CHAPTER 3

RESULTS

1. Determination of antibody titer

Each group of antiserum was titered by tube agglutination.

1.1 Antibody titer against B. anthracis

The titers of antiserum obtained from rabbit immunization with Bacillus anthracis vaccine and two types of bivalent vaccine of B. anthracis and P. multocida were shown in Fig 1 page 60.

The highest antibody titers of monovalent vaccine and bivalent vaccine with alum were 1:512 while bivalent vaccine without alum was 1:256.

1.2 Antibody titer against P. multocida

The titers of antiserum obtained from rabbit immunization with Pasteurella multocida vaccine and two types of bivalent vaccine of B. anthracis and P. multocida were shown in Fig 2 page 61.

The highest antibody titer of each vaccine was about 1:2048 at day 58 and the level was clearly significantly declined at day 140 (week 20)

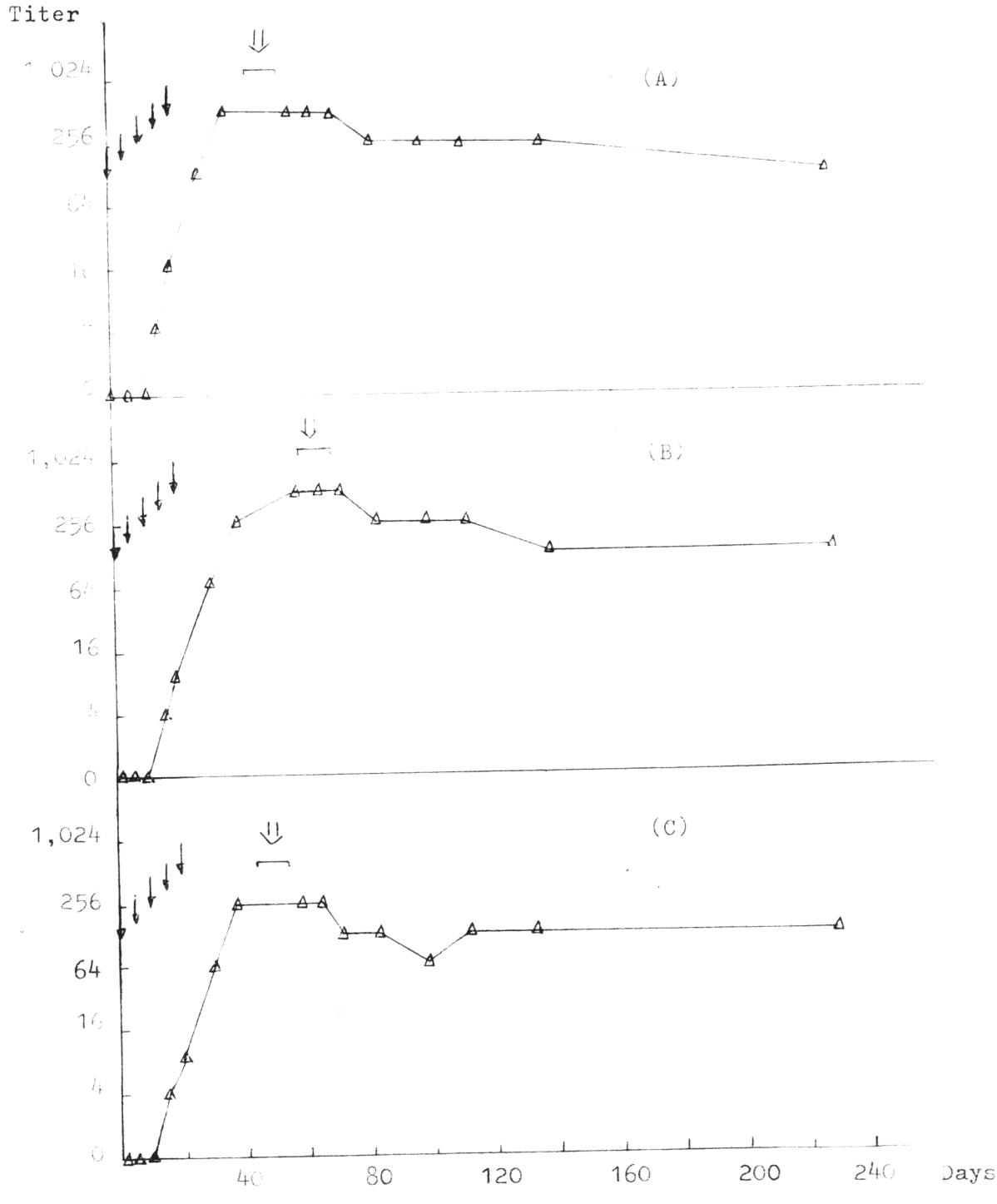
2. Determination of the protein content and antibody titer of immunoglobulin

