by Ahmed and Nada (1992), who reported significant increase in lymphocytes, significant decrease in neutrophils and no changes in PCV, Hb content, RBCs count and basophils, while, disagree with the same authors who reported no significant changes in ESR, WBCs count, monocytes and eosinophils.

The present results disagree with Wernery and Kaaden (1995), who mentioned that the haematological parameters remained within normal limits in brucella-infected camels.

Scrum proteins electrophoresis in brucella positive sero-reactor camels (Table, 4) recorded a significant increase in scrum albumin accompanied with a significant decrease in total scrum globulins (- globulins and globulins). The decreased globulins level inspite of brucella infection may be attributed to stressful conditions (long-distances and starvation) during transportation. This significant decrease in globulins level may be the cause of occurrence of high incidence of brucellosis in our study. Also, the decreased globulins level and increased incidence of brucellosis may be due to the high corticosteroid

level associated with stress factors (Davis et al., 1966), disease process or changes in animal metabolism (Sykes et al., 1980). In addition, the decreased trace elements especially copper and iron associated with brucellosis may result in decrease of their binding proteins (El-Tohamy and Salama, 1985). These changes may be attributed to stimulation of phagocytic cells by invading microorganisms to produce hormone like substance known as leucocytic endogenous mediator, which initiates a number of changes in the host's trace element metabolism, thus causing a swift of serum iron and many amino acids into the liver (Beisal et al., 1974). However, our results agree with those mentioned by Thanaa (1990) and Ahmed and Nada (1993) who reported non significant changes in total serum proteins in camels infected with brucellosis, while disagree with El-Sawalhy et al. (1996) who reported a significant decrease in albumin level, A/G ratio and a significant increase in globulins level in brucella positive camels.

One of the main objectives of this study was to use blood protein loci as genetic markers to clarify the expected relationships between specific alleles and susceptibility to infection with brucella. The genetic relationship between some blood protein loci and both productive and reproductive traits are based on protein coding loci (Barker et al., 1997). Analysis of allelic variation of scrum protein loci could potentially be used to evaluate temporal changes in genetic diversity (Kantanen et al., 1999). In the present study, all blood protein loci are polymorphic and this result confirms the fact that loci were defined as polymorphic when the frequency of the most common allele was less than 0.95 (Kantanen et al., 1999). The equal distribution of most genotypes in our study was led to increasing of heterozygosity and this result also has been reported by Leberg (1992), who recorded that average expected heterozygosity has been increased due to more equal distribution of allele frequencies. Moreover, association of production, viability and fertility traits with blood groups and blood protein polymorphism have been reported in different animal species such as buffalo (Nicholas, 1996), cattle (Borozden and Kleeberg, 1990), Egyptian Friesian cows (Zaabal et al., 2000) and South America camelidae (Penedo et al., 1998).

The brucella positive sero-reactors in this study records high frequencies of Pal^A, Pr^B and Tf^A genes (Table 6, Fig. 2) and the condition may be due to a relation between these genes and susceptibility of animal to infection. Concerning the brucella negative ser-reactors, we found 10-17 protein bands in most cases and the most

frequent genes were catalase (CATB), α -globulin (F α_2^B) and Transferrin (TfD) (Table 5, Fig. 1). Therefore, we can suppose that these genes may be closely related to natural resistance against brucella infection. On the other hand, the frequency of some alleles in this study especially prealbumin, post-albumin-2, vitamin-D binding protein and catalase was not similar to those obtained by Penedo et al. (1998) of South American camelidae and the condition may be due to the genomic diversity between breeds.

In conclusion, camels play an important role in the epidemiology of brucellosis and act as important source of infection to other domestic animals and human. Therefore, camels should be included in surveying programs in Egypt for complete eradication of the disease and the possible use of suitable vaccine for camels should be tried. The imported camels must be quarantined and examined for brucellosis before introducing into Egypt for slaughtering, fattening or breeding purposes. Moreover, for breeding purposes, camel should be selected according to genetic markers that related to the natural resistance against infectious diseases such as CAT^B , $F\alpha_2^B$ and Tf^D genes.

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Table 1. Incidence of brucellosis among camels examined with different serological tests.

umber of xamined	RBPT	(Positive)	LO/ml and	(Reactors) at 40 [/ml and higher	ME.1 (Posit dilution a	nve) at 1:10 nd higher	difution 1:20	1:20 and higher
camels	z	%	z	9%	z	9%	Z	0%
123	30	24.39	23	18.69	26	21.13	29	23.57

Table 2. Absolute sensitivity and specificity of different used serological tests.

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Table 3. Haematological study on brucella positive sero-reactor camels.

Parameter		Brucella negative sero-reactor camels (Control negative group)	Brucella positive sero-reactor camel	
RBC	Cs count (10 ⁶ /µl)	8.60 ± 0.41	8.20 ± 0.30	
Hb c	content (g/dl)	13.10 ± 0.33	13.61 ± 0.48	
PCV	(%)	36.13 ± 0.46	36.65 ± 0.32	
ESR	(mm/12 hours)	7.00 ± 0.20	19.00** ± 0,36	
WBCs count (10³/µl)		9.94 ± 0.36	23.75** ± 0.65	
Differential feacocytic count	Neutrophils (%)	47.50 ± 1.65	13.75** ± 0.51	
	Lymphocytes (%)	41.00 ± 0.72	84.00** ± 1.36	
	Monocytes (%)	4.00 ± 0.43	0.50** ± 0.06	
	Eosinophils (%)	7.00 ± 0.27	1.50** ± 0.17	
	Basophils (%)	0.50 ± 0.10	0.25 ± 0.08	
Number of examined animals		30	30	

^{*} P<0.05 ** P<0.01

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Table 4. Serum proteins electrophoresis in brucella positive sero-reactor camels.

