Antioxidant status in pregnant ewes vaccinated with Rev 1 against brucellosis

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Abstract

The aim of this study was to examine the changes in the indicators of free radicals and antioxidant activity, represented by malondialdehyde and glutathione peroxidase in the sera of ewes vaccinated with Rev 1 vaccine. The experiment included 28 animals which were divided into four equal groups. Animals of the first and second groups were vaccinated subcutaneously with 2×10^9 and 2×10^7 colony forming units (CFU), respectively, whereas the animals of third and fourth groups were vaccinated conjunctively with 2×10^9 and 2×10^7 CFU, respectively. Sera were collected monthly for 6 months. Antibody responses were assessed by classical tests (Rose Bengal test, tube agglutination and 2-mercaptoethanol tests) in comparison with competitive ELISA. The antibody titers were higher and remained for along period in the subcutaneously vaccinated groups with the two doses compared those vaccinated conjunctively. There was a significant increase in serum glutathione peroxidase activity in the 8th week post vaccination in subcutaneously vaccinated groups and during the 12th week in those vaccinated conjunctively. Significant increase of serum malondialdehyde levels occurred during the 4th week in those vaccinated conjunctively and in 8th week in those vaccinated subcutaneously. This study concluded that the route of administration of the vaccine affects glutathione peroxidase activity and malondialdehyde level, which act as indicators of oxidative stress response, more than the vaccine dose.

Keywords: Antioxidant; Ewe; Vaccine; Brucellosis.
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حالة مضادات الاكسدة في النعاج الحوامل الملقحة بريف ١ ضد داء البروسيلا

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الخلاصة

الهدف من هذه الدراسة معرفة التغيرات في مؤشرات فعالية الجذور الحرة ومضادات الاكسدة المتمثلة بالمالونديالديهايد والكلوتاثايون بيروكسيديز في امصال النعاج الملقحة بلقاح Rev1. إذ اجريت التجربة على 28 حيوان والتي قسمت الى 4 مجموعات متساوية، حيوانات المجموعتين الاولى والثانية لقحت تحت الجلد بجرعة 2×10^9 و2×10^7 وحدة مكونة للمستعمرات على التوالي، بينما حيوانات المجموعتين الثالثة والرابعة لقحت في الملتحمة بجرعة 2×10^9 و 2×10^7 وحدة مكونة للمستعمرات على التوالي. تم جمع عينات المصل شهرياً ولمدة 6 اشهر، وتم تقييم استجابة الاضداد باستخدام الاختبارات التقليدية (اختبارات وردية البنكال، التلازن الانبوبي و 2-المركبتوايثانول) بالمقارنة مع اختبار الاليزا التنافسي. معايير الاضداد كانت مرتفعة وبقيت لمدة اطول في المجموعات الملقحة بطريقة تحت الجلد وبالجرعتين مقارنة بالمجموعات الملقحة عن طريق الملتحمة ، وكذلك وجود زيادة معنوية في مستويات خميرة الكلوتاثايون بيروكسيديز في الاسبوع 8 بعد التلقيح في الحيوانات الملقحة تحت الجلد وخلال الاسبوع 12 في الحيوانات الملقحة عن طريق الملتحمة بينما ازدادت معنوياً مستويات المالونديالديهايد خلال الاسبوع 4 في الحيوانات الملقحة عن طريق الملتحمة وفي الاسبوع 8 في الحيوانات الملقحة تحت الجلد. نستنتج من هذه الدراسة بأن لطريقة إعطاء اللقاح تأثير في زيادة مستويات خميرة الكلوتاثايون بيروكسيديز والمالونديالديهايد والتي تعد كمؤشرات لحدوث الإجهاد التأكسدي اكثر من جرعة اللقاح.

Introduction

Brucellosis is a highly contagious zoonotic and economically important bacterial disease of animals worldwide (1). The disease is caused by various species of the genus *Brucella* which are facultative, intracellular bacteria capable of surviving and replication inside the cells of mononuclear phagocytic system (2). The capacity of *Brucella* to induce disease is dependent on their ability to survive and to replicate within both host professional and non- professional phagocytes (3).

