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The Role of Transferrin Allele in Resistance to Brucellosis in Camels (*Camelus dromedaries*)

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Abstract: The main goal of the present investigation was to study the possible relation between transferrin locus "as gene marker " 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 the most prominent allele in positive sero- reactors for brucellosis camels is Tf^{B} (0.714) while Tf^{A} allele was predominated in negative sero-reactor 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: Camel · Transferrin · Gene Marker · Brucellosis

INTRODUCTION

Camels one of the most important animals of the Arab countries and it is deeply imbedded in their culture. The number of camels around the world is 11.24 millions and 61% of them are located in the Arab countries. Camels are important producers of meat (9%), milk (24%) and wool (8%).

Sudan is among the largest countries containing camels (4 millions =26.3%) of Arab camel population [1].

More than 200 plasma proteins have been described and estimated in human and animals. Many of these proteins changes markedly in disease conditions and with age. The major site of their synthesis in the liver but also the immune system consisting of monocytes - macrophages –lymphocytes and plasma cells. Because the proteins of an individual or of a species are synthesized under genetic control, it is to be expected that variations in proteins would occur between individuals and between species [2].

Transferrin comprise class of monomeric glycoproteins found in all vertebrates, whose function is iron sequestration and transport [3]. Fractionation and genotyping of transferrin in camels has been widely reported either to study the genetic constitution or to evaluate some economic traits [4-9].

Several studies have shown that transferrin has many biological functions such as antibacterial activity against abroad spectrum of Gram-positive and Gram-negative bacteria [10-14]. Electrophretic patterns of serum proteins in relation to brucellosis in camels has been reported [6, 15-17]. Moreover, El Fakharany *et al*. [12], Mahmood *et al*. [18] and Othman *et al*. [19]analyzed the lactoferrin locus on the molecular genetic level.

The present study was planned to analyze the possible relationship between transferrin genotype and resistant and/ or susceptibility to brucellosis in camels.

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.

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Rose Bengal plate test (RBPT), tube agglutination test (TAT), mercaptoethanol test (MET) and Rivanol test were done according to A1ton *et al*. [20]. All antigens were obtained from Veterinary 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 a higher is considered as brucella positive sero-reactor [21].

The total serum protein (TSP) was electrophoretically fractionated on one dimensional polyacrylamide gel electrophoresis (PAGE) [22, 23]. Genotyping of transferring (Tf) locus and estimation of its gene frequency was done according to Mercoreva [24].

RESULTS

Resuls of present study revealed that the transferrin migrated in gel as two fractions fast one (Transferrin F) and slow one (Transferrin S), each of them controlled by two autosmal alleles A and B. Results in Table 1 showed that negative sero-reactor camels characterized by high frequency of slow Tf^B allel (0.671),while positive sero-reactor camels were characterized by high frequency of fast Tf^Aallel (0.714).The genotyping analysis of transferrin locus showed predominance of homozygoticgenotypes.

DISCUSSION

The main object of present study was to clarify the possible expected relationship between transferrin locus and susceptibility to infection to brucellosis. The concept of genetic relation between blood protein loci and both productive and reproductive traits is based on the theory of protein coding loci [25]. In the same time, Kantanen*etal*. [26] reported that theanalysis of allelic variation of some protein loci could potentially be used to evaluate temporal changes in genetic diversity. In the same time, Mehta *et al*. [27] used microsatellite markers for characterization, conservation, individual identification, parentage testing and production enhancement. Transferrin family is a group of proteins including serum transferrin Tf, ovatransferrin, lactotransferrin and melantotransferrin (MTF). Most family members contain two lobes (N and C). In the present study, two fractions of serum transferrin were obtained (Fast fraction F-Tf and slow one S-Tf), each fraction is controlled by two autosomal alleles A and B. This result is in line with those recorded by Chaudary *et al*. [2] and Sargent *et al*. [27] but disagree with the finding of Ghazi *et al*. [6] who found three fractions of transferrin (A-E-D) and Soichi *et al*.[4] who found one only Tf molecule in camel and the condition may attributed to the presence of high –abundant proteins in the plasma. Unless high-abundant proteins are depleted from the plasma specifically, many low-abundant proteins will not detectable with even the most sensitive mass spectrometer[30]. In this respect, Alyamany *et al*. [8] reported that 56.8% protein non-depleted of camel plasma. Antimicrobial peptides are host defense moleculeand the presence of such molecules across the phylogenetic spectrum reflects a common necessity to defend against microbial pathogenesis [11]. In vertebrates, serum transferrin has a well-known physiological functions as an iron-transport protein for delivery to cells. Other iron-binding transferrin are involved in local iron homeostasis and other biological activities, such as induction of cell proliferation, regulation of gene expression and defense against infection [31]. ElFakharany*etal*. [12] reported that the highly anti-infectivity of transferrin (Especially lactoferrin) was demonstrated in camel. In the present study the positive sero-reactor camels are characterized by predominance of fast Tf^A (0.714). Similar result was recorded by Ghazi *et al*. [6] while negative sero-reactor camels showed predominance of slow Tf^B allel, these results may indicate the role of specific alleles in susceptibility or natural resistance to brucellosis in camel. In this respect, Sargent *et al*. [29] reported that the immunoglobulin isotypes are the most prominent of predict proteins in diseased and exposed animals and the numbers of these immunoglobulin isotypes are depending on the numbers of functional genes. In conclusion, we have to select animal for breeding according to gene resistant marker because if the genetic cause remain undetected, then it will get propagated and increase the occurrence of undesirable genes in the breeding populations.

