

Cross protection studies after the use of live-attenuated IBV vaccines Nobilis[®] IB 4-91 and Nobilis[®] IB Ma5 (Massachusetts type).

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1. Introduction

Infectious Bronchitis (IB) continues to be recognised as a common cause of production losses in the poultry industry worldwide. The existence of a vast diversion in serotypes has been reported since the disease was first described in the United States in the early 1930s. The number of IB serotypes appears to have increased in recent years (1,2). Molecular studies have shown that a new IB serotype can emerge as a result of only a very few amino acid changes in the S1 part of the spike genome of the virus (3). The emergence of a new serotype may result in some cases in a lack of appropriate protection after the use of currently available vaccines. But there are cases in which the existing IB vaccines will be able to provide a good measure of cross protection against IB strains not belonging to the same serotype. The reason for this cross protection may lay in the fact that much of the virus genome has remained unchanged. From a practical point of view, it may, therefore, be more relevant to think in terms of protectotypes (12) rather than serotypes.

Despite the evidence of frequent cross protection between IB serotypes, there are occasions when existing IB vaccines do not provide adequate protection against newly emerging serotypes. One such example of this was the recent emergence of the 4-91 (793B) serotype (10,13). This serotype causes disease

problems in Massachusetts-vaccinated chickens in Europe and many other parts of the world (5) and a new live-attenuated IB vaccine has been developed to control infections caused by this serotype.

With the continual emergence of new IB serotypes worldwide, it seems prudent to evaluate the level of cross protection achievable by the use of currently available IB vaccines (6,9), since it is not always either possible or reasonable to develop a vaccine for each new serotype which emerges.

The problems caused by the emergence of IB serotype 4-91 (793B) have been already described in the previous VSD newsletter number 15. With the frequent presence of new antigenic types there is a clearly need to expand and complement the information existing with regard to the protection provided by different vaccination programs using different types of vaccines against IB isolates from different parts of the world. This was the purpose of the work presented here.

In this work, the ability was investigated, of two registered Intervet vaccines to protect against challenge with different IB isolates from many parts of the world. Protection against challenge was assessed by means of the ciliostasis test (4,7).

The trachea is equipped with a defence mechanism designed to protect the body against the intrusion of pathogens via this route. This mechanism is referred to

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as the mucociliary apparatus. The surface of the trachea is covered with specialised epithelial cells, which are lined with numerous, motile, hair-like structures called cilia. These structures look like a field of wheat in the wind due to their capacity to move. Located between the cilia are secretory cells called "Goblet cells". The mucus produced by these secretory cells serves to trap the foreign agents and thus, with the aid of the coordinated uni-directional motion of the beating cilia, the foreign material is removed. The importance of this mucociliary apparatus in controlling secondary respiratory pathogens (*E. coli*, *Aspergillus* sp., etc.) is considerable. The loss of cilia (deciliation) results in a major disruption in the defence capability of the respiratory system. Accordingly, a vaccine virus should have little adverse effect on this important disease protection mechanism.

By means of the ciliostasis test the effect of a virus on the tracheal mucosa can be measured. The test is also used to evaluate protection after vaccination. A vaccinated bird that is subsequently challenged, if protected, will have an active cilia layer.

2. Materials and Methods

2.1. Virus strains

The IBV field strains used in the challenge experiments included in this report were isolated from outbreaks of respiratory disease in commercial chickens by conventional methods (11). Each of them was shown by serum neutralisation tests in tracheal organ cultures (8) to be antigenically distinct from the two IB vaccines used, Nobilis® IB 4-91 and Nobilis® IB Ma5 (Massachusetts type). They were used in the challenge experiments at a dose of approximately \log_{10} 3.0 median ciliostatic doses (CD_{50}), administered by eye drop in 0.1 ml.

2.2. IB vaccines.

Nobilis® IB 4-91 and Nobilis® IB Ma5 (Massachusetts type) were used, administered by eye drop according to the recommendations of Intervet International BV.

2.3. Experimental design.

Groups of 10 specified pathogen free (SPF) chicks were used in each experiment. Group 1 was vaccinated with Nobilis® IB Ma5 at one day of age, group 2 with Nobilis® IB 4-91 at 14 days of age, whilst group 3 was vaccinated both at day-old with Nobilis® IB Ma5 (Massachusetts type) and 14 days with Nobilis® IB 4-91; group 4 was the negative control group and was left unvaccinated. All groups were challenged at 5 weeks of age. Protection was assessed 5 to 7 days later using the ciliostasis test. In all experiments, groups of vaccinated chicks challenged with the homologous IB serotype were included.

