

# Duration of immunity acquired by vaccination with the live attenuated vaccine Avishield IB H120 against infectious bronchitis virus in SPF chickens



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## Abstract

The most effective means of controlling infectious bronchitis in poultry is by vaccination. Live attenuated vaccines based on the H120 strain (Mass serotype) are the most commonly used vaccines. Since vaccination is often performed in the hatchery, long duration of immunity is preferred. Avishield IB H120, a live attenuated vaccine based on the strain H120 is registered across the European Union and other countries, and has a declared immunity period of up to 5 weeks after vaccination. This study presents the

results of an additional survey demonstrating protection against challenge with the IBV strain M41 up to 8 weeks after a single vaccination with Avishield IB H120. Seven weeks after vaccination, 94% of chickens vaccinated by spray were protected against the challenge. Eight weeks after vaccination, 80% of spray vaccinated chickens and 75% of orally vaccinated chickens were still protected against the challenge with virulent IBV.

**Key words:** *infectious bronchitis virus; vaccination; duration of immunity*

## Introduction

Though discovered 80 years ago, infectious bronchitis virus (IBV) still causes pronounced economic losses in the poultry industry worldwide (Toro et al., 2012). Both live and inactivated vaccines are used to vaccinate chickens against

IBV (Jordan, 2017). The most widely used vaccine strain is H120 (Cook et al., 2012), which belongs to the Massachusetts serotype and has been used all over the world for more than 50 years (Bijlenga et al., 2004).

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Recently, a new classification system based on the sequence of the S1 gene of IBV has been proposed, combined with an unambiguous lineage nomenclature (Valastro et al., 2016). Accordingly, 32 lineages belonging to 6 different IBV genotypes have been identified for both the H120 and M41 strains classified within the GI-1 genotype.

Many studies have shown cross-protection of H120 strain against heterologous field strains, either in combination with vaccines belonging to other serotypes (e.g. variant strains) (Mohammadi et al., 2014; Bru et al., 2017; Habibi et al., 2017) or even alone (Bijlenga et al., 2004). Given the fact that field strains of the Massachusetts serotype still cause disease in many areas of the world (Jackwood, 2012; Jackwood and Wit, 2013; de Wit et al., 2018), and relying on cross protection, vaccines based on the H120 strain are still the basis for immunoprophylaxis against IBV.

Avishield IB H120 is a live, attenuated vaccine against IBV, based on the strain H120. It has been registered across Europe since 2017 for use in broilers and future layers/breeders from the first day of life.

When developing a vaccine, the manufacturer should follow the requirements of the European Pharmacopoeia and Directive 2001/82/EC, which lays down the tests and defines the criteria that a vaccine needs to comply with to be granted marketing authorisation in the EU.

Amongst other tests, onset and duration of immunity provided by the vaccine should be tested and specified in the summary of product characteristics.

The onset of protection provided after vaccination with Avishield IB H120 is confirmed already at 10 days after vaccination, reaching full protection (100%) 21 days after vaccination (Boelm et al., 2018). The same level of protection was observed at 35 days after vaccination.

Since this vaccine is suitable for use in long living birds as well as in broilers, a longer duration of immunity was also investigated. Here we report the results of duration of immunity achieved by vaccination with Avishield IB H120 given to day-old chicks by spray and to 7-day-old chicks by oral administration route.

## Materials and Methods

### Viruses

A live, attenuated vaccine against avian infectious bronchitis virus, based on the H120 strain was used in this study (Avishield IB H120, Genera Inc., Part of Dechra Pharmaceuticals PLC Group). The vaccine is produced in compliance with the European Pharmacopoeia and Directive 2001/82/EC concerning the safety, quality and efficacy of live poultry vaccines.

For demonstration of the duration of immunity, the vaccine was diluted in an appropriate diluent, depending on the route of inoculation, to provide 3.5  $\log_{10}$  50% embryo infectious dose ( $EID_{50}$ ) per bird, which is the minimum dose recommended for vaccination according to the manufacturer's specification. Challenge of chickens was performed using the virulent IBV strain M41 in a dose of 3.0  $\log_{10}$   $EID_{50}$  per bird.