The intracellular environment of phagocytic cells is, however, potentially hostile for microorganism, threatening, their viability by oxidative (Myeloperoxidase-H_2O_2-halide) or non-oxidative (cationic protein, proteases, lactoferritin, and lysozyme (4). In study of (5) found that *Brucella* infection induced oxidative stress and lipid peroxidation in human, cattle (6) and in rat (7). Reactive oxygen species, such as superoxide, hydrogen peroxide and hydroxyl radical are released by neutrophils and have been shown to play an important role in inflammation and cell injury (8). Cytotoxic effects of oxidants include protein oxidation, lipid peroxidation, DNA damage and the inhibition of cellular metabolic pathways (9). Catalase, superoxide dimutase and glutathione peroxidase are part of intracellular defense systems against oxidation (10). The control of brucellosis in small ruminants in most countries are dependent on using vaccination programmes using attenuated live vaccine of *Br. melitensis* strain Rev.1 (11). Subcutaneous vaccination induced a generalized infection by 2 weeks after vaccination, being then restricted to the prescapular target lymph node (12). Vaccination with Rev.1 vaccine induced abortion, shedding of bacteria in vaginal discharges and milk of the vaccinated animals (13).

In this study, we aimed to assess reactive oxygen products and antioxidant enzyme activities by exploring the changes in serum malondialdehyde and glutathione peroxidase in ewes vaccinated against brucellosis.

Materials and methods

The study was conducted on 28 pregnant ewes (between 8^{th}, 12^{th} weeks of gestation), aged 2-3 years (Animals were from the field of the College of Veterinary Medicine, University of Mosul).The animals were divided into four groups equally. Both animals of the first and second groups were vaccinated subcutaneously with Rev.1 strain of *Brucella melitensis* vaccine (live attenuated vaccine, CZ Veterinary Company, Spain) at a dose of 2×10^9 and 2×10^7 Colony Forming Units (CFU) per ml, respectively. While both animals of third and fourth groups were vaccinated intra-conjunctively with a dose of 2×10^9 and 2×10^7 CFU per ml respectively.

Blood from all of the vaccinated animals were collected by venipuncture to obtain serum before vaccination (zero time) and at 4, 8, 12, 16, 20, 24, 28 weeks post vaccination. Sera were examined for antibrucella antibody titer, and also were used for determination of Glutathione peroxidase activity and Malondialdehyde level.

The Rose Bengal and cELISA tests were used to detect antibodies in the vaccinated animals. The Rose Bengal test (Chemelex Company, Spain) was performed as described by (14). A commercially available cELISA kits (Svanovir®, Sweden) were used according to manufacturer's instructions.

The tube agglutination and 2-mercaptoethanol tests were used to detect titeration of antibodies in the vaccinated animals, the tube agglutination test was performed as described by (14) and 2-mercaptoethanol tests according to (15).

The Glutathione peroxidase activity was measured using the modified method which was reported by (16) which originally performed by (17). Serum malondialdehyde levels were measured as the levels of malondialdehyde reacting with thiobarbituric acid (TBA) in the sera according to (18).

Data were analyzed statistically using SPSS statistical software (SPSS, 2005), by one way analysis of variance, the level of significance was at $P< 0.05$ (19).

Results

Clinically the body temperature of the vaccinated animals did not change from its normal values after 24 and 48 hours post vaccination, Abortion occurred only in one ewe from the second group and the remaining pregnant vaccinated ewes delivered normally at the end of pregnancy.

The animals in all vaccinated groups were seropositive to the Rose Bengal and Competitive ELISA tests between first and second weeks post vaccination.

The antibody titers reached the highest values in the second week post vaccination in all vaccinated animals. Afterwards, antibody titers fell gradually to the end of the study (Tables 1 and 2).

The results of comparing the average antibody titers between groups showed that the animals of the first group had highest antibody titer since 2 week postvaccination until 24 weeks compared with other groups, then animals of second group. In the animals of third group the antibody titers decreased significantly along 4, 20, 24 weeks compared with titers in the animals of the first group. The animals of the fourth group had the lowest antibody titers along periods of the study compared with the other groups (Tables 1 and 2).

The Glutathione peroxidase activities gradually increased from 4th weeks to 12th weeks post vaccination in all vaccinated animals, and its reached a highest values at 12th week post vaccination in first, third and fourth groups and at 8th week post vaccination in second group. Afterward, glutathione peroxidase activities decreased rapidly in all vaccinated groups (Table 3). The glutathione peroxidase activities showed a significant increase at the 12th week in the 1st, 3rd and 4th groups and at the 8th week in the animals of second group.

