

EVALUATION OF SOME BIOCHEMICAL AND IMMUNOLOGICAL STUDIES IN RABBITS VACCINATED WITH RADIATED AND FORMALIZED INACTIVATED *PASTEURELLA MULTOCIDA* VACCINES

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Abstract

Pure cultures of *P. multocida* type 5.A virulent strains were treated with doses of 103 k.rad by Gamma radiation. The treated cells lost their ability to grow but retained immunogenic activities as determined by serological and challenge tests, were considered as "a vaccine" and used to immunizing rabbits twice (15 days apart) with a dose level of 1ml s/c.(GrII) as compared with killed formalized oil adjuvant vaccine (GrIII) and control non - vaccinated (GrI). It could be considered that the immune response after 15, 25, 35, 45 after 1st dose of vaccination was excellent by both irradiated and formalized vaccines without any significant harmful side effects on liver, kidney, and the immune response functions. The immune response against irradiated vaccine was high in potency and longer in duration than formalized vaccine.

It could be concluded that the gamma irradiated inactivated vaccine of *P. multocida* type 5. A is safe and recommended for use as a protective vaccine against *P. multocida*.

INTRODUCTION

Pasteurellosis remains a common disease problem in commercially produced rabbits. It is responsible for severe economical losses. Antibiotics were only partially successful in controlling infection. There is no effective vaccine to prevent infection by this organism (Jarvinen *et al.* 2000).

Ionizing radiation could introduce a possible mean to overcome such problem and it seems that the progress in the application of ionizing radiation is in preparation of inactivated *P. multocida* type 5: A vaccine. Since toxins are very rich in proteins, it was of interest to know if one can get rid of bacterial toxins and retain the immunogenicity of the toxin by suitable choice of radiation types, doses strength (Kankonkar *et al.*

1975). Pure cultures of *P. multocida* type 5 : A were irradiated by doses of 103 k.rad by gamma radiation, the treated cells lost their ability to growth but retained immunogenic activities (Arkhipov *et al.* 1969) and (Shaker *et al.* 1992). Total globulins and specific gamma globulins were increased after vaccination by irradiated or Formalized vaccines in poultry. (Shaker *et al.* 1992). Also, serum immunoglobulins, IgG, IgM and IgA were highly increased in case of vaccinated rabbits by *P. multocida* toxins (PMT) Jarvinen *et al.* 2000).

Nerkar *et al.* (1977) recorded the advantage of irradiated vaccine over the conventional one as the radiation not only kills the bacteria but detoxifies the endotoxin within the cells. Bhat *et al.* (1984) found no significant change in serum bilirubin level in lambs immunized with two doses of gamma irradiated larvae of *Dityocaulus filaria*. Shaker *et al.* (1992) in case of vaccinated chicken by two doses of irradiated vaccine of *P. multocida*, found no significant change in liver and kidney functions.

The present work was performed to study the influence of ionizing radiation on *P. multocida* in order to obtain a vaccine and evaluation of safety of the vaccine on liver, kidney functions and immune response.

MATERIALS AND METHODS

Experimental animals

Seventy - two males newzealand rabbits of 8 weeks old were used in the experimental work.

Experimental design

The animals were divided into three groups (1,2,3) each group composed of 24 rabbits and housed in separate metal cages and provided with water and fed on constant growth ration of Atmida company.

Gr 1. unvaccinated control, Gr.2. vaccinated by irradiated *P. multocida* type 5.A vaccine, Gr 3. vaccinated by formalin killed *P. multocida* type 5. A vaccine at a dose level of 1ml per rabbit s/c, and a booster dose was given 15 days later to the initial one by the same dose and route of injection.

All groups were s/c challenged thirty day after the 2nd vaccination with 0.1ml of virulent strain containing 10^9 CFU of *P. multocida* (Okermen and Spanoghe, 1981). The blood samples were collected from all animal groups before and after vaccination

at intervals of 15, 25, 35 and 45 days from the initial dose of vaccination. Sera were separated and kept in a deep freeze at - 20°C until used for the biochemical, immunological and serologica investigations.

Serological test

Indirect haemagglutination test (IHA): it was done according to Carter and Rappy (1967).

Biochemical test

Serum protein Fractions

It was done according to Laemmli (1970).

Serum immunoglobulins (IgG, IgA and IgM)

It was done according to Erhard *et al.* (1992).