### Experimental design

#### *Duration of immunity after spray administration*

Two groups of day-old SPF chickens were vaccinated using the minimum dose of Avishield IB H120 by the spray administration route. A third group of day-old SPF chicks was kept as a non-vaccinated control. Each group was housed in a separate HEPA-filtered isolator for chickens. To demonstrate the duration of immunity, 20 chickens from each vaccinated group were challenged with IB M41. Group 1 was challenged 49 days (7 weeks) after vaccination, while

**Table 1.** Experimental design of duration of immunity studies for Avishield IB H120 vaccine

	Spray administration				Oral administration	
Study day	Group 1 20 animals	Group 2 20 animals	Group 3 10 animals		Group 4 20 animals	Group 5 5 animals
D0	Hatching, Identification					
	Vaccination Avishield IB H120 Spray administration method		/		/	/
D7	/		/		Vaccination Avishield IB H120 Oral administration route	/
D49	Challenge with IB M41	None	G3A	G3B	None	None
			Challenge with IB M41	None		
D53	Euthanasia, Ciliary activity of tracheal explants	/	Euthanasia, Ciliary activity of tracheal explants	/	/	/
D56		Challenge with IB M41		Challenge with IB M41	/	/
D60		Euthanasia, Ciliary activity of tracheal explants		Euthanasia, Ciliary activity of tracheal explants	/	/
D63					Challenge with IB M41	Challenge with IB M41
D67					Euthanasia, Ciliary activity of tracheal explants	Euthanasia, Ciliary activity of tracheal explants

group 2 was challenged 56 days (8 weeks) after vaccination. Group 3 (control group, unvaccinated chickens) was divided in two subgroups. The first subgroup (G3A) was used as the control group for Group 1 and received the challenge on the same day as Group 1, while the second subgroup (G3B) was used as the control for Group 2 and received the challenge on the same day as Group 2. Each control subgroup contained 5 chickens.

To assess the protection acquired by vaccination, ciliary activity was

examined and scored four days after the challenge as recommended by Ph.Eur. monograph 04/2013:0442, section 2-4-3 Immunogenicity (Ph.Eur., 2013).

#### *Duration of immunity after oral administration*

Duration of immunity after oral administration of Avishield IB H120 was also tested in a separate study. The outline of the study followed essentially the outline presented above, though chickens in the vaccination group (Group

4) received vaccination by the oral administration route at 7 days of age (Table 1). The challenge was performed in a single testing point, 56 days (8 weeks) after vaccination. A control group of 5 SPF chickens (Group 5) received the challenge on the same day. The level of protection acquired by vaccination was determined by assessment of ciliary activity in the tracheal rings four days after the challenge. The experimental design details are summarized in Table 1.

### Ciliary activity

The ciliary activity of 10 tracheal explants per challenged chicken was examined 4 days after the challenge. Shortly after euthanizing the chickens, the trachea of each chicken was extirpated by removing the skin of the neck. Subsequently, the trachea was disconnected from the surrounding tissues and cut just beneath the head and just above the thorax. Transverse sections of 0.5-1.5 mm of the trachea were made using a scalpel blade. The transverse tracheal sections were put in wells of a 24-well microtitre plate covered with 0.5 mL DMEM or MEM cell culture medium prewarmed at 35–40 °C and examined for ciliary activity under the microscope. Ciliary activity of three sections of the upper part, four sections of the middle part and three sections of the lower part of the trachea were examined under 100X magnification. The activity of the cilia in each tracheal section was scored according to Ph.Eur. monograph 04/2013:0442, section 2-4-3 Immunogenicity (Ph.Eur., 2013), as follows:

Score 0:  $\geq 50\%$  of the tracheal section shows cilia movement

Score 1:  $< 50\%$  of the tracheal section shows cilia movement

A chicken was considered not affected / protected if at least 9 out of 10 tracheal rings showed normal ciliary activity (a score of 0). For each chicken, the number of affected tracheal sections was

calculated. For each group, the number of affected chickens was calculated.

### Animal studies

In both studies, specific pathogen free (SPF) chickens were used. Animal experiments were conducted in accordance with national and European Union regulations regarding the use and protection of animals used for scientific purposes (Directive 2001/82/EC, Directive 2010/63/EU, Directive 86/609/EEC, Croatian law OG 102/17, Croatian regulation OG 55/13).

### Statistical analysis

Differences between the levels of protection between vaccinated and non-vaccinated groups were assessed using Fisher's exact *p* one-tailed test in Tibco Statistica 13.3.