Table 1: Average antibody titers in sera of the vaccinated pregnant ewes against brucellosis with Rev 1 vaccine by two different doses and routes of administration by using tube agglutination test.

weeks	First group (2×10^9) CFU/ml subcutaneous	Second group (2×10^7) CFU/ml subcutaneous	Third group (2×10^9) CFU/ml intra-conjunctival	Fourth group (2×10^7) CFU/ml intra-conjunctival
2	160± 0 ^{A,a}	105± 34 ^{a}	80.0± 40 ^{a}	23.3± 8.8 ^{a,B}
4	102± 27.6 ^{A,a}	80.0± 11.5 ^{a}	44.3± 10.2 ^{B,b}	15.0± 2.9 ^{a,B}
8	51.1± 14.9 ^{A,c}	28.9± 3.5 ^{b}	36.6± 9.5 ^{b}	32.0± 7.5 ^{b}
12	26.7± 4.4 ^{c}	21.1± 2.6 ^{b}	18.3± 4.8 ^{b}	20.0± 0 ^{a}
16	25.0± 6.3 ^{c}	20.0± 0 ^{b}	10± 0 ^{b}	10± 0 ^{a}
20	23.3± 8.8 ^{A,c}	13.3± 2.1 ^{b}	10± 0 ^{B,b}	7.5± 0.6 ^{a,B}
24	20.0± 6.3 ^{A,c}	10± 0 ^{B,b}	5.0± 0.2 ^{B,b}	2.5± 0 ^{a,B}

The values expressed as mean ± S.E. Different small letters vertically and different capital letters horizontally indicate significant differences at level (P<0.05).

Table 2: Average antibody titers in sera of the vaccinated pregnant ewes against brucellosis with Rev 1 vaccine by two different doses and routes of administration by using 2-mercaptoethanol test.

weeks	First group (2×10^9) CFU/ml subcutaneous	Second group (2×10^7) CFU/ml subcutaneous	Third group (2×10^9) CFU/ml intra-conjunctival	Fourth group (2×10^7) CFU/ml intra-conjunctival
2	120± 23 ^{A,a}	90± 25 ^{A,a}	80± 10 ^{a}	23.3± 8.8 ^{a,B}
4	96.6± 22.2 ^{A,a}	58± 22.6 ^{a,b}	30± 11 ^{B,b}	16.7± 3.3 ^{a,b,B}
8	83.3± 25.5 ^{A,a}	50± 10 ^{a,b}	23.3± 5.6 ^{B,b}	13.3± 3.3 ^{a,b,B}
12	46.7± 17.6 ^{A,a}	30± 10 ^{b}	13.3± 0.3 ^{b}	10± 0 ^{B,b}
16	43.3± 20.3 ^{b}	16.7± 3.3 ^{b}	10± 0 ^{b}	10± 0 ^{b}
20	30± 10 ^{A,b}	13.3± 3.3 ^{b}	^{B,b} 10± 0	6.7± 0 ^{B,b}
24	16.6± 3.3 ^{A,b}	10± 0 ^{B,b}	3.3± 0 ^{B,b}	3± 0 ^{B,b}

The values expressed as mean ± S.E. Different small letters vertically and different capital letters horizontally indicate significant differences at level (P<0.05).

Table 3: Glutathione peroxidase levels (Micromole/liter) in the sera of the vaccinated pregnant ewes against brucellosis with Rev 1 vaccine by two different doses and routes of administration.

