Cairo University

Faculty of Veterinary Medicine

Department of Microbiology



# Immunological studies on camels (Camelus dromedarius) in Egypt

A Thesis

Presented by

#### **Ehab Ali Mohamed Mohamed Fouad**

B.V.Sc. (2007), M.V.Sc. (2011) Faculty of Veterinary Medicine, Cairo University

For the degree of

Ph.D. in Veterinary Medical Sciences

**Microbiology** 

(Bacteriology, Immunology and Mycology)

Under the Supervision of

#### Prof. Dr. / Mahmoud Essam Hatem Ahmed

Professor of Microbiology,

Faculty of Veterinary Medicine,

Cairo University

#### Prof. Dr. / Jakeen Kamal Abdel-Halem El-Jakee

Professor of Microbiology,

Faculty of Veterinary Medicine,

Cairo University

### Prof. Dr. / Nagwa Sayed Sayed Mohammed Ata

Professor of Microbiology and Immunology,

National Research Centre

2015

Name : Ehab Ali Mohamed Mohamed Fouad

Nationality: Egyptian.

Date of birth: 25/09/1985.

Specialization: Microbiology.

**Degree**: Ph.D. in Microbiology.

Title of thesis: Immunological studies on camels (Camelus dromedarius) in Egypt.

**Supervision:** 

Prof. Dr. / Mahmoud Essam Hatem Ahmed

Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University

Prof. Dr. / Jakeen Kamal Abdel-Halem El-Jakee

Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University

Prof. Dr. / Nagwa Sayed Sayed Mohammed Ata

Professor of Microbiology and Immunology, National Research Centre.

Key words: Camel, Camelus dromedarius, Immunoglobulin G, HRP

#### **Abstract**

As the labeled anti-camel Igs with enzymes for ELISA are unavailable in commercial level, the present investigation was directed for developing labeled anti-camel IgG with horseradish peroxidase. For purification of camel IgG whole molecule, camel sera were preliminary precipitated with 50% saturated ammonium sulphate and dialyzed against diluted PBS then concentrated. This preparation was followed by Protein A Sepharose affinity column chromatograhy for purification of camel IgG. The purity of the eluted camel IgG was tested by SDS-PAGE. Four bands of molecular weight 63, 52, 40 and 33 kDa, represent camel IgG, were detected. Anti-camel IgG was prepared by immunization of goats and rabbits separately, with purified camel IgG. Then anti-camel IgG was purified by loading on Protein A Sepharose affinity column chromatography after precipitation and concentration. Whole molecule anti-camel IgG was conjugated with horseradish peroxidase. The obtained results showed that in fact glutraldehyde was effective and suitable reagent for producing protein-enzyme complexes that retained a part of their enzymatic and immunological specificity. Sensitivity of prepared conjugated secondary antibodies was detected using positive camel serum samples reacted with different antigens in ELISA compared with Protein A horseradish peroxidase. The recorded sensitivity is 100%. The specificity of the prepared conjugates was determined using negative serum samples in ELISA. The specificity is also 100% compared to 58-75% of protein A horseradish peroxidase. The conjugates are stable for one year at -20°C as proved by ELISA. Collectively, the current study introduces goat and rabbit anti-camel IgG whole molecules with simple, inexpensive method, with 100% sensitivity, 100% specificity and stability up to one year at -20°C. The important facet of the current study is saving hard currency. Future investigations are necessary for preparation of IgG subclasses.



"وَعَلْمَكَ مَا لَمْ تَكُن تَعْلَمُ وَكَانَ فَضْلُ اللهِ عَلَيْكَ عَظِيمًا"



سورة النساء / الآية - 113

## **DEDICATION**

To my father, my mother, my wife, my sons (Mohamed & Abdel Rhman), my brothers and my sister Whom I am indebted to them for my happiness in my life.

Thanks

Ehab

#### **Acknowledgment**

First of all, I would like to express my prayerful gratitude and great thanks to The Merciful **ALLAH** whose help I always seek, and without his will, I shall achieve nothing.

