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MONITORING OF IMMUNE RESPONSE AGAINST *BRUCELLA MELITENSIS* REV- 1 VACCINE FOLLOWING ADMINISTRATION OF SOME ANTIBIOTICS

(With 4 Tables and 4 Figures)

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قياس رد الفعل المناعى ضد لقاح البروسيلا مليتنسز ريف-١ عقب استخدام بعض المضادات الحيوية

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الأوكسى تتراسيكلين والاستربتوميسين والانروفلوكساسين اكثر المضادات الحيوية شيوعاً واستعمالاً لعلاج كثير من أمراض الحيوان. لذا فقد اجريت هذه الدراسة بهدف معرفة تأثير هذه المضادات الحيوية على الحالة المناعية للحيوانات المحصنة واستخدمت خنازير غينيا المحصنة بلقاح ريف-١ كمثال لذلك. تم تحصين عدد ٣٥٠ حيوان من خنازير غينيا بلقاح الريف-١ ثم تقسيمهم إلى ٧ مجموعات كل مجموعة تحتوى على ٥٠ حيوان. المجموعة الأولى بعد تحصينها تم تركها بدون أى معاملات أخرى واستخدمت كضابط للتجربة. أما المجموعة الثانية والثالثة والرابعة فقد أعطيت الأوكسى تتراسيكلين والاستربتومايسين والانروفلوكساسين على الترتيب بالتوافق مع التحصين بجرعات محددة لمدة ٣ أيام فى حين أن المجموعة الخامسة والسادسة والسابعة قد تم معالجتها بنفس رجيم العلاج ولكن عند بلوغ رد الفعل السائلى قمته (٤ أسابيع بعد التحصين). تم ذبح ٥ حيوانات من كل مجموعة أسبوعياً لمدة ٨ أسابيع هى فترة التجربة. تم تجميع عينتا دم من كل حيوان مذبوح أحدهما على مانع للتجلط (هيبارين) بهدف قياس رد الفعل المناعى السائلى والخلوى لهذه الحيوانات. أظهرت النتائج أن الأوكسى تتراسيكلين أكثر المضادات الحيوية تأثيراً ضاراً على رد الفعل المناعى كذلك الاستربتومايسين ولكنه أقل تثبيطاً من الأوكسى تتراسيكلين فى حين أن الانروفلوكساسين ليس له مثل هذه التأثير على رد الفعل المناعى سواءاً الخلوى أو السائل. وعليه فقد خلصت هذه الدراسة إلى استنتاج مفادة أن الحيوانات المريضة المحصنة بلقاحات البروسيلا يحظر علاجها بالأوكسى تتراسيكلين أو الاستربتومايسين وأن الأنروفلوكساسين هو العلاج الأمثل لمثل هذه الحالات.

SUMMARY

The humoral and cellular immune response in guinea pigs vaccinated with Rev-1 vaccine and simultaneously treated with antibiotics (oxytetracycline, streptomycin or enrofloxacin) or 4 weeks post-vaccination were evaluated in the present study. Groups vaccinated and simultaneously treated with oxytetracycline or 4 weeks post-vaccination showed delayed or abrupt reduction respectively in antibody production, lymphocyte transformation, phagocytic percent and intra-cellular killing one week post administration of this antibiotic while groups vaccinated and simultaneously treated with streptomycin or 4 weeks post-vaccination showed abrupt reduction in antibody titer and moderate reduction in phagocytic percent, intra-cellular killing and lymphocyte transformation. Treatment by enrofloxacin simultaneously with *Brucella melitensis* Rev-1 vaccine or at peak of vaccination (4 weeks post-vaccination) cause non-significant variation in both humoral and cellular immune response. Therefore oxytetracycline and streptomycin should not be recommended for animal treatment during exposure to *Brucella melitensis* Rev-1 vaccine. In contrast enrofloxacin exhibited a satisfactory margin of safety upon the humoral and cellular immune responses to *Brucella melitensis* Rev-1 vaccine.

Key words: *Brucellosis, Brucella melitensis, vaccination, antibiotics.*

INTRODUCTION

Brucellosis is a zoonotic disease that causes heavy economic losses and human suffering. Under most conditions vaccination and serological identification and culling of infected animals are the only practical means to achieve its eradication (Monreal *et al.*, 2003). Until now the best vaccine available for sheep has been the smooth (S) *Brucella melitensis* Rev-1 strain (Marine *et al.*, 1999). Several authors have concluded that vaccination with *Brucella melitensis* Rev-1 vaccine is effective immunizing agent against *Brucella* organisms irrespective of challenge strain (El-Bauomy 1993 and EL-Gibaly *et al.*, 1995). Protection against the facultative intra-cellular *Brucella* organism depends mainly on cellular response following vaccination, whereas humoral response is not indicative of good immunization (Nicoletti, 1990).

Therefore any exogenous agents which result in crucial depression of cellular response to the given *Brucella* vaccine will consequently affect the overall process of immunization against infection and leave non immune host. Since the use of chemotherapeutic agents was widely introduced in veterinary field many author have studied their influence on the immune response against animal disease. Immune suppression still represent the worst indirect adverse reaction that may be induced by chemotherapies, such agents have the capability to suppress the immune system even at therapeutic levels (Nagwa *et al.*, 2005). Immunosuppressive properties of some antibiotics are effective in inhibition of both cellular and humoral immune responses to a variety of vaccines (Shalaby, 1989).

