STUDIES ON THE ETIOLOGY OF INFECTIOUS ATROPHIC RHINITIS OF SWINE

V. EXPERIMENTAL *BORDETELLA BRONCHISEPTICA* INFECTION IN CONVENTIONAL PIGLETS

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(Received for Publication August 19, 1972)

In their previous studies^{6,7,9)}, the authors suggested that *Bordetella bronchiseptica* might be one of the most important agents being associated with infectious atrophic rhinitis of swine in Japan. Several workers^{1,2,5,11,13)} have reported that inoculation of *B. bronchiseptica* into nasal cavity of SPF or HPCD piglets results in turbinate atrophy. 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 present paper deals with the host-parasite relationship in experimentally infected colostrum-fed conventional piglets.

MATERIALS AND METHODS

Animals: Twenty-nine 5- or 6-day-old piglets born from 3 conventional sows of a crossbred between Large White and Landrace were used. The sows had an agglutinating antibody titer of 1 : 320 just after parturition, but no organism of *B. bronchiseptica* was detected in their nasal cavities. The young were fed colostrum and reared together with their mothers in a conventional environment during the first 5 days of life. Then, each litter was placed separately into an isolate house. The halves of the piglets were kept separately in other isolate pens as uninoculated controls. Animals were fed with a special ration prepared by Nisshin Flour Milling Co., Ltd. The ration contained no antibiotics and sulfa drugs.

Bacterial strain: A strain S-19 of *B. bronchiseptica* which had been isolated from nasal cavity of a naturally infected pigs was used. The phase variation of the organism was determined in the same manner, as described by NAKASE⁸⁾. Fairly stable Phase I organisms grown on trypto-soy agar medium (Eiken) containing 5% horse blood were inoculated into trypto-soy broth (Eiken), and incubated at 37°C for 18 hours with continuous agitation. Viable cell count was made before inoculation to piglets.

Culture media: MacConkey agar medium (Eiken) containing both 25 µg per ml of furazolidone and 1% of glucose was used for the selective isolation of *B. bronchiseptica* from nasal cavities of pigs. Tryptso-agar medium (Eiken) containing 5% horse blood was also used for the antigen preparation and the detection of nasal flora of piglets. The isolation of organisms from nasal cavities was conducted by the swab method as described

previously⁹⁾.

Agglutination test: Serum agglutinating antibody titer was examined after an improved method devised by the present authors¹⁰⁾ using formalin-killed organisms.

RESULTS

1. Inoculation of *B. bronchiseptica*

At 5 and 6 days of age, 10 piglets were inoculated intranasally with 0.5 ml of tryptsoy broth culture of *B. bronchiseptica* containing about 10^9 organisms per ml. All the piglets were examined for the recovery of *B. bronchiseptica* from the nasal cavities and the demonstration of agglutinin at intervals of about 1 to 2 weeks. One or 2 inoculated and controlled piglets were killed respectively every 2 or 3 weeks between 3 to 29 weeks of age. At autopsy, the turbinate bone and lungs were examined. Nasal, tracheal and ethmoid exudate were sampled with a sterile swab, and cultured onto modified MacConkey agar medium and tryptsoy agar medium containing 5% horse blood. Liver, spleen, lung and kidney were also cultured.

The infected piglets showed sneezing and purulent nasal discharge. Some of them coughed occasionally at 4 to 10 weeks of age but the symptoms disappeared thereafter. Wrinkles on the nose side and so called eye patches were seen in all the piglets at 5 to 7 weeks of age, and nasal distortions were recognized clearly in 3 piglets at 3 to 7 weeks of age.

Before inoculation, no *Bordetella* organism was isolated from the nasal cavities of all piglets. Within 1 week postinoculation, the organisms were found to be established in the nasal cavities of all the piglets. However, the number of organisms recovered from the nasal cavity declined gradually at 16 or more weeks of age, disappearing at 22

Table 1. Recovery of *B. bronchiseptica* from Various Organs, Gross Lesions and Agglutinin Titers of Piglets Killed at Intervals