2.4. Assessment of protection.

In our experiments the ciliostasis test was carried out as follows. Briefly, at between 5 and 7 days post challenge, the chicks were killed by administration of an intravenous overdose of barbiturate and their tracheas removed. Ten thin rings were prepared (3 from the top and bottom and 4 from the middle) and examined by low power microscopy. Ciliary activity was scored on a scale from 0 (all cilia beating) to 4 (no cilia beating). The ciliostasis score for each bird was the total score for all 10 rings examined, giving a maximum score of 40, if complete ciliostasis was observed. A score of less than 20 indicates protection. A percentage ciliostasis score may also be calculated by dividing the mean group score for the vaccinated-challenged group by the score for the corresponding challenge control group x 100. The lower the score, the higher the degree of cross protection provided by the vaccination

program used (7). In order to present the final percentage protection score in a clearer way, in this publication the percentage ciliostasis score (obtained as mentioned above) was subtracted from 100. In such a case the ciliostasis score obtained in the unvaccinated chicks represents a percentage of protection equal to zero - 0 -.

3. Results

Protection was complete in the vaccinated groups challenged with the homologous strain (data not shown). The results of the cross-protection studies are presented in table 1 and the following graphs. In the case of the heterologous challenges, it is clear that in the majority of cases the use of both a Massachusetts-type (Ma5) and the 4-91 IB vaccine in the vaccination program provided much better cross-protection than did the use of either vaccine alone. Two clear examples of this phenomenon can be seen in the experiments including the challenge strains from South Africa and Taiwan. The South African strain was isolated in 1980 and is still known to be present in that country. The isolate from Taiwan had previously been shown not to be protected against by the H120 vaccine (14).

In both cases the single vaccines were not able to confer a good level of protection whereas an excellent cross-protection level was achieved when both vaccines (Ma5 and 4-91) were included in the vaccination program. The same is true for the Dutch isolate D1466. This strain, belonging to the D212 serotype was originally isolated in the Netherlands at the beginning of the 1980s and is presently being considered responsible for most of the IB problems in this country (de Wit, personal communication). This strain is also known not to be closely related to other known IB serotypes. This low relatedness results in a poor level of cross-protection when using vaccines from other serotypes. In our experiments the best level of cross-protection against D1466 could also be obtained when both the Ma5 and 4-91 were included in the program. Considering the characteristics of the D212/D1466 strain and the average challenge score of <20 obtained in the experiments, the challenged group can be considered as protected.

In other cases either IB Ma5 (e.g. against the Arkansas, Brazilian and Japanese isolates) or IB 4-91 (e.g. against the Italian and Japanese isolates) alone were able to provide excellent protection against the antigenically different challenge strains.

Table 1:

Heterologous cross protection provided by live-attenuated IB vaccines.

Country of origin of challenge strain	Protection following challenge at 5 weeks of age of SPF chicks vaccinated with								
	Unvaccinated		Vaccinated groups						
			Group 1		Group 2		Group 3		
			1 day	14 days	1 day	14 days	1 day	14 days	
		Ma5	-	-	4-91	Ma5	4-91		
		Score*	%**	Score	%	Score	%	Score	%
Italy- (nephropathogenic)	39.4	-	35.8	9.1	5.6	85.8	3.4	91.4	
The Netherlands- D1466	39.6	-	31.8	22.2	29.4	25.8	16.1	59.3	
USA - Arkansas	39.1	-	9.8	74.9	11	71.9	5.8	85.2	
Brazil	40	-	7.1	82.3	7.8	80.5	5.3	86.8	
South Africa	39.6	-	21.2	46.5	31.5	20.5	4.5	88.6	
Taiwan	36.5	-	17.3	52.6	20.7	43.3	3.5	90.4	
Japan - TM86	37.5	-	7.8	78.6	10.9	70.1	2.5	93.2	
Japan - FB3	38	-	8.9	76.6	2.8	92.6	2.2	94.2	

* Mean ciliostasis score for 10 chicks/group

** Percentage of protection of the challenged group. The lower the mean ciliostasis score, the higher the level of cross protection

