Immune Responses Of Camels To Vaccination With *Brucella abortus* S19 Vaccine

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ABSTRACT

Throughout the present work , the humeral and cellular immune responses to *Brucella Abortus* 19 (S19) vaccine were evaluated in camels. In a group of 10 camels, of about 6 months of age, a dose of 6.2 x 10^9 CFU was inoculated subcutaneously in each camel while a group of another 5 camels was kept without vaccination as control. Rose Bengal test (RBT) revealed that vaccinated camels began to response to S19 vaccine by the 2nd week post – vaccination showing detectable antibody levels that began to decrease by the 12th week post – vaccination to reach a zero level by 22nd week post – vaccination. The results of Indirect ELISA and complement fixation test (CFT) indicated such observation while non vaccinated animals remain sero–negative allover the experimental period. It was noticed that camel cellular immune response to *B. Abortus* S19 vaccine showed gradual increase through 10 weeks post – vaccination but this response still poor as expected during this period. This study showed that camel behave as cattle for both humeral and cellular immune response to *B. Abortus* S19 vaccine.

INTRODUCTION

In many countries, brucellosis is still a serious economic problem with regard to livestock and a major public health hazard for human beings. Among 176 countries that responded to questionnaires distributed through the FAO, WHO and OIE organizations concerning disease occurrence and control in 1987 , the disease was recorded in 140 countries *(1)*. Animal brucellosis has been recorded in Egypt since 1939 and the prevalence of serological reactors on limited surveys has varied from on survey to another with a range between 16.5% to 23% in cattle and 7% to 10% in buffaloes. During the sixties , with importation of Friesian cows, the prevalence on some farms became very high especially in areas with high animal densities. Although *B. Abortus* was the most common isolate during early investigations, and *B. melitensis* has been recorded to be the cause of brucellosis in Egypt in 1970 *(2),* especially *B. melitensis* biovar 3 *(3)* and Brucella also has been demonstrated in camels , equines and swine where *B. melitensis* biovar 3 is the causative agent *(3).*

Camels possess an economic importance especially among Egyptian farm animals in Egypt as well as in several other countries allover the world. In Egypt their numbers were estimated as 102327 camels *(3, 4)* according to Animal population in Near East Countries (FAO Statistics, 1998). Nowadays camels are considered as one of the main sources of animal protein in some provinces in Egypt. Control and eventual eradication of brucellosis depends upon strict implementation of a test and slaughter program combined with massive vaccination of the susceptible population. In Egypt this policy is applicable only for cattle, buffaloes, sheep and goats wherever camel brucellosis was recorded in Egypt by many authors with variable incidence as 10.92% by *(5-9)* and 7.48% *(3)*. Camels could play a role in transmission of brucellosis to farm animals beside the public health significance. So, the present study was designed to evaluate the humeral and cellular immune response of camels to S19 using Indirect ELISA (ELISA), Complement Fixation Test (CFT), Rose Bengal test (RBT) and lymphocyte proliferation test.

MATERIAL AND METHODS

1. Animals

A total number of 15 brucellosis–free camels of 6 month of age were divided into 2 groups.

1. Groups 1 of 10 camels were inoculated S/C with the cattle dose of S19 vaccine.
2. Group 2 of 5 camels were kept as control.

2. Samples

1. Blood samples were collected from all animals every 2 weeks up 22 weeks post–vaccination.
2. Blood samples were collected from all animals on heparin every week for 12 weeks post-vaccination.

3. Vaccine

Brucella Abortus S19 vaccine was supplied by SZ Veterinaria S.A., Pontevedra , Spain.

4. Antigen

Rose Bengal antigen prepared from S99 was supplied by Veterinary Serum and Vaccine Research Institute –Abassia –Cairo – Egypt.

5. Lipopolysaccharide (LPS)

S-LPS antigen was extracted from freeze – dried , heat killed *B. Abortus* strain 11119 by the hot water / hot phenol method ***(10)***.

6. Conjugates

Anti-bovine IgG a (whole molecule) peroxidase conjugate (antibody developed in rabbit –IgG fraction of antiserum) was supplied by Sigma company.

7. Amboceptor

It was supplied by Dade Behring-Martburg GmbH-D-35041 Martbug/ Germany.

8. Complement

Guinea pigs fed on green food and their sera were free from burcella antibody.

9. Mitogens

Phytohaemagglutinin (PHA) was supplied by Biochrom KG, Leon Renstr, 2-6-D-1224 , Berlin, Germany. It was used in the lymphocyte blastogenesis assay after its dilution in Roswel Park Memorial Institute (RPMI-1640) complete medium according to the manufacturer directions.

