Prophylaxis of *Bordetella bronchiseptica* Infection in Guinea Pigs by Vaccination

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Abstract. *Bordetella bronchiseptica* vaccines, which were killed by formalin, merthiolate or heat and emulsified with Freund's incomplete adjuvant, were examined on their protective effects in guinea pigs. Results indicated that merthiolate-killed vaccine was most effective among the 3 vaccines and almost all guinea pigs received 10–20 mg of the vaccine were protected from the establishment of carrier state, as well as formation of pneumonic lesions, when challenged intranasally with 10^6 cells of a virulent strain. Formalin-killed vaccine was also effective but not so potent as merthiolate-killed vaccine. No protective effect was demonstrated in a vaccine heated at 100°C for 1 hour.

Protective action afforded by merthiolate-killed vaccine appeared 20 days after vaccination, became greatest 50 to 80 days and then decreased gradually, but persisted for at least 150 days. Serum agglutinins appeared earlier than the development of protective action and remained at high titers for at least 180 days. Although there was a general correlation between protective potencies and serum agglutinin titers, discrepancies were observed in some guinea pigs. Therefore, agglutinin production was not always accepted as evidence of protective potency.

Remarkable protective effects were demonstrated in all virulent strains of *B. bronchiseptica* originated from guinea pigs. However, no protective effects was shown in an avirulent variant derived from a virulent strain by subculturing 20 times on MacConkey agar.

Offspring delivered from mothers in good immunity showed evident protection against challenge inoculation and high agglutinin titers for 4 weeks after birth.

In a previous report [11], the authors revealed that about 80 per cent of guinea pigs suffered from natural infection of *Bordetella bronchiseptica* resulted in recovery of the infection within 54 weeks, carrying no organisms in any parts of the respiratory system, and acquired nasal and tracheal resistance against the reinfection of the organisms. These results suggest the usefulness of vaccination for prophylaxis of *B. bronchiseptica* infection in guinea pigs.

In the present study, prophylactic value

of *B. bronchiseptica* vaccine prepared by several different methods are assessed, and in addition, such problems as effective duration of administrated vaccine and transmission of the immunity from mother to young are also examined. Knowledges of these problems are necessary in applying vaccination to breeding guinea pig colonies.

Materials and Methods

Guinea pigs: Male and female guinea pigs of Hartley strain, weighing 250–300 g, were used

throughout the present study, except the work of transmission of immunity in which animals of exact age were selected. All the guinea pigs were obtained from a breeding colony which had been proved to be free of *B. bronchiseptica* contamination by repeated checkings over 6 years. They were placed in metal cages on bedding of straw and fed with commercial pellets and tap water ad libitum, supplying sometimes green feed.

B. bronchiseptica strains: Strain 64L originated from pneumonic lesions of a naturally infected guinea pig was mainly used for vaccine preparation. This strain showed so high virulence that all guinea pigs were suffered from the infection when inoculated intranasally with 10^4 organisms [7]. Two additional strains, N2-88 and 1088, were also used for vaccine preparation. These strains had been isolated from lung lesions of naturally infected guinea pigs and subcultured 3 times on nutrient agar before use. Infectivities of the strains to guinea pigs were almost same as that of strain 64L.

A vaccine was also made from an avirulent strain, 64L-M, which had been delivered from strain 64L by subculturing 20 times on MacConkey agar and lost the infectivity to guinea pigs [7].

Vaccine preparation: The following 3 different killed vaccines were prepared from 48-hour nutrient agar culture of strain 64L.

a) Formalin-killed vaccine . . . The bacterial growth was suspended into 1% formalin saline and the suspension was incubated at 37°C for 5 days with occasional shaking. After washed twice with saline, inactivated cells were resuspended in saline at the concentration of 100 mg/ml.

b) Merthiolate-killed vaccine . . . This vaccine was made by the same procedures as in the formalin-killed vaccine, except that instead of formalin saline, 0.1% merthiolate saline was used for inactivation of bacterial cells and two final suspensions of different cell concentrations, which contained 100 mg and 200 mg of cells per 1 ml saline, were prepared.

c) Heat-killed vaccine . . . Bacterial cells suspended in saline were heated at 100°C for 1 hour and then washed twice with saline. The cell concentration in the final suspension was adjusted to 100 mg/ml.

Equal parts of each of the above bacterial suspensions and Freund's incomplete adjuvant (Iatron, Tokyo) were emulsified thoroughly by use of a syringe. The resulted "water in oil" emulsions were stored at 4°C and used within 10 days after preparation.