The concentrated immunoglobulins from 2.4 were determined

the protein contents by method described by Lowry *et al*[96] and bovine serum albumin was used as the standard protein. The antibody titers of immunoglobulins were retitrated by method as described in 2.3.

The protein contents of four immunoglobulin solutions resulted from using *Bacillus anthracis* vaccine, *Pasteurella multocida* vaccine, bivalent vaccine of *B. anthracis* and *P. multocida* with alum and bivalent vaccine of *B. anthracis* and *P. multocida* without alum were 27.8, 29.5, 25.0 and 26.4 mg/ml respectively.

The antibody titers of each immunoglobulin obtained from rabbit immunization with *Bacillus anthracis* vaccine, bivalent vaccine with alum and bivalent vaccine without alum against *B. anthracis* were 1:1024, 1:1024 and 1:512 respectively whereas the antibody titers of each immunoglobulin obtained from using *Pasteurella multocida* vaccine, bivalent vaccine with or without alum against *P. multocida* were 1:4096.



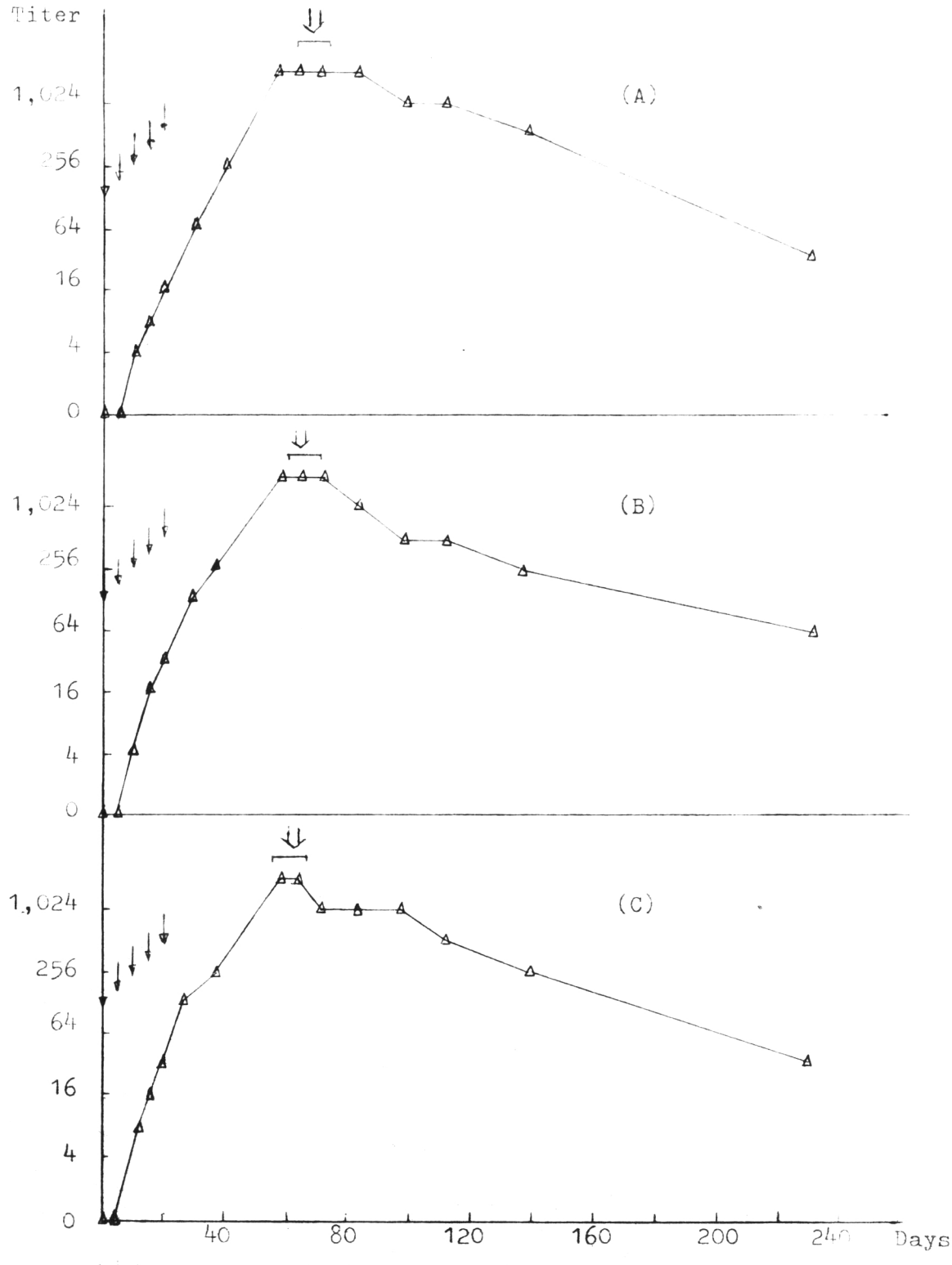
(⇓) represented the vaccine injections.
(⊔) represented the heavy bleeding for passive protection test.

