

Evaluation of Cross Protective Efficacy of Commercial Vaccines against *Mannheimia haemolytica* in Mice

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Summary

Mannheimia haemolytica together with *Pasteurella multocida* represents as a major bacterial causative agent of cattle, sheep and goats respiratory diseases and its one of the most important causes for economic losses to these animals. Commercially available vaccines were used to prevent infections caused by *P. multocida* and *M. haemolytica*. Thus, the aim of the present study was to evaluate the cross protection efficacy of two vaccines to protect mice against *M. haemolytica*, studying humeral immunity, using Enzyme-Linked Immunosorbent Assay. Forty five mice were divided into three equal groups, group one and two were inoculated subcutaneously 4µl JOVAPAST® and 1µl of Al-kindy vaccines respectively, while the third group was with 0.5 ml sub cutaneous PBS. LD50 for *M. haemolytica* was estimated as 2×10^6 cfu/ml and challenge test was conducted by dropping 0.05 ml 2×10^6 cfu/ml intranasally after three weeks of immunization for the three groups. The results of Enzyme-Linked Immunosorbent Assay, showed significant increase of antibody titters at ($P < 0.01$) in (group 1 and 2) after first and second weeks post immunization, in comparison with control group. Also, the re-isolation of *M. haemolytica* from lungs tissue of all groups after challenged were positive with significant difference between control and immunized group, control group was 4×10^8 cfu/ml which was higher than immunized group one and group two, which were 2.5×10^4 cfu/ml and 3.5×10^5 cfu/ml respectively after 24 hour of vaccine. In conclusion, the two commercial vaccines showed good cross protection efficacy against *M. haemolytica*, but JOVAPAST® vaccine showed higher efficacy than Alkindy vaccine, as that it contain two heterologous killed strains and providing the basis for production a vaccine from the two pathogen of local strains.

Keywords: *P. multocida*, *M. haemolytica*, vaccine, ELISA.

Introduction

Pneumonic pasteurellosis caused by *Mannheimia haemolytica*, which is commensals in the nasopharynx of many domestic and wild animals and its one of the major problems in sheep, goats and cattle. It is responsible for considerable economic losses to these animals and other livestock industries in many parts of the world (1 and 2). In Iraq, *Mannheimia haemolytica* and *Pasteurella multocida* were main causative agent in outbreak of pneumonic pasteurellosis in mountain goats, gazelles and deer's in a social sector field, (*M. haemolytica* and *P. multocida*) were isolated from nasopharynx swab of infected animals and apparently healthy goats (3). Because *M. haemolytica* remains extracellular in pneumonic pasteurellosis, humeral immunity is probably most important in protection against infection (4). The B cells

play a large role in the humoral immune responses which make antibodies identify and neutralize invading pathogens. It is well known that immunoglobulins functions as opsonins for a great number of phagocytosis resistant bacteria (5).

The cross protection between *Pasteurella* species have been demonstrated and judged by gel diffusion precipitation analysis (6). Mukkur., (1977) reported, that there is a cross protection between *P. multocida* type A and *P. haemolytica* serotype 1, because they possess sharing common immunogenic antigens(s), this study deals with immunizing mice with either the KSCN extract or formularized whole cells of *M. haemolytica* followed by a challenge with *P. multocida* to determine cross-protection, it was exciting that mice immunized with 1.0 OD unit of *P. haemolytica* KSCN extract protected 100% than the

immunized with formularized *P. haemolytica* vaccine (7). Also, (8) study the Immunogenicity of *Pasteurella multocida* and *Mannheimia haemolytica* outer membrane vesicles (OMV and OM), the study revealed similar protein profiles between the respective OMV and OM preparations of *P. multocida* and *M. haemolytica*. Also (9) study the Immunogenicity of *M. haemolytica* outer membrane proteins (OMPs) and Lipopolysaccharide (LPS), compared with a commercial vaccine (contain killed *P. multocida* and *M. haemolytica*) by ELISA and challenged test with *M. haemolytica*, thus demonstrating cross protection between the two pathogens. According to the above Knowledge's and little information's were reported on cross protection, so this study suggested to evaluate the cross protection between the *P. multocida* and *M. haemolytica*. So, the aim of this study was to evaluate the cross protective efficacy of two commercial vaccines against heterologous or homologous strain.