My sincere thanks and gratitude for the kindness of spirit of **Prof. Dr. / Mahmoud Essam Hatem Ahmed,** Professor of Microbiology,

Faculty of Veterinary Medicine, Cairo University, for his stimulating supervision, heartily encouragement, great help, support, criticism and valuable discussion during the course of present work.

It is great pleasure to record my great thanks to **Prof. Dr. / Jakeen Kamal Abdel-Halem El-Jakee,** Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University, for her stimulating, helpful supervision, cooperation and valuable encouragement.

I am deeply grateful to **Prof. Dr. / Nagwa Sayed Sayed Ata,** Professor of Microbiology, National Research Centre, for her supervision and encouragement in this work.

I would like to express my deep appreciation and sincere thanks to **Prof. Dr. / Eman Hussein Abel-Rhman,** Professor of parasitology and immunology, National Research Centre, for her stimulating supervision, cooperation and supplying facilities required for the practical work, kind guidance, valuable help and continuous interest and advice. No words enough to explain her effort to help me to be researcher.

It is great pleasure to record my deep and great thanks to all members of Microbiology and Immunology Department as well as Department of Parasitology and Animal diseases, National Research Centre for valuable help and advices during the work.

#### List of abbreviations

Ab antibody
Abs antibodies
Ag antigen

AGID agargel immunodiffusion BoNT/A Botulinum complex toxoid A

BSA bovine serum albumin

CC Constant chain

CH<sub>1</sub>, CH<sub>2</sub>, CH<sub>3</sub> first, second and third constant domain of H-

chain

ELISA Enzyme linked immunosorbent assay

Fig. Figure gram

HC heavy chain

HCAbs heavy chain antibodies HRP horseradish peroxidase IEP immuno-electrophoresis

IEX ion-exchange Igs immunoglobulins IgG immunoglobulin G

kDa kilo daltons

KLH Keyholl limpet hemocyanin

LC light chain min Minutes ml Militer mM millimolar µg Microgram

NAbs Natural antibodies

No. Number

NSS Normal saline solution
PBS Phosphate buffered saline

PBST Phosphate buffered saline Tween 20

Photo. Photograph Percentage

RT Room temperature

SDS-PAGE Sodium dodecyl sulphate-polyacrylamide gel

electrophoresis

Spp. Species

VC variable chain

VH Variable domain of H-chain VHH Variable domain of H-chain

## **List of contents**

No.	Title	Page
1.	Introduction	1
2.	Review of literature	7
2.1.	Antibody structure	7
2.1.1.	Classical antibodies	7
2.1.2	Camel Immunoglobulin G (IgG)	9
2.1.2.1.	Camel IgG structure	9
2.1.2.2.	Camel IgG importance	13
2.2.	Preparation of conjugated camel IgG	16
2.2.1.	Separation and Purification of IgG	16
2.2.2.	Preparation of anti-camel IgG	27
2.2.3.	Labeling of anti-species IgG with horseradish peroxidase	31
2.2.4.	Sero-diagnostic techniques for evaluation of conjugation	32
2.3	Sero-diagnosis of camel diseases by ELISA	37
3.	Materials & Methods	42
3.1	Materials	42
3.1.1.	Laboratory Animals	42
3.1.1.1.	Rabbits	42
3.1.1.2.	Goats	42
3.1.1.3.	Samples	42
3.1.2.	Blood collection for serum preparation	43
3.1.3.	Solutions and buffers used for precipitation of Igs	43
3.1.4.	Chemicals for purification of immunoglobulins	43

3.1.5.	Solutions and buffers used for dialysis			
3.1.6.	Solutions and buffers used for affinity chromatography			
3.1.7.	Solutions and buffers used for protein determination	44		
3.1.8.	Solutions and buffers used for SDS-PAGE	45		
3.1.9.	Solutions and buffers used for preparation of goat and rabbit anti-camel IgG	45		
3.1.10.	Solutions and buffers used for coupling anti-camel IgG with horseradish peroxidase	46		
3.1.11.	Antigens	46		
3.1.12	Bacteriological Media	47		
3.1.12.1.	Media used for transportation of samples	47		
3.1.12.2.	Media used for isolation of the bacteria	47		
3.1.12.3.	Media used for detection of haemolysis of the isolates	47		
3.1.12.4.	Media used for biochemical identification of isolates	48		
3.1.12.5.	Media used for preservation of bacterial isolates	49		
3.1.12.6.	Citrated rabbit plasma	49		
3.1.12.7.	Reagents	49		
3.1.12.8.	Stains	50		
3.1.13.	Solutions and buffers used for ELISA	50		
3.1.14.	Apparatus& Equipments	52		
3.2.	Methods	53		
3.2.1.	Collection of camel blood samples	53		
3.2.2.	Separation and storage of serum	53		
3.2.3.	Precipitation of Igs with ammonium sulphate solution	53		