Figure 1 The antibody level of rabbit immune serum obtained from immunization with various vaccines against B. anthracis

(A) monovalent vaccine of B. anthracis

(B) bivalent vaccine of B. anthracis and P. multocida with alum

(C) bivalent vaccine of B. anthracis and P. multocida without alum



(↓) represented the vaccine injections.
(⇓) represented the heavy bleeding for passive protection test.

Figure 2 The antibody level of rabbit immune serum obtained from immunization with various vaccines against P. multocida:
(A) monovalent vaccine of P. multocida
(B) bivalent vaccine of B. anthracis and P. multocida with alum
(C) bivalent vaccine of B. anthracis and P. multocida without alum

3. Determination of the LD_{50} in mice

The LD_{50} determination was conducted by applying the procedure described by Litchfield et al.[97]

3.1 LD_{50} of B. anthracis

The ten fold concentration of spore suspension ranging from 1.25 x 10 to 1.25 x 10^5 spores per mouse were given intraperitoneally to each group The detail data was shown in Table 6 page 63. The line as shown in Fig 3 page 64 was tested for a good fit by Chi-square test. The LD_{50} of B. anthracis read from the graph was 3.55 x 10^2 spores and confidence limits of the LD_{50} was 7.85 x 10 to 1.60 x 10^3 spores.

3.2 LD_{50} of P. multocida

The two fold concentration of bacterial suspension ranging from 1 to 32 cells per mouse were given intraperitoneally to each group.The detail data was shown in Table 7 page 65 . The line as shown in Fig 4 page 66 was also tested for good fit by Chi-square test. The LD_{50} of P. multocida was 6 cells and and confidence limits of LD_{50} was 3 to 9 cells.

Table 6 Estimation of LD_{50} per mouse of Bacillus anthracis, the spore suspension was diluted to ten-fold dilution and administered into each group of 10 mice and mortality was observed for 14 days.

Inoculum dose (spores / mouse)	Number of mice		
	Total	Died	Survived
1.25×10	10	0	10
1.25×10^2	10	3	7
1.25×10^3	10	8	2
1.25×10^4	10	9	1
1.25×10^5	10	10	0

LD_{50} = 3.55×10^2 spores
(range 7.85×10 to 1.60×10^3)

