# The Synergistic Effect of BCG and *Pasteurella multocida* Vaccines in Local rabbits

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## Abstract

The study was aimed to determine the humoral and cellular immune responses of different *Pasteurella multocida* vaccines that synergist with BCG vaccine. Twenty five local bread rabbits had been used which divided randomly into five groups, each group contains five animals. The first group immunized with killed whole cells of *P. multocida* (3×10^ cell /ml) and BCG vaccines, The second group had been immunized with sonicated whole cell *P. multocida* and BCG vaccines, The third group immunized with killed whole cell *P. multocida* vaccine, the fourth group immunized with sonicated whole cell *P. multocida* vaccine and the fifth group as control (negative group). The humoral passive haemagglutination test (PHA) and cellular delayed type hypersensitivity (DTH) immune responses were determined in 35 and 28days respectively and for three months after immunization. Our result showed a higher significant differences ($P<0.05$) of antibodies titer had been recorded in the first and third groups (1945.6 ± 389.8, 1792.4 ± 444.6 respectively) in the third month while in the second and the fourth groups (2257.4 ± 308, 1894.4 ± 377.3 respectively). Also these was a significant differences ($P<0.05$) in the diameter of erythema and skin in duration which had high levels in the second and the fourth groups (4.6 ± 0.5 mm, 3.9 ± 0.6 mm & 4.1 ± 0.3 mm, 4.3 ± 0.5 mm) respectively compared with the first and the third groups (3.34 ± 0.3 mm, 2.22 ± 0.3 mm and 2 ± 0.03 mm, 2.76 ± 0.3 mm) after 24 and 48 hours respectively.

# تأثير الفعل التأزري للقاحي BCG وجرثومة *Pasteurella multocida* في الأرانب المحلية

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## الخلاصة

هدفت الدراسة إلى تقييم بعض الاستجابة المناعية الخلطية والخلوية للقاحات جرثومة *Pasteurella multocida* مع لقاح BCG. إذ اختير 25 أرنبا محلي، قسمت عشوائيا إلى خمس مجاميع، كل مجموعة تضم خمسة أرانب. المجموعة الأولى منعت بلقاح الخلية الكامل المقتول لجرثومة الباستوريلا مالتوسيدا مع لقاح BCG والمجموعة الثانية منعت بلقاح الخلية الكامل المتكسر لجرثومة الباستوريلا مالتوسيدا مع لقاح BCG أما المجموعة الثالث فقد منعت بلقاح الخلية الكامل المقتول بجرثومة الباستوريلا مالتوسيدا. أما المجموعة الرابعة منعت بلقاح

الخلية الكامل المتكسر لجرثومة الباستوريلا مالتوسيدا أما المجموعة الخامسة فقد عدت مجموعة سيطرة سالبة. قيست الاستجابة المناعية الخلطية والخلوية باختبار التلازن الدموي غير المباشر وفحص الحساسية الجلدي المتأخر بعد مرور 35 و28 يوم على التوالي ولمدة ثلاثة أشهر من التمنيع. أظهرت النتائج ارتفاع المعيار الحجمي للأجسام المضادة وبفرق معنوي ($P<0.05$) في المجموعتين الأولى والثالثة 444.6± 1792.4 ,389.8 ± 1945.6 على التوالي في الشهر الثالث بينما أظهرت المجموعة الثانية والرابعة 377.3± 1894.9 ,308± 2257.9 على التوالي في الشهر الأول. كما لوحظ أن هناك فرقا معنويا ($P<0.05$) في قطر الاحمرار وتثخن الجلد (فحص الحساسية الجلدي المتأخر) والذي ظهر بمستوى عالٍ في المجموعتين الثانية والرابعة 4.1 mm, 3.9± 0.6 mm, 4.6 ±0.5 mm ± 0.3 mm, 4.3 ±0.5 mm على التوالي مقارنة بمعدلات المجموعتين الأولى والثالثة 2.22 mm, 3.34 ± 0.3 mm, 2.76 ± 0.3 mm , 2 ± 0.03 mm ± 0.3 mm) خلال 24 و48 ساعة على التوالي.