## Results and discussion

Previously, we showed that the onset of immunity after vaccination with Avishield IB H120 already started 10 days after vaccination and reached full protection (100%) at 21 days after vaccination (Boelm et al., 2018). Complete protection was also demonstrated 5 weeks after vaccination.

The objective of this study was to investigate the duration of immunity provided by vaccination with the minimum dose of Avishield IB H120 when applied by spray administration method to day-old chicks and by oral administration method to 7-day-old chicks. There were no clinical signs of disease in the period between vaccination and challenge in any of the groups. During the four days post-challenge, no visual clinical signs or mortality were observed in any of the challenged chickens in the control group for spray-vaccinated chickens.

However, in the control group for orally-vaccinated chickens, 3 out of 5

**Table 2.** Protection rates 4 days after challenge with  $10^{3.0}$  EID<sub>50</sub> of IB M41

GROUP	Age at vaccination	Age at challenge	Protection rate
Vaccinated by spray with Avishield IB H120	1 day	49 days	94.4%
Non-vaccinated control for spray vaccinated group	n/a		0%
Vaccinated by spray with Avishield IB H120	1 day	56 days	80%
Non-vaccinated control for spray vaccinated group	n/a		0%
Vaccinated orally with Avishield IB H120	7 days	63 days	75%
Non-vaccinated control for orally vaccinated group	n/a		0%

chickens exhibited tracheal rales. There were no clinical signs nor mortality after challenge in any of the vaccinated groups of chickens.

In comparison with the ciliary activity of tracheal explants determined four days after challenge, 94.4% of chickens vaccinated by spray at 1 day-old were protected 49 days after vaccination, while 56 days after vaccination, 80% of spray vaccinated chickens and 75% of orally vaccinated chickens were still protected. Conversely, none of the unvaccinated, control chickens were protected against the challenge and complete ciliostasis was demonstrated (Table 2). Statistical analysis showed there is a significant difference in protection between the vaccinated and control groups of chickens.

In conclusion, Avishield IB H120 provides protection for chickens up to 8 weeks after vaccination, corresponding to the published data for the vaccines based on the H120 vaccine strain (Gough and Alexander, 1979) and for vaccines belonging to the variant serotype GI-13 (Kutle et al., 2020). However, it can be observed that the level of protection decreases when compared to protection rates observed 3 and 5 weeks after vaccination (Boelm et al., 2018), and therefore re-vaccination is often performed in long living birds (Jordan, 2017).

## Conclusions

The Avishield IB H120 live attenuated vaccine based on H120 vaccinal strain provides protection against M41, a virulent strain of IBV up to 8 weeks after vaccination when administered to day-old chicks by spray or to 7-day-old chicks by oral administration route. The present study showed that adequate protection against Mass serotype IBV field strain can be achieved after a single vaccination with the H120 vaccine and that the acquired protection may last up to 8 weeks post-vaccination. Taking the short lifespan of broiler chickens into account, it can be concluded that only one vaccination with Mass serotype vaccine is needed for broilers. For broader protection, introducing a second IBV vaccine of distinct serotype is usually recommended.

## Disclaimer

Lana Ljuma Skupnjak, Anto Vrdoljak and Nikol Očuršćak are employed by Genera Inc., part of Dechra Pharmaceuticals PLC, manufacturer of the Avishield IB H120 vaccine.

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## Trajanje imunosti nakon cijepjenja s Avishield IB H120, cjepivom protiv ptičjeg zaraznog bronhitisa

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Cijepjenje je najučinkovitiji način kontrole zaraznog bronhitisa u peradi. Najčešće korištena cjepiva su živa atenuirana cjepiva temeljena na soju H120 (pripadaju Mass serotipu). Budući da se cijepjenje često provodi već u valionici, poželjno je dugo trajanje imunosti. Avishield IB H120, živo atenuirano cjepivo koje sadrži cjepni soj H120, registrirano je u brojnim zemljama Europske

unije i na drugim tržištima s deklariranim trajanjem imunosti od 5 tjedana nakon cijepjenja. U ovom radu prikazani su rezultati dodatne studije gdje smo dokazali zaštitu od izazivačke infekcije s patogenim virusom M41 i 8 tjedana nakon cijepjenja s Avishield IB H120 putem spreja ili vode za piće.

**Ključne riječi:** virus zaraznog bronhitisa, cijepjenje, trajanje imunosti