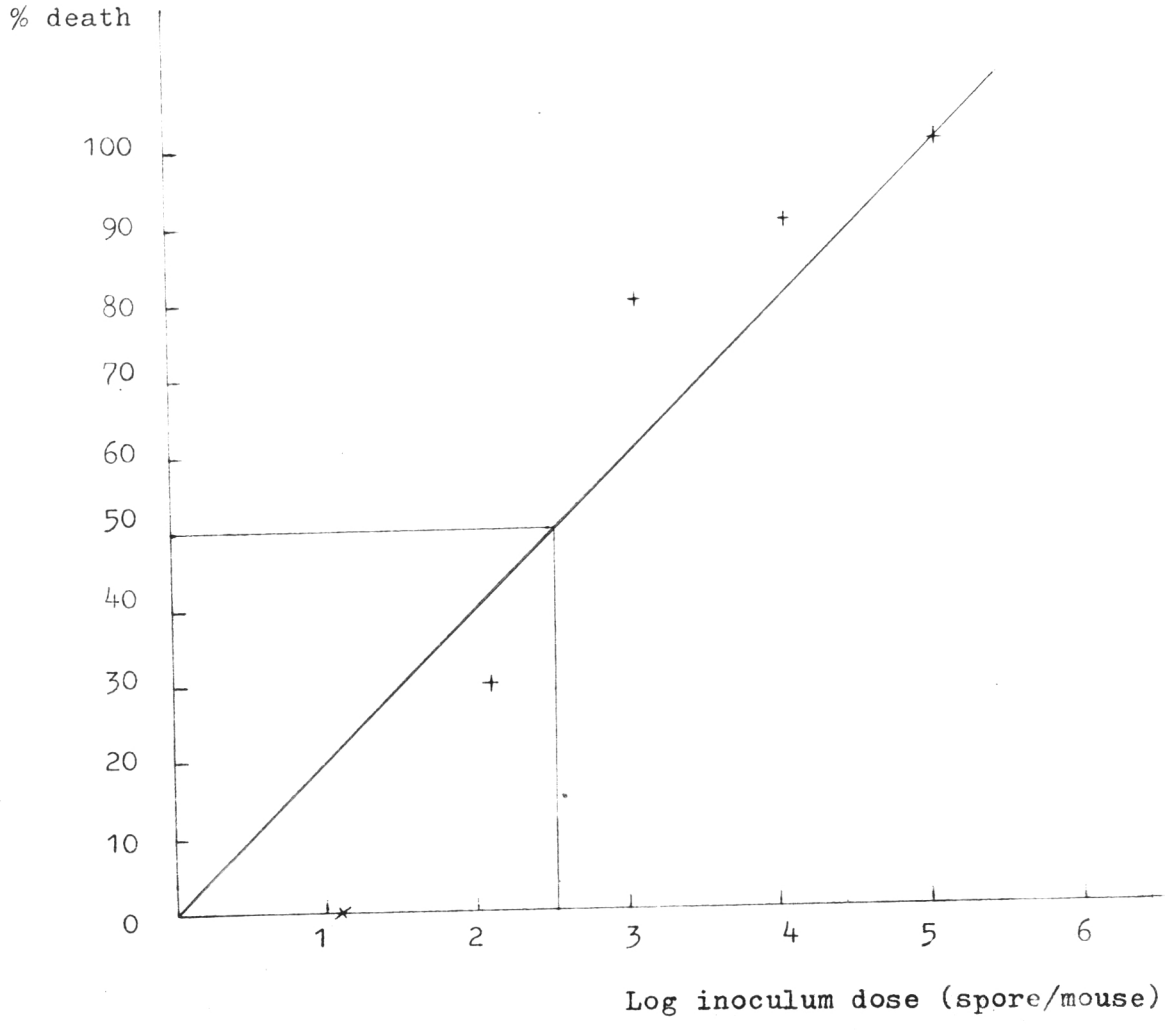


Figure 3 Estimation of LD_{50} per mouse of Bacillus anthracis culture

Table 7 Estimation of LD_{50} per mouse of Pasteurella multocida culture, the bacterial suspension was diluted to two-fold dilution and administered into each group of 10 mice and mortality was observed for 3 days.