As regard to strains N2-88, 1088 and 64L-M, emulsions of merthiolate-killed vaccine with Freund's incomplete adjuvant (final cell concentration was adjusted to 100 mg/ml) were only prepared follow-

ing the procedures above described.

Inoculation of vaccines: Each guinea pig received a single intramuscular injection of 0.2 ml vaccine at the inside of the thigh. This volume of vaccine was calculated to contain 10 mg of bacterial cells in formalin- and heat-killed vaccines and 10 mg or 20 mg in merthiolate-killed vaccine.

Challenge inoculation: Prophylactic value of the vaccines was assessed by challenge inoculation with the virulent strain 64L. The external nares of unanesthetized guinea pigs were smeared with 10^8 or 10^4 viable cells of 18-hour trypticase soy broth culture and the guinea pigs were autopsied 15 days after inoculation for cultivation of the challenged organisms in the respiratory system and for serum agglutination test. Procedures for these examinations were reported in detail elsewhere [6].

Results

1. Protective effects of vaccines prepared by three different procedures

Twenty-five guinea pigs were divided into 5 groups, consisting 5 each. Animals of four groups received 10 mg of formalin-killed vaccine, 10 mg and 20 mg of merthiolate-killed vaccine and 10 mg of heat-killed vaccine, respectively. The remaining one group served as unvaccinated control. Challenge inoculation of 3×10^6 organisms was performed to all the animals 30 days after vaccination and at the same time, serum agglutinin titers were checked. Fifteen days later, the animals were killed for detection of the organisms in the respiratory system, including the external nares, nasal cavity, upper and lower tracheas and lung. Results are shown in Table 1.

Although no remarkable difference was obtained in agglutinin production by the 3 vaccines, merthiolate-killed vaccine was found to be most effective for protection of *B. bronchiseptica* infection. In four of 5 guinea pigs received 10 mg of this vaccine, the challenged organisms were not recovered from any parts of the respiratory tract. In the exceptional one guinea pig, growth of the organisms limited to the nasal cavity and upper trachea and no organisms were

Table 1. Protective effects of three different vaccines

Treatment of vaccine	Dose of vaccine inoculated	Animal no.	Agglutinin titer	Recovery of the organisms on the 15th day after challenge*					Pneumonic lesion
				Ext. nares	Nasal cavity	Trachea		Lung	
						Upper	Lower		
Formalin	10 mg	1	1 : 1280	+++	+++	+++	—	—	—
		2	1 : 1280	—	+	—	—	—	—
		3	1 : 1280	+++	+++	+++	—	—	—
		4	1 : 2560	—	—	—	—	—	—
		5	1 : 1280	—	—	+	—	—	—
Merthiolate	10 mg	1	1 : 1280	—	—	—	—	—	—
		2	1 : 1280	—	—	—	—	—	—
		3	1 : 2560	—	—	—	—	—	—
		4	1 : 1280	—	+++	+++	—	—	—
		5	1 : 1280	—	—	—	—	—	—
	20 mg	1	1 : 20480	—	—	—	—	—	—
		2	1 : 20480	—	—	—	—	—	—
		3	1 : 10240	—	—	—	—	—	—
		4	1 : 10240	—	—	—	—	—	—
		5	1 : 10240	+	+++	+++	++	—	—
Heat	10 mg	1	1 : 320	+++	+++	+++	+++	—	—
		2	1 : 1280	++	+++	++	—	—	—
		3	1 : 640	++	+++	+++	—	—	—
		4	1 : 1280	+++	+++	+++	+++	++	+
		5	1 : 640	+++	+++	+++	+++	—	—
Unvaccinated control		1	<1 : 10	+++	+++	+++	+++	+++	+
		2	<1 : 10	+++	+++	+++	+++	++	+
		3	<1 : 10	+++	+++	+++	+++	+++	+
		4	<1 : 10	+++	+++	+++	+++	++	+
		5	<1 : 10	+++	+++	+++	+++	+++	+

* +++: more than 100 colonies, ++: 10–100 colonies, +: less than 10 colonies, —: negative.