Parameter		Brucella negative sero-reactor camels (Control negative group)	Brucella positive sero-reactor camels	
TSP (g/dl)		7.19 ± 0.27	7.79 ± 0.11	
	%	46.62 ± 1.39	50.83* ± 0.90	
Albumin	g/dl	3.35 ± 0.18	3.96* ± 0.12	
00 1 1 1 1 1 1	%	53.38 ± 1.27	49.17** ± 0.67	
Total globulins	g/dl	3.84 ± 0.24	3.83 ± 0.16	
A/G ratio		0.87	1.03	
	%	24.51 ± 0.76	18,01** ± 0.40	
α-globulins	g/dl	1.76 ± 0.10	1,40** ± 0.06	
0	%	19.84 ± 0.83	15.05** ± 0.34	
β-globulins	g/dl	1.43 ± 0.17	1.17 ± 0.07	
	%	9.03 ± 0.36	16.11** ± 0.26	
γ-globulins	g/dl	0.65 ± 0.04	1.26** ± 0.06	
Number of examined animals		30	30	

^{*} P<0.05 ** P<0.01

Table 5 Distribution of genotypes of blood protein loci and their gene

frequencies of brucella negative sero-reactor camels. Distribution of genotypes protein loci Genotype Observed Expected frequencies Prealbumin $Pr^{A} = 0.571$ $Pr^{B} = 0.428$ AB 6 13.7 8.8 (n=28) BB 5.1 Albumin 4.5 AA 6 $AI^{A} = 0.407$ $AI^{B} = 0.592$ Al AB 10 13.0 1.3 (n=27) BB 11 9.5 Post 4.7 AA 8 $Pal-1^{A} = 0.411$ $Pal-1^{B} = 0.589$ albumin-1 AB 13.6 6.6 Pal-1 ВВ 13 9.7 (n=28) Post 12 10.0 $Pal-2^A = 0.586$ $Pal-2^B = 0.413$ albumin-2 AB BB 10 14.0 2.4 Pal-2 7 4.9 (n=29) Vitamin-D 6 5.6 14.7 binding 00 $GC^{O} = 0.433$ $GC^{C} = 0.566$ OC CC 0.06 protein GC 10 9.5 (n=30) Catalase CAT 2.4 11.2 6 AA $CAT^{A} = 0.296$ $CAT^{B} = 0.703$ AB 10.9 (n=27)BB 17 13.4 5 5 19 α-globulin AA 1.9 $F\alpha_2^{\ A}=0.258 \\ F\alpha_2^{\ B}=0.741$ $F\alpha_2$ AB 11.0 8.7 (n=29)BB 16.0 AA AB γ-głobulin 11 8.5 $S\alpha_2^A = 0.533$ $S\alpha_2^B = 0.466$ $S\alpha_2$ 10 14.9 3.2 BB 9 6.5 (n=30) Transferrin AA 0.0 1.8 TF AD 6.0 6 9 5 8 $TF^{A} = 0.25$ $TF^{D} = 0.40$ $TF^{E} = 0.35$ (n=30)AE 5.2 5.2 D_1D_2 4.8 DE 8.4 EE 3.6

Table 6. Distribution of genotypes of blood protein loci and their gene

Blood	Distribution of genotypes			- 2	Gene
protein loci	Genotype	Observed	Expected	χ^2	frequencies
Prealbumin Pr	AA AB BB	2 3 5	1.2 4.5 4.2	0.85**	$Pr^{A} = 0.350$ $Pr^{B} = 0.650$
Albumin Al	AA AB BB	0 5 5	2.5 5.0 2.5	5	$Al^A = 0.500$ $Al^B = 0.500$
Post albumin Pal	AA AB BB	4 6 0	4.9 4.2 0.9	1.48*	$Pal^{A} = 0.700$ $Pal^{B} = 0.300$
α-globulin Fα ₂	AA AB BB	3 4 3	2.5 5.0 2.5	0.4	$F\alpha_2^A = 0.500$ $F\alpha_2^B = 0.500$
γ-globulin Sα ₁	AA AB BB	2 2 6	1.6 4.8 3.6	3.3	$S\alpha_2^A = 0.400$ $S\alpha_2^B = 0.600$
β-globulin (Transferrin) TF	AA AD AE D ₁ D ₂ DE EE	3 2 3 1	3.0 2.75 2.2 0.6 1.0 0.4	1.1*	$TF^{A} = 0.55$ $TF^{D} = 0.25$ $TF^{E} = 0.20$

N=10 * P<0.05 ** P<0.01

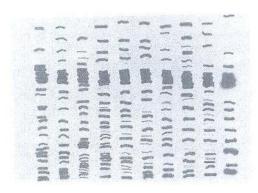


Fig. 1: Fractionation and different genotypes of serum proteins in normal healthy brucella negative sero-reactor camels.

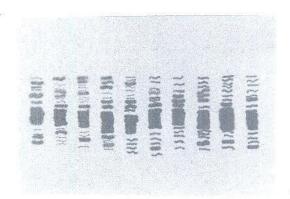


Fig. 2: Fractionation and different genotypes of scrum proteins in brucella positive sero-reactor camels.