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HOST SPECIFIC CROSS PROTECTION USING PASTEURELLA MULTOCIDA BACTERIN PREPARED FROM IN-VIVO PROPAGATED STRAIN

(With 2 Tables and 4 Figures)

By

HASSAN ABD EL-MAKSOUD HASSAN; HALA AHMED FADL and WAFAA ALY GHONIEMY

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إحداث حماية نسيجيه بلقاح الباستيريلا ملتوسيدا الميت

حسن عبد المقصود حسن ، هالة احمد فضل ، وفاء على محمد غنيمى

أظهرت تلك الدراسة أن تحصين الكتاكيت بلقاح كوليرا الطيور الميت والمحضر من كبد الكتاكيت المحقونة بعترة ٥:أ يهيىء لها الحماية النسيجيه عند تعرضها للعدوى بأى من عترات الباستيريللا ملتوسيدا سواء المتماثلة أنتيجينياً مع عترة اللقاح (٥:أ) أو المختلفة معها (٨:أ ، ٩:أ). وعلى العكس فقد ثبت أن اللقاح المحضر من نفس العترة والسابق استزراعها معملياً على المستنبتات الغذائية يهيىء الحماية الجيدة للطيور عند تعرضها للعدوى بالعترة المتماثلة فقط مع عترة اللقاح (٥:أ). والحماية المزدوجة التى يوفرها النوع الاول من اللقاح تحدث من خلال عوامل أنتيجينية تسمى عوامل الحماية التبادلية والتى يتم تخليقها بجسم الكائن الحى المصاب وهذه العوامل لها صفة الخصوصية بمعنى أنها توفر الحماية التبادلية فقط لنفس نوع الكائن الحى المخلقة بجسمه. وقد ثبتت عملياً ذلك بحدوث حماية تبادلية لفئران التجارب المحقونة بلقاح محضر من أنسجة الفئران المصابة وليس باللقاح المحضر من أنسجة الكتاكيت المصابة حيث هذا اللقاح الأخير يحمى الفئران فقط من الإصابة بالعترة المتماثلة مع عترة اللقاح.

SUMMARY

Fowl cholera bacterin prepared from liver of chicken infected with strain 5:A induced satisfactory cross-protection in chickens against challenge exposures to either homologous strain (5:A) or heterologous strains of *P. multocida* belonged to the same capsular serogroup A (8:A and 9:A). A bacterin prepared from bacteria grown on laboratory media induced only homologous protection against challenge with the vaccinal strain (5:A). It appeared that the cross-protecting factors expressed in-vivo grown *P. multocida* were host-specific. This was evident as the chicken tissue bacterin induced only a homologous protection in mice whereas mouse tissue bacterin induced both homologous and heterologous protection in mice.

Key words: *Host specific, Cross protection, P.multocida, bacterin.*

INTRODUCTION

Fowl cholera (avian pasteurellosis), is an avian disease of major importance. It is caused by *Pasteurella multocida*. Serologic typing of the organism have been detected both capsular serogroups and sixteen somatic serotypes. Rhoades and Rimler (1987) indicated that capsular group A and D strains (commonly infect avian species) are associated with acute and chronic fowl cholera, respectively. Inactivated bacterins and/or live attenuated vaccines can be used to control fowl cholera (Suzan, 1992). Rimler and Rhoades (1981) reported that protection by inactivated bacterin is generally specific to those serotypes contained within the bacterin (homologous protection). Hofacre et al. (1989) pointed out that in live vaccines, protection generally crosses over to strains not necessarily incorporated into the vaccine (heterologous protection). Heddleston and Rebers (1972); Ibrahim and Sawada (1998) found that *P. multocida* bacterins prepared from tissues of infected turkey or chicken embryos can induce protection in turkeys or chickens respectively against different strain challenge exposures.