10. MTT

It was supplied by Sigma Chemical Company. It was used to measure the activity of dehydrogenase enzymes in the active mitochondria in the activating lymphocytes.

11. Media

Tryptose Soya Agar (TSA) was used for determining CFU and TSA supplied with the selective media (OXOID) for culturing of blood .

12. Determination of colony count of S19 vaccine

Determination of colony count of S19 vaccine was carried out ***(11)*** and this applied to confirm that the colony count of the vaccine batch is satisfactory. Colony count must be not less not more than $6\text{-}10 \times 10^9$ CFU/ dose.

13. Animals vaccination

As described previously.

14. Rose Bengal test

Rose Bengal test was carried out ***(11)***.

15. ELISA

ELISA was carried out ***(11,12)*** and the cut off value and the calculation of OD of tested serum were performed ***(12)*** but due to the lack of anti-camel conjugate which is the main reason to hamper the application of the ELISA in diagnosis of camel brucellosis so, Anti-bovine IgG peroxidase conjugate ***(13)*** was used in this work. Serum of brucella infected Camel was used as control positive serum and a serum of brucellosis free Camel was used as negative control serum. The optical density was determined at 490 nm using ELISA reader (Molecular Devices Corporation, Sunnyvale , California , USA).

16.Complement fixation test (CFT)

The CFT was performed by the Australian standard method *(14)*. Complement fixation at a dilution of log 2 (1:8) , showing the level recommended by the Australian Bureau of Animal Health, was regarded as a positive reaction. Serum samples were titrated 1:4 to 1:128, well beyond the point of significance. Titers determined by the CFT were expressed as log 2 of the reciprocal of the last dilution at which a positive reaction occurred *(15)*.

17.Evaluation of the cell mediated immunity

The cell mediated immunity was evaluated in vaccinated animals using lymphocyte blastogensis assay using PHA as a mitogen *(16)*.

RESULTS AND DISCUSSION

There is a fact that could not be neglected, that is there is no determined dose of *B. Abortus* S19 vaccine for camels and there is no available data discuss camel vaccination against brucellosis. So , in the present study which aim to investigate the immune response of camels to such vaccine, it was of interest intelligence to use the cattle dose which determined by the vaccine manufacturer (Quality control of vaccine indicate that the dose was 62 CFU / dose).

Rose Bengal revealed that all the serum samples obtained from the vaccinated camels showed positive reactions by the 2nd week post- vaccination (1st samples used for rose Bengal test) recording maximum possivity (+++) up to 8th week post –vaccination then began to decrease by the 12th week post – vaccination to reach zero level by the 22nd week post-vaccination in the 7 vaccinated camels while the other 3 vaccinated camels did not response immunologically to the applied vaccination as shown in Table (1) and Fig. (1).

Table 1. Results of rose Bengal test on vaccinated camel sera

Camel groups	Mean RB reaction / weeks post-vaccination											
	0	2	4	6	8	10	12	14	16	18	20	22
Vaccinated camels	N	+4	4	+4	+4	+4	+3.5	+3	+2.5	+2	+1	*N
Control group	N	N	N	N	N	N	N	N	N	N	N	N

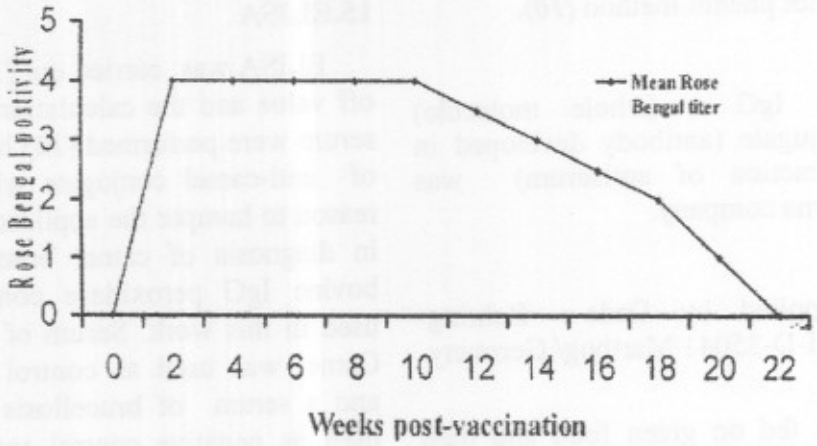

Fig. 1. Average rose Bengal titers of ten S19 vaccinated camels

Table 2. Results of ELISA assay on vaccinated camels sera

Camel groups	Number of positive reacted camels											
	0	2	4	6	8	10	12	14	16	18	20	22
Vaccinated camels	0 (0%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	7 (70%)	20 (70%)	0 (0%)
Control group	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

