Korean J. Vet. Res., Vol. 18, No. 1 (1978)

Studies on Protective Immunity Against *Bordetella bronchiseptica* Infection

Byong-Kyu Kang, D.V.M., M.S., Ph.D.

Department of Veterinary Medicine, College of Agriculture
Jeonnam National University

Introduction

Results of research on swine respiratory disease since 1956 have proved *B. bronchiseptica* to be major etiologic factor in the disease commonly called the infectious atrophic rhinitis of swine (AR).[1~4,6,11~14,20,21]

In their previous paper,[11,12,25] the authors observed that the detection of *B. bronchiseptica* by the isolation culture was gradually difficult when the age of animals was increased, and that agglutinating-antibodies became detectable in naturally infected piglets about 3 months of age. These results suggested that the organism present in the nasal cavities decreased gradually in numbers and that it was one of a model of infection in relation to establishment of infection and the immune response of the host. However, many problems still remain to be solved, especially on the specific relationship between the establishment of the agent in the nasal cavity and apparition of symptoms, lesions and immune response.

The purpose in the present experiments is to evaluate the prophylactic value of *B. bronchiseptica* vaccine prepared by several different method including assessment of the biological characters of used strain originated from infected swine, and is to obtain further information concerning the host-parasite relationship in experimentally infected mice.

Materials and Methods

Bacterial Strain: A strain of W-1029 of *B. bronchiseptica* which had been isolated from the nasal cavity of a naturally infected pigs with turbinate atrophy was used (designated virulent W-1029 strain, Phase I). An isolate of *B. bronchiseptica* which was originally isolated from the above source and subcultured on the pepton water for several times was also used (designated low-virulent strain H-969, Phase III). The phase variation of the organisms was determined as described by Nakase.[17-19]

Culture Media: MacConkey agar medium (Eiken) containing 1% of glucose was used for the selective isolation of *B. bronchiseptica*. Trypto-soy agar medium (Eiken) containing 5% horse blood was also used for vaccine preparation and the detection of the flora of various organs from mice. Trypticase soy broth (Eiken) was used to obtain the challenge living organism for intranasal inoculation.

Experimental Animal: White ddS-line, male mice weighing 15 g of body weight, were distributed into cages as a group. Two mice selected at random from each cage were employed to confirm Bordetella-free state by cultivating the materials from the respiratory organs as well as by serological agglutination test.

Experimental Procedure:

Experiment 1.-

Virulence and mouse protection tests were conducted by the method described by Nakase[19] with slight modification. Each organisms of Phase I and III cultured on the Trypto-soy agar plate medium for 48 hours at 37C were suspended in the pepton water in the concentration of 1 mg of harvested organism per ml and 0.5 ml of serial ten-fold dilution was inoculated intraperitoneally and intracerebrally. Death or survive was recorded for 14 days and LD_{50} was calculated by the Behren's-Kärber and/or probit method.

Comparisons of the protective potency between the *B. bronchiseptica*, Phase I and III was also done with the challenge of virulent W-1029, Phase I organism in the dose of 1 mg per ml inoculated intraperitoneally. Death or survive was also recorded for 14 days.

To confirm the toxicity of virulent W-1029 strain, Phase I organism, mouse toxicity test was done with the sonic extracted and the fraction obtained by the DEAE-cellulose column chromatography as described previously[24].

Experiment 2.-

Vaccine Preparation: The organism (virulent W-1029 strain, Phase I) for preparing a whole culture bacterin was grown on the tryptose blood agar base medium, incubated at 37 C for 20~24 hours and harvested with 0.85% saline solution (1mg per ml). This bacterin containing 10^7 colony forming units (CFU) per ml was kept at 37 C for one week or 0 C for 10 to 14 days untill it was inactivated in the presence of formalin (0.5%) or merthiolate (1 : 10,000). The sterility was checked by inoculating 1 ml of bacterin into 10 ml of the trypticase soy broth or on the tryptose blood agar plate medium and after inactivation, all bacterins were kept at 4 C until used. From the above preparation of bacterins, the following four different killed vaccines were designated as merthiolate-killed at 4 C, merthiolate-killed at 37 C, formalin-killed at 4 C and formalin-killed at 37 C vaccine respectively.

Inoculation of Vaccine: A group of 30 mice received a single intraperitoneal injection of 0.3 ml vaccine. This volume of vaccine was calculated to contain either 0.3 mg of organism harvested from grown cells or 3×9^9 CFU.