3.2.3.1.	Preparation of 50% saturated SAS 53			
3.2.3.2.	Precipitation of immunoglobulins 5			
3.2.4.	Purification of the precipitated immunoglobulins 5			
3.2.5.	Concentration of immunoglobulins 5			
3.2.6.	Separation of camel IgG by protein A affinity chromatography			
3.2.7.	Estimation of protein contents of camel IgG 5.			
3.2.8.	Electrophoresis of camel IgG in SDS-PAGE	56		
3.2.9.	Preparation of camel IgG anti-species in goats and rabbits	56		
3.2.10.	Separation of goat and rabbit anti-camel IgG	57		
3.2.11.	Evaluation of anti-camel IgG antibody production by ELISA	57		
3.2.12.	Labeling of enzyme to rabbit and goat IgGs	58		
3.2.13.	Isolation and Identification of bacteria 5			
3.2.13.1.	For Gram-positive coccobacilli 5			
3.2.13.2.	For Gram-positive cocci			
3.2.13.3.	For Gram-negative bacteria 59			
3.2.14.	Evaluation of anti-camel IgG conjugate 6			
3.2.15.	Comparison of the prepared conjugate (goat and rabbit anticamel IgG horseradish peroxidase) with commercial protein-A conjugate			
4.	Results	62		
4.1.	Protein content of purified camels, goats and rabbits IgG 62			
4.2.	SDS-PAGE profile of camel IgG 62			
4.3.	Humoral responses of goats and rabbits to camel IgG by	63		

## ELISA

4.4.	Isolation and Identification bacterial isolates	
4.4.1.	Coccobacilli isolates	66
4.4.2.	Cocci isolates	66
4.4.3.	Gram-negative isolates	67
4.5.	Potency of prepared goat and rabbit anti-camel HRP in ELISA	67
4.6.	Sensitivity of prepared conjugates in comparison with commercial protein A peroxidase	82
4.7.	Specificity of prepared conjugates in comparison with commercial protein A peroxidase	84
4.8.	Stability of prepared conjugates	86
5.	Discussion	88
6.	Conclusion	96
7.	Summary	97
8.	References	100
9.	Arabic Summary	1

## **List of Tables**

No	Title			
1	Estimated camel populations of Africa and the world			
2	Estimated camel populations, milk and meat productions of Africa and the world			
3	Protein content of Igs and IgG of camel, goat and rabbit			
4	Mean OD values represent binding activities in goat serum samples from 3 <sup>rd</sup> week to 8 <sup>th</sup> week post immunization with camel IgG	64		
5	The binding activities of rabbits serum samples from 3 <sup>rd</sup> week to 8 <sup>th</sup> week post immunization with camel IgG	65		
6	Potentials of goat anti-camel HRP to detect reaction of camel serum samples with <i>E. granulosus</i> protoscolex antigen	68		
7	Potentials of rabbit anti-camel HRP to detect reaction of camel serum samples with <i>E. granulosus</i> protoscolex antigen	69		
8	Potentials of goat anti-camel HRP to detect reaction of camel serum samples with <i>E. granulosus</i> fluid antigen	70		
9	Potentials of rabbit anti-camel HRP to detect reaction of camel serum samples with <i>E. granulosus</i> fluid antigen	71		
10	Potentials of goat anti-camel HRP to detect reaction of camel serum samples with <i>E. granulosus</i> wall antigen	72		
11	Potentials of rabbit anti-camel HRP to detect reaction of camel serum samples with <i>E. granulosus</i> wall antigen	73		
12	Potentials of goat anti-camel HRP to detect reaction of camel serum samples with C. <i>pseudotuberclosis</i> antigen	75		
13	Potentials of rabbit anti-camel HRP to detect reaction of camel serum samples with C. <i>pseudotuberclosis</i> antigen	76		
14	Potentials of goat anti-camel HRP to detect reaction of camel serum samples with <i>S. aureus</i> antigen	77		
15	Potentials of rabbit anti-camel HRP to detect reaction of camel serum samples with <i>S. aureus</i> antigen	78		
16	Potentials of goat anti-camel HRP to detect reaction of camel serum samples with <i>E. coli</i> antigen	79		
17	Potentials of rabbit anti-camel HRP to detect reaction of camel serum samples with <i>E. coli</i> antigen	80		
18	Comparison between sensitivity of protein A peroxidase, goat and rabbit anti-camel HRP based on mean OD values of positive camel serum samples reacted with <i>E. granulosus</i> antigens	82		
19	Comparative specificities of protein A peroxidase, goat and rabbit anti-camel HRP using negative camel serum samples and <i>E. granulosus</i> antigens	84		
20	Stability of stored conjugates at -20 °C for one year	86		