The aim of this work was to determine:

1. Whether cross-protection could be induced in chickens with *Pasteurella multocida* bacterin prepared from in-vivo-grown organism (Exp. I).
2. Whether this protection is associated with host-specific tissue in which the organism was grown (Exp. II).

MATERIAL and METHODS

1. Bacterial strains:

Four local strains of *P. multocida*, 5:A (x-73), 8:A (P-1059), 9:A, and 2:D, were obtained from Vet. Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. They were immunologically and serologically different (Collins, 1977; Carter, 1972). A bacterin prepared with one strain did not protect birds against the other strain (Heddleston, 1966).

2. Bacterin:

Oil-adjuvant bacterin was prepared with strain 5:A, which was propagated in-vitro on laboratory media as described by Heddleston (1966).

3. Tissue bacterin:

Chicken or mouse tissue bacterin was prepared with strain 5:A using the technique described by Rimler et al. (1979a) and Heddleston and Rebers (1972, 1974).

4. Experimental chickens and mice:

6-week-old Arbor Acres chickens were obtained from the General Poultry Company. Birds were free of antibodies to *P. multocida* as confirmed serologically (Heddleston et al., 1972). Careworth white mice were obtained from Laboratory Animal Dept. Serum and Vaccine Research Institute, Abbasia, Cairo.

5. Challenge of immunity:

Chickens and mice were challenged intramuscularly (IM) and intraperitoneally (IP) respectively as described by Heddleston and Rebers (1969).

Experimental Design

The following experiments were undertaken:

Experiment (I): Two-hundred 6-week susceptible chickens were divided into three groups (A, B and C). Chickens of group A and B (of 80 chickens each) were injected IM at 6 and 9-weeks of age with 0.5 ml dose of oil-adjuvant bacterin (containing $3.4X10^7$ CFU/dose) and chicken tissue bacterin (containing $3.8X10^8$ CFU/dose) respectively. Forty chickens (group C) were kept as non-vaccinated controls.

Two-weeks after the second injection, each chicken group was subdivided into four equal subgroups (1, 2, 3 and 4). At 11-weeks of age all chickens including controls were challenged by IM injection of 0.1ml dose of either virulent homologous or heterologous strain containing 10^5 viable organisms (Table 1). Birds were observed daily for ten days post-challenge where clinical signs, mortality rate patterns, and median death times (MDT) were recorded. Post-mortem examination was performed on chickens that died of challenge infection. Lesions were graded qualitatively from 3+ for severe, 2+ for moderate, and 1+ for mild.

Attempts were also made to re-isolate the challenge organism from liver, heart, and bone marrow. The protection index (PI) was calculated using the following formula as described by Timms and Marshall (1989):

$$PI = \frac{\text{\% mortality in controls - \% in vaccinates}}{\text{\% in controls}} \times 100$$

Experiment (II): Ninety-mice were divided into three equal groups. Mice in group I and II were injected twice (12-days apart) with 0.1ml dose of mouse and chicken tissue bacterin, respectively. Mice in group III were kept as non-vaccinated controls. Two weeks after the last injection, each group was subdivided into three equal subgroups. The 1st, 2nd, and 3rd subgroup belonged to each group, were challenged with 0.1ml dose of virulent strain 5:A, 8:A and 9:A, respectively (containing 10^8 viable organisms). Mice were observed for five days post-challenge and mortalities were recorded. Trials for recovery of challenged organism, were also performed from dead challenged mice.

RESULTS

Experiment (I):

As shown in table (1), oil-adjuvant bacterin prepared from strain 5:A (grown in-vitro) induced only homologous protection (PI:80%) whereas chicken tissue bacterin prepared with the same strain induced both homologous (PI:80%) and heterologous protection. The heterologous protection index was 75% to strain 8:A, 70% to strain 9:A and only 20% to strain 2:D.