Challenge Inoculation: Prophylactic values of the vaccines were assessed by challenge inoculation with the virulent strain W-1029, Phase I. After two weeks, the immunized mice were challenged with the dose of 10, 200 and 250 LD_{50}. This volume of challenge organisms was calculated as 10^6 CFU in 1 LD_{50}. Procedures for these examinations were reported in detail elsewhere.[16,19,22]

Experiment 3.-

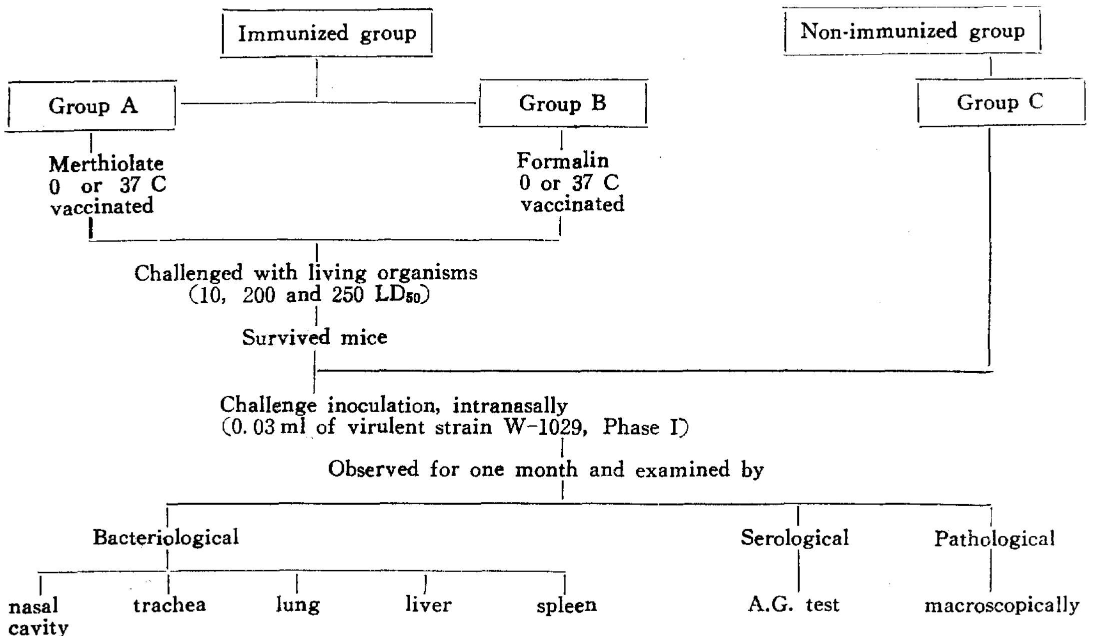


Fig. 1. Experimental procedure to determine the effects of vaccines on mice after intranasal challenge.

Table 1. Comparisons of Virulence between *B. bronchiseptica*, Phase I and Phase III Organisms on Mice

Strain Used	Phase Variation	Inoculation Route	LD_{50} (mg/ml)
W-1029, Virulent	I	Intracerebral Intraperitoneal	$10^{-3.5}$* $10^{-4.5}$**
H-969, Low-virulent	III	Intracerebral Intraperitoneal	$10^{-1.0}$* $2\times10^{11.8}$**

* Calculated by the method of Behren's—Kärber method.
** Calculated by the method of probit method.
LD_{50} was shown as mg per ml in 0.03ml intracerebral and in 0.5ml intraperitoneal inoculation, respectively.

Table 2. Comparisons of Protective Potency with *B. bronchiseptica*, Phase I and Phase III Organisms to Mice

Strain Used	Dosis (0.5ml)		Mouse
	Immunized(mg/ml)	Challenged (mg/ml)*	Survived/Immunized
W-1029, Virulent	10^{-4}	1.0	5/5
	10^{-5}	1.0	5/5
H-969, Low-virulent	10^{-4}	1.0	0/5
	10^{-5}	1.0	0/5

* Challenge was done with virulent strain W-1029, Phase I inoculated intraperitoneally.

Schematic diagram of experimental procedure to clarify the prophylactic value of the vaccines and the host-parasite relationship in relation to the immunological response of mice after challenged intranasally was shown in Fig. 1.