When initiating medication you must select not only the most biologically efficient therapeutic agent but also selecting an efficacious and safe drug which does not induce direct or indirect adverse reaction (Morris, 1995 and Harkness and Wanger, 1995). So the present study was designed to clarify the effect of medication with some antibiotics extensively used in veterinary medicine for treatment of several diseases namely oxytetracycline, streptomycin and enrofloxacin on both humoral and cellular immune response of guinea pigs vaccinated with *Brucella melitensis* Rev-1 vaccine.

The effect of medication was studied during vaccination and shortly after vaccination. As treatment of animals may become obligatory in certain instances therefore this study was also done to determine which of the used antibiotics is less harmful to the immune status of the animals and consequently can be used safely in concomitant with the vaccination process.

MATERIALS and METHODS

Vaccine: *Brucella melitensis* Rev-1 was obtained from Rhone Merieux Lyone, France.

Experimental animals:

A total of 350 guinea pigs (weighing 200 to 300g) free from any clinical signs were strictly selected and purchased from a trustable well established lab. animals unite of a well known institute. They were allocated randomly into 7 groups (50 animals/ each). Animals of all groups were vaccinated with *Brucella melitensis* Rev-1vaccine in a dose of 2×10^8 CFU. The 1st group received the vaccine alone and served as control group. The 2nd group was injected I/M with oxytetracycline in a

therapeutic dose 5 mg daily for 3 consecutive days simultaneous with the vaccination process. The 3rd group injected with streptomycin in a dose of 10 mg daily for 3 consecutive days simultaneous with the vaccination process. The 4th group was injected with enrofloxacin in dose of 10 mg for 3 consecutive days simultaneous with the vaccination process.

The 5th group was inoculated with oxytetracycline with the same dose used for group 2 for 3 successive days at the 4th week post vaccination. The 6th group was inoculated with streptomycin with the same dose used for group 3 for 3 successive days at the 4th week post vaccination. Whereas the last 7th group was inoculated with enrofloxacin with the same regime used for group 4 at the 4th week post vaccination .

Blood samples: 5 guinea pigs from each group were sacrificed prior vaccination and weekly during 8 weeks post vaccination. Two samples (serum and heparinized blood) were collected from each sacrificed animal.

1- Humoral immune response:-

A - Conventional serological test:

Antibody titers in collected serum samples were measured using Serum agglutination (SAT), Mercapto-ethanol (ME) and Rivanol (Riv.T) tests. Serum agglutination and mercapto-ethanol tests were done according to the methods adopted by Alton *et al.* 1975 whereas Rivanol test was carried out according to the procedures of the National Vet Services Laboratories, Ames, Iowa, USA 1984 (Alton *et al.*1988)

B - ELISA:

ELISA technique was performed by the method of Bodwen *et al.*1999. Briefly, polystyrene plates sensitized by 5µg of *Brucella melitensis* biovar 3 lipopolysaccharide prepared by phenol extraction of killed bacteria (Grain Bastuj *et al.* 1990) and blocked with 3% skim milk in phosphate buffer saline (PBS). The plates were washed with PBS 0.05% Tween 20 and the sera under study were added (diluted 1: 100) in PBS- 0.05% Tween 20 containing 1% skim milk. After incubation, the plates were washed and incubated with 100 µl of 1: 10.000 diluted horse radish peroxidase conjugated goat anti guinea pig IgG (Htt; zymed, S. Sanfrancisco, Calif.) the reaction was developed with o-phenylenediamine (2µg/ml) and 0.03 H_2O_2 in 0.1 M citrate phosphate buffer (ph 5) and was stopped with 4 N H_2SO_4. The resulting colour was read at 492 nm O.D Negative and positive controls were assayed in the same run.

2- The cell mediated immune response:

A- Phagocytic activity: Polymorph nuclear (PMN) cells were isolated from blood by the method described by Rouse *et al.* 1980. The mixture of PMN and bacteria (Staphylococcus aureus) were incubated at 37°C for 2 hour with regular stirring and then the mixture was centrifuged at 20 X g for 5 minutes at 4°C. The supernatant was used to estimate the percentage of bacteria phagocytised. The mixture of bacteria and PMN were treated with one cycle of freezing and thawing and the percentage of bacteria killed was estimated according to the formula described by Woldehiwet and Rowan 1990.

B- Lymphocyte transformation: Lymphocyte proliferation was measured by a blastogenesis assay using 3-(4, 5 dimethylthiazol 1,2) - 2,5 diphenyl tetrazolum bromide (MTT) (Sigma USA) as a tetrazolium dye. MTT is reduced to a blue Formazan compound by succinate dehydrogenase Mitochondrial enzyme produced by liver cells. MTT blastogenesis microassay was conducted as described by Denizot and Lange, 1991 with some modifications. Maslak and reynlods 1995. Two hundred µl culture medium containing mitogen phytohaemagglutinine (PHA) at 25 µg/ml or no mitogen (control well) was dispensed into each 96 well flat bottom tissue culture plate. Ten µl of cell suspension containing 10^5 lymphocyte was added to each well. Plates were incubated at 37°C for 72 hours in humid CO_2 incubator. Two hours before the termination of incubation 20 µl MTT 10 µg/ml was added to each well. At the end of incubation period 10% SDS in PBS was used to lysis the cells (50µl/ well). Then absorbance value was detected in an automated microtitre plate reader at wave length of 550 nm. The difference in reading between treated and control wells were subjected to statistical analysis.