As shown in fig. (2) and (3) and table (1), mortality in the unvaccinated controls were 100%; the majority died within 24-48 hours of challenge. Gross lesions found in dead birds are typical of acute fowl cholera (Harshfield, 1967; Rimler and Glisson, 1997). Mortality in vaccinates with oil-adjuvant bacterin occurred within 2-3 days of challenge and lesions were generally moderate. Mortality in vaccinates with chicken tissue bacterin started on the third day of challenge or birds remained in a morbid condition for an extended period of five days before deaths occurred. The majority of dead birds had mild lesions and few showed moderate ones.

P. multocida were frequently re-isolated from liver, heart, and bone marrow of all dead challenged birds.

Experiment (II):

Table (2) and Fig. (4) showed that tissue-protection was observed only in mice vaccinated with mouse tissue bacterin and not in mice vaccinated with chicken tissue bacterin. Both bacterins did induce homologous protection in vaccinated mice.

DISCUSSION

The results indicate that a bacterin prepared from a single strain of *P. multocida* that grown in-vivo can induce broad-spectrum immunogens against exposure to different serotypes whereas that grown in-vitro can not. Similar observations were recorded in turkeys (Heddleston and Rebers, 1974; Rimler et al., 1979a) and in chickens (Ibrahim and Sawada, 1998). These workers concluded that *P. multocida* produces a wider spectrum of

immunogens (cross-protecting factors, CPF) in live birds than on laboratory media. Glisson and Cheng (1991); Rimler and Rhoades (1989); Brogden and Rimler (1982) indicated that the CPF are proteins of the outer membrane of *P. multocida* which were found in the lysate of in-vivo-propagated organism and has been soluble in various detergents. This cross-protective characteristic is gradually reduced upon continuous subculture on artificial medium (Rimler et al., 1979b; Rebers and Heddleston, 1977). In passive immunization studies, antisera directed against CPF protected poults and chickens against heterologous serotype challenge exposure (Rimler, 1987; Rebers et al., 1975; Ibrahim and Sawada, 1998).

Our results showed that chicken tissue bacterin (CTB) of strain 5:A induced good protection against both homologous (5:A) and the two heterologous strains belonged to the same capsular group A (8:A and 9:A). Differences in protection levels between them could be attributed to differences in their somatic antigens as indicated by Namioka and Murata (1964). Consequently, the little protection level induced against challenge with strain 2:D could be attributed to differences in both somatic and capsular antigens of that strain relative to the vaccinal one. In this respect, Rimler et al. (1979a) also reported that the level of CPF immunity required for protection is different between different strains of *P. multocida*. Moreover, Rimler (1987) found that a higher volume (ml) of CPF antiserum was required to protect poults against challenge with *P. multocida* strain belonged to different capsular group and somatic antigen.

It is worth to state that chicken tissue bacterin (CTB) was as protective as the oil-adjuvant bacterin (OAB) prepared with the same strain (5:A) upon homologous challenge. This finding could be of great economical importance since it appears that there is no need for adjuvanting this type of bacterin. Our finding is in accordance with those of Rebers and Heddlestone (1977). They pointed out that *P. multocida* tissue bacterin do not require potentiation with adjuvant to induce good protection as do in-vitro-grown pasteurellas. In fact, addition of adjuvant to tissue bacterin is deleterious to protection.

Dead challenged chickens vaccinated with CTB showed the longest median death times and the least lesion scores. This observation is of great immunological interest since it means development of partial resistance in

these birds (although not fully protected) relative to controls or OAB vaccinates. Similar finding was recorded by Rimler et al. (1979a).

In experiment II the influence of the infected host on expression of CPF was studied. It appeared that the CPF was host-specific because mouse tissue bacterin (MTB) but not CTB protected the mouse against heterologous serotype challenge exposures. Our result coincided with those of Heddleston and Rebers (1972) and (1974). They similarly found that fowl cholera bacterin prepared from infected turkey induced immunity in turkey (though not in mice) against different immunogenic type of *P. multocida*. On the other hand, Rimler et al. (1979b) mentioned that *P. multocida* grown in bovine blood did not express CPF for turkeys. This observation suggested that expression of CPF for specific host occurred only when *P. multocida* was grown in that host.