Guinea pigs were challenged with 3×10^6 cells of strain 64L 30 days after vaccination.

recovered from the lower trachea and lung. A similar degree of protection was also obtained in guinea pigs received 20 mg of this vaccine, in spite of the fact that serum agglutinin titers of these animals were higher about 10 times than those of guinea pigs treated with 10 mg of the vaccine. Among 5 guinea pigs injected with 10 mg of formalin-killed vaccine, 4 animals were suffered from the infection by challenge inoculation. In the affected animals, however, the organisms were only recovered from upper parts of the respiratory tract, and the lower trachea and lung were pro-

tected from the invasion of the organisms. On the contrary to these findings, all the 5 guinea pigs received 10 mg of heat-killed vaccine were affected by challenge inoculation as in unvaccinated control animals. Namely, the organisms were recovered abundantly from the upper respiratory tract of all guinea pigs and also from the lower trachea or lung of 3 animals. All guinea pigs of unvaccinated control harbored the organisms abundantly throughout the respiratory tract and lung, suffering from bronchopneumonia.

To study secondary reactions of the vac-

cines, all guinea pigs used in this experiment were examined daily on the body weight, temperature and local reactions for 3 weeks after vaccination. No significant changes were observed in an increase of body weight and a fluctuation of temperature of guinea pigs received any vaccines, comparing with those of unvaccinated controls. In vaccinated guinea pigs, however, a slight induration probably due to adjuvant was formed in the site of inoculation and remained for a long time.

It was apparent from these findings that the merthiolate-killed vaccine was best protective to *B. bronchiseptica* infection and harmless to guinea pigs, and therefore, all subsequent works were carried out by injecting 20 mg of this vaccine.

2. Time of development and duration of protective activity by vaccination

Guinea pigs received 20 mg of merthiolate-killed vaccine were challenged intranasally with 3×10^6 or 10^4 viable organisms of strain 64L at intervals after vaccination

Table 2. Time of development and duration of protective activity by vaccination

Days after vaccination	No. of organisms challenged	Animal no.	Agglutinin titer	Recovery of the organisms on the 15th day after challenge*					Pneumonic lesion
				Ext. nares	Nasal cavity	Trachea		Lung	
						Upper	Lower		
10	3×10 ⁶	1	1 : 320	+++	+++	+++	+++	++	+
		2	1 : 320	+++	+++	+++	+++	+++	+
		3	1 : 1280	+++	+++	+++	+++	—	—
		4	1 : 320	+++	+++	+++	+++	++	+
		5	1 : 640	+++	+++	+++	+++	—	—
20	3×10 ⁶	6	1 : 5120	—	—	—	—	—	—
		7	1 : 5120	—	—	—	—	—	—
		9	1 : 2560	—	+++	—	—	—	—
		10	1 : 640	—	—	—	—	—	—
30	3×10 ⁶	11	1 : 20480	—	—	—	—	—	—
		12	1 : 10240	—	—	—	—	—	—
		13	1 : 10240	—	—	—	—	—	—
		14	1 : 10240	+	+++	+	—	—	—
		15	1 : 20480	—	—	—	—	—	—
50	3×10 ⁶	16	1 : 10240	—	—	—	—	—	—
		17	1 : 10240	—	—	—	—	—	—
		18	1 : 10240	—	—	—	—	—	—
		19	1 : 10240	—	—	—	—	—	—
		20	1 : 20480	—	—	—	—	—	—
80	3×10 ⁶	21	1 : 5120	—	—	—	—	—	—
		22	1 : 5120	—	—	—	—	—	—
		23	1 : 5120	—	—	—	—	—	—
		24	1 : 20480	—	—	—	—	—	—
		25	1 : 2560	—	—	—	—	—	—
100	3×10 ⁶	26	1 : 20480	+	+++	+++	—	—	—
		27	1 : 20480	++	++	—	—	—	—
		28	1 : 5120	++	+++	++	—	—	—
		29	1 : 20480	+	+++	++	—	—	—
		30	1 : 20480	+++	+++	+++	+++	+++	+

Table 2. (Continued)