RESULTS

Animals of the 1st group vaccinated with Rev-1 and not treated gave positive results to the current serological tests used (SAT,ME and Riv.T) and ELISA starting at 1st week post vaccination reached their peak at the 5th week then started to decline gradually till the end of the experiment (8 weeks post vaccination). Whereas animals vaccinated and simultaneously treated with oxytetracycline or streptomycin (Groups 2 and 3 respectively) showed significant reduction in antibody production as measured by conventional tests and ELISA.

Animals of groups 5&6 which treated at the expected peak of immunization titer with oxytetracycline or streptomycin showed abrupt decline in antibody titers. These decline in antibody titers occurred one week post administration of antibiotics while vaccinated animals simultaneously treated with enrofloxacin or 4 weeks post-vaccination (Groups 4&7) showed no significant differences in antibody titers in comparison to vaccinated non treated group (Tables 1& 2 and Figure 1).

Animals vaccinated and simultaneously treated with oxytetracycline or 4 weeks post-vaccination (Groups 2&5) displayed significant reduction in phagocytic percent and intra-cellular killing. Also animals vaccinated and simultaneously treated with streptomycin or 4 weeks post-vaccination (Groups 3&6) showed reduction in phagocytic and intra-cellular killing percent, but less than that occurred in oxytetracycline treated groups. This reduction observed one week after administration of antibiotics. In contrast enrofloxacin simultaneously treated group or 4 weeks post-vaccination disclosed no reduction in phagocytic activity comparing to vaccinated non treated group (Table 3 and Figures 2&3).

Simultaneously oxytetracycline treated group with vaccine or 4 weeks post-vaccination showed significant reduction in lymphocyte transformation after administration of antibiotics by one week. While streptomycin treatment cause moderate reduction (among treated groups) in lymphocyte transformation. Whereas treatment with enrofloxacin simultaneously with the vaccine or 4 weeks post vaccination showed no effect on lymphocyte transformation (Table 4 and Figure 4).

Table 1: Serological profiles of Rev-1 vaccinated and vaccinated treated animals with oxytetracycline, streptomycin or enrofloxacin.

Weeks post-vaccination	Test used	Animal groups						
		1	2	3	4	5	6	7
Pre-vaccination	SAT	0	0	0	0	0	0	0
	MET	0	0	0	0	0	0	0
	Riv.T	0	0	0	0	0	0	0
1week	SAT	14*	0	0	0	10	10	10
	MET	10	0	0	0	0	0	0
	Riv.T	0	0	0	0	0	0	0
2 week	SAT	26	0	0	20	20	20	26
	MET	14	0	0	10	10	10	10
	Riv.T	15	0	0	15	20	15	25
3 week	SAT	112	20	26	112	112	96	96
	MET	48	10	10	52	48	48	48
	Riv.T	50	0	0	35	35	35	50
4 week	SAT	160	26	20	96	160	112	160
	MET	144	10	10	52	80	48	112
	Riv.T	100	5	15	100	90	80	90
5 week	SAT	192	26	20	112	26	26	160
	MET	160	14	14	52	10	20	120
	Riv.T	100	15	15	80	5	10	80
6 week	SAT	112	40	52	112	20	18	120
	MET	96	20	26	96	10	10	80
	Riv.T	80	25	25	50	5	5	90
7 week	SAT	96	26	26	80	20	26	96
	MET	48	10	18	40	14	18	52
	Riv.T	35	15	15	35	10	10	35
8 week	SAT	30	18	26	30	10	10	20
	MET	20	10	10	14	0	0	10
	Riv.T	25	15	20	10	0	0	25

* Mean antibody titre.

1- Vaccinated non treated (control).
2- Vaccinated simultaneously treated with oxytetracycline.
3- Vaccinated simultaneously treated with streptomycin.
4- Vaccinated simultaneously treated with enrofloxacin.
5- Vaccinated and treated at peak (4 weeks post immunization) with oxytetracycline.
6- Vaccinated and treated at peak (4 weeks post immunization) with streptomycin.
7- Vaccinated and treated at peak (4 weeks post immunization) with enrofloxacin.

Table 2 : Evaluation of humoral immune response using ELISA test in Rev-1 vaccinated and vaccinated treated animals with oxytetracycline, streptomycin or enrofloxacin.