In our study, the homologous immunity induced in mice with either MTB or CTB is not-host specific. This finding was observed previously by Heddleston and Rebers (1974).

As a conclusion, a monovalent fowl cholera bacterin prepared from in-vivo-grown *P. multocida*, could be of great immunological value specially in endemic areas to protect birds against different immunotypes commonly encountered in the field.

REFERENCES

Brogden, K.A. and Rimler, R.B. (1982): Lysates of turkey-grown *Pasteurella multocida*: partial solubilization of the cross-protection factor (5). Am. J. Vet. Res., 43 : 1781 – 1785.

Carter, G.P. (1972): Simplified identification of somatic variants of *Pasteurella multocida* causing fowl cholera. Avian Dis., 16 : 1109 – 1114.

Collins, F.M. (1977): Mechanisms of acquired resistance to *Pasteurella multocida* infection.: A review. Cornell Vet. 67 (1) Jan.

Glisson, J.R. and Cheng, I.H.N. (1991): In-vivo antigen expression by *Pasteurella multocida.* Avian Dis., 35 : 392 - 396.

Harshfield, G.S. (1967): Fowl cholera in "Disease of Poultry". Edited by *Biester, H.E.* and *Schwarte, L.H.*, Fifth edition, 359 – 373.

Heddleston, K.L. (1966): Immunologic and serologic comparison of three strains of *Pasteurella multocida.* Cornell Vet., 56 : 235 – 241.

Heddleston, K.L. and Rebers, P.A. (1969): Pasteurella multocida: immune response in chicks and mice. Proc. 73rd Ann. Meeting of USA, HA : 280 – 284.

Heddleston, K.L. and Rebers, P.A. (1972): Fowl cholera : cross-immunity induced in turkeys with formalin killed in-vivo propagated *Pasteurella multocida.* Avian Dis., 16: 578-586.

Heddleston, K.L. and Rebers, P.A. (1974): Fowl cholera bacterins: host-specific cross-immunity induced in turkeys with *Pasteurella multocida* propagated in embryonating turkey eggs. Avian Dis., 18: 213-219.

Heddleston, K.L.; Gallagher, J.E.; Rebers, P.A. (1972): Gel diffusion precipitin test for serotyping Pasteurella multocida from avian species. Avian Dis., 16 : 925 – 936.

Hofacre, C.L.; Glisson, J.R.; Kleven, S.H.; Brown, J. and Rowland, G.N. (1989): Evaluation of *Pasteurella multocida* mutants of low virulence. I. Development and pathogenicity. Avian Dis., 33 : 270 – 274.

Ibrahim, R.S. and Sawada, T. (1998): Studies on cross-protection induced in chickens with *Pasteurella multocida* prepared from infected chick embryos. Proc. 5th, Sci. Conf. Egypt. Vet. Poult. Assoc.: 99-109.

Namioka, S. and Murata, M. (1964): Serological studies on *Pasteurella multocida.* V. Some epizootiological findings resulting from "O" antigenic analysis. Cornell Vet., 54 : 520 – 534.

Rebers, P.A. and Heddleston, K.L. (1977): Fowl cholera: induction of cross-protection in turkeys with bacterins prepared from host-passaged *Pasteurella multocida.* Avian Dis., 21 : 50–56.

Rebers, P.A.; Heddleston, K.L.; Wright, B. and Gillette, K. (1975): Fowl cholera: cross-protective turkey antisera and IgG antibodies induced with *Pasteurella multocida*-infected tissue bacterin. Carbohydrate Res., 40: 99-110.

Rhoades, K.R. and Rimler, R.B. (1987): Capsular groups of *Pasteurella multocida* isolated from avian hosts. Avian Dis., 31: 895–898.