Days after vaccination	No. of organisms challenged	Animal no.	Agglutinin titer	Recovery of the organisms on the 15th day after challenge*					Pneumonic lesion
				Ext. nares	Nasal cavity	Trachea		Lung	
						Upper	Lower		
150	3×10^4	31	1 : 2560	—	—	—	—	—	—
		32	1 : 10240	—	—	—	—	—	—
		33	1 : 1280	—	—	—	—	—	—
		34	1 : 5120	++	++	++	++	—	—
		35	1 : 5120	++	++	++	++	—	—
	3×10^6	36	1 : 1280	++	++	++	++	+	+
		37	1 : 1280	++	++	++	++	—	—
		38	1 : 640	++	++	++	++	—	—
		39	1 : 1280	++	++	+	—	—	—
		40	1 : 5120	++	++	++	++	+	+
180	3×10^4	41	1 : 1280	++	++	++	++	—	—
		42	1 : 320	++	++	++	++	—	—
		43	1 : 1280	++	++	++	++	—	—
		44	1 : 320	++	++	++	+	—	—
	3×10^6	46	1 : 1280	++	++	++	—	—	—
		47	1 : 320	++	++	++	++	—	—
		48	1 : 2560	++	++	++	++	—	—
		49	1 : 640	++	++	++	++	++	+
		50	1 : 1280	++	++	++	++	—	—
		Unvaccinated control	3×10^4	51	<1 : 10	++	++	++	++
52	1 : 10			++	++	++	++	—	—
53	<1 : 10			++	++	++	++	++	+
54	<1 : 10			++	++	++	++	+	+
55	<1 : 10			++	++	++	++	++	+
3×10^6	56		<1 : 10	++	++	++	++	++	+
	57		<1 : 10	++	++	+	++	++	+
	58		<1 : 10	++	++	++	++	++	+
	59		<1 : 10	++	++	++	++	++	+
	60		<1 : 10	++	++	++	++	—	—

* Remarks are the same as in Table 1.

All guinea pigs, except unvaccinated controls, received 20mg of a merthiolate-killed vaccine.

and killed 15 days after challenge inoculation. Results are shown in Table 2.

Although effective protection was not developed in guinea pigs within 10 days after vaccination, it appeared distinctly between days 20 and 80. Namely, the inoculated organisms could not grow in any parts of the respiratory tract of almost all or all guinea pigs when they were challenged on days 20, 30, 50 and 80 after vaccination. The pro-

TECTIVE potency was gradually reduced thereafter, so that the organisms established themselves in upper parts of the respiratory tract of all guinea pigs vaccinated 100 days before challenge inoculation. The protectivity, however, seemed to retain till days 150, because when challenged with 10^4 organisms, 3 of 5 guinea pigs in this vaccination period were resistant completely to the infection, differing from unvaccinated con-

Table 3. Protective effects of merthiolate-killed vaccines prepared from two virulent and one avirulent strains of *B. bronchiseptica*

Strains used for vaccine	Animal no.	Recovery of the organisms on the 15th day after challenge*					Pneumonic lesion	
		Ext. nares	Nasal cavity	Trachea		Lung		
				Upper	Lower			
Virulent	N2-88	1	—	—	—	—	—	—
		2	—	—	—	—	—	—
		3	++	++	++	—	—	—
		4	—	—	—	—	—	—
		5	++	+++	+++	—	—	—
	1088	6	++	++	++	—	—	—
		7	—	—	—	—	—	—
		8	—	—	—	—	—	—
		9	—	—	—	—	—	—
		10	++	+++	+++	—	—	—
Avirulent	64L-M	11	++	+++	+++	+++	+++	+
		12	++	+++	+++	+++	+++	+
		13	+++	+++	+++	+++	+++	+
		14	+++	+++	+++	+++	+++	+
		15	+++	+++	+++	+++	+++	+
		16	+++	+++	+++	+++	+++	+
Unvaccinated control		17	+++	+++	+++	+++	+	+
		18	+++	+++	+++	+++	+	+
		19	+++	+++	+++	+++	+	+
		20	+++	+++	+++	+++	—	—
		21	+++	+++	+++	+++	+++	+

* Remarks are the same as in Table 1.

All guinea pigs were challenged with 4×10^6 cells of strain 64L 50 days after injecting 20 mg of vaccines.

trols, in which the infection occurred without exception by inoculation of 10^4 organisms.

The serum agglutinins were detectable already 10 days after vaccination, and a rise of titer continued to the 30th day when the highest titer of 1:20,480 was obtained. From days 150, the titer decreased gradually, but showed 1:320 to 1:2,560 even on the 180th day.

Degrees of the protective potency were not always in parallel with agglutinin titers. For example, no. 10 guinea pig was strongly resistant to challenge inoculation, in spite of showing such a low agglutinin titer as 1:640, and in contrast to this, nos. 14, 26,

27, 29 and 30 guinea pigs were suffered from the infection, though their agglutinin titers were 1:10,240 and 1:20,480.