Table 1: Genotyping and gene frequency of transferrin locus of brucellosis positive and negative sero- reactor camels (N= 70 in each groups)

	Brucella positive sero-reactor camels					Brucella negative sero-reactor camels				
	Genotyping					Genotyping				
Blood protein loci	AA	AB	BB	X^2	Gene frequency	AA	AB	BB	X^2	Gene frequency
Fast fractions	45 (35.7)	10 (28.5)	15 (5.7)	29.6**	Tf^A (0.714) Tf^B (0.285)	14 (7.4)	18 (30.8)	38 (31.5)	12.4**	Tf^A(0.328) Tf^B (0.671)
Slow fractions	25 (19)	23 (34.8)	22 (15.9)	8.2**	Tf^A (0.521) Tf^B (0.478)	23 (14.2)	17 (34.6)	30 (21.2)	17.9**	Tf^A(0.45) Tf^B(0.55)

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

REFERENCES

1. Layer, A.P., M. Albaikand I. Baghallab, 2014. Mastitis in camels in Africa and Middle East countries. J. Bacteriol. Parasitol., 5: 188.
2. Chaudhary, Z., J. Iqbal and J. Rashad, 2003. Serum protein electrophoretic patterns in young and adult camels. Aust. Vet. J., 81: 625-6.
3. Mingxuan, Z., R. Dimtry, A. Igar and B. Anne, 2004. Indirect detection of protein –metal binding :Interaction of serum transferring with In^{3+} and Bi^{3+}. Journal of the American society for Mass Spectrometry, 15(11): 1658-1664.
4. Soichi, T., K. Hideaki, M. Yasohero, F. Toyokazu, N. Iwao, A. Takashi and N. Takao, 1984. Phylogenetical and ontogenetical studies on the molecular weight heterogenecity of bovine serum transferring. Biochemical Genetics, 22(11):1127-1143.
5. Junrja, R., M. Pendo, H. Larsson, B. Ghane and A. Bowling, 1989. Tow-dimentional electrophoresis of the plasma proteins of Alpacas and llamas: Genetic polymorphism of alpha 1beta-glycoprotein and three other proteins. Animal Genetics, 20(1): 395-406.
6. Ghazi, Y.A., M.M. Zaabal and A.A. Ghazy, 2001. Studies on camel brucellosis with preliminary report on some immunogenetic markers. Assiut Vet. Med. J., 45(89): 144-160.
7. Al-yamani, M., M. Shehata, M. Al-Dubai, A. Alshanqeet, M. Al fageeh, A. Al Naeem, K. Al-Busdah, K. Al harbi, M. Hashad, O. Mahmoud and A. Al Swailem, 2011. Comparison of different strategies for Arabian camel (*Camelus dromedaries*) plasma. J of food, Agriculture and Environment, 9(3/4): 309- 314.
8. Soharbi, S., A. Niazi, M. Chahrdoo and F. Aram, 2014. Isolation and expression of antimicrobial camel lactoferrin (cLf) gene in tobacco. Plant Omics, 7(5): 298-30.
9. Lisa, D., A. Sascha and N. Heinrich, 2012. A review on camel brucellosis : A zoonosis sustained by ignorance and indifference. Pathog. Glob. Health, 106(3): 144-149.
10. Nannette, Y., T. Maria, J. Andres, F. Joes and R. Michael, 2007. The y-core motif correlates with antimicrobial activity in cysteine– containing kaliocin -1 originating from trnsferrin. Biochemica et BiophysicaActa (BBA), 1768(11): 2862-2872.
11. El Fakharany, E., L. Sanchez, H. Al Mahdar and E. Radwan, 2013. Effectivness of human, camel bovine and sheep lactoferrin on the hepatitis C virus cellular infectivity: Comparison study. Virology Journal, 10: 199.
12. Shereen, Y., Y. Safaa, M. Wahid, M. Soad, A. Eman, M. Khaled and H. Azza, 2015. Vector identification and clinical, hematological, biochemicaland parasitological characteristics of camel (*Camelus dromedaries*) theileriosis in Egypt. Tropical Animal Health and Production, 47(4): 649-656.