Inoculum dose (cells / mouse)	Number of mice		
	Total	Died	Survived
1	10	0	10
2	10	2	8
4	10	3	7
8	10	6	4
16	10	9	1
32	10	10	0

LD_{50} = 6 cells

(range 3 to 9 cells)

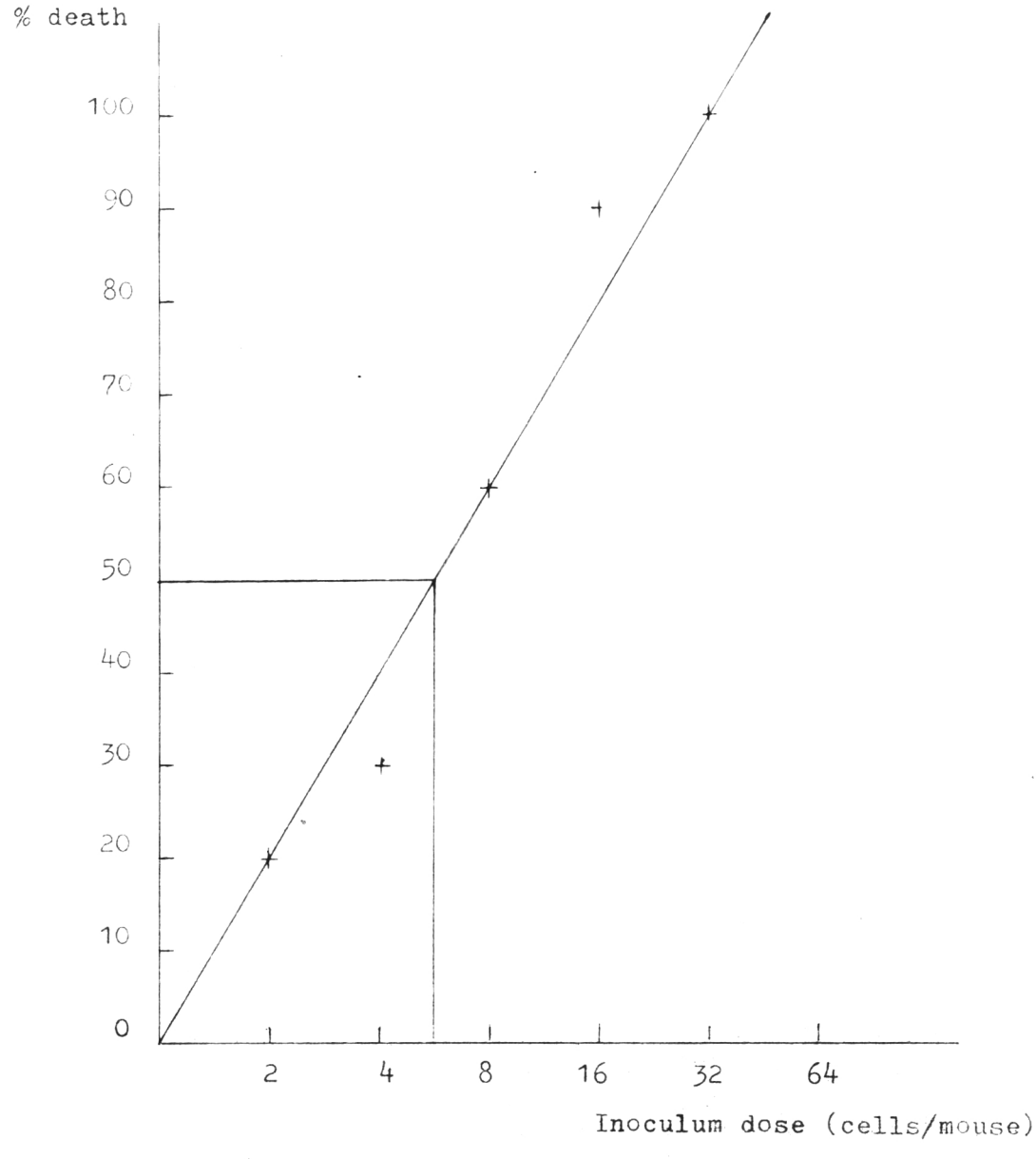


Figure 4 Estimation of LD_{50} per mouse of **Pasteurella multocida** culture.

4. Determination of the protective dose in mice

4.1 Protective dose to B. anthracis

The data was shown in Table 8 page 68. The selected doses for passive protection test of immunoglobulin prepared from immunization with Bacillus anthracis vaccine and two types of bivalent vaccine to each group of three rabbits were 10 mg per mouse.