## **List of Figures**

No	Title			
1	Schematic representation of the structural organization of the IgGs in			
	different fractions as obtained from protein A and protein G			
	chromatography of serum of camelids.			
2	SDS-PAGE profile of camel IgG. Lane St: Molecular weight marker			
	Lane A: Camel IgG.			
3	Time based goats antibody response to camel IgG measured by	64		
	ELISA			
4	Time based rabbits antibody response to camel IgG measured by	<b>65</b>		
	ELISA	68		
5	Potency of goat anti-camel HRP in detection of IgG in camel serum			
	samples reacted with <i>E. granulosus</i> protoscolex antigen			
6	Potency of rabbit anti-camel HRP in detection of IgG in camel serum	69		
	samples reacted with <i>E. granulosus</i> protoscolex antigen	70		
7	Potency of goat anti-camel HRP in detection of IgG in camel serum samples reacted with <i>E. granulosus</i> fluid antigen	<b>70</b>		
	samples reacted with E. granutosus fluid antigen			
8	Potency of rabbit anti-camel HRP in detection of IgG in camel serum	71		
O	samples reacted with <i>E. granulosus</i> fluid antigen	/1		
9	Potency of goat anti-camel HRP in detection of IgG in camel serum	72		
	samples reacted with <i>E. granulosus</i> wall antigen	12		
	δ			
10	Potency of rabbit anti-camel HRP in detection of IgG in camel serum	73		
	samples reacted with E. granulosus wall antigen			
11	Comparative evaluation of goat anti-camel HRP potentials in	74		
	detection of IgG in camel serum samples react with different E.			
	granulosus antigens			
12	Comparative evaluation of rabbit anti-camel HRP potentials in	<b>74</b>		
	detection of IgG in camel serum samples react with different E.			
4.0	granulosus antigens			
13	Potency of goat anti-camel HRP in detection of IgG in camel serum	<b>75</b>		
1.4	samples reacted with <i>C. pseudotuberclosis</i> antigen	7.		
14	Potency of rabbit anti-camel HRP in detection of IgG in camel serum samples reacted with <i>C. pseudotuberclosis</i> antigen	<b>76</b>		
15	Potency of goat anti-camel HRP in detection of IgG in camel serum	77		
15	samples reacted with <i>S. aureus</i> antigen	11		
16	Potency of rabbit anti-camel HRP in detection of IgG in camel serum	79		
10	samples reacted with <i>S. aureus</i> antigen	1)		
17	Potency of goat anti-camel HRP in detection of IgG in camel serum	78		
1,	samples reacted with E. coli antigen	70		
18	Potency of rabbit anti-camel HRP in detection of IgG in camel serum	80		
	samples reacted with E. coli antigen			
19	Comparative evaluation of goat anti-camel HRP potentials in	81		
	detection of IgG in camel serum samples react with different bacterial			
	antigens			
20	Comparative evaluation of rabbit anti-camel HRP potentials in	81		