The immunized mice which were included Experiment 2 were challenged with 0.03ml of virulent strain W-1029, Phase I. And the non-immunized mice were also challenged as control. The external nares of unanesthetized mice were smeared with 10^6 or 10^4 CFU of 18 hour cultures in the trypticase soy broth and one or two mice were autopsied at 5-day intervals for a month after inoculation for cultivation of the challenged organisms in the respiratory system and internal organs and for serum agglutination test. Gross examinations were performed especially on the lung and the methods of cultivation and agglutination test were reported in detail previously[11,25)

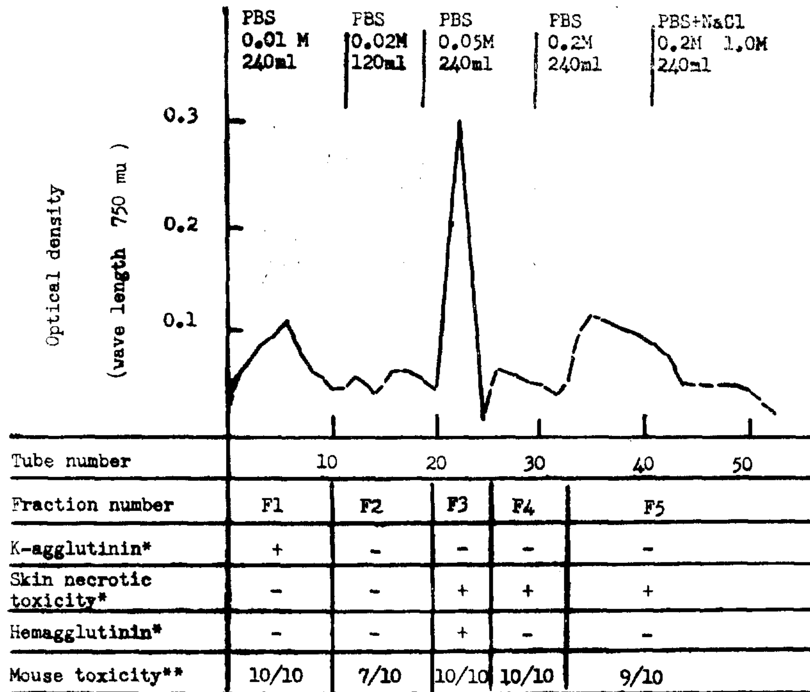
Results

Experiment 1: The virulence of strains used in this experiment was tested and a desirable LD_{50} calculated from the results. The virulence of strains used are shown in Table 1.

In intracerebral inoculation, the LD_{50} of Phase I organism was approximately $10^{-3.5}$ mg per ml and that of Phase III was $10^{-1.0}$ mg per ml. The LD_{50} of Phase I was $10^{-4.5}$ mg per ml when inoculated intraperitoneally and the viable cell counts were 1×10^7 CFU per ml and that of Phase III was $2\times10^{11.8}$ mg per ml.

To confirm the protective potency of the organisms used by the phase variation, mouse protection test was carried out. As shown in Table 2, the mice immunized with virulent W-1029, Phase I organism survived completely. On the contrary, none of mice immunized with the low-virulent H-969, Phase III organism survived when they were challenged with a virulent strain of Phase I organism.

Toxicity of obtained fractions from the sonic extracted crude or starting material by diethylaminoethyl (DEAE)-cellulose column chromatography was



Tube number	10	20	30	40	50
Fraction number	F1	F2	F3	F4	F5
K-agglutinin*	+	-	-	-	-
Skin necrotic toxicity*	-	-	+	+	+
Hemagglutinin*	-	-	+	-	-
Mouse toxicity**	10/10	7/10	10/10	10/10	9/10

Fig. 2. Chromatography of *B. bronchiseptica*, Phase I cells on a DEAE-cellulose column. Sonic extracted crude antigen (50mg/ml in 0.005M PBS at pH 7.4) was chromatographed on a column (1.3×23cm). The flow rate was 10 ml per hr. Total recovery of protein was 89% as measured by the Lowry-Folin reaction.
* Methods were described in a previous paper[24].
** Mouse died/mouse inoculated

tested on mice by intraperitoneal inoculation with a dose of 0.5ml of each fraction, and obtained results which included the various characters such as K-agglutinin, skin necrotic toxicity and hemagglutinability described in a previous paper[24] are shown in Fig. 2.

With the dosage employed, 80 to 100% of the mice died one to 6 days after inoculated and no differences between the fractions were observed although the K-agglutinin, skin necrotic toxicity and hemagglutinability showed difference in each fractions.