Serum Alanine aminotransferase (ALT)

It was done according to Colomb *et al.* (1974),

Serum Aspartate aminotransferase (AST)

It was done according to Reitman and Frankel (1957).

Serum Bilirubin and creatinine

It was done according to Tietz (1976).

RESULTS AND DISCUSSION

The obtained data (Table 1) shows that the dose of gamma rays 103 k rad lead to complete inactivation of *P. multocida*. The geometric mean titres (GMT) in serum of rabbits vaccinated with formalized and gamma irradiated vaccines as measured by (IHT) are 1156.25 and 1337 compared to control (5.25) (Tobl 2 and Fig 1). The protection % in case of challenge in rabbits vaccinated by formalized and irradiated vaccines was 100% compared to control rabbits which showed 100% mortality (Table 3).

Serum total globulins reveled that significant increase (table-4 and Fig 3) and serum Albumin / Globulin A/G ratios were signifcant decreased. (Table 5, Fig 4).

Serum gamma globulin and immunoglobulins IgG, IgA and IgM in all vaccinated rabbits showed a significant increase along the experiment (Table 6) and table 9. The Biochemical test of liver and kidney function showed no significant changes in all vaccinated rabbits Table 10 , 11 ,12 and 13).

Dose of gamma radiation inactivation on *P. multocida* type 5.A was 103 krad as recorded by Arkhipov *et al.* (1969) and Shaker *et al.* (1992). The results of indirect hemagglutination titers in all vaccinated groups were increased and reached their highest value on the 45 day after the initial dose of vaccination and the protection rate was 100% in all vaccinated groups. The highest titer was recorded in groups vaccinated by irradiated vaccine and this is related to the effect of vaccine on the immune responses as recorded by Nerkar *et al.* (1977) who suggested that the radiation, not only kills the bacterial cells of salmonella, but also significantly detoxifies the endotoxin within the cells.

The biochemical results showed that there was a significant increase in serum total globulins levels, specially, gamma globulins and immunoglobulins (IgG, IgA and IgM) in all vaccinated groups. This significance was high in rabbits vaccinated by irradiated vaccine. (Tables 4, 6, 7, 8 and 9). This increase is due to the induction of high immune response in rabbits due to vaccination as recorded by (Jarvinen *et al.* (2000). Also, these results showed that there was no any significant change in liver and kidney functions as recorded by Shaker *et al.* (1992). (Tables 10 , 11, 12 and 13).

It could be concluded that rabbit *pasteurella multocida* type 5.A vaccine inactivated by irradiation is highly immunogenic with 100% protection and safe.

Table 5. Protection in rabbits vaccinated with *Pasteurella multocida* vaccine as recorded by homologous challenge (100 MICID₅₀) 10 days post 2nd vaccination

Group	Number of rabbits	Dead/ survived	Total No. survived	Protection %
1	10	0/10	10	100
2	10	0/10	10	100
3	10	0/10	10	100
4	10	0/10	10	100
5	10	0/10	10	100
6	10	0/10	10	100
7	10	0/10	10	100
8	10	0/10	10	100
9	10	0/10	10	100
10	10	0/10	10	100
11	10	0/10	10	100
12	10	0/10	10	100
13	10	0/10	10	100

Table 1. Effect of gamma irradiation on inactivation of pasteurella multocida type (P.4)

Bottle No.	Dose rate Mrad/hr	Time of irradiation / minutes	Dose of irradiation (Krad)	Bacterial activity	Bacterial count / G
1	0.618	2.5	25.75	Active	9×10^8
2	0.618	5	51.5	Active	9×10^8
3	0.618	7.5	77.25	Active	5.69×10^8
4	0.618	10	103	Inactive	-
5	0.618	12.5	128.75	Inactive	-

K- rad= 1000 rad
Kgy= 100 K. rad

Gray (Gy) = 100 rad
Mega= 10 K. Gy

Table 2. Geometric mean (GM) of antibody titers as measured by IDA test in sera of rabbits vaccinated by irradiated and formal P. multocida vaccines (n=24).

Type of vaccine	Pre-vaccination	Days post vaccination after 1 st vaccine				(G.M.T)
		15	25	35	45	
Irradiated vaccine	5	485	844	1949	2079	1337
Formalized vaccine	11	391	735	1589	1815	1158.25
Control non vaccinated	5	5	5	5	5	5.25

GMT = geometric mean titers

Table 3. Protection in rabbits vaccinated with pasteurella multocida vaccines as determined by Homologous challenge (100 MLD50/ 0.10 ml) at 30 days post 2nd vaccination.