3. Protective effects of vaccines prepared from additional two virulent and one avirulent strains of *B. bronchiseptica*

Merthiolate-killed vaccines were prepared from strains N2-88 and 1088, both of which had been isolated from lung lesions of naturally infected guinea pigs and had infectivities comparable to strain 64L. No epizootic incorporation had existed absolutely each other among infections due to these two strains and strain 64L. Guinea pigs injected with 20 mg vaccine of each of strains N2-88 and 1088 were challenged in-

Table 4. Transmission of the immunity from mother to young by vaccination

Weeks after birth	Animal no.	Agglutinin titer	Recovery of the organisms on the 15th day after challenge*					Pneumonic lesion
			Ext. nares	Nasal cavity	Trachea		Lung	
					Upper	Lower		
2	1	1 : 640	+++	+++	+++	—	—	—
	2	1 : 1280	—	—	—	—	—	—
	3	1 : 640	+	+++	+++	—	—	—
	4	1 : 1280	—	—	—	—	—	—
	5	1 : 2560	++	+++	+++	—	—	—
4	6	1 : 1280	+	+++	+++	—	—	—
	7	1 : 2560	++	+++	+++	—	—	—
	8	1 : 5120	—	—	—	—	—	—
	9	1 : 640	+++	+++	+++	+++	—	—
	10	1 : 1280	++	+++	+++	—	—	—
6	11	1 : 160	+++	+++	+++	+++	+	+
	12	1 : 40	+++	+++	+++	+++	—	—
	13	1 : 40	+++	+++	+++	+++	—	—
	14	1 : 40	+++	+++	+++	—	—	—
	15	1 : 320	++	+++	+++	—	—	—
8	16	1 : 20	+++	+++	+++	+++	+++	+
	17	1 : 40	+++	+++	+++	++	++	+
	18	1 : 20	+++	+++	+++	+++	+++	+
	19	1 : 80	+++	+++	+++	—	—	—
	20	1 : 80	+++	+++	+++	—	—	—
10	21	1 : 10	+++	+++	+++	+++	—	—
	22	1 : 10	+++	+++	+++	+++	+++	+
	23	1 : 10	+++	+++	+++	+++	—	—
	24	<1 : 10	+++	+++	+++	+++	+	+
	25	<1 : 10	+++	+++	+++	+++	++	+
2-week-old weanlings from unvaccinated mothers	26	<1 : 10	+++	+++	+++	+++	+++	+
	27	<1 : 10	++	+++	+++	+++	++	+
	28	<1 : 10	++	+++	+++	+++	++	+
	29	<1 : 10	+++	+++	+++	+++	+++	+
	30	<1 : 10	+++	+++	+++	+++	+++	+

* Remarks are the same as in Table 1.

All guinea pigs were challenged with 4×10^6 cells of strain 64L.

transally with 4×10^6 cells of strain 64L on the 50th day after vaccination.

It is apparent from Table 3 that 3 guinea pigs in each group vaccinated with strain N2-88 or 1088 acquired strong resistance to challenge inoculation of strain 64L, indicating no growth of the inoculated organisms in any parts of the respiratory tract. Although the infection occurred in 2 guinea pigs in each group, growth of the organisms

limited in only the nose and upper trachea but invasion of the organisms to the lower trachea and lung was prevented. All of 5 unvaccinated controls suffered from the infection of challenged organisms and the pneumonic lesions were formed in 4 guinea pigs.

Prophylactic effect was also examined on a vaccine prepared from strain 64L-M which had lost the infectivity to guinea

pigs by subculturing 20 times on MacConkey agar. Six guinea pigs were injected with 20 mg of the vaccine and challenged with 4×10^6 cells of strain 64L 50 days later. Results are also shown in Table 3. All the guinea pigs were infected with the organisms used for challenge inoculation and suffered from the bronchopneumonia, as in unvaccinated controls, indicating no prophylactic effect of this vaccine.

4. Transmission of passive immunity from mother to young

To examine the transmission of immunity from mother to young, offspring of various ages, which had been born during 20 to 80 days after injected 20 mg merthiolate-killed vaccine of strain 64L to their mothers, were submitted to experimental infection and serum agglutination test. Namely, at 2, 4, 6, 8 and 10 weeks after birth, they were challenged with 4×10^6 cells of strain 64L immediately after taking 1 ml blood and autopsied for examination of the infection 15 days later. These animals were maintained with their mothers till 2 weeks of age and then weaned. Results are shown in Table 4.