No different lethal activities of the fractions and it was presumed that the toxic fraction could not be seperated by the DEAE-cellulose column chromatography and further rechromatography will be necessary to seperate the toxic fraction.

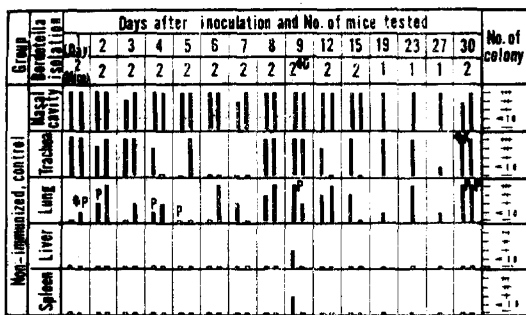
Obtained results from the above, the active, biological and immunological characters of virulent W-1029 strain, Phase I organism were confirmed and this strain was used in a further experiment.

Experiment 2: Protective effect of vaccines prepared by four different procedures was evaluated and results are shown in Table 3. There was a marked difference between the non-immunized, control and the active immunized groups; all mice were dead in the non-immunized, control group, whereas all survived in the immunized group when they were challenged with the 10, 200 and 250 LD_{50} doses of virulent organism. However, no difference was obtained in protecting potency by the four vaccines and it was apparent from these findings that the vaccines prepared by the merthiolate or formalin treatment showed protective effect to *B. bronchiseptica* infection and harmless to mice, and therefore, subsequent

Table 3. Protective Effects of Vaccines with Whole Cell Killed Organisms of *B. bronchiseptica*, Phase I

Strain Used	Treatment of Vaccine	Dose of Vaccine Inoculated*	Challenge Dose (LD_{50})**	Survival Rate***
W-1029 Virulent	Merthiolate at 0 C	3×10^9	250	9/10
			200	10/10
			10	10/10
	Formalin at 0 C	3×10^9	250	10/10
			200	10/10
			10	10/10
	Merthiolate at 37 C	3×10^9	250	10/10
			200	10/10
			10	10/10
	Formalin at 37 C	3×10^9	250	8/10
			200	10/10
			10	10/10
Control (Non-immunized)	—	—	250	0/10
			200	0/10
			10	2/10

* Viable organisms in colony counts.
** 1 $LD_{50}=1\times10^7$ CFU.
*** Numbers of mice survived/challenged.



■ : Nos. of colony from which *B. bronchiseptica* was isolated (卌=more than 100).
□ : :B. bronchiseptica was not isolated.
D : Die out. P: Lesions of pneumonia was recognized. K : Klebsiella sp. isolated.

Fig. 3. Sequential detection of *B. bronchiseptica* in Experiment 3(non-immunized, control).

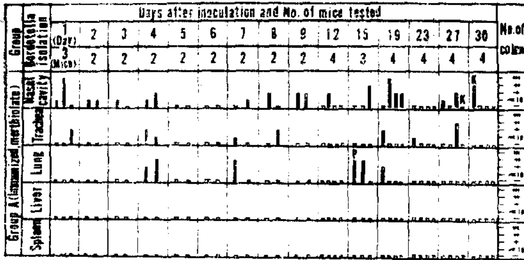
work to clarify the host-parasite relationship were carried out.

Experiment 3: To determine whether or not there exists a relationship between the antibody response and protection against *B. bronchiseptica* infection, further intranasal infection was conducted on the survived mice in Experiment 2 and results are shown in Figs 3, 4 and 5.

In the non-immunized group of mice, a definite respiratory wheeze persisted for several hours after the inoculation. During the next 24 hours, the mice became less active, refused food, lost body weight, and showed rough coat. But in the immunized group of mice, clinical symptoms were not recognized.

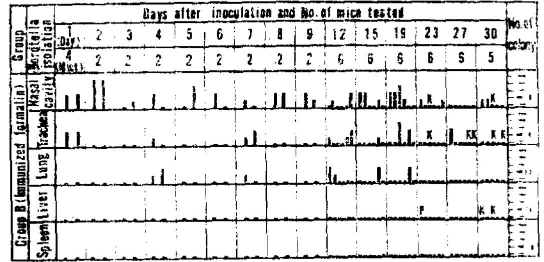
Necropsy findings of the non-immunized group of mice are as follows. There was often a small amount of free bloody fluid in the serous cavities and petechial hemorrhages were recognized in the peritoneum and pleura. The liver, spleen and kidneys were enlarged. The lungs were distended and diffuse hemor rhagic areas were noted, particularly on the apical regions. Bronchi contained frothy exudate. On the contrary, typical signs were not observed in the immunized, control group of mice.