Post-vaccination	Animal groups						
	1	2	3	4	5	6	7
Pre-vaccination	0.122±0.002	0.122±0.002	0.121±0.002	0.109±0.003	0.121±0.005	0.124±0.002	0.121±0.006
1 week	0.290±0.012	0.112±0.004**	0.191±0.003**	0.281±0.002	0.292±0.002	0.254±0.012	0.286±0.002
2 week	0.321±0.002	0.181±0.003**	0.204±0.005**	0.318±0.004	0.322±0.002	0.319±0.002	0.320±0.003
3 week	0.594±0.003	0.201±0.002**	0.221±0.002**	0.593±0.005	0.601±0.003	0.593±0.002	0.600±0.003
4 week	0.931±0.002	0.295±0.004**	0.342±0.006**	0.928±0.006	0.932±0.004	0.928±0.002	0.930±0.002
5 week	0.982±0.006	0.393±0.007**	0.351±0.004**	0.979±0.007	0.311±0.003**	0.329±0.004**	0.981±0.003
6 week	0.722±0.007	0.401±0.002**	0.422±0.006**	0.721±0.005	0.366±0.002**	0.325±0.003**	0.719±0.002
7 week	0.725±0.002	0.321±0.004**	0.391±0.004**	0.721±0.002	0.321±0.002**	0.403±0.003**	0.720±0.003
8 week	0.439±0.006	0.305±0.002**	0.396±0.006**	0.425±0.002	0.304±0.004**	0.302±0.002**	0.421±0.002

1- Vaccinated non treated (control).
2- Vaccinated simultaneously treated with oxytetracycline.
3- Vaccinated simultaneously treated with streptomycin.
4- Vaccinated simultaneously treated with enrofloxacin.
5- Vaccinated and treated at peak (4 weeks post immunization) with oxytetracycline.
6- Vaccinated and treated at peak (4 weeks post immunization) with streptomycin.
7- Vaccinated and treated at peak (4 weeks post immunization) with enrofloxacin.
* Significant at $P < 0.01$ using t-student test comparing with control (G1)
** Significant at $P < 0.001$ using t-student test comparing with control (G1)

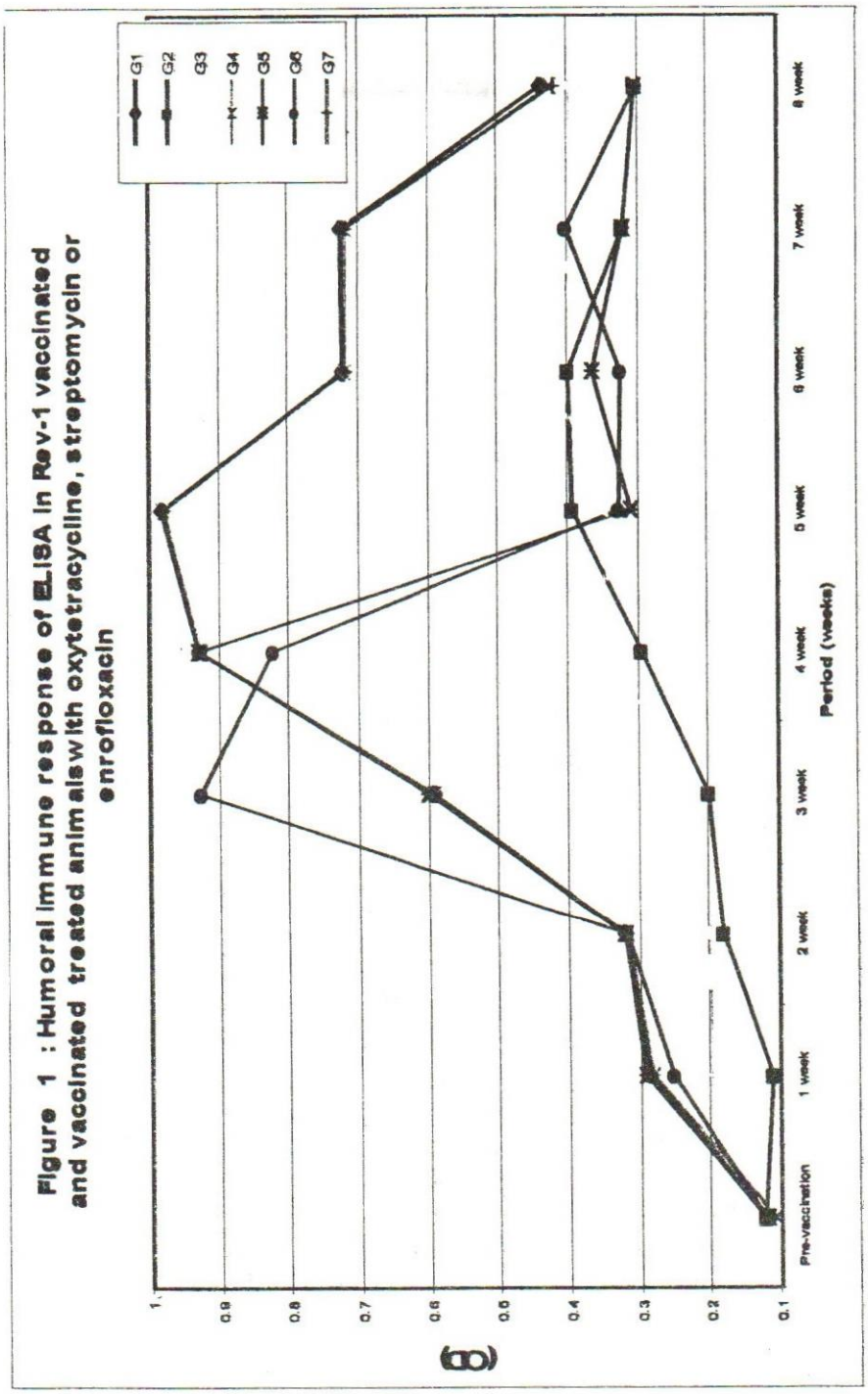

Figure 1 : Humoral immune response of ELISA in Rev-1 vaccinated and vaccinated treated animals with oxytetracycline, streptomycin or enrofloxacin

Table 3 : Phagocyctic activity in Rev-1, vaccinated and vaccinated treated animals with oxytetracycline, streptomycin or enrofloxacin.