4.2 Protective dose to P. multocida

The data was shown in Table 9 page 69. The selected doses for passive protection test of immunoglobulin prepared from immunization with Pasteurella multocida vaccine and two types of bivalent vaccine to each group of three rabbits were 0.625 mg per mouse.

The minimum dose that gave 100% mouse passive protection was 0.312 mg per mouse.

Table 8 Determination of immunoglobulin dosages for passive protection in mice against 75 LD_{50} of the spore suspension of the virulent strain of Bacillus anthracis

Immunoglobulin obtained from rabbit immunized with	Dose (mg) of immuno -globulin per mouse	Number of mice		Selected dose for passive protection
		Total	Survived	
Bacillus anthracis	10	10	4	
vaccine	5	10	2	10
	2.5	10	0	
Bivalent vaccine*	10	10	4	
with alum	5	10	2	10
	2.5	10	0	
Bivalent vaccine*	10	10	3	
without alum	5	10	1	10
	2.5	10	0	
Control group	-**	10	0	
(no immunization)				

* combined vaccine of B. anthracis and P. multocida

** applying the same volume of saline in place of immunoglobulin

Table 9 Determination of immunoglobulin dosages for passive protection in mice against 100 LD_{50} of the virulent culture of Pasteurella multocida

Immunoglobulin obtained from rabbit immunized with	Dose (mg) of immuno-globulin per mouse	Number of mice		Selected dose for passive protection
		Total	Survived	
Pasteurella multocida	1.250	10	10	
vaccine	0.625	10	10	
	0.312	10	10	0.625
	0.156	10	8	
	0.078	10	2	
Bivalent vaccine*	1.250	10	10	
with alum	0.625	10	10	
	0.312	10	10	0.625
	0.156	10	8	
	0.078	10	5	
Bivalent vaccine*	1.250	10	10	
without alum	0.625	10	10	
	0.312	10	10	0.625
	0.156	10	6	
	0.078	10	4	
Control group	-**	10	0	
(no immunization)				

* combined vaccine of B. anthracis and P. multocida

** applying the same volume of saline in place of immunoglobulin

5. Mouse passive protection tests

5.1 Passive protection test of *B. anthracis*

Selected dose (10 mg / ml) of each immunoglobulin for passive protection test against *B. anthracis* was used.

The results of single dose and a booster dose used of each immunoglobulin were shown in Table 10 page 71 and the profiles that showed the percent survival of mice observed in each day were in Fig 5 page 72 and Fig 6 page 73.

5.2 Passive protection test of *P. multocida*

Selected dose (0.625 mg / ml) of each immunoglobulin for passive protection test against *P. multocida* was used and the results were shown in Table 11 page 74.

Table 10 Passive protection of mice with rabbit immunoglobulins (10 mg /mouse) against 75 LD_{50} of the spore suspension of the virulent strain of Bacillus anthracis

Immunoglobulin obtained from rabbit immunized with	Number of mice		P***
	Total	Survived	
Single dose of immunoglobulin			
Bacillus anthracis vaccine	20	6	< 0.05
Bivalent vaccine* with alum	20	6	< 0.05
Bivalent vaccine* without alum	20	3	> 0.05
Control group** (no immunization)	20	0	
A booster dose of immunoglobulin			
Bacillus anthracis vaccine	20	12	< 0.05
Bivalent vaccine* with alum	20	10	< 0.05
Bivalent vaccine* without alum	20	6	< 0.05
Control group** (no immunization)	20	0	