Groups	Number of rabbits	Died/ survived	Total No. survived	Protection %	Lesion score
Irradiated V.	8	0/8	8	100%	-
Formol. V.	8	0/8	8	100%	-
Non vaccinated	8	8/0	0	0%	+++

V= Vaccine.
(-) = non.
(+++)= severe

Table 4. Mean values of serum total globulins concentration in rabbits subjected to gamma irradiated and formol vaccine (g/L). (n=8)

Animal Groups Time post vaccination /day	Control	Irradiated vaccine	Formol vaccine	F Calculated	F Tabulated at P < 0.05	LSD	
						0.05	0.01
15 day	26.51 ^a ± 1.33	33.77 ^b ± 0.51	31.38 ^b ± 0.93	4.553	3.88	3.03	4.25
25 day	25.65 ^a ± 0.95	33.002 ^b ± 0.27	29.86 ^b ± 0.36	13.815		3.51	4.92
35 day	26.62 ^a ± 0.78	32.12 ^a ± 1.84	30.55 ^a ± 1.46	3.756		N.S.	
45 day	25.62 ^a ± 1.088	30.11 ^b ± 0.71	27.50 ^{ab} ± 0.95	5.850		2.87	4.03

LSD: Least significant difference.

N.S. non significant at P < 0.05

- Values have the same letters are non significantly different at P < 0.05

- Values have different letters are significantly at P ≤ 0.05.

Table 5. Mean values of serum albumin / globulin ratio in rabbits subjected to gamma irradiated and formol vaccine (n=8).

Animal Groups Time post vaccination /day	Control	Irradiated vaccine	Formol vaccine	F Calculated	F Tabulated at P < 0.05	LSD	
						0.05	0.01
15 day	1.42 ^a ± 0.089	1.092 ^b ± 0.004	1.16 ^b ± 0.049	5.427	3.88	0.098	0.137
25 day	1.52 ^a ± 0.053	1.084 ^b ± 0.033	1.17 ^b ± 0.088	10.798		0.225	0.328
35 day	1.41 ^a ± 0.08	1.16 ^a ± 0.057	1.23 ^a ± 0.085	3.873		N.S.	
45 day	1.5 ^a ± 0.060	1.268 ^b ± 0.028	1.392 ^c ± 0.047	27.009		0.073	0.106

LSD: Least significant difference.

N.S. non significant at P < 0.05

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Table 6. Mean values of serum gamma globulin concentrations (gm/L) in rabbits subjected to gamma irradiated and formol vaccine (gm/L). (n=8).

Animal Groups Time post vaccination /day	Control	Irradiated vaccine	Formol vaccine	F Calculated	F Tabulated at P < 0.05	LSD	
						0.05	0.01
15 day	7.84 ^a ± 0.66	12.07 ^b ± 0.21	11.02 ^b ± 0.75	28.441	3.88	1.812	2.539
25 day	7.99 ^a ± 0.322	13.55 ^b ± 0.26	11.53 ^c ± 0.52	77.424		1.186	1.663
35 day	7.98 ^a ± 0.15	13.27 ^b ± 0.74	11.42 ^c ± 0.36	50.870		1.491	2.089
45 day	7.86 ^a ± 0.15	13.23 ^b ± 0.28	10.58 ^c ± 0.48	22.181		1.021	1.432

LSD: Least significant difference.

N.S. non significant at P < 0.05

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Table 7. Mean values of serum immunoglobulin IgG concentrations in rabbits subjected either to gamma irradiated and formol vaccine (mg/dl). (n=8).

Animal Groups Time post vaccination /day	Control	Irradiated vaccine	Formol vaccine	F Calculated	F Tabulated at P < 0.05	LSD	
						0.05	0.01
15 day	1414.75 ^a ± 46.27	1864.07 ^b ± 44.91	1624.82 ^c ± 34.06	31.31	3.88	124.79	173.564
25 day	1381.74 ^a ± 8.075	1942.8 ^b ± 16.9	1881.6 ^c ± 30.31	202.68		63.399	88.888
35 day	1371.74 ^a ± 11.223	2103.54 ^b ± 40.35	1941.66 ^c ± 25.88	183.01		87.558	122.759
45 day	1464.04 ^a ± 28.024	1990.49 ^b ± 36.80	1867.53 ^c ± 15.41	60.805		86.744	121.616

LSD: Least significant difference.

