

# Mixed Infection with *Mycoplasma gallisepticum* and the B1 or TCND Strain of Newcastle Disease Virus in Chickens

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Chickens infected with *Mycoplasma gallisepticum* alone generally develop no clinical symptoms. However, it is known that they show chronic respiratory distress or serious respiratory lesions by complication with other infectious microbial agents.

Especially, in the field this phenomenon has been considered to be important not only in natural infection with various bacteria and viruses, but also in the application of live-virus vaccine against viral respiratory diseases of chickens.

In Japan, since 1965, the Asian type Newcastle disease (NDV) has been prevalent, and as a result, the traditional killed-virus vaccine, or live-virus vaccine made of NDV B1 or TCND strain which is of low virulency has been widely in use.

The present writer and colleagues examined the effects of these virus strains on the infection of *M. gallisepticum* in chickens. The following is the list of the studies.

## Mixed infection with *M. gallisepticum* and NDV-B1 strain

Forty-eight-day-old broiler chickens were inoculated intratracheally with *M. gallisepticum* 1RF ( $10^7$  organisms/0.1 ml) or NDV-B1 strain ( $10^5$  TCID<sub>50</sub>/0.1 ml) alone, or with both agents at the same time or one week apart. These chickens were observed clinically for 10–18 days after inoculation and then autopsied. The result is shown in Table 1. The development of respiratory symptoms is

especially tabulated in Table 2.

From the result of Table 1, it was seen that only in the case of the mixed-infection group with *M. gallisepticum* and NDV-B1 (Groups 1, 5, and 8) did respiratory symptoms appear, and compared with the single infection group (Groups 2, 4 and 7), the frequency of isolation of *M. gallisepticum*, appearance of gross lesions and agglutinin titer against *M. gallisepticum* tended to be higher.

As seen in Table 2, in groups 1 and 5, which were inoculated with *M. gallisepticum* and NDV-B1 one week apart, it was observed that most of the chickens coughed comparatively for a short period of time. Group 8, which was inoculated with both at the same time, showed respiratory symptoms, accompanied not only with cough but also r le for a considerable period of time.

In this group, there was a stronger tendency observed for such gross lesions as congestion, an increase of mucus, thickening of the mucous membrane in the trachea compared with the former two groups.

This bears out the belief that the order or the timing of infection is an important factor for the development of mixed infection with *M. gallisepticum* and other infectious microbial agents<sup>9</sup>.

Nonomura *et al.*<sup>9</sup> observed the multiplication of NDV-B1 and *M. gallisepticum* in the respiratory tract and pathological changes in time sequence in order to clarify the role of microbial agents which contribute to the mixed infection. Fig. 1 shows the multiplica-

Table 1. Single and dual intratracheal inoculation of 48-day-old broiler chickens with *M. gallisepticum* (MG) and Newcastle disease virus (NDV)-B1

Group of chickens	Inoculum* <sup>1</sup>			No. of chickens examined	Serological response* <sup>2</sup>		No. of positive chickens* <sup>3</sup>						
	Sequence and course of infection in days				MG	NDV	Isolation of MG		Respiratory symptoms	Cross lesions			
	0	7	19				T	AS		T	AS	+	+
1	MG ↓	NDV ↓	‡	5	121	80	4	0	4	2	3	2	0
2	MG ↓		‡	5	15	—	2	2	0	0	1	0	0
3		NDV ↓	‡	5	—	242	0	0	0	0	0	0	0
4	MG ↓	‡		5	20	—	3	0	0	0	1	1	0
5	NDV ↓	MG ↓	‡	5	60	106	5	0	2	3	2	0	2
6	NDV ↓		‡	5	—	96	0	0	0	0	0	0	2
7		MG ↓	‡	5	46	8	2	2	0	0	1	1	1
8		MG+NDV ↓	‡	5	138	40	5	0	5	5	0	0	0

\*<sup>1</sup> MG: Modified Hofstad's broth-propagated IRF strain of MG, 10<sup>7</sup> organisms/0.1 ml  
NDV: Freeze-dried B1 strain, 10<sup>8</sup>TCID<sub>50</sub>/0.1 ml

\*<sup>2</sup> MG: Geometric mean agglutinin titer.—: <10  
NDV: Geometric mean HI titer.—: <5

\*<sup>3</sup> T: Trachea, Lu: Lung, AS: Air sac, ++: Severe, +: Slight, NE: Not examined

Table 2. Course of development of respiratory symptoms in the mixed-infection groups

Group	inoculum	Days after dual inoculation of chickens											
		1	2	3	4	5	6	7	8	9	10	11	12
1	<i>M. gallisepticum</i> —NDV-B 1* <sup>1</sup>	—			C								
		—		C		C	C	C		C			
		—		C	C	C	C						
5	NDV-B1 — <i>M. gallisepticum</i> * <sup>2</sup>	—											
		—						C	C				
		—											
8	<i>M. gallisepticum</i> +NDV-B 1* <sup>3</sup>	—							C				
		—					C	C	C				
		—		C	CR	CR	CR	CR	CR	C	C	C	C

C: Cough CR: Cough and r le

\*<sup>1</sup> NDV-B 1 was inoculated 1 week after inoculation with *M. gallisepticum*.