The recovery of the organisms from the respiratory system in the immunized group of mice decreased remarkably in number by the isolation culture in comparison with the non-immunized group of mice,



Note: Same as Fig. 3.

Fig. 4. Sequential detection of *B. bronchiseptica* in Experiment 3 (immunized with the merthiolate-killed vaccines)



Note: Same as Fig. 3.

Fig. 5. Sequential detection of *B. bronchiseptica* in Experiment 3 (immunized with the formalin-killed vaccines).

Table 4. Summary of Incidence of *B. bronchiseptica* Recovered from Organs of Immunized and Non-immunized, Control Mice

Group	*B. bronchiseptica* Isolated from					
	Nasal Cavity		Trachea		Lung	
	Positive	%	Positive	%	Positive	%
A&B (Immunized)	47/97*	48.5	23/97	23.6	14/97	14.5
C(Non-immunized)	30/30	100.0	20/30	66.7	20/30	66.7

* Numbers of mice from which Bordetella recovered/numbers of mice tested.

but a long-term harbouring of the organism was recognized until the experiment ended in some cases of the immunized group of mice. Summary of incidence of *B. bronchiseptica* recovered from the various organs of the immunized and non-immunized, control groups of mice are presented in Table 4.

Results of the serum agglutinin response detected against *B. bronchiseptica* antigen after the intranasal challenge inoculation in Experiment 3 are shown in Fig. 6. The average serum agglutinin titers produced by the immunized groups of mice (group A & B) ranged from 1 : 40 to 1 : 2,560 and persisted continuously during the experimental period, while the titers of he non-immunized, control group of mice were negative or from 1 : 10 to 1 : 80, which appeared from 4 days after the intranasal inoculation.

Discussion

Antigenic fractions of bacterial cells concerning the protective action were not particularly demonstrated in the present study, even though they were seperated from the sonicated extracted materials to the fractions of agglutinogen, necrotizing factor and hemagglutinin by DEAE-cellulose column chromatography[24]. However, it was suggested that antigenic fractions were located in capsule antigens of cells, because a vaccine heated at 100 C for 1 hour lost the protective ability[16,17]. These considerations may be suported by the fact that no protective ability was demonstrated in an avirulent or Phase III organism (Table 2)[17,18].

On the other hand, Fetter et al.[4] reported that *B. bronchiseptica*, in producing atrophic rhinitis, must release substance which diffuse into the tissue and elicit changes in the osseous core without inciting an inflammatory reaction and that the endotoxin of *B. bronchiseptica* possibly was the initiating agent. Harris et al.[9] also reported that Boivin extracts of *B. bronchiseptica* inhibited or uncoupled the energized process of bovine heart and porcine heart mito-

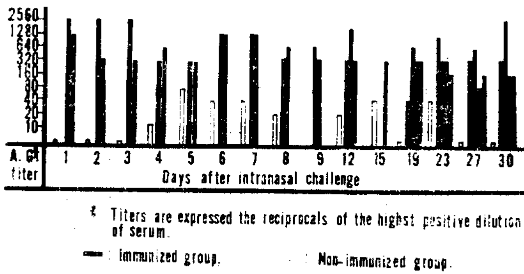


Fig. 6. The average serum agglutinin response detected against *B. bronchiseptica* antigen in nonvaccinated and vaccine injected mice after intranasal inoculation with virulent strain (Experiment 3).

chondria, and elaboration of endotoxin or other factor(s) could have resulted in a direct cytotoxic effect on bone cells.

As shown in Table 1 and 2, low-virulent H-969, Phase III obtained by subculturing showed lower virulence compared with the virulent strain of W-1029, Phase I and completely losted the protective ability. Nakase[18] reported that, although the organism originally obtained from pneumonic lesions of infected mice was Phase I, it easily varied to Phase II, Phase III or rough phase within 3 passages on blood agar. On the other hand, Nakagawa et al.[16] reported that the 20th subcultures of strain 64 L of *B. bronchiseptica* originated in guinea pig had no remarkable effects of subculturing on the infectivity and ability to form pneumonic lesions of the organism. Further investigation will be needed to clarify the difference of virulence and infectivity among the strain originated from various sources, such as the mouse, rabbit, guinea pig, dog and pig.