Rimler, R.B. (1987): Cross-protection factors(s) of *Pasteurella multocida*: Passive immunization of turkey against fowl cholera caused by different strains. Avian Dis., 31 (4) : 884 - 887.

Rimler, R.B. and Glisson, R. (1997): Fowl cholera in "Diseases of Poultry", Tenth Edition, edited by *Calnek, B.W.*; Page 143-159.

Rimler, R.B. and Rhoades, K.R. (1981): Lysates of turkey-grown *Pasteurella multocida:* protection against homologous and heterologous serotype challenge exposures. Am. J. Vet. Res., 42 (12) : 2117 – 2121.

Rimler, R.B. and Rhoades, K.R. (1989): Solubilization of membrane associated cross-protection factor(s) of *Pasteurella multocida.* Avian Dis., 33 : 258-263.

Rimler, R.B.; Rebers, P.A. and Rhoades, K.R. (1979a): Fowl cholera: cross-protection induced by *Pasteurella multocida* separated from infected turkey blood. Avian Dis., 23 : 730 – 741.

Rimler, R.B.; Rebers, P.A. and Rhoades, K.R. (1979b): Modulation of cross-protection factor(s) of avian *Pasteurella multocida.* Avian Dis., 24 (4) : 989 – 998.

Suzan, F.G. (1992): Comparison of the efficiency of different pasteurella vaccines used for protection of ducks against fowl cholera in Egypt. Ph. D. Thesis, Assiut Univ., Egypt.

Timms, L.M. and Marshall, R.N. (1989): Laboratory assessment of protection given by experimental *Pasteurella anatipestifer* vaccine. Br. Vet. J., 145: 483 – 493.

Table (1) : Response of chickens that were vaccinated with P. multocida bacterin prepared from strain 5:A grown either in-vitro or in-vivo and challenge exposed with different strains.

Group	Bacterin Prepared From strain 5:A	Sub-Group	Challenge Strain	No. deaths on (days post challenge)										No. dead / Total	% Of mortality	Protection Index (PI)	Median Death Time (Days)	Lesion Score
				1	2	3	4	5	6	7	8	9	10					
A	Grown In-vitro	a1	5:A		2	2								4/20	20	80	2.5	1+to2+
		a2	8:A		12	1								17/20	85	15	2.3	1+to2+
		a3	9:A		12	6								18/20	90	10	2.3	1+to2+
		a4	2:D		12	7								19/20	95	5	2.4	2+
B	Grown In-vivo	b1	5:A			1	2	2						4/20	20	80	4.2	1+
		b2	8:A			1	1	3						5/20	25	75	4.4	1+
		b3	9:A			1	4	1						6/20	30	70	4.0	1+
		b4	2:D			5	7	4						16/20	80	20	3.9	1+to2+
C	Unvaccinated controls	c1	5:A	9	1									10/10	100	-	1.1	3+
		c2	8:A	6	4									10/10	100	-	1.4	3+
		c3	9:A	6	3	1								10/10	100	-	1.5	3+
		c4	2:D	3	5	2								10/10	100	-	1.9	3+

Table (2) : (Experiment II, mice) Response of mice that were vaccinated with mouse or chicken tissue bacterin of P. multocida strain 5:A and challenge exposed with different strains.

Bacterin prepared from (strain 5:A)	Challenge exposure*		
	Homologous strain	Heterologous strain	
	(5:A)	8:A	9:A
1. Mouse tissue	9/10 (90)	7/10 (70)	7/10 (70)
2. Chicken tissue	8/10 (80)	1/10 (10)	0/10 (0)
Non (Controls)	0/10 (0)	0/10 (0)	0/10 (0)

* No. survived / No. challenged (% survived).