Group	Piglet No.	Age in weeks	Detection of <i>B. bronchiseptica</i>						Turbinate atrophy	Pneumonia	Agglutinin titer
			Nasal cavity	Ethmoid	Trachea	Lung	Liver	Kidney			
Inoculated with <i>B. bronchiseptica</i>	B-10	3	+++	+++	+++	+++	—	—	—	+	ND
	B-1	4	+++	+++	+++	++	—	—	—	+	1:80*
	B-13	7	++	++	+++	—	—	—	++	—	1:20*
	A-3	9	+++	+++	+++	—	—	—	+	+	<1:10*
	B-11	10	+++	+++	++	—	—	—	++	+	1:160
	B-9	13	+++	+++	—	—	—	—	+	—	1:80
	B-14	19	++	+	—	—	—	—	—	—	1:40
	A-2	22	+	+++	—	—	—	—	—	+	1:80
	B-7	25	—	—	—	—	—	—	—	—	1:80
Exposed to carriers	A-4	29	—	—	—	—	—	—	++	+	1:160
	C-10	22	+++	+	++	—	—	—	—	—	1:40
	B-5	25	++	+++	—	—	—	—	—	—	1:320
	C-4	25	—	—	—	—	—	—	—	—	1:80
	C-1	27	—	—	—	—	—	—	—	+	1:160
	B-2	28	—	+	—	—	—	—	—	+	1:320
	A-5	29	—	+	—	—	—	—	—	—	1:160
	A-6	29	—	++	—	—	—	—	—	+	1:160

*: Titer is probably of maternal origin.

Fig. 1. Sequential Demonstration of *B. bronchiseptica* and Agglutinin in Piglets

* Inoculated with *B. bronchiseptica*.

** At this age, 2 control piglets were exposed to carrier animals.

+, ++, +++: Less than 10 colonies, 10–100 colonies and more than 100 colonies were produced on a plate, respectively.

Fig. 2. Sequential Demonstration of *B. bronchiseptica* and Agglutinin in Piglets

* Inoculated with turbinate suspensions containing no *Bordetella* organism.

** At this age, each piglet was exposed to carrier animals.

Fig. 3. Sequential Change of Symptom, Establishment of Organism, Antibody Response and Pathologic Lesion in Experimentally Infected Piglets

to 24 weeks of age. *B. bronchiseptica* was also recovered from ethmoid, trachea and lungs of piglets. No organism was isolated from liver, kidney and spleen in any cases including control piglets (Table 1).

At 5 days of age, agglutinating antibodies probably of maternal origin were demonstrable at titers of 1 : 320 in all piglets. Such titers were declined gradually being 1 : 10 or less at 7 to 8 weeks of age. Around this time, a significant antibody response was detected only in infected piglets. The titers attained 1 : 80 to 1 : 160 in a few weeks, remaining at these levels throughout the experiment as shown in Fig. 1.

Mild or moderate atrophy in the scrolls of the ventral turbinates were observed macroscopically in 5 of 10 infected piglets, while the remaining 5 had no macroscopic lesion. The uninfected control piglets had normal turbinate. Six of 10 infected piglets had pneumonia. *Bordetella* organisms were purely recovered in large numbers from the lung lesions in 2 piglets killed at 3 to 4 weeks of age. There was no detectable lesion in the liver, kidney, spleen and lymph node.

2. Infection with turbinate suspension

Six piglets were inoculated intranasally at 6 days of age with 2.0 ml of emulsified suspension of turbinate bone from a naturally infected pig. This suspension was shown to contain *Pasteurella multocida* but no *B. bronchiseptica*.

B. bronchiseptica did not become established in the nasal cavities of piglets, whereas *P. multocida* was transiently recovered during the experimental period. Maternal antibodies to *B. bronchiseptica* were detected in the sera of all piglets at titers of 1 : 320 at 6 days of age, and 1 : 10 or less at 9 to 12 weeks of age. In some of piglets, mild snuffles and severe coughings were observed at 9 to 12 weeks of age. At autopsy, turbinate atrophy was not observed macroscopically, while extensive pneumonic lesions were observed in most of these piglets. *P. multocida* was isolated purely from the pneumonic lesions, but any *B. bronchiseptica* was not (Fig. 2).

3. Infection by exposure to infected piglets

At 13 to 18 weeks of age, 7 uninoculated control piglets were exposed to infected piglets which had been shown to have *B. bronchiseptica*. These piglets were reared in an isolation unit and subjected to bacteriological, serological and pathological examination.

Within a week after the exposure, *B. bronchiseptica* was found to be established in the nasal cavity of the piglets. Agglutinating antibodies against *B. bronchiseptica* were demonstrated with titers ranging from 1:40 to 1:320 2 weeks after the exposure. Neither clinical sign nor turbinate atrophy was recognized, while pneumonias were observed in 3 of 7 piglets at autopsy.