Among 5 guinea pigs received challenge inoculation at 2 weeks of age, 2 protected absolutely the infection. Although 3 guinea pigs were suffered from the infection, the organisms were recovered only from the upper respiratory tract but not from the lower trachea and lung. Similar protective potency was also observed in guinea pigs aged 4 weeks. Serum agglutinin titers in guinea pigs of these ages were so high as 1:640 to 1:5,120. From 6 weeks after birth, no significant protection was demonstrated, but serum agglutinin titers still remained positive till 8 weeks of age. All of control weanlings from unvaccinated mothers showed high sensitivities to challenge inoculation and negative titers for serum agglutinins.

Discussion

Up to the present time, only a few workers have studied on prophylactic effects of vaccines for *Bordetella bronchiseptica* infection. Harris and Switzer [3] demonstrated accelerated nasal clearance of *B. bronchiseptica* in pigs by injecting subcutaneously sonicated cells of *B. bronchiseptica* strain or pertussis vaccine for human use. Ganaway et al. [2] reported that the administration of formalin-killed vaccine to breeder guinea pigs resulted in complete protection of *B. bronchiseptica* infection, including carrier state, when challenged under natural colony conditions. In the present study in which prophylactic effects of 3 different vaccines were compared, it was demonstrated that although both vaccines killed by formalin and merthiolate were effective, the latter gave stronger protective ability than the former. No infection occurred in almost all guinea pigs received merthiolate-killed vaccine, when challenged intranasally with so large dose as 10^6 cells of virulent strain 64L, while 4 of 5 guinea pigs received formalin-killed vaccine were suffered from the infection. It was also suggested in pertussis vaccine for human use that merthiolate-killed vaccine might be more effective in protective action than formalin-killed vaccine [4], but the reason for this phenomenon is still obscure.

Antigenic fractions of bacterial cells concerning the protective action were not particularly investigated in the present study. However, they were suggested to locate in capsule antigens of cells, because a vaccine heated at 100°C for 1 hour lost the protective ability (Table 1), and in addition, a part of capsule antigens of *B. bronchiseptica* was indicated to be destroyed at 100°C by Nakase [8]. These considerations may be supported by the fact that no protective

ability was demonstrated in an avirulent strain 64L-M which had been obtained by subculturing virulent strain 64L on Mac-Conkey agar 20 times and probably varied to phase III lacking the capsule [8, 9]. On the other hand, however, Wickert et al. [10] reported that administration of a vaccine heated at 60°C for 30 minutes protected rats considerably from the death due to pneumonia caused by *B. bronchiseptica*. Therefore, it is assumed that the protective antigens are destroyed at 100°C but not at 60°C. Further studies will be needed to solve these problems.

For estimation of degrees of the protection in guinea pigs, there is no way other than the intranasal challenge inoculation of virulent strains. Although there was a general correlation between protection against challenge inoculation and agglutinin response, discrepancies were observed in some guinea pigs. A similar phenomenon was shown in guinea pigs which acquired protection by natural infection of *B. bronchiseptica* [11] and also in children received pertussis vaccines [5].

For practical use of vaccine in breeding guinea pig colonies, it is necessary to learn not only protective effects of the vaccine but also the duration and the transmission from mother to young of the immunity. Ganaway et al. [2] suggested that guinea pigs received formalin-killed vaccine emulsified with Freund's incomplete adjuvant would protect *B. bronchiseptica* infection for at least 7 months after vaccination under natural colony conditions. In the present study, the duration of immunity in vaccinated guinea pigs was examined by intranasal challenge inoculation of so large dose as 10^6 or 10^4 cells of a virulent strain, and the protective action was shown to last for at least 5 months. Considering that guinea pigs are usually used for breeding

till 18 to 24 months of age, the durations of immunity described above are not long enough to give protective activity throughout the breeding life of guinea pigs. Two to three booster injections of vaccine will be needed to keep guinea pigs in a condition of immunity.

Protective activity afforded by vaccination was proved to be transmitted from mother to young. Namely, offspring delivered from mothers in good immunity showed considerable degree of protection against challenge inoculation till 4 weeks after birth, resulting in no growth of the challenged organisms in the respiratory tract or no invasion of the organisms into the lung. Serum agglutinins were also transmitted from mother to young and detectable till 8 weeks of age. Such a transmission of immunity probably occurred before birth but did not after birth [1].

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