Post vacc.	Animal groups													
	1		2		3		4		5		6		7	
	Ph%	K%	Ph%	K%	Ph%	K%	Ph%	K%	Ph%	K%	Ph%	K%	Ph%	K%
Pre-vacc	76.5±7.11	72.21±4.11	77.11±8.22	73.02±2.11	79.01±5.21	75.00±3.08	78.00±2.91	73.44±1.83	76.05±5.11	70.72±3.54	81.05±2.22	76.00±3.91	76.66±4.22	71.03±3.11
1	78.10±2.01	73.15±2.15	65.21±2.44*	61.33±1.11*	73.01±3.42	69.21±1.94	78.90±3.39	75.22±3.03	78.90±3.55	71.42±1.71	82.19±1.48	77.14±4.09	77.04±6.11	71.93±1.73
2	81.51±2.44	79.22±1.97	61.52±2.11**	58.01±2.99**	71.03±1.11*	67.27±1.21**	80.31±3.51	78.91±5.10	80.31±2.81	75.84±2.48	84.00±2.93	78.08±1.29	82.00±3.42	78.33±4.21
3	83.20±3.51	80.01±2.48	53.21±3.82**	51.31±4.05**	70.21±1.18*	65.00±1.91*	82.45±8.09	79.33±6.01	82.45±7.19	76.63±1.91	84.03±6.11	78.83±3.01	83.94±3.90	79.00±5.03
4	88.09±2.11	83.21±2.90	58.00±3.18**	56.54±2.20**	75.33±.2.91*	69.32±2.11*	86.91±3.92	81.92±3.37	86.91±8.15	81.34±4.22	85.10±3.22	80.00±2.83	89.08±4.51	85.32±2.21
5	85.22±2.49	80.91±1.80	58.33±4.51**	55.03±1.42*	69.22±2.82*	55.08±5.88*	86.00±5.44	82.03±2.42	56.00±2.88**	53.21±3.41*	69.22±2.15*	65.00±1.51**	86.11±5.09	81.94±3.03
6	89.31±4.22	83.21±3.50	59.01±5.11*	56.00±2.83*	70.82±3.01*	65.61±1.03	88.92±4.92	82.34±1.97	58.41±3.33**	54.02±2.83*	68.81±3.21*	64.94±0.91**	85.92±6.13	81.00±4.10
7	83.11±2.14	79.25±6.11	70.92±1.49*	68.00±3.11	71.00±2.41*	67.22±2.45	85.93±2.92	80.41±2.80	58.93±3.1**	55.04±1.94	70.01±2.81	65.62±2.39	81.94±4.33	76.98±3.13
8	79.01±6.14	74.92±1.88	74.44±5.17	69.02±2.09	74.00±3.91	64.42±1.09	80.08±3.81	75.01±1.89	71.08±3.31	66.15±3.21	71.94±4.21	65.83±3.50	77.04±3.21	73.91±3.26

Ph % = phagocytic percent. K% = intra-cellular killing percent.

1- Vaccinated non treated (control).

2- Vaccinated simultaneously treated with oxytetracycline.

3- Vaccinated simultaneously treated with streptomycin.

4-Vaccinated simultaneously treated with enrofloxacin.

5- Vaccinated and treated at peak (4 weeks post immunization) with oxytetracycline.

6- Vaccinated and treated at peak (4 weeks post immunization) with streptomycin.

2- Vaccinated and treated at peak (4 weeks post immunization) with enrofloxacin.

* Significant at $P < 0.01$ using t-student test comparing with control (G1)

** Significant at $P < 0.001$ using t-student test comparing with control (G1)

Figure 2 :Phagocyctic activity of Rev-1 vaccinated animals without and with oxytetracycline,streptomycin or enrofloxacin treatment.

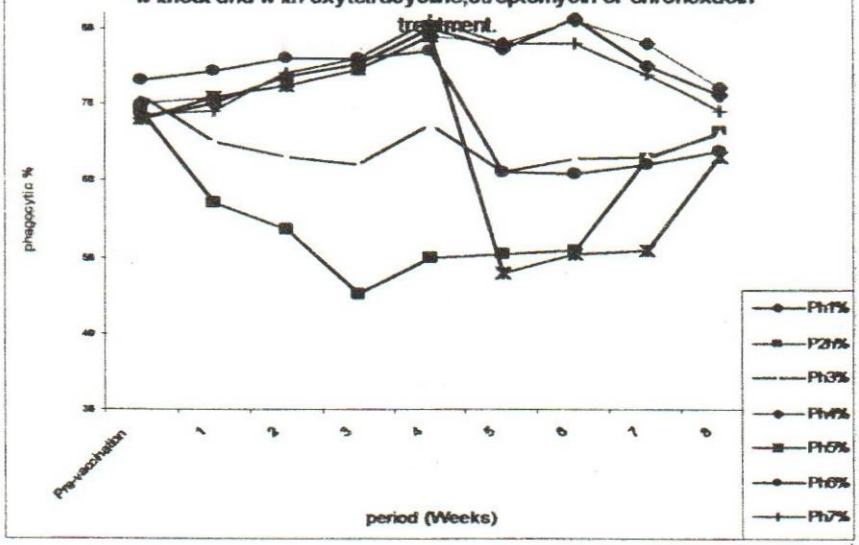

Figure 3 :Intracellular killing precent of Rev-1, vaccinated animals and vaccinated animals treated with oxytetracycline, streptomycin and nerofloxacin.