Sequential changes of symptoms, establishment of organisms, antibody response and pathologic lesions demonstrated in the experimental infection are summarized in Fig. 3.

DISCUSSION

In recent years, several workers^{1,2,5,11-13}) have reported that turbinate atrophy was produced by experimental inoculation of *B. bronchiseptica* into nasal cavities of susceptible baby piglets. In natural infections, however, there might be some influence of maternal antibodies on the occurrence of turbinate atrophy in piglets. In the present studies, it was demonstrated that *B. bronchiseptica* could be established and persisted in the nasal cavities of piglets having some level of the maternal antibodies. The titers of the maternal antibodies were shown to be 1:320 at the age of 5 days, declining gradually with age, and being 1:10 or less at the age of 8 weeks. Only after 8 weeks of age, a specific antibody response was demonstrable to *B. bronchiseptica* infection, suggesting that the maternal antibody may have blocked the production of antibody. But it is necessary to consider that very young piglets can not react sufficiently to the organisms having been established on the surface of the nasal epithelium. Furthermore, the authors⁷) have reported that agglutinating antibodies became detectable in naturally infected piglets about after 3 months of age.

As shown in the present experiment, the antibody detectable in a few-week-old piglets may be of maternal origin. On the contrary, around 10 weeks of age agglutinin was not demonstrable in spite of the presence of *B. bronchiseptica* in nasal cavities. Therefore, the detection of agglutinating antibodies should be evaluated carefully for the diagnosis of *B. bronchiseptica* infection.

The organisms persisting in the nasal cavities declined gradually in numbers after 16 weeks of age and became undetectable at 22 to 24 weeks of age, whereas agglutinating antibody titers were 1:80 or 1:320 until 28 weeks of age. These results were also similar to those reported in naturally infected pigs^{7,9}). 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 responses.

In 5 of 10 piglets inoculated with *B. bronchiseptica*, no macroscopic turbinate atrophy was observed, though the organisms were recovered in large numbers from the nasal cavities. This can not be explained at the present state.

B. bronchiseptica did not become established in the piglets inoculated with the emulsified suspension of turbinate containing no Bordetella organisms, while *Pasteurella multocida* was isolated only transiently from the piglets of this group. None of these piglets, however, showed noticeable turbinate atrophy. GWATKIN et al.^{3,4}) reported that a pure culture of *P. multocida*, type B, produced turbinate atrophy in baby piglets. On the other hand, HARRIS et al.⁵) observed that *P. multocida*, type D, was not capable of becoming established in normal swine, while this strain became established and produced severe turbinate atrophy in association with *B. bronchiseptica*. These observations lead to a suggestion that *P. multocida* never play an important role as the primary agent for infectious atrophic rhinitis of swine. In the present study, the serological typing of

P. multocida isolated from the nasal cavities of piglets was not conducted.

B. bronchiseptica became established easily in the nasal cavities of 13- to 18-week-old piglets which were exposed to carrier animals. Agglutinating antibodies became detectable at 1 to 2 weeks after exposure in these piglets. These results suggest that horizontal infection with *B. bronchiseptica* may occur naturally among piglets. None of the piglets of such an advanced age, however, showed any gross lesions in the turbinate bone.

Details of the microscopic findings of the lesions will be described later.

SUMMARY

An experimental *Bordetella bronchiseptica* infection was carried out by using colostrum-fed conventional piglets at 5 or 6 days of age. They were inoculated intranasally with either *B. bronchiseptica* culture or emulsified suspension of turbinate bone from a naturally infected pig, and subjected to bacteriological, serological and pathological examinations. The results obtained are as follows.

Clinical signs were seen in all the infected piglets though transiently. *B. bronchiseptica* became established within a week after instillation into the nasal cavities of the piglets, being undetectable at 22 to 23 weeks of age. The titers of maternal antibodies in piglets were shown to be 1:320 at 5 days of age, declining 1:10 or less at 8 weeks of age when a significant immune response to *B. bronchiseptica* infection became detectable.

At autopsy, turbinate atrophy was observed macroscopically in only 5 of 10 piglets inoculated with *B. bronchiseptica*.

No specific nasal lesion was produced in piglets inoculated with an emulsified suspension of turbinate bone from a naturally infected piglet containing no *Bordetella* organisms.

