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# Observations on Brucellosis in Male Camels (*Camelus dromedaries*) with Emphasis on Genetic Polymorphism of Some Blood Protein Loci

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**Abstract:** Brucellosis is a serious disease that causes direct and indirect losses in all primates through abortion, stillbirth, metritis and reduction in the milk production. Diagnosis of brucellosis is based on both bacteriological and serological examinations. Recently, immunogenetic studies and genetic polymorphism are used to investigate the susceptibility and / or resistance of animals to infectious diseases particularly brucellosis. The main goal of the present investigation was to study the possible relation between some blood protein loci and resistance and /or susceptibility to brucellosis in male camels. A total number of 200 blood samples was aseptically collected from slaughtered mature male camels (*Camelus dromedaries*). These animals were over 5 years old, came from Sudan and slaughtered at abattoirs nearby Cairo. Sera were separated and serologically examined for brucellosis with tube agglutination test (TAT), Rose Bengal plate test ( RBPT ) and Rivanol test (Riv.T). 140 serum samples from positive sero-reactor (N=70) and negative sero-reactors (N=70) were used in immunogenetic investigations. Electrophoretic patterns were used for genotyping of 4 blood protein loci. Results revealed that 35% of 200 examined cases were positive sero- reactors for brucellosis. Homozygotic genotypes were predominated in positive sero-reactors animals, especially $Gc^C$ and $S\alpha_2^B$ gene markers while, $AI^A$, $F\alpha_2^B$ and $S\alpha_2^A$ were predominated in negative sero-reactors camels. It was concluded that these genetic markers can be used for identification of animals naturally bearing susceptibility and / or resistance to brucellosis in selection programs.

**Key words:** Brucellosis · Camels · Genetic Polymorphism

## INTRODUCTION

Brucellosis is a serious zoonotic disease that affect the animal wealth as well as the national economy in many countries of the world. In livestock, brucellosis causes direct and indirect losses through abortion, stillbirths, metritis and up to 25% reduction in the milk production. This affection is an important remerging communicable disease in the Middle East and Mediterranean countries [1].

Camelids are not known to be primary or main hosts of brucella species, but they are susceptible to both *B. abortus* and *B. melitensis* [2,3]. In the same time, brucellosis in camels has not received much attention from researchers and scientists [4].

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 animal by periodic examination of blood for presence of specific antibodies and elimination of positive reactors [5]. This can be achieved by using both bacteriological and serological examinations [6]. Bactriological isolation is not safe and time consuming. Serological tests such as RBPT and ELISA are wildly used to detect antibodies against brucellosis[2] with some sort of confliction among tests. so there is a special need for more accurate diagnostic tools[7].

Immungenetic studies on camel brucellosis are still limited. Genetic polymorphism was used to evaluate the resistance of animals to infectious diseases [8,9]. Recently, some studies were carried out on camel genetics, especially in the aspect of susceptibility and / or resistance to infectious diseases and brucellosis in particular [10-12].

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Genetic resistance to brucellosis is a vital question to detect the genes of resistance and genes of susceptibility to the disease. In this respect, Tatiane *et al*. [13], Rodrigo *et al*. [14] and Rebeca *et al*. [15] discussed the polymorphism of SlC11a1 coding region on resistance gene to brucellosis as well as the genotyping of MLVA gene marker and the role of NRAMP gene in natural resistance to brucellosis in cattle. In this respect, Elaine *et al*. [16] reported that multiple - locus variable tandem repeat anaylsis (MLVA) is a useful tool to epidemiological trace back in *Brucella abortus* infection.

This study was planned to investigate the present incidence of brucellosis in camels and characterize some blood protein genetic markers which may be correlated with natural susceptibility and/or resistance to camel's brucellosis.

## MATERIALS AND METHODS

This study was carried out on adult male camels (*Camelus dromedaries*) imported from Sudan to be slaughtered in some abattoirs nearby Cairo. A total number of 200 blood samples was aseptically collected. Serum samples were serologically examined for identification of positive and negative reactors.

Rose Bengal plate test (RBPT), tube agglutination test (TAT), mercaptoethanol test (MET) and Rivanol test were done according to A1ton *et al*. [17]. All antigens were obtained from Veterinary Serum and Vaccine Resaech 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 a higher is considered as brucella positive sero-reactor [18].

The total serum protein (TSP) was electrophoretically fractionated on one dimensional polyacrylamide gel electrophoresis (PAGE ) [19,20]. Genotyping of blood protein loci and gene frequency were done according to Mercoreva [21]. Four serum protein: albumin (AI), vit.D binding protein (Gc), Alpha globulin ($F\alpha_2$) and Gamma globulin ($S\ \alpha_2$) were analysed in the present study.

Data were computed and statistically analyzed using SPSS.

## RESULTS

Results revealed that the incidence of brucellosis in camels with different serological tests was 70 (35%), 36 (31.5% ), 60 (30%) and 65 (32.5%) with RBPT, TAT, MET and Rivanol, respectively (Table1).