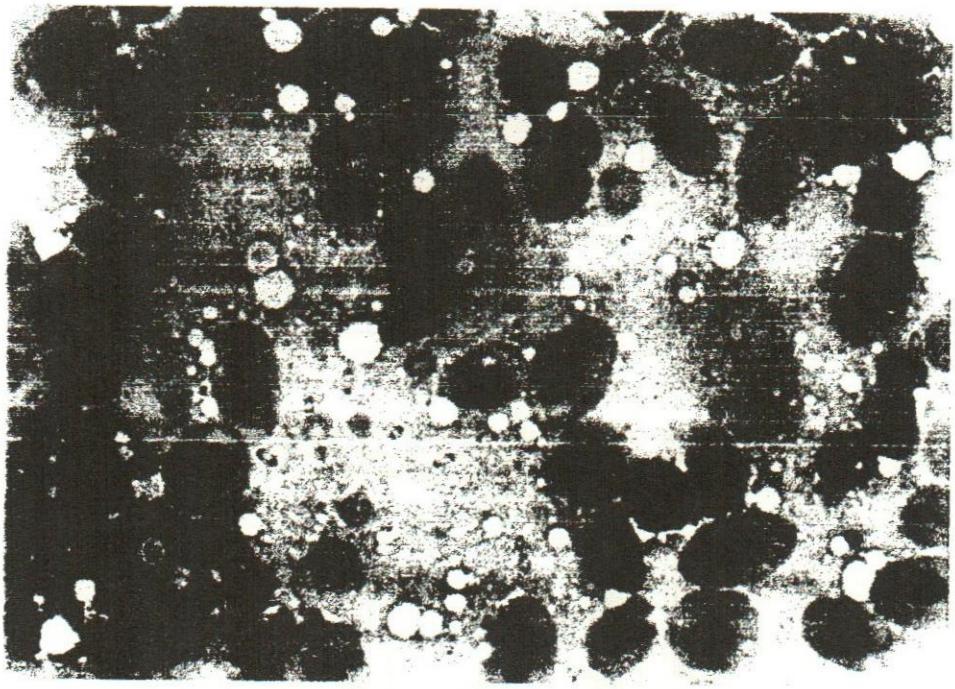

Fig. 1: *Pasteurella multocida* in liver imprints from chicken with acute fowl cholera (note bipolarity) (X1500).

Fig. (2) : Comparison of protective indices in chickens vaccinated with either in-vitro-grown or in-vivo-grown P. multocida (strain 5:A) and challenged with different strains.

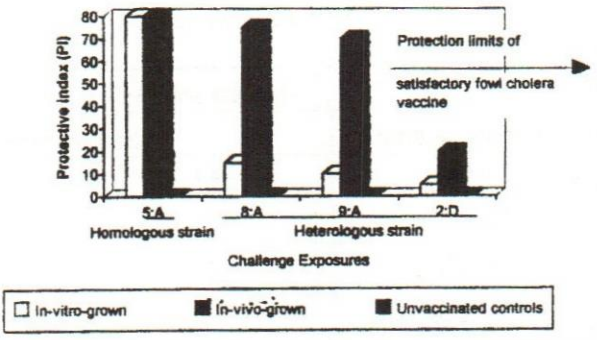

Fig. (3) : Comparison of median death time in chickens vaccinated with either in-vitro or in-vivo grown P. multocida (strain 5:A) and challenged with different strains.

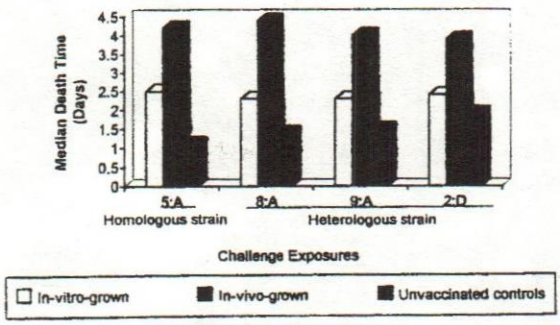

Fig. (4) : Comparison of protective indices in mice vaccinated with either in-vitro-grown or in-vivo-grown P. multocida (strain 5:A) and challenged with different strains.