Within a week after exposure to carrier animals, *B. bronchiseptica* became established in the nasal cavities of 13- to 18-week-old piglets, showing significant antibody titers but no turbinate atrophy.

REFERENCES

- 1) CROSS, R. F. and CLAFLIN, R. M. (1962): *J. Amer. vet. med. Ass.*, **141**, 1467~1468.
- 2) DUNCAN, J. R., ROSS, R. F., SWITZER, W. P. and RAMSEY, F. K. (1966): *Amer. J. vet. Res.*, **27**, 457~466.
- 3) GWATKIN, R., DZENIS, L. and BYRNE, J. L. (1953): *Canad. J. comp. Med.*, **17**, 215~217.
- 4) GWATKIN, R. and DZENIS, L. (1953): *Canad. J. comp. Med.*, **17**, 454~464.
- 5) HARRIS, D. L. and SWITZER, W. P. (1968): *Amer. J. vet. Res.*, **29**, 777~785.
- 6) KANG, B. K., KOSHIMIZU, K. and OGATA, M. (1970): *Jap. J. vet. Sci.*, **32**, 295~306.
- 7) KANG, B. K., KOSHIMIZU, K. and OGATA, M. (1971): *Jap. J. vet. Sci.*, **33**, 17~23.
- 8) NAKASE, Y. (1957): *Kitasato Arch. exp. Med.*, **30**, 57~72.
- 9) OGATA, M., KOSHIMIZU, K., KANG, B. K., ATOBE, H. and YAMAMOTO, K. (1970): *Jap. J. vet. Sci.*, **32**, 185~199.
- 10) OGATA, M., KODAMA, Y. and KOSHIMIZU, K. (1973): *Jap. J. vet. Sci.*, **35**, 149~155.
- 11) ROSS, R. F., DUNCAN, J. R. and SWITZER, W. P. (1963): *Vet. Med.*, **58**, 566~570.
- 12) ROSS, R. F., SWITZER, W. P. and DUNCAN, J. R. (1967): *Canad. J. comp. Med.*, **31**, 53~57.
- 13) SHIMIZU, T., NAKAGAWA, M., SHIBATA, S. and SUZUKI, K. (1971): *Cornell vet.*, **61**, 696~705.

豚の伝染性萎縮性鼻炎の病原学的研究

V. 子豚 (conventional) を用いた *Bordetella bronchiseptica* 感染実験

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(昭和47年8月19日受付)

母豚の初乳から移行抗体 (1:320) を得た子豚 (conventional) 3 腹29頭を用い, *Bordetella bronchiseptica* の感染実験を行なった. このうち, 2 腹10頭の子豚には, 生後5日および6日齢において *B. bronchiseptica* I 相菌のトリプトソイ・ブイヨン培養菌液 (生菌数 10^9 /ml) 0.5 ml を, また残りの1腹6頭の子豚には萎縮性鼻炎自然例の甲介骨乳剤 2 ml を鼻腔内接種した. 以後, 臨床症状, 菌の定着性, 凝集抗体の推移および病変の発現などについて経時的な検査を行なった. 生後13~18週齢の時点において, 未接種対照豚を感染豚と同居させ, その感染経過を同様に検討した. 成績を要約すると, 次のとおりである.

1. *B. bronchiseptica* 接種群では, 臨床症状は, 4~10週令において一過性に認められた. *B. bronchiseptica* は, 接種後1週以内ですべての子豚の鼻腔内に定着したが, 16週齢頃より菌数

が減少し, 22~24週齢において検出不能となった. 子豚の移行抗体は5日齢時に 1:320 を示したが, 8週齢まで次第に減少し 1:10 あるいはそれ以下となった. これと前後して感染による特異抗体が出現した. その凝集抗体価は 1:80 ないし 1:320 まで上昇し, 29週齢のと殺時まで維持された.

2. 鼻甲介骨乳剤接種群においては, *B. bronchiseptica* は定着せず, *Pasteurella multocida* が一過性に検出された. 鼻甲介骨の萎縮は認められなかった.

3. 生後 13~18 週齢時に同居感染させた群では, *B. bronchiseptica* は同居後1週以内で鼻腔内に定着した. 凝集抗体価は2週後に 1:40 ないし 1:320 を示した. しかしながら症状および甲介萎縮は肉眼的に全く認められなかった.