## Introduction

*Pasteurella multocida* is one of the notorious animal pathogen causing wide spread infections in various domestic animals, pneumonia and hemorrhagic septicemia in cattle, sheep and goats, fowl cholera, sever respiratory disease in pigs such as atrophic rhinitis and pneumonic pasteurellosis and snuffles in rabbits (1). It was considered to be the most economic important disease of livestock in South East Asia and cause a significant economic losses in India and Africa (2) Cattle and buffalo are the most common host but pigs, sheep, goats, deers and camels also are susceptible to infections (3). Due to the peracute nature of disease and the failure of antibiotic therapy because of resistant nature of bacteria, so the effective control measures of disease could only be achieved by vaccination to face the new epidemic of disease. Various vaccine types have been developed e.g: broth bacterin, oil adjuvant vaccine, double emulsion vaccine and live vaccine (2). BCG (Bacillus Caimette Guerin) vaccine is used for immunization against tuberculosis and leprosy in humans and animals (4). Some studies showed that BCG vaccine could improve and potentiate both humoral and cellular immune responses as non specific immuno - stimulatory agent in experimental animals (5) This study was aimed to investigate the role of synergistic effect of BCG to different *P. multocida* vaccine as immuno potentiation.

## Materials and Methods

- **Bacterial isolate and vaccines:**

A. *Pasteurella multocida* isolate was supplied by Al-Kindi company for veterinary Drugs and Vaccines production, Baghdad– Iraq and the biochemical test were done for this isolate to confirm diagnosis according to (6).

B. *Pasteurella multocida* vaccine (alum- precipitated killed whole cell vaccine) supplied by Al-Kindi company for veterinary Drugs and Vaccines productions, Baghdad – Iraq.

C. BCG (Bacillus Calmette Guerin) vaccine supplied by Respiratory and Chest Diseases Institute – Baghdad – Iraq.

- **Experimental design:**

A. Antigen preparation: killed whole cell antigen prepared according to (7).

B. Animals: Twenty five healthy local breed rabbits of both sexes, their weight were ranged between 1.25 kg were randomly divided into five groups: The first and second groups were immunized by 0.1ml of BCG Intradermal after 7 days. The first group was immunized by 1 ml (3× 10^)of killed whole cell *P. multocida* vaccine subcutaneously. The second group immunized by 1 ml (43.8 mg/ml) of sonicated whole cell *P.*

*multocida* vaccine. The third group was immunized by injection 1ml of killed whole cell *P. multocida* vaccine .The fourth group immunized by 1ml (43.8mg/ml) sonicated whole cell *P.multocida* vaccine and the fifth group was inject by 1 ml of phosphate buffer saline (PBS) and considered as the control group.

All animals of immunized groups were given a booster dose (antigen preparation) after 14 days from the first dose of immunization. After 28 days post immunization, skin test were done .Blood was collected from all animals every two weeks for three months to determine the antibodies titers by using passive haemagglutination test (8). The titer of antibodies were measured by the test,1,2 and 3 months.

- **Statistical analysis:** The results obtained are expressed as mean ± SE student's t-test that used to compare the means of the groups statements of statistical significance are based on $P<0.05$ (9).

## Results

- **Humoral immune response (Passive haemagglutination-PHA):** The humoral immune response showed increase of the antibodies titers in the first and third groups in the second and third months after immunization with a significant differences ($P<0.05$) while in the second and fourth groups the high antibodies titers were showed after one month and decline in second and third months that are statistically significant ($P<0.05$). Also there were a significant differences between the first and third groups as compared with the second and fourth groups in the first and third months after immunization (Table-1).