Materials and methods:

M. haemolytica strain was obtained from the Microbiology Department /College of Veterinary Medicine. The strain (Acc.No:MG897458) was transferred from brain heart infusion broth containing 15% glycerol, sub cultured on blood agar and incubated for 24hr at 37°C, and then the strain was inoculated in mice to get a fully encapsulated virulent form, and the virulent organisms isolated from mice organs streaked on blood agar and MacConkey agar incubated 24 hr at 37 °C. Growth was checked and tested for purity by staining with Gram's stain.

Two commercial vaccine were used in this study, (JOVAPAST®) was supplied by Jordan Industrial Biological Center inactive vaccine contain *P. multocida* serovars A , B and *M. haemolytica* serovars A1, second vaccine was supplied by Al-Kindy company for the production of vaccines and veterinarian drugs in Baghdad. This vaccine contains *P. multocida* serovars B.

Bacterial count and Lethal Dose 50 (LD50): Viable cell bacterial count was conducted according to (10) by transferring (0.05 ml per

drop) from each diluted tube (10-1 to 10-10) and culture on blood agar plate, then incubation at 37°C for 18–24 hours. The CFU was estimated using five dilutions corresponding to 2×10^6 , 2×10^5 , 2×10^4 , 2×10^3 and 2×10^2 to estimate the dilution that gave countable number of colonies. While the LD50 was estimated according to (11). Thirty mice were divided into five groups (n=6), each group was intraperitoneally injected with 0.3 ml of five dilutions, all groups were monitored for 12 days to calculate total live and dead mice.

The efficacy of vaccine which was evaluated by ELISA (12) and by the challenge test (9). Evaluation of humeral immunity by ELISA test: Forty five mice were divided randomly into three equal groups, first group was inoculated SC with 4µl of JOVAPAST® vaccine and the second group was inoculated 1µl of Al-Kindy vaccine, while the third group was inoculated 0.5 ml\Sc PBS. Blood was collected, after anaesthetization and aspirated blood by insulin syringe from the heart of mice of each animal of the groups, before and post immunization for three weeks, and serum was separated, allowed to clot overnight at 4°C then centrifuged at 3000 rpm for 10 min. The separated sera were stored at -20°C until used for measuring antibody titer by ELISA test kit (XpressBio\USA) which was used according to manufactures instructions.

After third week post immunization of mice, remaining mice thirty two were divided into three groups equally, all groups were challenged with (2×10^6 cfu/ml) LD50 of *M. haemolytica*. The mice were inoculated with 0.05 ml intranasally by dropping with micro titter pipette, mice were monitored every 6 to 8 hours for any signs of clinical illness (13).

Results and Discussion

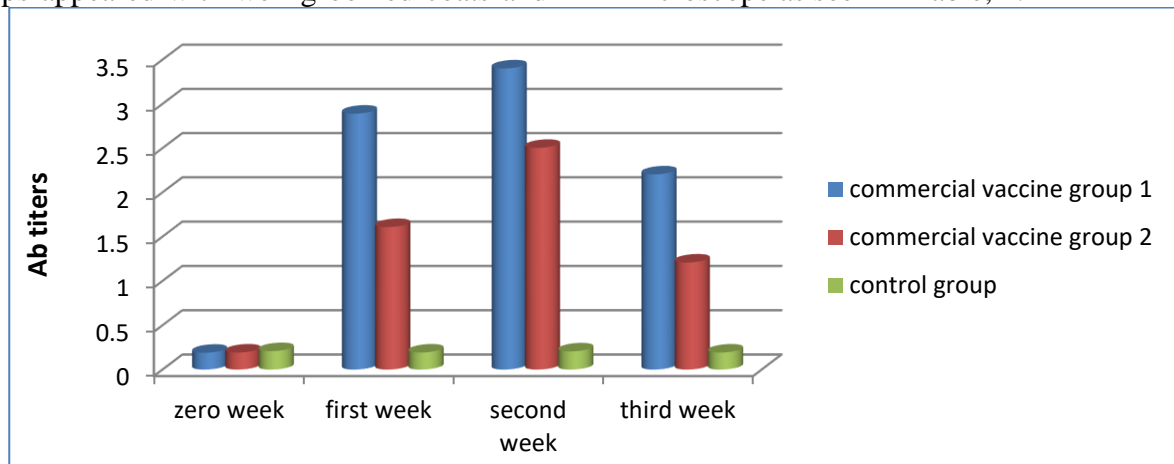
Determination of Lethal Dose 50: The result of estimation showed mortality 100% in group one which was injected with (2×10^7 cfu/ml) and 50% mortality in group two which was injected with (2×10^6 cfu/ml). The clinical signs observed in group with mortality only, while in other groups no clinical signs were observed. In current study the LD50 of