Harris and Switzer[10] demonstrated accelerated nasal clearence of *B. bronchiseptica* in pigs by injecting subcutaneously sonicated cells of *B. bronchiseptica* strain or pertussis vaccine for the prevention of whooping cough in man. Ganaway et al.[5] reported that, in guinea pigs, the administration of formalized bacterin emulsified in Freund's incomplete adjuvant prevented *B. bronchiseptica* infection completely. Nakagawa et al.[16] reported that formalin and merthiolate killed vaccines were effective to prevent *B. bronchiseptica* infection in guinea pigs and merthiolate killed vaccine was most effective compared with heat or formalin treatment and same results were obtained in pertussis vaccine for human use[15]. In the present study in which prophylactic effects of four different vaccines were compared, it was demonstrated that no difference between the treatments of formalin or merthiolate and 0 C or 37 C inactivation methods, and that almost all mice survived when challenged with large dose (250 LD_{50}) of virulent strain.

Although it is commonly demonstrated that most naturally or artificially infected AR pigs showed the agglutinin in the sera by the agglutination test, the nature of the immunity especially in relation to prevention of *B. bronchiseptica* infection has never been clearly defined. Harris and Switzer[7] reported that the serum of four swine intramuscularly inoculated with whole culture *B. bronchiseptica* formalized bacterin contained high titers of agglutinating antibody but resistance against nasal infection did not occur and the serum of swine intranasally inoculated with low-virulent strain did not contain detectable titers of agglutinating antibody. They also described that the simultaneous intranasal inoculation of both virulent and low-virulent strains did not accelerate the clearence of the infection[8]. Recently, however, Harris and Switzer[10] demonstrated accelerated nasal clearence of *B. bronchiseptica* in pigs by injecting subcutaneously sonicated cells of *B. bronchiseptica* strains or pertussis vaccine for human use and finally they concluded that this differs from the resistance against reinfection produced in pigs given a live low-virulent strain of *B. bronchiseptica*.

On the other hand, Yoda et al.[23] reported that agglutinins were demonstrable in a few recovered guinea pigs submitted to experimental infection and that these animals were highly resistant to the challenge infection; most of the recovered guinea pigs had acquired nasal and tracheal resistance against the reinfection of the organism. Nakagawa et al.[16] reported that there was a general correlation between protective potencies and serum agglutinin

titers, although discrepancies were observed in some guinea pigs and agglutinin production was not always accepted as evidence of protective potency.

It is particularly interest to note that the age of animals was related to resistance. It is difficult to accept that the resistance was heightened with age, because no differences in susceptibility had been found among guinea pigs[23] or swine[7].

Recently, Koshimizu et al.[13] reported that *B. bronchiseptica* could be established and persisted in the nasal cavities of piglets having some level of the maternal antibodies and suggested that the maternal antibody may have blocked the production of antibody.

As shown in Fig. 4, 5, 6 and Table 4, agglutinin was demonstrable in the immunized group of mice and these animals were highly resistant to the challenge infection. However, the organisms which were inoculated into the nasal cavities or respiratory organs of the immunized mice decreased gradually in numbers and became undetectable at 30 days after inoculation. The authors[12] reported that agglutinating antibodies became detectable in naturally infected piglets about 3 months of age when the causative organism were gradually decreased in number and undetectable by the isolation culture. Consequently, it will be reasonable to conclude that the present experiment may be regarded as a model of natural infection, especially in relation to the establishment of infection and the immune response. Similar observations were also made by Koshimizu et al.[13,14] in conventional piglets. And it is assumed that the favorite localization sites of the organism are the mucous membrane of the respiratory tract as was already stated by Ganaway et al.[5] Harris et al.[9] and Fetter et al.[4] Further studies are needed to find any factor of the organism responsible for the resistance of the respiratory system in case of either natural infection or vaccination.

Conclusion

An experimental *Bordetella bronchiseptica* infection was carried out by using the ddS-line mice to evaluate the immunogenecity of the organisms originated from the naturally infected atrophic rhinitis of swine(AR) and to clarify the host-parasite relationship.

Results obtained are summarized as follows:

In the virulence and protective potency test, the phase I strain (virulent, W-1029) showed strong letheal toxicity and high protective potency, while the phase III strain (low-virulent, H-969) lacked it. This indicated that the virulent, phase I organism is the best and perhaps the only antigen for the preparation of active immunity in mice.