**Table (1) Titers of antibodies of different *P. multocida* vaccines in rabbits (PHA)**

| No. | Groups | Months (mean ± SE) | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 1. | Killed whole cell *P. multocida* with BCG vaccines | 819.2±96.04 Bb | 1843.2±348.2 Aa | 1945.6±389.8 Aa |
| 2. | Sonicated whole cell *P. multocida* with BCG vaccines | 2252.4±308 Aa | 1024±140.6 Ba | 563±51.3 Cc |
| 3. | Killed whole cell *P. multocida* vaccines | 742.5±208.3 Cb | 1100.8±379.8 Ac | 1792 ± 444.6 Aa |
| 4. | Sonicated whole cell *P. multocida* vaccines | 1894.4±377.3 Aca | 972.8±235.3 Bc | 409 ± 48 Bc |
| 5. | Control PBS | 8.8 ± 1.9 | | |

**Mean 5 animals of each group SE = standard error.**
**Capital letters denote a significant differences (P<0.05)**
**Small letter denote a significant differences (P<0.05) between groups**

- **Cellular immune response (Delayed type hypersensitivity-DTH):** Skin reaction was determined after 28 days of immunization which characterized by erythema and indurations (Thickness). This was evaluated by making the delayed type hypersensitivity test (DTH).

A. Erythema: The diameters of skin erythema (mm) after 24 hours of Intradermal injection with sonicated whole cell *P. multocida* concentration (43.8 mg/ml) 1:2, 1:4 and 1:8 showed increase in second and fourth groups compared with the first and third groups. Also same results were showed after 48 hours (Table -2).

B. Indurations (skin thickness): The indurations of flank skin area (mm) after 48 hours of Intradermal injection of *P. multocida* sonicated antigen of concentration (43.8mg/ml) 1:2, 1:4, and 1:8 showed a significant differences ($P<0.05$) of second and fourth groups

(sonicated whole cell *P. multocida* with BCG and sonicated whole cell *P. multocida* vaccines). Comparison to first and third groups (killed whole cell *P. multocida* with BCG, killed whole cell *P. multocida*) at 48 and 72 hours. (Table-3).

**Table (2) Erythema (mm) of skin test of different *P. multocida* vaccines vaccines in immunized rabbits**

| No. | Groups | Erythema (mm) mean ± SE 24 hr | | | Erythema (mm) mean ± SE 48 hr | | |
|---|---|---|---|---|---|---|---|
| | | 1:2 | 1:4 | 1:8 | 1:2 | 1:4 | 1:8 |
| 1. | Killed whole cell *P. multocida* with BCG vaccines | 3.34±0.3 Ab | 2.2±0.2 Ba | 1.64±0.12 Cb | 2.44±0.3 De | 1.56±0.2 Ee | 1.34±0.09 Feh |
| 2. | Sonicated whole cell *P. multocida* with BCG vaccines | 4.6±0.5 Aa | 3.26±0.4 Bb | 2.32±0. 2 Ca | 3.62±0.5 Ed | 2.94±0.4 Ed | 2.1±0.2 Cd |
| 3. | Killed whole cell *P. multocida* vaccines | 2.0±0.03 Ac | 1.48±0.12 Bc | 1.28±0.07 Cc | 1.62±0.13 Df | 1.28±0.08 Be | 1.28±0.05 Ce |
| 4. | Sonicated whole cell *P. multocida* vaccines | 4.1±0.3 Aa | 2.96±0.3 Bab | 2.34±0.21 Ca | 3.7±0.3 Dd | 2.32±0.2 Bd | 1.88±0.3 Cdh |
| 5. | Control PBS | 0±0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |

**Mean 5 animals of each group, SE = standard error.**
**Capital letters denote a significant differences (P<0.05)**
**Small letter denote a significant differences (P<0.05) between groups.**