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

| Total number of examined animals | RBPT + ve | | TAT at 40 IU/ ml and higher | | MET +ve at dilution 1: 10 and higher | | Rivanol + ve at dilution 1:20 and higher | |
|---|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | N | % |
| 200 | 70 | 35 | 63 | 31.5 | 60 | 30 | 65 | 32.5 |

Table 2: Genotyping of blood protein loci and their gene frequencies of brucellosis positive and negative sero- reactor camels (N= 70 in each groups)

| | Brucello positive sero-reactor camels | | | | | Brucello negative sero-reactor camels | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Genotyping | | | | | Genotyping | | | | |
| Blood protein loci | AA | AB | BB | $X^2$ | Gene frequency | AA | AB | BB | $X^2$ | Gene frequency |
| Albumin | 25 | 30 | 15 | | $AI^A$ 0.571 | 17 | 21 | 32 | | $AI^A$ 0.392 |
| (AI) | (22.8) | (34.2) | (12.8) | 1.09 | $AI^B$ 0.428 | (10.7) | (33.3) | (25.8) | 12.3** | $AI^B$ 0.607 |
| Vit.D binding protein | OO | OC | CC | 10.9** | $Gc^o$ 0.385 | OO | OC | CC | 5.0** | $Gc^O$ 0.443 |
| Gc | 17 | 20 | 33 | | $Gc^c$ 0.614 | 20 | 22 | 28 | | $Gc^c$ 0.557 |
| | (10.4) | (33.1) | (26.4) | | | (13.7) | (34.5) | (21.7) | | |
| α globulin | AA | AB | BB | | $F\alpha_2^A$ 0.492 | AA | AB | BB | | $F\alpha_2^A$ 0.314 |
| F $\alpha_2$ | 28 | 13 | 29 | | $F\alpha_2^B$ 0.507 | 12 | 20 | 38 | 7.8** | $F\alpha_2^B$ 0.685 |
| | (16.90) | (34.9) | (17.9) | | | (6.9) | (30.1) | (32.8) | | |
| □ globulin | AA | AB | BB | | $S\alpha_2^A$ 0.271 | AA | AB | BB | | $S\alpha_2^A$ 0.643 |
| $S\alpha_2$ | 10 | 18 | 42 | 7.9** | $S\alpha_2^B$ 0.728 | 35 | 20 | 15 | 9.8** | $S\alpha_2^B$ 0.357 |
| | (5.1) | (27.6) | (37.0) | | | (28.9) | (32.1) | (8.9) | | |

** P < 0.01, In brackets, the theoretical number of genotypes

The immunogenetic analysis of serum protein loci of male camels was recorded in Table 2. Results showed that all studied loci were polymorphic and the most predominant gene markers in positive sero-reactor camels were $Gc^C$ (0.614) and $S\alpha_2^B$ (0.728), while in negative sero-reactor camels were distinguished by high frequency of $F\alpha_2^B$ (0.685), $S\alpha_2^A$ (0.643) and $AI^B$ (0.607).

## DISCUSSION

Brucellosis presents a serious problem in developing countries whereas it causes huge economic losses and health hazard. Imported camels may be a source of infection transfer to local community. Males play a major role in transfer of infection through natural copulation. In this study, a trial was conducted to throw light on the present incidence of brucellosis in imported male camel with special reference to possible use of some genetic markers as a tool for identification of positive reactors.

Results revealed that the incidence of brucellosis in camels ranged between 30-35% by different serological tests. In previous studies, Ghazi *et al*. [9] reported an incidence of brucellosis as 24.39, 18.69, 21.13 and 23.57% for RBPT, TAT, MET and Rivanol, respectively. While, Lisa *et al*. [22] recorded the prevalence of brucellosis using RBPT and Elisa tests as 60% in Sudan, 40% in Lybia and 32.3% in Egypt. Such variation of incidence results may be due to the course of the disease in camels, locality, rate of exposure to infection and the used diagnostic tool.

In the present study, four serum protein loci (AI, Gc, $F\alpha_2$ and S $\alpha_2$) were analyzed and the results showed that all these loci were polymorphic and the most predominant gene markers in positive sero-reactor camels were $Gc^C$ and $S\alpha_2^B$, while in negative sero-reactor camels were distinguished by high frequency of $F\alpha_2^B$, $S\alpha_2^A$ and $AI^B$. These allelic variations of blood protein loci in the present study confirm the finding of Kantanen *et al*. [23] which could potentially be used to evaluate the temporal changes in genetic diversity. Moreover, in this study the majority of genotypes are approximately equal and this result agreed with the finding of Leberg [24]. The high frequency of $Gc^C$ and $S\alpha_2^B$ gene markers in positive sero-reactor camels in the present study may be due to the possible relation between these gene markers and susceptibility of animal to infection. This result agreed with those obtained by Ghazi *et al*. [9] for $S\alpha_2^B$ markers. The high frequency of $S\alpha_2^A$, $F\alpha_2^B$ and $AI^B$ in negative sero-reactor camels in the present study may be due to the responsibility of these gene markers in the natural resistance of camels to brucellosis. This result was in line with those reported by Ghazi *et al*.[9] specially for $F\alpha_2^B$ gene marker.

The finding of genotyping of globulin fraction in the present study agreed in general with the finding of Chaudhary *et al*. [.25] except of absence of alpha1 in the present study and Boid *et al*.[26], especially to the higher gamma globulin and consequently the high gene frequency of $S\alpha_2^A$. In this respect it could be concluded that both alpha globulin ($F\alpha_2^B$) and gamma globulin ($S\alpha_2^A$) may play a principle role in natural resistance phenomena in camel.

## CONCLUSION

The incidence of brucellosis is high in imported male camel which may present an epidemiological hazard to our local livestock. Therefore, the imported camels must be quarantined and examined. Moreover, for breeding purposes, camels should be selected according to gene resistance to brucellosis.

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