Whole cell vaccines (virulent, Phase I, W-1029) killed by formalin and merthiolate produced much effective protection potency and no differences were found by the inactivation methods.

There was a general correlation between the post-challenged bacterial recovery of the reinfected organisms from the respiratory organs or protective potency and serum agglutinin levels, although discrepancies were observed in some mice during a long-term observation. Therefore, agglutinin production was not always accepted as evidence of protective potency.

References

1. Cross, R.F. and Claflin, R.M.: *Bordetella bronchiseptica*-induced porcine atrophic rhinitis. J. Am. Vet. Med. Ass. (1962) 141 : 1467.
2. Duncan, J.R., Ross, R.F., Switzer, W.P. and Ramsey, F.K.: Pathology of experimental *Bordetella bronchiseptica* infection in swine: atrophic rhinitis. Am. J. Vet. Res. (1966) 27 : 457.
3. Dunne, H.W. and Leman, A.D.: Diseases of swine. 4th ed., The Iowa State University, Ames. Iowa (1975) p. 687.
4. Fetter, A.W., Switzer, W.P. and Capen, C.C.: Electron microscopic evaluation of bone cells in pigs with experimentally induced Bordetella rhinitis (Turbinate osteoporosis). Am. J. Vet. Res. (1975) 36 : 15.
5. Ganaway, J.R., Allen, A.M. and McPherson, C.W.: Prevention of acute *Bordetella bronchiseptica* pneumonia in a guinea pig colony. Lab.

Animal Care (1965) 15 : 156.

6. Harris, D.L. and Switzer, W. P.: Turbinate atrophy in young pigs exposed to *Bordetella bronchiseptica*, *Pasteurella multocida* and combined inoculum. Am. J. Vet. Res. (1968) 29 : 777.
7. Harris, D. L. and Switzer, W. P.: Nasal and tracheal resistance of swine against reinfection by *Bordetella bronchiseptica*. Am. J. Vet. Res. (1969) 30 : 1161.
8. Harris, D.L.: Resistance to *Bordetella bronchiseptica* infection in swine. Ph.D. Thesis, Iowa State University, Ames, Iowa (1970).
9. Harris, D.L., Switzer, W.P. and Haris, R.A.: Suggested mechanism for the pathogenesis of infectious atrophic rhinitis. Can. J. Comp. Med. (1971) 35 : 318.
10. Harris, D.L. and Switzer, W.P.: Immunization of pigs against *Bordetella bronchiseptica* infection by parenteral vaccination. Am. J. Vet. Res. (1972) 33 : 1975.
11. Kang, B.K., Koshimizu, K. and Ogata, M.: Studies on the etiology of infectious atrophic rhinitis of swine. II. Agglutination test on *Bordetella bronchiseptica* infection. Jap. J. Vet. Sci. (1970) 32 : 295.
12. Kang, B.K., Koshimizu, K. and Ogata, M.: Studies on the etiology of infectious atrophic rhinitis of swine. III. Field survey by agglutination test in relation to incidence of *B. bronchiseptica* and turbinate atrophy. Jap. J. Vet. Sci. (1971) 33 : 17.
13. Koshimizu, K., Kodama, Y. and Ogata, M.: Studies on the etiology of infectious atrophic rhinitis of swine. V. Experimental *Bordetella bronchiseptica* infection in conventional piglets. Jap. J. Vet. Sci. (1973) 35 : 223.
14. Koshimizu, K., Kodama, Y. and Ogata, M.: Studies on the etiology of infectious atrophic rhinitis of swine. VI. Effect of vaccination against nasal establishment of *Bordetella bronchiseptica*. Jap. J. Vet. Sci. (1973) 35 : 411.
15. Munoz, J. and Hestekin, B.M.: Antigens of *Bordetella pertussis*. IV. Effect of heat, merthiolate and formaldehyde on histamin-sensitizing factor and protective activity of soluble extracts from *Bordetella pertussis*. J. Bacteriol. (1966) 91 : 2175.
16. Nakagawa, M., Yoda, H., Muto, T. and Imazumi, K.: Prophylaxis of *Bordetella bronchiseptica* infection in guinea pigs by vaccination. Jap. J. Vet. Sci. (1974) 36 : 33.
17. Nakase, Y.: Studies on *Hemophilus bronchisepticus*. I. The antigenic structures of *H. bronchisepticus* from guinea pig. Kitasato Arch. Exp. Med. (1957) 30 : 57.
18. Nakase, Y.: Studies on *Hemophilus bronchisepticus*. II. Phase variation of *H. bronchisepticus*. Kitasato Arch. Exp. Med. (1957) 30 : 73.
19. Nakase, Y.: Studies on *Hemophilus bronchisepticus*. III. Differences of biological properties between Phase I and Phase III of *H. bronchisepticus*. Kitasato Arch. Exp. Med. (1957) 30 : 79.
20. Ross, R.F., Duncan, J.R. and Switzer, W.P.: Turbinate atrophy in swine produced by pure culture of *Bordetella bronchiseptica*. Vet. Med. (1963) 58 : 566.
21. Ross, R.F., Switzer, W.P. and Duncan, J.R.: Comparison of pathogenecity of various isolates of *Bordetella bronchiseptica* in young pigs. Canad. J. Comp. Med. (1967) 31 : 53.
22. Standfast, A.F.B. and Dolby, J.M.: A comparison between the intranasal and intracerebral infection of mice with *Bordetella pertussis*. J. Hyg. Camb. (1961) 59 : 217.
23. Yoda, H., Nakagawa, M. Muto, T. and Imaizumi, K.: Development of resistance to reinfection of *Bordetella bronchiseptica* in guinea pigs recovered from natural infection. Jap. J. Vet. Sci. (1972) 34 : 191.
24. 康炳奎 : *Bordetella bronchiseptica*의 菌體成分에 關한 硏究. 特히 DEAE-cellulose chromatography에 依한 分劃精製에 對하여. 大韓獸醫學會誌 (1973) 13 : 47.
25. 尾形 學, 輿水 馨, 康炳奎, 跡部ヒサエ, 山本孝史 木津野南夫, 池田讚成: 豚の傳染性萎縮性鼻炎の 病原學的硏究. I. 鼻腔內細菌叢と疾病との關係. 日本獸醫學會誌 (1970) 32 : 185.