\*<sup>2</sup> *M. gallisepticum* was inoculated 1 week after inoculation with NDV-B 1.

\*<sup>3</sup> *M. gallisepticum* and NDV-B 1 were inoculated at the same time.

tion of *M. gallisepticum* and NDV-B1 in the trachea, in the groups which were inoculated

with both NDV-B1 and *M. gallisepticum* simultaneously, and the groups which were

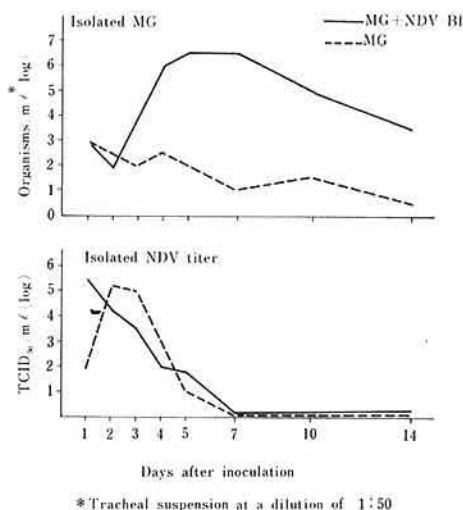


Fig. 1. Multiplication of *M. gallisepticum* (MG) and Newcastle disease virus (NDV)-B1 in trachea of 90-day-old chickens.

inoculated with either agent alone.

There was hardly any difference in the multiplication of NDV-B1, between the mixed infection group and the group infected with NDV-B1 alone. In either case, after one day or two, virus titer reached around  $10^4$  TCID<sub>50</sub>, then decreased in number, and the virus could not be isolated on the 7th day or so.

When a group of chickens were inoculated with *M. gallisepticum* alone, *M. gallisepticum* tended to decrease as time passed but in the mixed-infection group around the third day of inoculation, the number of *M. gallisepticum* increased rapidly and about 4-7 days after inoculation, the number of organisms reached  $10^6$ - $10^7$  then gradually decreased.

The histopathological lesions in the tested chickens showed in the case of the group infected with *M. gallisepticum* alone slight thickening of the mucous membrane, and in the group infected with NDV-B1 alone, thickening of the mucous membrane, loss of cilia and slight increase of mucus.

In the mixed-infection group, similar histopathological lesions were observed in a stronger degree. Fig. 2 evaluates these lesions in varying degrees in time sequence. In the

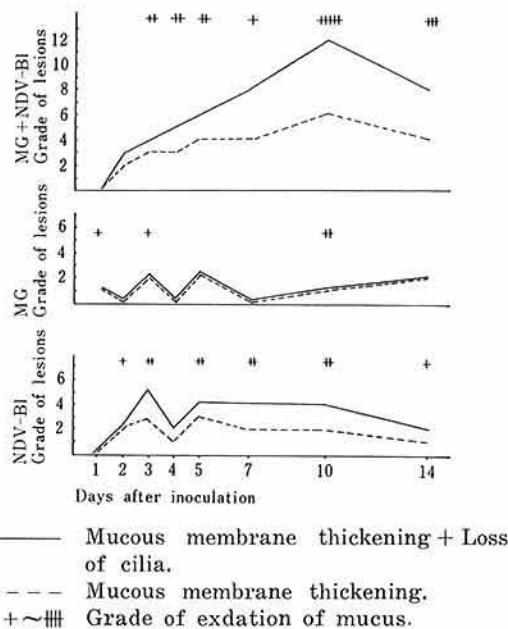


Fig. 2. Development of pathological lesions of trachea.

cases of NDV-B1 infection and in the mixed-infection group, lesions were observed from the second day of inoculation, and in the former, after 3-5 days as the peak, and then tended to be reorganized.

However, in the mixed-infection group, the lesions aggravated up to the 10th day, and slightly reorganized after two weeks on.