13. El rashdy, M., A. Nawal, M. Ahmed, N. Mohammed, H. Baeshen, E. Abdulrahman, M. Abu-Bakr, B. Saad, A. Fohad, E. Isam and N. Vladimir, 2016. Significant antibacterial activity and synergistic effects of camel lactoferrin with antibiotics against methicillin –resistant staphylococcus aureus (MRSA). Research in Microbiology, 167(6): 480-491.
14. Farhad, S.A., S. Shain and M. Hassan, 2012. Detection and segregation of brucellaabortus and brucellamelitensis in aborted bovine, ovine, buffalo and camelide fetuses by application of conventional and real-time PCR. Thai. J. Vet. Med., 42(1): 13-20.
15. Carwar, A., B. Sashi, J. Padnaja, N. Verra, B. Bendo, K. Virk and R. Girish, 2016. Genetic characterization and comparative genome analysis of *Brucella melitensis* isolate from India. Int. J. Genomics, pp: 1-13.
16. Zaabal, M.M., M.A. Ahmed, H.M. Emtenan and H.S. Elkhadrawy, 2016. Observations on Brucellosis in Male Camels (*Camelus dromedaries*) with Emphasis on Genetic Polymorphism of Some Blood Protein Loci. Middle East Journal of Scientific Research, 24(10): 3121-312.
17. Mahmood, C.H., A. Niazi, F. Aram and S. Soharbi, 2016. Expression of recombinant Arabian camel lactoferrin – related peptide in pichiapastori and its antimicrobial identification. J. Sci. Food and Agriculture, 96(2): 569-575.
18. Othman, A.O., A.M. Nowier and M.E. El-Denary, 2016. Genetic variation in two casen genes Among Maghrabi camels reared in Egypt. Biosci. Biotech. Res. Asia, 3(1): In press.
19. Alton, G.G., L.M. Jones, R.D. Angus and J.M. Verger, 1988. Techniques for brucellosis. Laboratory institute.Nationale de la Recherche Agronomique, 174 rue de l'universite,75007 Paris 4.
20. Pinsent,R.J. and C.J. Fuller, 1977. Outline of clinical diagnosis in the horse. Blackwell Science Ltd.,London, 2nd edition, P."Omoc128.

21. Lammeli, U.K., 1970. Cleavge of structural proteins during the assembly of head of bacteriophage T. Nature, 227: 680-685.
22. Carlstrom, A. and B. Johnson, 1983. Electrophoresis immunofixation. Scand. J. Immunnol., 17: 23-27.
23. Mercoreava, E.K., 1977. Genetic basis in farm Animals. Text book, 1st edition, Moscow,Coloc.
24. Barker, J.S.F., S.G. Tan, O.S. Selvaraj and T.K. Mukherjee, 1997. Genetic variation within and relationships among populations of Asian water buffalo (*Bubalus bubalus*). Animal Genetics, 28: 1-13.
25. Kantanen, J., I. Osker, S. Adalsteinsson, K. Sandberg, E. Eyhorcdottir, K. Pirhonen and E. Holml, 1999. Temporal changes in genetic variation of North European cattle breed. J. Anim. Gen., 30: 16-27.
26. Mehta, S.C., A. Goyal and M.S. Sahani, 2007. Microsatellite markers for genetic characterization of Kachchhi camel. Indian J. Biotechnology, 6(7): 336-339.
27. Sargent, P., S. Farnaudand R. Evans, 2005. Structure / function Overview of proteins involved in iron storge and transport. Current Medical Chemistry, 12(33): 2683-2693.
28. Bjorhall, K.M. and P. Davidsson, 2005. Comparison of different depletion strategies for improved resolution in proteomic analysis of human serum samples. Proteomics, 5(1): 307-317.
29. Baker, H., B. Andersson and E. Baker, 2003. Dealing with iron :Common structural principles in proteins that transport and heme. Natl. Acad. Sci. USA, 100: 3583.