N.S. non significant at P < 0.05

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- Values have different letters are significantly at P ≤ 0.05.

Table 8. Mean values of serum immunoglobulin (IgA) concentrations in rabbits subjected to gamma irradiated and formol vaccine (mg/dl). (n=8).

Animal Groups Time post vaccination /day	Control	Irradiated vaccine	Formol vaccine	F Calculated	F Tabulated at P < 0.05	LSD	
						5%	1%
15 day	20.076 ^a ± 0.642	23.028 ^a ± 0.948	23.43 ^a ± 1.04	2.973	3.88	N.S.	
25 day	20.336 ^a ± 0.639	25.39 ^b 1.07	24.900 ^b ± 0.94	21.391		1.965	2.859
35 day	22.482 ^a ± 0.783	29.316 ^b ± 2.32	25.69 ^{ab} ± 0.84	4.618		5.189	7.550
45 day	22.704 ^a ± 0.860	24.42 ^a ± 1.096	23.52 ^a ± 0.92	0.6259		N.S.	

LSD: Least significant difference.

N.S. non significant at P < 0.05

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Table 9. Mean values of serum immunoglobulin (IgM) concentration in rabbits subjected either to gamma irradiated or formol vaccine (mg/dl). (n=8).

Animal Groups Time post vaccination /day	Control	Irradiated vaccine	Formol vaccine	F Calculated	F Tabulated at P < 0.05	LSD	
						5%	1%
15 day	157.38 ^a ± 2.21	170.72 ^b ± 1.43	167.73 ^b ± 1.22	13.226	3.88	6.276	9.131
25 day	158.25 ^a ± 1.14	204.83 ^b ± 2.07	201.64 ^b ± 5.92	38.991		13.52	19.77
35 day	160.72 ^a ± 1.17	180.50 ^b ± 1.56	177.91 ^b ± 4.12	27.368		6.716	9.771
45 day	157.45 ^a ± 2.015	158.59 ^a ± 10.19	161.63 ^a ± 0.499	1.983		N.S.	

LSD: Least significant difference.

N.S. non significant at P < 0.05

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Table 10. Mean values of serum Alanine aminotransferase (ALT) activity in rabbits subjected to gamma irradiated and formol vaccine (u/L). (n=8).

Animal Groups	Control	Irradiated vaccine	Formol vaccine	F Calculated	F Tabulated at P < 0.05	LSD
Time post vaccination /day						
15 day	61.20 ^a ± 2.99	61.19 ^a ± 3.59	61.19 ^a ± 2.89	0.056	3.88	N.S.
25 day	61.22 ^a ± 4.79	61.18 ^a ± 2.51	61.20 ^a ± 4.43	0.364		N.S.
35 day	61.19 ^a ± 3.96	61.18 ^a ± 2.1	61.19 ^a ± 4.55	0.0086		N.S.
45 day	61.20 ^a ± 1.12	61.19 ^a ± 1.7	61.15 ^a ± 1.52	1.132		N.S.

LSD: Least significant difference.

N.S. non significant at P < 0.05

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Table 11. Mean values of serum Aspartate aminotransferase activity (AST) u/L in rabbits subjected to gamma irradiated and formol vaccine. (n=8).

Animal Groups	Control	Irradiated vaccine	Formol vaccine	F Calculated	F Tabulated at P < 0.05	LSD
Time post vaccination /day						
15 day	39.09 ^a ± 0.83	39.43 ^a ± 1.55	38.72 ^a ± 0.78	0.219	3.88	N.S.
25 day	39.37 ^a ± 0.48	39.71 ^a ± 0.22	39.45 ^a ± 2.19	0.18		N.S.
35 day	38.40 ^a ± 1.21	38.61 ^a ± 0.20	38.85 ^a ± 0.83	0.525		N.S.
45 day	39.17 ^a ± 0.45	38.02 ^a ± 0.58	39.16 ^a ± 0.45	0.041		N.S.

LSD: Least significant difference.