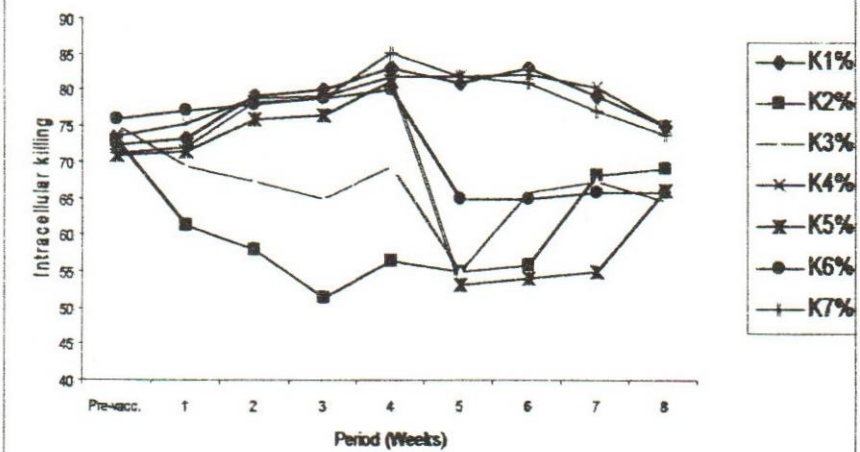

Table 4 : Lymphocyt transformation using MTT in Rev-1 vaccinated and vaccinated treated animals with oxytetracycline, streptomycin or enrofloxacin.

Weeks post-vaccination	1	2	3	4	5	6	7
Pre-vaccination	1.85±0.17	1.96±0.13	1.69±0.13	1.77±0.08	1.74±0.09	1.91±0.21	1.66±0.19
1	2.33±0.11	1.09±0.09**	1.31±0.26*	2.31±0.11	2.32±0.12	2.12±0.15	2.41±0.14
2	2.59±0.11	1.09±0.18**	1.43±0.23*	2.51±0.21	2.51±0.18	2.53±0.21	2.52±0.12
3	2.59±0.09	1.06±0.19**	1.31±0.29*	2.51±0.18	2.50±0.11	2.55±0.11	2.54±0.11
4	2.51±0.17	1.14±0.12**	1.21±0.24*	2.55±0.11	2.58±0.21	2.51±0.22	2.51±0.09
5	2.57±0.10	1.08±0.13**	1.21±0.29*	2.50±0.09	1.84±0.11**	2.06±0.03*	2.51±0.10
6	2.49±0.10	1.18±0.09**	1.31±0.27*	2.54±0.10	1.36±0.21**	1.82±0.04*	2.49±0.12
7	2.41±0.21	1.26±0.07**	1.35±0.11*	2.40±0.12	1.49±0.11**	1.63±0.04*	2.42±0.13
8	2.12±0.18	2.04±0.12	2.07±0.10	2.15±0.14	1.80±0.12	2.11±0.19	2.18±0.26

1- Vaccinated non treated (control).
2- Vaccinated simultaneously treated with oxytetracycline.
3- Vaccinated simultaneously treated with streptomycin.
4- Vaccinated simultaneously treated with enrofloxacin.
5- Vaccinated and treated at peak (4 weeks post immunization) with oxytetracycline.
6- Vaccinated and treated at peak (4 weeks post immunization) with streptomycin.
7- Vaccinated and treated at peak (4 weeks post immunization) with enrofloxacin.
* Significant at $P < 0.01$ using t-student test comparing with control (G1)
** Significant at $P < 0.001$ using t-student test comparing with control (G1)