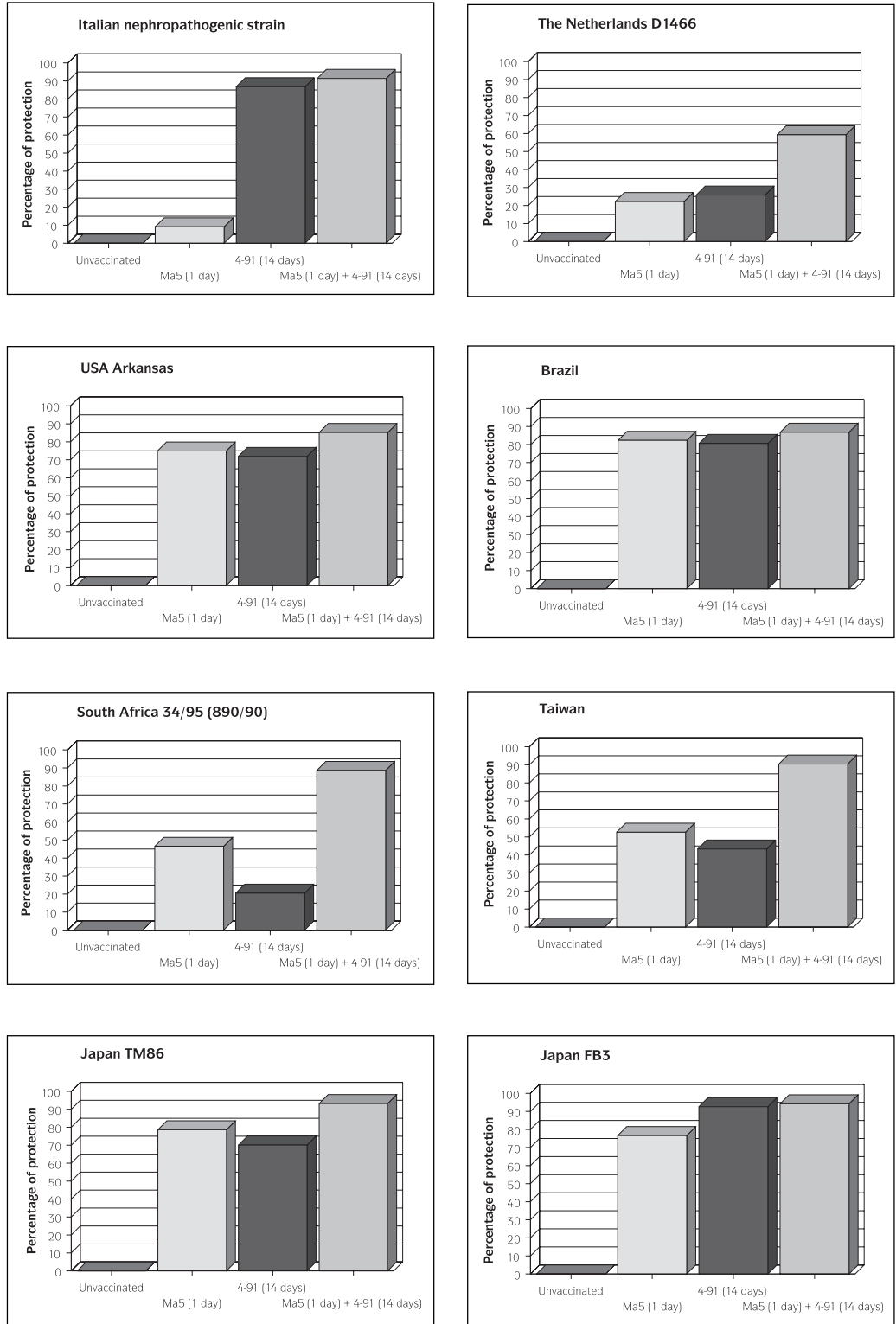
4. Conclusions

In recent years, much effort has been applied to determining the particular serotype of new isolates of IB virus. This information is of considerable value for research purposes and particularly for understanding the epidemiology of IB infections. With the increasing prevalence of different antigenic types of IB virus worldwide, the problem of designing vaccination programs to control each new serotype becomes increasingly difficult. Furthermore, it is both undesirable and unnecessary to consider developing a new live-attenuated vaccine for each new emerging IB serotype as shown in the results presented in this article. These results confirm the validity of the concept of protectotypes, which from a practical point of view is more relevant, and show that, with IB, in vivo protection is much broader than the results of in vitro serum neutralisation tests might suggest.

The results presented here clearly show that the use of the two live-attenuated IB vaccine strains 4-91 and Ma5, developed from different IB serotypes, considerably broaden the protection achieved against challenge with a wide variety of antigenically different IBVs prevalent worldwide. The enhancement of cross-protection against isolates belonging to antigenically different serotypes may occur particularly if revaccination is carried out at approximately 2 weeks of age, using a licensed IB vaccine of a different serotype than the one used initially. In broilers vaccination against IB is usually carried out at day old. The protection provided by a single vaccination may not be enough to cover for the entire production period. Application of a second IB vaccination may well be beneficial in such situations, not only to prolong the duration of the protection obtained but also to broaden the spectrum of such protection.

Figure:

Percentage of cross-protection against different IBV serotypes.



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