* combined vaccine of B. anthracis and P. multocida

** applying the same volume of saline in place of immunoglobulin

*** Chi-square test

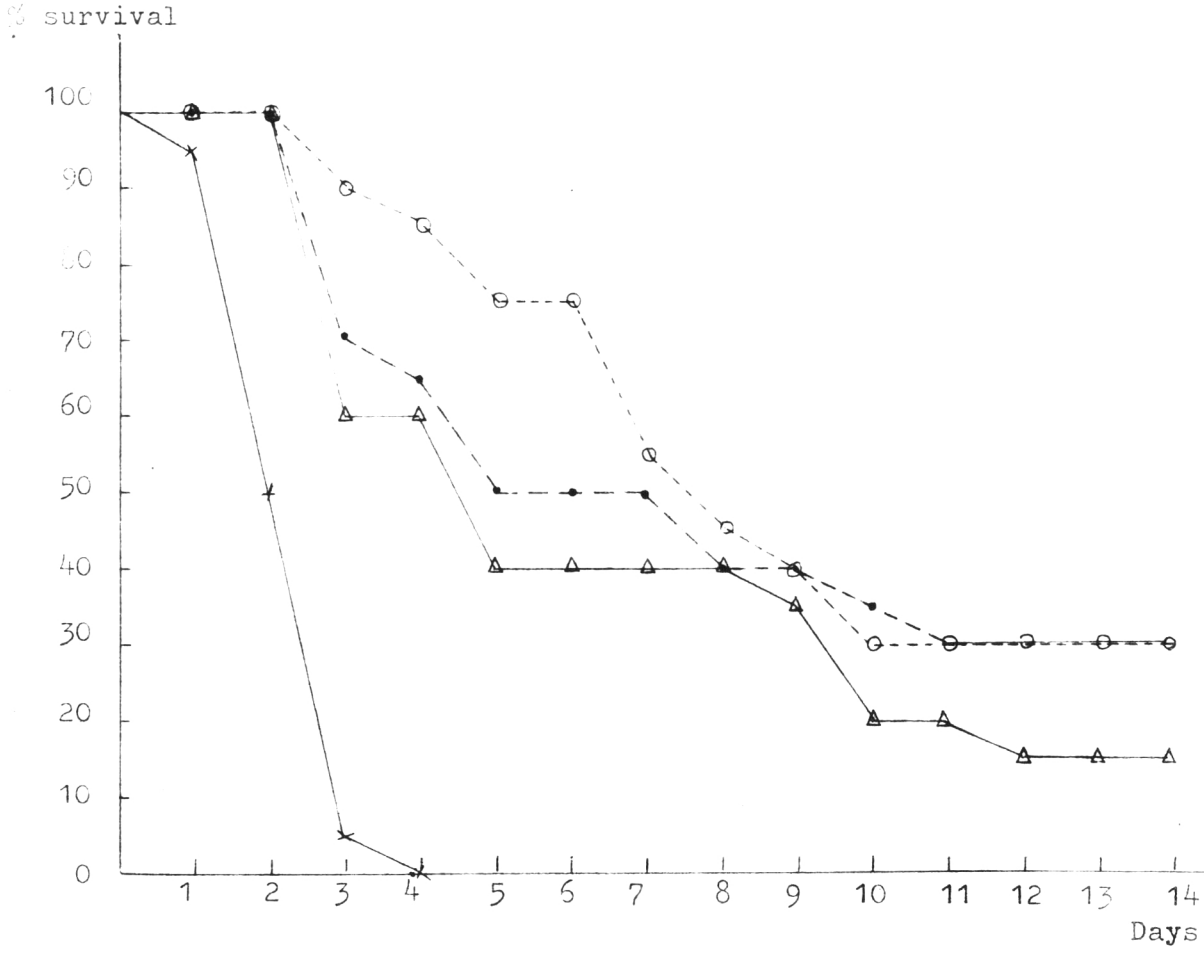


Figure 5 Observed responses of the immunized mice challenged intraperitoneally with B. anthracis for 14 days when a single dose of immunoglobulin (10 mg/mouse) obtained from rabbit immunization with various vaccines was used.

- control
- monovalent vaccine of B. anthracis
- bivalent vaccine with alum
- bivalent vaccine without alum