*Bordetella bronchiseptica*의 感染免疫에 關한 硏究

康 炳 奎

全南大學校 農科大學 獸醫學科

抄 錄

돼지의 傳染性萎縮性鼻炎의 주요한 病原菌이라고 생각되는 *Bordetella bronchiseptica*(以下 B菌) 自然感染豚分離由來菌株를 사용하여 mouse 鼻腔內定着性에 對한 死菌免疫의 效果를 검토하고자, 먼저 相變異에 따르는 菌의 毒力과 感染防禦性을 비교함과 동시에, I 相菌不活化豫防液의 免疫原性에 對한 기초적인 검토를 실시하였고, 이에 계속하여 生菌鼻腔內接種에 따르는 菌의 定着性과 血中凝集抗體의 經時的인 推移를 검토하였다. 얻어진 성적을 요약하면 다음과 같다.

1. B菌 I 相菌(W-1029株)의 毒力은 Ⅲ相菌(H-969株)에 비하여 毒力이 강하였으며, I 相菌은 感染防禦性을 보유하고 있었으나 Ⅲ相菌은 感染防禦性이 전혀 없었다.

2. B菌 I 相菌死菌免疫에 있어서 formalin과 merthiolate 處理 및 不活化溫度(0° 및 37°C)間에는 현저한 免疫原性의 차이는 인정할 수 없었다.

3. 死菌免疫을 실시한 mouse群에 生菌의 經鼻接種에 의하여 經時的으로 菌의 呼吸氣道內의 定着性과 血中凝集抗體價의 推移를 보았던 바, 非免疫對照群에 비하여 免疫群은 현저한 免疫效果를 나타내었으나, 免疫群에서 間歇的인 排菌이 상당기간에 걸쳐 인정되었으며 또한 凝集抗體價의 상승에 따르는 鼻腔內定着菌의 完全消失은 다소 곤난함이 인정되었다.

以上의 成積으로 B菌死菌抗原을 사용하여 高度의 免疫을 시킴으로서 鼻腔內에 있어서의 B菌의 定着을 阻止할 수 있는 可能性이 示唆되나 抗體價의 上昇에 따르는 菌이 鼻腔內에서 완전 消失이 다소 곤난한 점은 本菌의 呼吸器道粘膜感染의 實態를 示唆하는 것으로 보아진다.