	detection of IgG in camel serum samples react with different bacterial			
	antigens			
21	Comparative sensitivities of protein A peroxidase, goat and rabbit			
	anti-camel HRP in detection of IgG in positive camel serum samples			
	specific to <i>E. granulosus</i> protoscolex antigen			
22	Sensitivity evaluation of goat and rabbit anti-camel HRP compared 83			
	with protein A peroxidase in detection of IgG in positive camel serum			
	samples reacted with E. granulosus fluid antigen			
23	Sensitivity evaluation of goat and rabbit anti-camel HRP compared to	84		
	protein A peroxidase in detection of IgG in positive camel serum			
	samples reacted with E. granulosus wall antigen			
24	Specificity evaluation of goat and rabbit anti-camel HRP compared 85			
	with protein A peroxidase using negative camel serum samples and			
	E. granulosus protoscolex antigen			
25	Specificity evaluation of goat and rabbit anti-camel HRP compared	85		
	with protein A peroxidase using negative camel serum samples and			
	E. granulosus fluid antigen			
26	Specificity evaluation of goat and rabbit anti-camel HRP compared 86			
	with protein A peroxidase using negative camel serum samples and			
	E. granulosus wall antigen			
27				
	-20 °C			
28	Significant stability of stored rabbit anti-camel HRP for one year at	87		
	-20 °C			

#### 1. Introduction

The camel occupies a unique position among animals which man has failed to exploit adequately and undoubtedly have unexplored and unrealized potential. Although the camel is neglected by science, largely ignored in technical literature, unappreciated and even unfavorable in the countries which makes its greatest contribution. Arabian camels have been domesticated for approximately 1500 years BC and have been long valued as pack animals David (2006). From a global perspective, the economic significance of camel production is minimal in comparison with that of other domestic animals so that camels breeding could be done to overcome Egyptian's needs for animal protein and its by-products. Certain breeds of camel can live in more humid environments the main breed is the dromedary of the Nile Delta of Egypt (Nawito et al., 1967). Dromedaries form an integral part of the desert environment, where their ability to survive and produce milk, meat, fibre and provide transport for nomadic peoples is unique (Azwai et al., 1995a). The camel plays such an important role in Arab culture that there are over 160 words for camel in the Arabic language. There were 11.24 million camels in the Arab world which represent 61% of camel numbers in the world (Farid, 1990) and 15% of the total number of animal species. The amount of meat and milk produced from camels is 289.2 and 213 thousand tons, respectively (Wardeh, 1990). Hamam (1993) reported that the camel represents a national wealth and source of income to the majority of citizens particularly in desert areas. Improvement of camel breeds production and health would preserve the recent increasing demand for camel meat and milk of distinguished quality (Wernery and Kaaden, 1995). Elagamy et al. (1996) refer to camel's milk is an important component of the diet of certain Arabian peoples. It has been noted that despite the lack of refrigeration, camel's milk remains unspoiled for several days: this may be due to the antibacterial activity of certain minor proteins contained in camel's milk. Camels are used for military uses as well as freight animals instead of horses and mules. In World War I, the British Army also created the Egyptian Camel Transport Corps, which consisted of a group of Egyptian camel drivers and their camels. The Corps supported British war operations in Sinai, Palestine, and Syria by transporting supplies to the troops (David, 2006).

The distribution and economic potential of camels according to **FAOSTAT** (2001) are about 19 million camels in the world, of which 15 million are found in Africa and 4 million in Asia. Of this estimated world population, 17 million are believed to be one-humped dromedary camels (*Camelus dromedarius*) and 2 millions two-humped (*Camelus bactrianus*). Approximately 11 million dromedaries, representing two thirds of the world's camel population, are in the arid areas of Africa, particularly in North East Africa, i.e. Somalia, Sudan, Ethiopia and Kenya.

**Table (1)**: Estimated camel populations of Africa and the world (**FAOSTAT**, 2001)

Country	Camel population (x 10 <sup>3</sup> )	Country	Camel population (x 10 <sup>3</sup> )
Algeria	240	Egypt	120
Morocco	36	Ethiopia	1070
Chad	725	Somalia	6200
Niger	415	Kenya	830
Djibouti	70	Sudan	3200