M.haemolytica was determined as a 2×10^6 cfu /ml.

ELISA: The results of antibody titer values of immunized groups (commercial vaccine 1 and 2) showed significant increase in antibody titers at ($P < 0.01$) after (first and second weeks) post immunization in comparison with (control group), while they showed significant decrease of antibody titers at ($P < 0.01$) after third week as appeared in (Fig.1 and Table, 1):

Challenge Test: No clinical signs were observed on the challenged mice after 6 hr, all groups appeared with well-groomed coats and

normal activity. While after 24 hour the mice of control positive group appeared dull with scruffy coat and abnormal activities while other groups appeared to be unaffected. After that *Mannheimia haemolytica* has been isolated from lungs of all challenged group's at 24 and 48 hr post inoculation. Also as seen in (Table, 2) ,the bacteria were isolated from lungs tested culturally and biochemically according to (14), the results of these tests showed typical cocobacilli, G-ve and bipolar with methylene blue stain under light microscope as seen in Table, 2.



Figure, 1: Antibody titers in the immunized and control groups in ELISA test.

Table, 1: Antibody titers in the immunized and control groups in ELISA test.

The group	Zero week	First week	Second week	Third week
Commercial vaccine group 1	0.19 0 b	2.89 0 a	3.40 1 a	2.203 ab
Commercial vaccine group 2	0.19 2 b	1.60 8 ab	2.50 3 a	1.208 ab
Control negative group	0.20 8 b	0.19 2 b	0.20 5 b	0.192 b
LSD value	2.26070169**			

- Similar small letters represent no significant differences.
- Different small letters represent significant differences at level of $P < 0.01$ **.

Table, 2: Re-isolation of *M.haemolytica* from lung after intranasal challenge.

The group	Bacterial load	
	24 hr	48 hr
Commercial vaccine (group1)	2.5×10^4	1×10^4
Commercial vaccine (group1)	$3,5 \times 10^5$	2.5×10^4
Control positive	4×10^8	3×10^8

Determination of (LD₅₀) of *M. haemolytica* was determined as (2×10^6 cfu/ml), this result is agree with (9), who estimated the same value for *M. haemolytica* which was isolated from nasal mucous of slaughtered goats. Previous investigation reported cross-reactivity between different strains of *P. multocida* (7 and 15). In this study, the results showed that immunized mice with the two commercial vaccines stimulated immunity compared with control group (Table, 1), with an increase in antibody titer value in immunized groups after first and second week post immunization in comparison with the control group. That's due to similar protein profiles between the outer membranes vesicles (OMVs) of *P. multocida* and *M. haemolytica*, this result is in agreement with (16) who found that the Bactrian vaccine for *P. Multocida* and *M. haemolytica* (one dose) could be efficient for increasing the antibodies level than normal produce. This characteristic feature of OMVs has also been described for another member of the Pasteurellaceae family (8). As well as (7) demonstrated a cross-protection between *P. multocida* type A and *M. haemolytica*, serotype 1, who immunized the mice with the potassium thiocyanate extract of *M. haemolytica* serotype 1, and showed resistant to a challenge infection with *P. multocida* type A.