From the above results, in the mixed-infection with *M. gallisepticum* and NDV-B1, it seems that due to the NDV-B1 infection, a slight catarrhal lesion occurs on the mucous membrane of the respiratory tract, and as a result of stimulated multiplication of *M. gallisepticum*, lesions in the respiratory tract aggravated, and caused symptoms of respiratory distress.

#### Effect of administration of tylosin on mixed-infection with *M. gallisepticum* and NDV-B1 strain

When NDV-B1 is given to the chickens infected with *M. gallisepticum*, as described above, there is the danger of inducing respira-

**Table 3** Effect of tylosin on development of respiratory symptoms in chickens infected with *M. gallisepticum* (MG) and Newcastle disease (NDV)-B1

	Exp-1	Exp-2	Exp-3
Age of chickens (days old)	25	30	23
Mean body weight (g)	185	270	179
Inoculum* {			
MG CFU/0.1 ml	10 <sup>7</sup>	10 <sup>6.6</sup>	10 <sup>7.5</sup>
NDV-B1 TCID <sub>50</sub> /0.1 ml	10 <sup>6.5</sup>	10 <sup>3.5</sup>	10 <sup>3.5</sup>
Administration of tylosin tartrate (40 mg /0.5 ml/bird/day)			
None	4/5**	5/5	2/5
1 day	1/5	0/5	.
2 days	2/5	0/10	0/5
3 days	.	0/10	0/5

\* Chickens were inoculated with MG 3 days prior to inoculation with NDV-B1

\*\* Denominator: No. of chickens tested, Numerator: No. of chickens developed symptoms over 5 days,

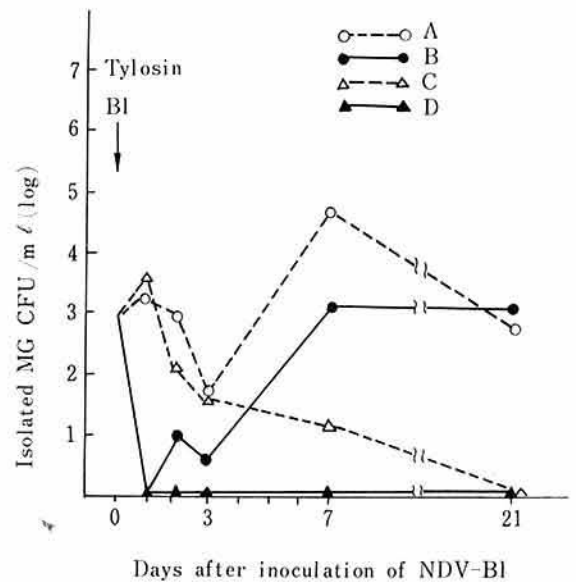
tory distresses. Therefore, in order to prevent it, it is necessary to give some drug effective against *M. gallisepticum*.

The result of investigation on the effect of tylosin tartrate dissolved in water given orally to the group of White Leghorns 23 to 30 days old infected with both *M. gallisepticum* and NDV-B1 is shown in Table 3.

Through the experiments repeated three times, after the intratracheal inoculation of NDV-B1, on the third or the fourth day, such main symptoms of respiratory distress as r le or cough appeared among the group of mixed infection and the group infected with only NDV-B1 there were many cases among the mixed-infection group which was not treated with tylosin to continue these symptoms for 3-4 weeks.

On the other hand, most of the chickens in the group which were infected with NDV-B1 alone and the mixed-infection group to which tylosin was given recover within 4 days after developing these symptoms, but sometimes, as in the case of experiment, there were some which continued to have respiratory symptoms for 1-2 weeks even after 40 mg a day dosage of tylosin for 2 days.

Multiplication of *M. gallisepticum* in the trachea among these groups is shown in Fig. 3. *M. gallisepticum* could not be detected any more after one dosage of tylosin among the group infected with *M. gallisepticum* alone.



Days after inoculation of NDV-B1

- A: Infected with MG and NDV-B1.
- B: Infected with MG and NDV-B1, administered with tylosin (40 mg) for one day.
- C: Infected with MG.
- D: Infected with MG, administered with tylosin (40 mg) for one day.

**Fig. 3.** Effect of tylosin on the multiplication of *M. gallisepticum* (MG) in the trachea of (21-day-old) chickens.

However, among the mixed-infection group, directly after the dosage of 40 mg, even though the presence of *M. gallisepticum* could

not be proved any more, the multiplication of *M. gallisepticum* itself showed almost the same rate of multiplication as in the group which was not treated with tylosin after one week.

The result seems to indicate that in the case of mixed infection, *M. gallisepticum* decreases remarkably by administration of tylosin but when there is a slight degree of catarrhal lesions on the mucous membrane of the respiratory tract by infection of NDV-B1, the micro organisms start to multiply after the medicine given to the chickens has been discharged out of the body. Therefore, when tylosin is given in the field, it would be desirable to give 40 mg of tylosin per chicken per day together with live-virus vaccine over two days<sup>2)</sup>.