weeks	First group (2×10^9) CFU/ml subcutaneous	Second group (2×10^7) CFU/ml subcutaneous	Third group (2×10^9) CFU/ml intra-conjunctival	Fourth group (2×10^7) CFU/ml intra-conjunctival
2	0.5 ±0.02 ^{a}	0.4± 0.01 ^{a,c}	0.6 ±0.01 ^{a}	0.5± 0.01 ^{a}
4	1.0 ± 0.4 ^{a}	1.2± 0.5 ^{c}	0.6 ±0.2 ^{a}	0.7± 0.01 ^{a}
8	2.1 ±1.0 ^{b}	3.3± 0.4 ^{A,b}	0.8 ± 0.03 ^{a,B}	0.8± 0.03 ^{a,B}
12	2.9 ± 2.1 ^{b}	0.8± 0.3 ^{b}	3.2 ±0.8 ^{b}	4.0± 1.6 ^{b}
16	0.3 ± 0.06 ^{a}	0.2± 0.06 ^{A,a}	0.2 ±0.05 ^{A,a}	0.5± 0.06 ^{a,B}
20	0.2 ± 0.05 ^{a}	0.2± 0.04 ^{a}	0.3 ± 0.08 ^{a}	0.3± 0.1 ^{a}
24	0.2 ± 0.06 ^{a}	0.15± 0.02 ^{a}	0.2 ± 0.03 ^{a}	0.2± 0.08 ^{a}

The values expressed as mean ± S.E. Different small letters vertically and different capital letters horizontally indicate significant differences at level (P<0.05).

The results of Malondialdehyde levels showed a significant increased in the 8th week post vaccination in the first group and in the 8th and 16th weeks in the second group and in the 4th and 24 weeks in the third group and in 4th week in the fourth group compared with the prevaccination levels (Table 4).

Table 4: Malondialdehyde levels (Micromole/liter) in the sera of the vaccinated pregnant ewes against brucellosis with Rev 1 vaccine by two different doses and routes of administration.

weeks	First group (2×10^9) CFU/ml subcutaneous	Second group (2×10^7) CFU/ml subcutaneous	Third group (2×10^9) CFU/ml intra-conjunctival	Fourth group (2×10^7) CFU/ml intra-conjunctival
2	0.2 ± 0.09^{a}	$0.3 \pm 0.08^{a,c}$	0.2 ± 0.01^{a}	0.2 ± 0.02^{a}
4	0.4 ± 0.06^{a}	$0.2 \pm 0.03^{A,a}$	0.5 ± 0.1^{b}	$0.9 \pm 0.3^{B,b}$
8	$1.1 \pm 0.4^{A,b}$	0.8 ± 0.3^{b}	0.3 ± 0.0^{a}	$0.2 \pm 0.1^{a,B}$
12	0.2 ± 0.06^{a}	0.2 ± 0.06^{a}	0.3 ± 0.1^{a}	0.2 ± 0.03^{a}
16	0.6 ± 0.06^{a}	$0.6 \pm 0.1^{b,c}$	0.4 ± 0.0^{a}	0.5 ± 0.09^{a}
20	0.4 ± 0.09^{a}	$0.3 \pm 0.1^{a,c}$	0.4 ± 0.1^{a}	0.4 ± 0.06^{a}
24	0.3 ± 0.03^{a}	$0.3 \pm 0.06^{a,c}$	0.5 ± 0.09^{b}	0.4 ± 0.16^{a}

The values expressed as mean ± S.E. Different small letters vertically and different capital letters horizontally indicate significant differences at level ($P<0.05$).

Discussion

The present study showed a significant increased in the glutathione peroxidase activity and malondialdehyde levels in pregnant ewes vaccinated against brucellosis with Rev 1 vaccine by two different doses and routes of administration.

There is a balance in animal body between production of free radicals and enzymatic and non–enzymatic anti-oxidant defense mechanisms (4). In the case of increase in reactive oxygen intermediates in cancer and various infections or decrease in antioxidant defence, free oxygen radicals react with macromolecules that contain protein, lipids and DNA and cause oxidative damage (20).

A study (5) showed elevation of antioxidant enzymes and malondialdehyde levels in patients with brucellosis, whereas a significant increase in malondialdehyde and nitric oxide levels were reported in cattle infected with brucellosis (6).

In our study, the elevation of glutathione peroxidase level may be due to high requirement of glutathione peroxidase at the presence of oxidative tissue damage at the elevation of reactive oxygen intermediate levels (4) this enzyme plays an important role in protection from oxidative stress (21), the vaccination with live attenuated Rev1 strain of *Brucella melitensis* vaccine had produced a mild infection in the vaccinated animals (13).

Increase in malondialdehyde level may be resulted from excessive production of free radicals secondary to brucellosis itself acting upon membrane lipids (5), and this elevation of serum malondialdehyde indicates oxidative stress (22).

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