The reduction in antibody level at third week indicated that a booster dose was required. The booster dose, might be delivered at 3rd week, significantly increased the antibody production for the next 2 weeks (Weeks 4 and 5) before they were challenged (17). In this study, the challenged test was conducted intranasally by dropping, that the intranasal infection was the effective route for experimental infection (13). The challenge with live *M. haemolytica* was carried out on day 28th post vaccination. While (18) showed that the challenge of animals in the vaccination trial was most suitably done at between Days 28 and 35 post vaccination, it acted as another booster to the immune system resulting in even higher antibody levels (18). The re-isolation from lung in all group showed variation in bacterial load. The reason for the clearance of *M. haemolytica* from mouse lungs following aerosol exposure has been documented to be

efficient due to the influx of neutrophils into pulmonary tissues after exposure to *M. haemolytica* is thought to be responsible for efficient elimination of the bacteria from the lungs of mice (19). In conclusion, the two commercial vaccines showed good protection against *M. haemolytica*, but JOVAPAST® showed higher efficacy than Al-Kindy vaccine, as that it contain two heterologous inactivated strains.

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تقييم كفاءة الحماية التصالبية للقاحات التجارية ضد جرثومة *M.haemolytica* في الفئران

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الخلاصة

تعد جرثومتي (*M.haemolytica* و *P.multocida*) من العوامل الرئيسية المسببة لأمراض الجهاز التنفسي في الأبقار والأغنام والماعز و أحد أهم أسباب الخسائر الاقتصادية التي لحقت بهذه الحيوانات. استخدمت اللقاحات المتاحة تجارياً للوقاية من الإصابات التي تسببها هاتين الجرثومتين ، لذلك كان الهدف من هذه الدراسة هو تقييم كفاءة الحماية التصالبية لنوعين من اللقاحات لحماية الفئران ضد جرثومة *M.haemolytica* وذلك من خلال دراسة المناعة الخلطية باستخدام اختبار الأليزا (ELISA). تم تقسيم خمسة وأربعين من الفئران وقسمت إلى ثلاثة مجاميع متساوية. تم تلقيح المجموعة الأولى والثانية بلقاحات JOVAPAST® 4µl 1µl، من لقاح الكندي تحت الجلد ، على التوالي، بينما لقت المجموعة الثالث 0.5 ml PBS \ كمجموعة سيطرة. حددت الجرعة القاتلة لنصف العدد (LD50) $(2 \times 10^6 \text{ cfu/ml})$ ، لـ *M.haemolytica* ، وتم إجراء اختبار التحدي عن طريق التقطير داخل الأنف لفئران المجاميع الثلاثة بجرعة 0.05 ml $(2 \times 10^6 \text{ cfu/ml})$ وبعد ثلاثة أسابيع من التمنيع تم أخذ نماذج من الحيوانات وإجراء اختبار الأليزا (ELISA). أظهرت نتائج الاختبار زيادة كبيرة في عيارية الأجسام المضادة ($P < 0.01$) في المجموعتين (الأولى والثانية) بعد (أسبوع – أسبوعين) بعد التمنيع للمقارنة مع مجموعة السيطرة. كما تم إعادة عزل جرثومة *M.haemolytica* من أنسجة الرئة لجميع المجاميع التي أظهرت إيجابية مع وجود فرق كبير بين مجموعة السيطرة وبقية المجاميع الممنعة، حيث كانت مجموعة السيطرة $(4 \times 10^8 \text{ cfu/ml})$ أعلى من المجموعة الممنعة الأولى والثانية $(2.5 \times 10^4 \text{ cfu/ml})$ و $(3.5 \times 10^5 \text{ cfu/ml})$ على التوالي بعد 24 ساعة . واستنتجت الدراسة بان اللقاحان التجاريان أظهرتا حماية جيدة ضد جرثومة *M.haemolytica* و أظهر لقاح JOVA PAST كفاءة أعلى من لقاح الكندي ، لاحتوائه عترتين متشابهتين مقتولتين.

الكلمات المفتاحية: باستريلا ملتيسيدا، مانيميا هيموليتيكا، لقاح ، الأليزا.