N.S. non significant at P < 0.05

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Table 12. Mean values of serum Bilirubin concentration in rabbits subjected either by gamma irradiated and formol vaccine (mg/dl). (n=8)

Time post - vaccination/day	Control	Irradiated vaccine	Formol vaccine	F Calculated	F Tabulated at P < 0.05	LSD
15 D.P.V.	0.69 ^a ± 0.083	0.68 ^a ± 0.073	0.70 ^a ± 0.06	0.016	3.88	N.S.
25 D.P.V.	0.72 ^a ± 0.07	0.78 ^a ± 0.075	0.73 ^a ± 0.04	0.558		
35 D.P.V.	0.62 ^a ± 0.023	0.57 ^a ± 0.035	0.57 ^a ± 0.04	0.598		
45 D.P.V.	0.67 ^a ± 0.017	0.67 ^a ± 0.010	0.66 ^a ± 0.02	0.037		

LSD: Least significant difference.

N.S. non significant at P < 0.05

- Values have the same letters are non significantly different at P < 0.05

- Values have different letters are significantly at P ≤ 0.05.

Table 13. Mean values of serum creatinine concentrations in rabbits subjected either to gamma irradiated and formol vaccine (mg/dl). (n=8).

Animal Groups	Control	Irradiated vaccine	Formol vaccine	F Calculated	F Tabulated at P < 0.05	LSD
Time post vaccination /day						
15 day	1.074 ^a ± 0.12	1.16 ^a ± 0.17	1.14 ^a ± 0.15	0.088	3.88	N.S.
25 day	1.34 ^a ± 0.1	1.09 ^a ± 0.12	1.25 ^a ± 0.13	1.217		
35 day	1.35 ^a ± 0.13	1.2 ^a ± 0.13	1.36 ^a ± 0.17	0.382		
45 day	1.29 ^a ± 0.13	1.26 ^a ± 0.05	1.32 ^a ± 0.11	0.071		

LSD: Least significant difference.

N.S. non significant at P < 0.05

- Values have the same letters are non significantly different at P < 0.05

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تقييم بعض الدارسات الكيميائية والمناعية على الأرناب المحصنة بلقاح الباستيريلا ملتوسيدا المُخمد بطريقة الإشعاع والفورمالين

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تم عمل تشييع لميكروب الباستيريلا ملتوسيدا (١:٥) من النوع الضارى بجرعة ١,٣ كيلو
راد باستخدام أشعة جاما ووجد أن هذا الميكروب عند هذه الجرعة قد فقد القدرة على التكاثر ولكن
استمرت قدرته على إحداث المناعة والتي تم معرفتها باختبار التحدى والسيرولوجى واعتبر كلقاح
مخمد يمكن استخدامه بدرجة أمان عالية حيث تم تحصين الأرناب المقسمة إلى ٣ مجموعات ١, ٢, ٣.
أما المجموعة الأولى تم تحصينها بجرعة أولى من اللقاح المشع والجرعة الثانية كانت عند ١٥ يوماً
من الجرعة الأولى ومقارنة ذلك باللقاح الفورمالينى الزيتى فى المجموعة الثانية وذلك بمقارنتها
بالمجموعة الثالثة الكنترول (لم تحصن)، وقد تم اعتبار أن الاستجابات المناعية بعد التحصين عند
١٥, ٣٥, ٤٥ من جرعة التحصين الأولى كانت جيدة بواسطة كل من اللقاحات المشعة والفورمالين
بالمقارنة بالمجموعة الثالثة (الضابطة)، بدون إحداث أية آثار جانبية على وظائف كل من الكبد
والكلى والجهاز المناعى. ولكن وجد أن الاستجابة المناعية للقاح كانت أكثر فاعلية وأعطت فترة
مناعة أطول من اللقاح الفورمالينى.

وبهذا يمكن استنتاج أن اللقاح المشع بأشعة جاما أفضل من اللقاح الفورمالينى واعتباره
أنه لقاح واقى ضد الباستيريلا ملتوسيدا وذو درجة أمان عالية.

الخلاصة والتوصيات:

اللقاح المشع (Irradiated vaccine) اقوى وأطول فى التأثير المناعى ضد ميكروب
الباستيريلا ملتوسيدا من نظيره اللقاح الفورمالينى الزيتى لذلك يوصى باستخدام اللقاح المشع
لتحصين الأرناب ضد مرض التسمم الدموى المتسبب عن ميكروب الباستيريلا ملتوسيدا فى
مزارع الأرناب.