**Table (3) Indurations (Thickness) of skin test of different *P. multocida* vaccines in immunized rabbits**

| No. | Groups | Indurations mean ± SE 24 hr | | | Indurations mean ± SE 48 hr | | |
|---|---|---|---|---|---|---|---|
| | | 1:2 | 1:4 | 1:8 | 1:2 | 1:4 | 1:8 |
| 1. | Killed whole cell *P. multocida* with BCG vaccines | 2.22±0.3 Ab | 1.82±0.14 Bc | 1. 4±0. 2 Ca | 3.54±0.5 Dd | 2.64±0.2 Ee | 1.82±0.2 Cf |
| 2. | Sonicated whole cell *P. multocida* with BCG vaccines | 3.9±0.6 Aa | 2.64±0.4 Bbd | 1.82±0. 4 Ca | 5.0±0.4 Dd | 3.14±0.6 Ee | 2.4±0.4 Ff |
| 3. | Killed whole cell *P. multocida* vaccines | 2.76± 0.3 Ab | 2.24± 0.3 Acd | 1.66± 0.2 Ca | 3.28± 0.2 Af | 2.62± 0.3 Bf | 1.96± 0.2 Ce |
| 4. | Sonicated whole cell *P. multocida* vaccines | 4.3±0.5 Aa | 3.32±0.3 Bab | 1.9±0.13 Ca | 6.3±0.6 Dd | 4.18±0.5 Bd | 2.28±0.13 Fe |
| 5. | Control PBS | 0±0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |

**Mean 5 animals of each group, SE = standard error.**
**Capital letters denote a significant differences (P<0.05)**
**Small letter denote a significant differences (P<0.05) between groups.**

## Discussion

Our results showed a higher level of antibodies titers in group that vaccinated with killed whole cell of *P. multocida* vaccine which agree with (10), who referred to induct of humoral immunity and active protection with this vaccine, principally IgG classes with only a mild IgM response. Also this vaccine induce a substantial B-cell response leading to copious plasma cell production and the release of specific antibodies into the blood stream (11). On the same hand, Modified alum precipitated vaccine produce satisfactory immunity up to 4 months post vaccination also the sera collected during 4 to 6 months post

vaccination induce an acceptable protection and enough potent to produce immunity against direct challenge (12). (13) found that a vaccine prepared from *P. multocida* A:3 outer membrane proteins provide a significant protection in rabbits against homologous challenge; while lipopolysaccharide-LPS alone induce only a partial protection (14). BCG vaccine act as a potent immune stimulator has revealed its ability to efficiently elicit both humoral and cellular immune response (15). Also BCG induces interluken-4 (IL-4) production from bone marrow precursor cells and B-Lymphoid precursor which can directly cause development of T-helper 2 (Th-2) immune response (16). On same hand, (17) mentioned that vaccination with BCG stimulate humoral and cellular responses and increased all types of immunoglobulins especially IgG. These result support our results that the antibodies titers increased in the first month in the first and second groups. Skin test was used to test if the prior exposure to an antigen had occurred when a small quantities of extracted antigen are injected Intradermal, an obvious mark and monocytic infiltration into the site of the lesion within 24 to 72 hours (18). The interferon and TNF-α stimulate lymphocyte immigration into the skin following Intradermal inoculation, the interferon and TNF –α and β all increase binding of lymphocytes to micro vascular epithelium presume these two phenomena are linked because the interferon recruit lymphocytes to inflammatory sites (19). The memory T cells proliferate more as a response to known antigens and produce a higher amounts of cytokines than naive T cells (20) and the ability and activity of DTH test depend on T-helper cell to recognize antigen and the secrete of IL-1 which enhanced proliferation and differentiation of other T cells into T-helper cells that secrete IL-2 as achematractive factor to attract macrophage around the area of activated T cell; also secrete INF – gamma which enhancing the cytolysis activity of accumulated macrophages leading into a skin indurations (21). Our results showed an increase in the diameter of indurations often 48hrs as considered a positive reaction and increased after 72hrs specially in the groups of sonicated whole cell *P. multocida* vaccines. These may be due to an antigen presenting cells which first take up a foreign protein. The protein antigen was broken into peptides and bound to an major histocompatibility- MHC class II molecules and acquire a conformation by the T- cell antigen receptor. T- cells that were committed to develop helper activity or lymphokines secretion (19). On the same way groups that vaccinated with BCG, the proteins of mycobacterium have been recognized play a major role in the host immunity and serve as a targets in the specific immune response. The mycobacterium cell wall was known to be a strong adjuvants (22) and also induce increases the costimalatory molecules CD80 and CD86 (23). Our conclusion explain that we can use both sonicated whole cell *P. multocida* either alone or with BCG vaccines for vaccinated livestock animals against hemorrhagic septicemia.

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