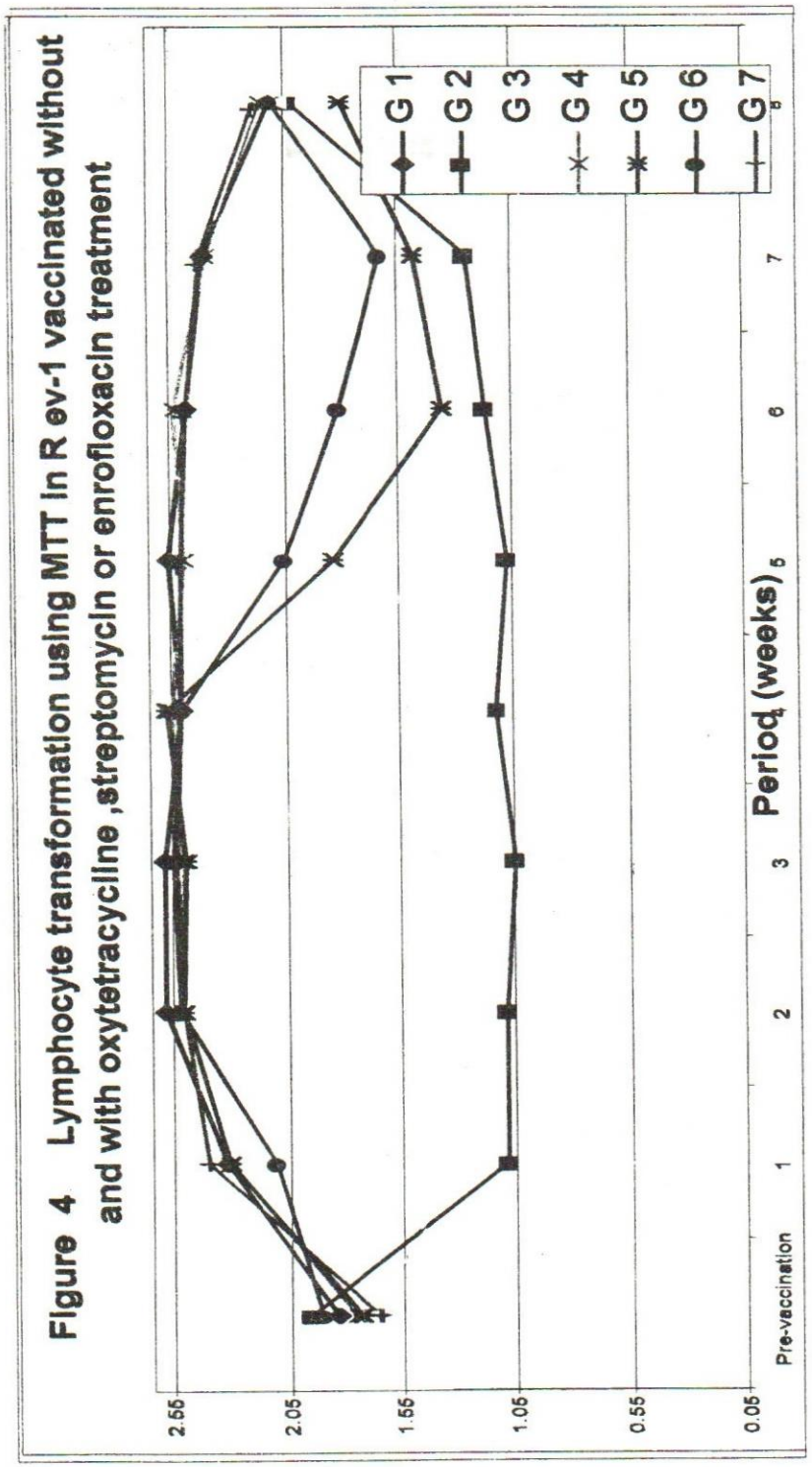

Figure 4 Lymphocyte transformation using MTT in R ev-1 vaccinated without and with oxytetracycline ,streptomycin or enrofloxacin treatment

DISCCUSION

Brucellosis is a chronic contagious disease that affects different animal species and humans. Control of the disease depends largely on slaughtering of diseased animals and immunizing of free animals with the suitable vaccine. The living attenuated *Brucella melitensis* Rev 1 vaccine still remains the most widely accepted immunizing agent used successfully in many countries against ovine and caprine brucellosis. Although protection against infection with Brucella organisms requires cell mediated response, however antibodies play an important role in this process restricting dissemination of the organism. Moreover the traditional antibody response of the vaccine indicates proper handling and administration of the vaccine.

In the present study the humeral immune response of Guinea pigs vaccinated with *Brucella melitensis* Rev 1 vaccine using serum agglutination (SAT), mercapto-ethanol (MET), Rivanol (Riv.T) (Table 1) and ELISA techniques (Table 2 & Figure 1) revealed that antigen antibody reaction started to appear one week post vaccination, quiet evident 15 days post vaccination and reached their peak 5 weeks post vaccination then declined rapidly throughout the experiment. Similar results were previously obtained by El-Bauomy (1993), El Gibaly *et al* 1995 and Hamdy *et al.* (2002)

Concurrent administration of oxytetracycline or streptomycin with *Brucella melitensis* Rev-1 vaccine or 4 week post-vaccination resulted in delay in antibody production and significant depression of antibody titers. This result correlated with the results obtained by Simith *et al.,* (1983); Mettias, (1984) and Hassan, (1994) who reported that simultaneous administration of oxytetracycline with *Brucella* vaccine reduced the percentage of animals with detectable humoral antibody titers.

The previous immuno-suppressive effect of oxytetracycline might be attributed to its ability to suppress the antigenicity of live *Brucella melitensis* Rev-1 as a result of simultaneous treatment with the antibiotic. On the other hand although *Brucella melitensis* Rev-1 is streptomycin resistant strain, the streptomycin caused decline in antibody titer which referred to suppressive effect of streptomycin on the reticuloendothelial system as has been reported by Zhakove *et al* (1980) who claimed that inhibition of haematopoietic activity resulted in acute depletion of the circulating B and T lymphocyte which consequently declining the antibody titers.