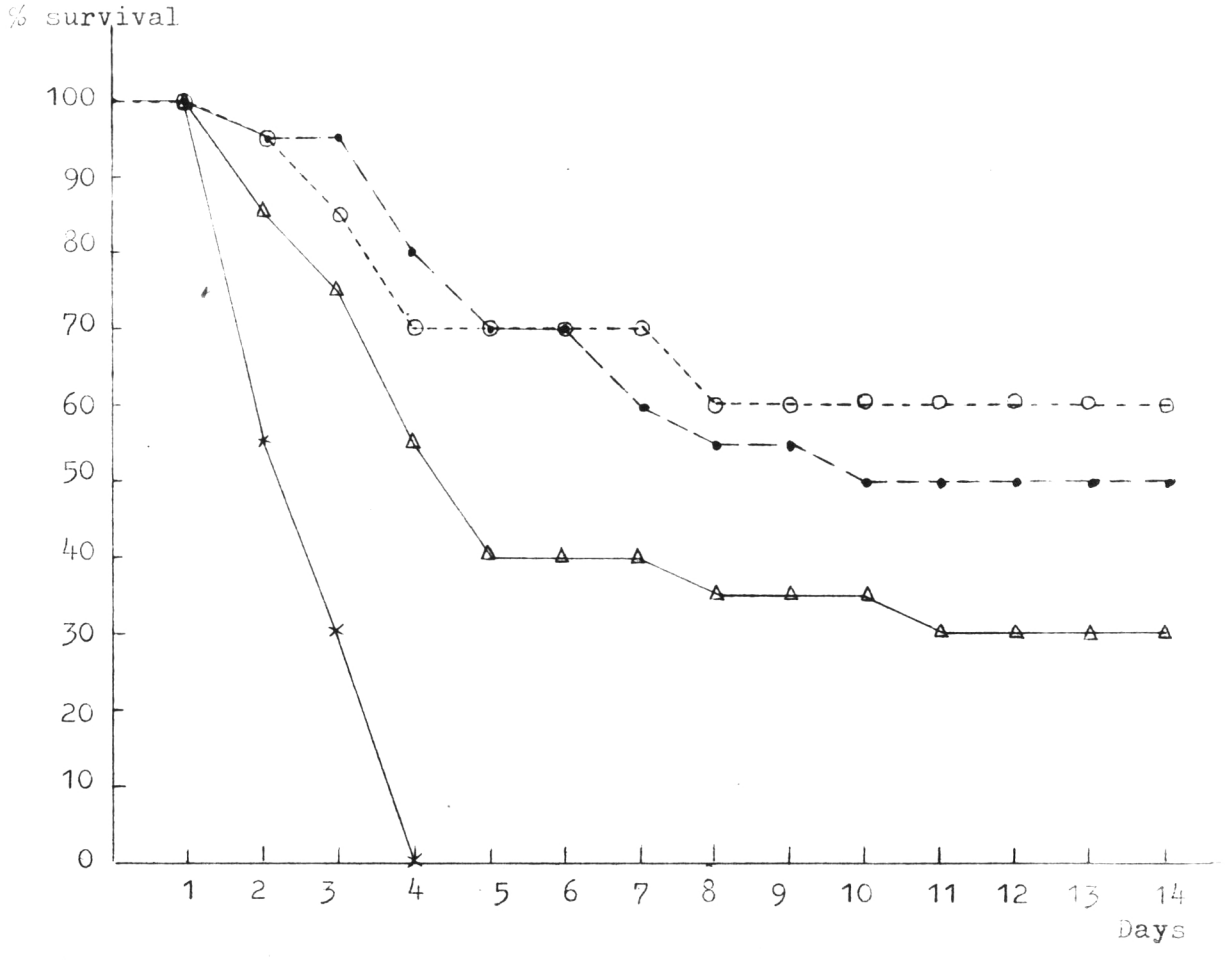


Figure 6 Observed responses of the immunized mice challenged intraperitoneally with B. anthracis for 14 days when a booster dose of immunoglobulin (10 mg/mouse) obtained from rabbit immunization with various vaccines was used.

- control
- monovalent vaccine of B. anthracis
- bivalent vaccine with alum
- bivalent vaccine without alum

Table 11 Passive protection of mice with rabbit immunoglobulin (0.625 mg / mouse) against 100 LD_{50} of the virulent culture of Pasteurella multocida

Immunoglobulin obtained from rabbit immunized with	Number of mice		p***
	Total	Survived	
Pasteurella multocida vaccine	20	20	< 0.01
Bivalent vaccine* with alum	20	20	< 0.01
Bivalent vaccine* without alum	20	20	< 0.01
Control group** (no immunization)	20	0	

* combined vaccine of B. anthracis and P. multocida

** applying the same volume of saline in place of immunoglobulin

*** Chi-square test