* N= negative

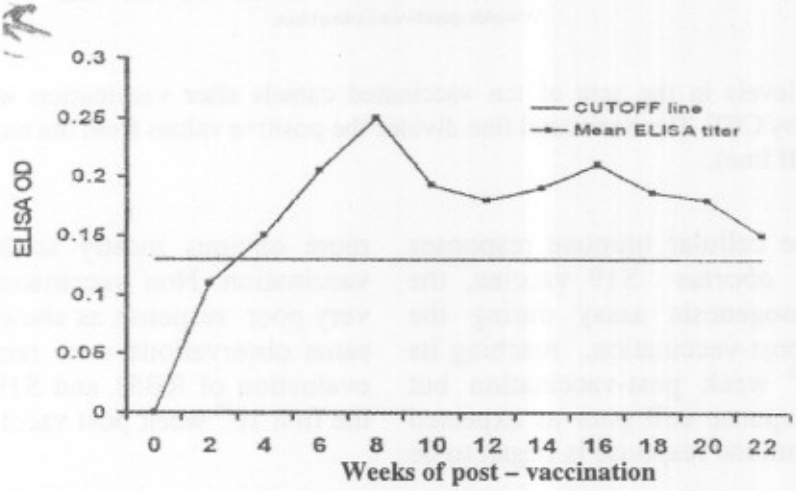

Fig. 2. Antibody levels in the sera of ten vaccinated camels after vaccination with S19 vaccine as measured by ELISA. The horizontal line divides the positive values from the negative values for the CFT (cutoff line).

Indirect ELISA showed that vaccinated camels with *B. abortus* S19 vaccine had significant brucella antibody response by the 3rd week post-vaccination and remaining up to the 22nd week post–vaccination as shown in Table (2) and Fig. (2).

Similar finding in RBT and ELISA were reported among calf hood and adult cattle vaccinated with the same vaccine via the same route used in the present work ***(17,18)*** and among Elk with RB51 vaccine ***(12).***

Complement fixation test (CFT) was assessed by determining the week number of which test positive results were detected. The test showed the highest positivisty (61og 2) on the 3rd week up to the 10 week post-vaccination then decreased to 51og2 on the 18th and 20th week post-vaccination recording its lowest level by the 22nd week post-vaccination as demonstrated in Table (3) and Fig. (3) . These findings can parallel to those of using the same vaccine in cattle ***(17)***.

Table 3. Results of CFT on vaccinated camel sera

Camel groups	Mean log of CFT reaction / weeks post-vaccination											
	0	2	4	6	8	10	12	14	16	18	20	22
Vaccinated camels	N	7	77	7	7	7	6	6	6	5	5	4.5
Control group	*N	N	N	N	N	N	N	N	N	N	N	N

*N- negative

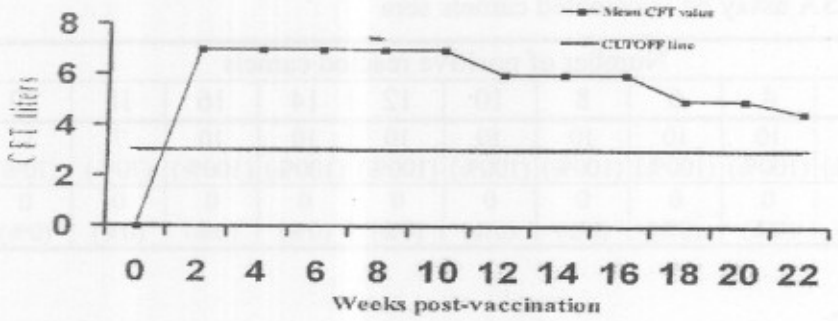

Fig. 3. Antibody levels in the sera of ten vaccinated camels after vaccination with S19 vaccine as measured by CFT. The horizontal line divides the positive values from the negative values for the CFT (cutoff line).

Recording the cellular immune responses of camels to *B. abortus* S19 vaccine, the lymphocyte blastogenesis assay during the first 10 weeks post-vaccination, reaching its peak by the 4th week post-vaccination but these immune response still poor as expected as the cellular immune response is began to be more obvious mostly at 20-24 weeks post-vaccination. Non vaccinated animals showed very poor response as shown in Fig. (4) . The same observations were recorded through the evaluation of RB51 and S19 vaccines during the first 10th week post vaccination of Elk ***(16)***.