#### Mixed infection of *M. gallisepticum* and NDV-TCND strain

Live-virus vaccine of NDV-TCND was given to chickens 2-5 weeks old and older mainly by intramuscular inoculation. The virus strain lacks affinity to the mucous membrane of the respiratory organs so that it is said that there is little danger of inducing respiratory diseases.

The writer and colleagues, in order to confirm this point, tried mixed infection with *M. gallisepticum*. Using White Leghorns, 21-70 days old, *M. gallisepticum* and NDV-TCND were inoculated into the trachea at the same time and observation was continued for 2 weeks. In either of the group, respiratory symptoms did not appear.

Compared with the group which was infected with *M. gallisepticum* alone, there was no appreciable difference observed as to the frequency of isolation of *M. gallisepticum* from the trachea, lungs and air sac, and appearance of lesions in the trachea, and air sac, or in the timing of appearance and strength of antibody against *M. gallisepticum*.

The results were compared among the group infected with NDV-TCND as well as NDV-B1 together with *M. gallisepticum* in 21-day-

old chickens.

Among the mixed-infection group with NDV-B1 as stated above, from the third day of inoculation, respiratory symptoms were observed and gross lesions in the trachea and air sac occurred with high frequency. The multiplication of *M. gallisepticum* and NDV strains in the trachea of these chickens are shown in Fig. 4. Among the chickens infected

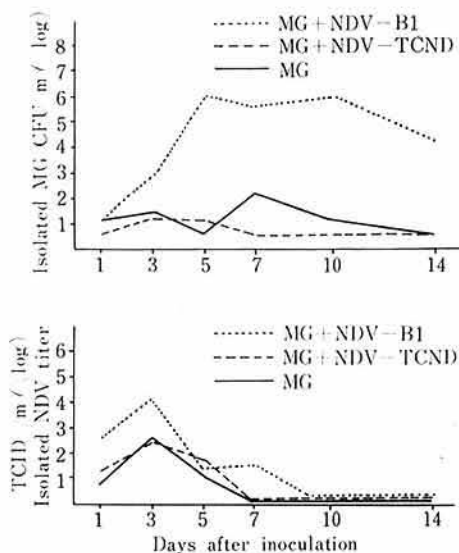


Fig. 4. Multiplication of *M. gallisepticum* (MG) and Newcastle Disease Virus (NDV) in the trachea of (21-day-old) chickens.

with both *M. gallisepticum* and NDV-B1, grade of multiplication of *M. gallisepticum* was remarkable, but among the mixed-infection group of NDV-TCND and *M. gallisepticum*, almost as in the case of chickens infected with *M. gallisepticum* alone, hardly any multiplication could be observed. As to multiplication of NDV, degree of multiplication of NDV-TCND was lower than that of NDV-B1, and although NDV-B1 multiplied to about  $10^4$  TCID<sub>50</sub>/ml, NDV-TCND multiplied to only  $10^{2.5}$  TCID<sub>50</sub>/ml.

When NDV-TCND was given to 21-day-old chickens by intramuscular inoculation, the presence of the virus in the trachea proved



to be negative.

From these results it was confirmed that compared with NDV-B1, NDV-TCND could not multiply on the mucous membrane of the respiratory tract. Neither did it promote the multiplication of *M. gallisepticum*.

### References

- 1) Nonomura, I., *et al.*: Multiplication of *Mycoplasma gallisepticum* and Newcastle disease virus (B1 strain) inoculated simultaneously into trachea of chickens. *Jap. J. Vet. Sci.* **30**, suppl. 139 (1968). [In Japanese. Summary of oral presentation.]
- 2) Nonomura, I., Sato, S. and Ando, K.: Effect of tylosin on experimental infection of *Mycoplasma gallisepticum* and Newcastle disease virus (B1) in chickens. *Jap. J. Vet. Sci.* **31**, suppl. 8-9 (1969). [In Japanese. Summary of oral presentation.]
- 3) Nonomura, I. and Sato, S.: Influence of TCND strain of Newcastle disease virus infection on multiplication of *Mycoplasma gallisepticum* in chickens. *Proc. Suiyokai*, **19**, 32-33 (1970). [In Japanese.]
- 4) Sato, S., *et al.*: Mixed infection with *Mycoplasma gallisepticum* and the B1 strain of Newcastle disease virus in chickens. *Nat. Inst. Anim. Hlth. Quart.* **10**, 58-65 (1970).