On observing the humoral immune response of guinea pig to *Brucella melitensis* Rev-1 vaccine, it could be noted that administration of enrofloxacin with or 4 week post-vaccination had no significant effect on antibody titre when compared with control group (vaccinated only) (Tables 1 & 2 and Figure 1). The present finding was consistent with that obtained by Shalit, (1991) who mentioned that most quinolones seem to have no direct effect on immunoglobulin production in vitro.In another study Chimura (1997) mentioned that there is no change in IgA or IgG antibody titer due to levofloxacin and enrofloxacin treatment.

Immunity against brucellosis is principally mediated by cellular immune response since it is an intra cellular pathogen. Cell mediated immunity is believed to play the major role in recovery from infection with Brucella. Polymorphnuclear cells represent corner stone of host defense mechanism against infection.

The data presented in Table (3) demonstrates increase in phagocytic percent and intra-cellular killing in group I following vaccination (vaccinated non treated group). In this respect it was reported that Brucellae are facultative intra-cellular bacteria that survive and replicate in both phagocytic and non phagocytic cells. Phagocytes play a key role in initiating T cell response by processing and presenting antigens. T cells play a major role in the acquired specific resistance to intra-cellular bacteria determine the resolution of bacteria.

In the present study it has demonstrated that administration of oxytetracycline with or 4 weeks post-vaccination evoked significant reduction in phagocytic activity of polymorphnuclear cells (Table 3 and Figures 2&3).

Hassan (1994) reported that buffalo calves injected with oxytetracycline produced reduction of phagocytic activity due to depress the synthesis of cytochrom oxidase and hence inhibiting metabolic process of phagocytic cells. It is also possible that effect of prolonged exposure of leukocytes membrane to oxytetracycline is responsible for the possible alteration in phagocytic function of polymorph phagocytes (Pious and Hawley, 1972). From the result illustrated in Table (3) it is clear that streptomycin moderately reduced phagocytic activity one week after its administration. This result correlated with the result obtained by Grass and Fietta (1991) who reported that aminoglycosides as streptomycin affected some leukocyte functions only at concentration higher than those achieved in therapy. In addition Labro (1993) reported that there is inhibitory effect of aminoglycosides at therapeutic concentration of RMN, chemotaxis, phagocytosis and intra-cellular

killing. Moreover, Sacha *et al* (1999) recorded that streptomycin inhibit rabbit peritoneal macrophage activity.

In this study it is clear that administration of enrofloxacin simultaneously with vaccine or 4 weeks post-vaccination had no effect on phagocytic activity. This result coincided with that reported by Vander Auwera *et al.* (1987) and Vander Auwera and Hassan (1989) who reported that no significant effect on phagocytic function (chemotaxis, oxidative metabolism phagocytosis or intra-cellular killing) by quinolones at therapeutic concentration. Moreover, Usluer *et al.* (1991) stated that non toxic doses of ciprofloxacin and enrofloxacin increase cellular and humoral immune response in mice.

In our study the effect of drug treatment was demonstrated by lymphocyte transformation in guinea pigs vaccinated by *Brucella melitensis* Rev-1 vaccine on lymphocyte incubated with PHA mitogen. For the results illustrated in Table (4) it is clear that oxytetracycline treated groups depicted sever reduction in lymphocyte transformation while streptomycin moderately reduced this transformation. This result agree with Hassan (1994) who recorded the reduction in lymphocyte transformation indices due to long acting oxytetracycline treatment in calves. The decline in lymphocyte stimulation index also referred to reduction of T cell function; DNA and protein synthesis.

Artismovish *et al.* (1991) reported that streptomycin induced inhibition in humoral and cellular immune response (lymphocyte transformation) so streptomycin should be recommended when inhibition of immunity is needed. In the present work it was found that enrofloxacin administration simultaneously with or 4 weeks post-vaccination caused non significant stimulation to lymphocyte transformation one week post administration in comparison to control group (vaccinated only). The recorded results may be attributed to the direct stimulant effect of the drug on the progenitor cells of lymphocytes in the bone marrow and/or its stimulant effect on DNA synthesis in the lymphocyte Forsgren *et al.* (1978). In addition Roche *et al.* (1987) stated that proliferation of mononuclear cells of human did not diminish or enhanced by using of ciprofloxacin at concentration of 5 to 125µg/ ml. Also, Pulverer *et al.* (1986) mentioned that enrofloxacin and ofloxacin doesn't affect cellular and humoral immune responses in vivo in BALB/C mice or in vitro human.

It is worthy to mention that what is presented here for the vaccine can be applied to the field infection. As serological diagnosis is the most reliable tool used almost exclusively for rapid detection of infected herd

or individual animal. This reliable method depends mainly on the recognition of antibodies arise due to infection. Therefore extensive abuse of bactericidal agents like antibiotics that affect the humeral response may hinder and compromise the whole process of routine diagnosis due to miss diagnosis of infected animals.

Conclusively, it seems that vaccination with *Brucella melitensis* Rev-1 vaccine during therapy with enrofloxacin is quite safe approach. In contrast oxytetracycline and streptomycin adversely affect humoral and cell mediated immune responses against *Brucella melitensis* Rev-1vaccine and hence should not be recommended during *Brucella melitensis* Rev-1 vaccination.

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