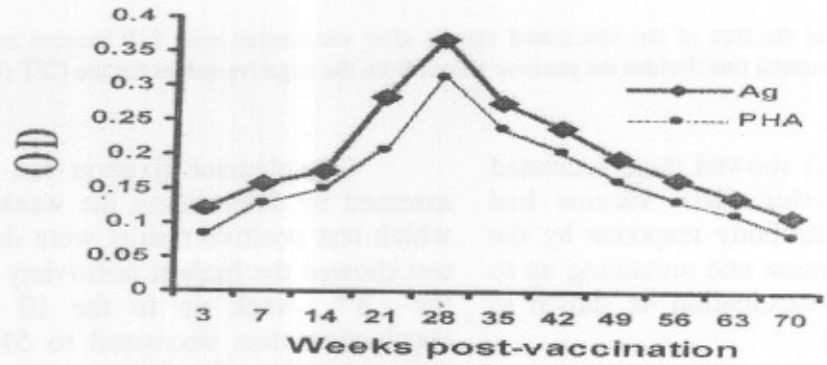

Fig. 4. Mean lymphocyte blastogenesis assay of camels vaccinated with S19 during the first 10 weeks post-vaccination in comparison with control samples.

The obtained results revealed that camels responded well to *B. abortus* S19 vaccine in both humeral and cellular manner, the thing which will help to control the disease in camels and prevent its transmission to other susceptible farm animals. But there is a need to further investigations to determine the most protective dose for camels to reach a maximum protection level as the negative immune response of the 3 camels in RBT could be attributed to the use of sub-protective dose. In addition, more studies are in need to compare between the potency and immune responses of other brucella vaccines as RB51, Rev-1 and S19 vaccines in camels to select the most potent one to eradicate or even to control the disease. Other studies also in need to evaluate the cellular immune responses for long periods, protection against brucellosis. The present study also spotted the light on the necessity to prepare anti- camel (protein–G) conjugated with horse reddish peroxidase as a specific anti-species to avoid the use of

heterogeneous anti-species which could affect the ELISA results leading to un-accurate results.

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الملخص العربى

رد الفعل المناعى للجمال المحصنة بعترة البروسيلا 19

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* المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية

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تم خلال العمل الحالى تقييم الاستجابة المناعية الخلطية والخلوية للجمال المحصنة بلقاح البروسيلا S19 حيث تم تحصين عشرة جمال تبلغ من العمر سته أشهر بالجرعة المستخدمة للأبقار وهى 6.2x109 مستعمرة بكتيرية لكل حيوان وتم حقنها تحت الجلد فى حين تم ترك خمسة جمال دون تحصين كضوابط. وقد تم جمع عينات دم وامصال من كل الجمال قبل وبعد التحصين كل اسبوعين حتى ٢٢ أسبوع . حيث أوضحت نتائج اختبار الروزبنجال أن الجمال المحصنة تظهر تفاعلا ايجابيا من الأسبوع الثانى بعد التحصين ثم تقل درجة هذا التفاعل من الأسبوع الثانى عشر لتصل لحد السلبية بالأسبوع الـ ٢٢ بعد التحصين أما الثلاثة جمال الأخرى فقد وصلت لحد السلبية من الاسبوع الثامن عشر بعد التحصين أما نتائج اختبارى الانزيم المرتبط المناعى الغير مباشرة والمثبت المتكامل فقد تبين أن الجمال المحصنة قد استجابت بصورة جيدة للقاح المستخدم مسجلة معايير مناعية عالية استمرت حتى الاسبوع الاخير من الدراسة فى حين أن اختبار المناعة الخلوية اظهر ارتفاعا تدريجيا فى دالة الكثافة الضوئية خلال العشرة اسابيع الاولى بعد التحصين ولكن هذا الارتفاع يعد بسيطا خلال هذه الفترة هذا وقد ظلت الحيوانات الغير محصنة تعطى نتائج سلبية مع كل الاختبارات المطبقة خلال الدراسة ومما سبق يتضح أن اختبارى الانزيم المرتبط المناعى الغير مباشر والمثبت المتكامل ادق وأكثر حساسية من الروزبنجال وان الجمال تستجيب خلطيا وخلويا باللقاح S19 بصورة مماثلة للابقار.