

COMPARISON OF REACTOGENICITY AND IMMUNOGENICITY OF LIVE VACCINES AGAINST INFECTIOUS LARYNGOTRACHEITIS

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SUMMARY

Humoral immune response and ciliary activity of tracheal mucosa of poultry, vaccinated against infectious laryngotracheitis using ciliostatic test, was studied. Regardless of the vaccination route the vaccines decreased the ciliary activity by 5–9% on Days 3 to 7 post vaccination. Herewith the vaccine ocular application in some chicks induced one-eye serous conjunctivitis, which resolved subsequently. Oral vaccination did not cause any clinical changes. Serological monitoring revealed an earlier and stronger immunity in poultry vaccinated by ocular route. The required seroprotection level in test groups was higher than the minimal value (80%) starting from Day 16 post vaccination. The period of immunity development after oral vaccination correlated with the vaccine dose volume. Moreover the domestic vaccine was highly competitive with foreign vaccines in immunogenicity and reactogenicity.

Key words: infectious laryngotracheitis, reactogenicity, ciliostatic test, seroconversion.

INTRODUCTION

Infectious laryngotracheitis (ILT) is caused by a highly contagious virus of *Gallid herpesvirus 1* type. ILT is a severe respiratory disease of chickens causing significant economic losses in industrial poultry production all over the world [4, 10]. As a rule, live vaccines are used to control and prevent ILT. Herewith the vaccine strains are able to transmit horizontally and reverse when passaged *in vivo* [4, 6, 8, 11].

The agents of such avian respiratory diseases as infectious bronchitis, Newcastle disease, metapneumovirus infection, respiratory mycoplasmosis and ILT affect regular beatings of cilia on tracheal mucosa. As a result an inflammatory exudate accumulates and blocks the lumen of a larynx and trachea, leading to death from choking. Secondary microflora or management of poultry under unfavourable animal health conditions (like draughts, heavy dust burden or gas contamination of the air in poultry houses) contribute to a higher lethality [2, 3, 5, 7, 9]. That's

why the lethality rate cannot be judged as an objective criterion of a field virus high virulence.

In this context the ciliostatic test, involving scoring of ciliary beat frequency on avian tracheal mucosa, is a more adequate evaluation, compared to lethality rate in the infected group. This technique is especially valuable when comparing residual reactogenicity of attenuated ILTV production strains and testing safety of vaccines, because the used strains do not kill poultry *a priori* [2].

Multiple challenge tests when chicks were challenged with ILTV Bogatishchevsky pathogenic strain demonstrated that there is a correlation between humoral immunity strength and vaccine protectivity. The level of sera antibodies ensuring protection of vaccinated poultry was justified; its value was twice bigger (or more) than the minimal value of positive/negative threshold used in the ELISA test-kit [1].

This study was aimed at the comparison of reactogenicity and immunogenicity of three live vaccines against ILT,



Fig. 1. Typically occurring eye reaction on Day 6 post ocular vaccination (right) compared to a healthy eye (left)

authorized in the RF using the ciliostatic test, clinical observations and serological monitoring.

MATERIALS AND METHODS

Live vaccine against ILT. Commercially available vaccines were used in this study; their basic characteristics are shown in the table below.

Test poultry. 180 35 day-old chicks of Hisex Brown cross were used for the study. The trial was performed in the aseptic room of the FGBl "ARRIAH" animal facilities. Birds were divided into groups and placed into glove boxes with temperature and filtered air pressure controllers and autonomous feed and water supply.

Ciliostatic test. Preparation of tracheal explants and assessment of their ciliary activity were performed according to the methods described before [2, 3], but slightly amended. Briefly: 3, 4 and 3 0.5–1.0 mm tracheal cross sections (rings) from upper, middle and lower parts from every bird were examined under inverted microscope using 60–150× magnification. Immobility value for each tracheal explant was scored using five point system from 0 to 4 point meant absence of ciliary beating in the area of not more than 5% of the section perimeter; 1 point – not more than 25%; 2 points – not more than 50%; 3 points not more than 75% and 4 points meant absence of ciliary movements up to 100% of the section perimeter. Then the

sum of points given for epithelial ciliostasis of 10 tracheal rings from each chick was calculated (Σc). The calculated sum of points (from 0 to 40) was transformed into percentage using the following formula

$$C, \% = 2,5 \times \Sigma c.$$

Sera testing. ILTV antibody titers were determined in chicken sera in solid-phase ELISA using ProFLOCK LT ELISA Kits ("Zoetis", USA), titers were expressed as logs (lg). The value of positive/negative threshold for the abovementioned test kit is 340 (2.531 lg).

Study design. 6 groups of chicks (30 birds per group) were formed. Groups 1 and 2 were vaccinated ocularly and orally using embryo vaccine against ILT based on strain O, Groups 3 and 4 were immunized ocularly and orally with Merial vaccine, Group 5 was vaccinated ocularly with Intervet-International B.V. vaccine according manufacturer's instructions. Birds were clinically observed every day for 28 days post vaccination.

To perform a ciliostatic test two chicks were randomly taken from each group on Days 1–7, 9 and 12 post vaccination. To test sera in ELISA for ILTV antibodies, blood samples were taken before vaccination and on Days 7, 16, 19, 24 and 28 post vaccination. Percentage of birds having sera with protective antibody levels was calculated (seroprotection level).

Statistical processing of results. Standard methods of statistical processing of variable sampling rates were used. The paper presents mean titres of sera antibodies and their standard deviations ($x \pm s$), determined by at least 10 sample measured values ($n = 10$). Calculations and diagrams were made using Microsoft Office Excel.

STUDY RESULTS

Clinical observation of birds after ocular and oral vaccination. Some test chicks (10–15%), vaccinated ocularly by three vaccines, demonstrated ocular reactions expressed as one-eye serous conjunctivitis on Days 5–7 (Fig. 1). These postvaccinal reactions resolved completely on Days 8–9 post vaccination.

Chicks immunized orally by ARRIAH and Merial vaccines did not demonstrate any postvaccinal reactions. During the whole observation period no other clinical signs were noted.

Evaluation of vaccination effect on ciliary activity in trachea. After vaccination chicks were studied for ciliary activity on tracheal mucosa (C, %).

Results given in the diagram (Fig. 2) show that vaccines under study are little different from each other in onset time, strength and length of ciliostatic effect re-

Table
Basic characteristics of three live vaccines against ILT

Criterion	Embryo vaccine based on O strain	Nobilis ILT	Gallivac LT
Strain	O	Serva	T-20
Manufacturer	ARRIAH, Russia	Intervet-International B.V., Netherlands	Merial, France
Viral material	Tissue homogenate, CAM and EEF of SPF chicken embryos	Tissue homogenate, CAM and EEF of SPF chicken embryos	EEF of SPF chicken embryos
Application route	Ocular and oral	Ocular	Ocular and oral
Virus dose in one vaccine inoculation dose, EID ₅₀	500 (ocular) 2,000 (oral)	320	500

SPF – category of animals free from specific pathogenic factors and antibodies against them; CAM – chorioallantoic membrane of chicken embryos; EID₅₀ – 50% embryo infecting dose; EEF – extra embryonic fluid of chicken embryos.

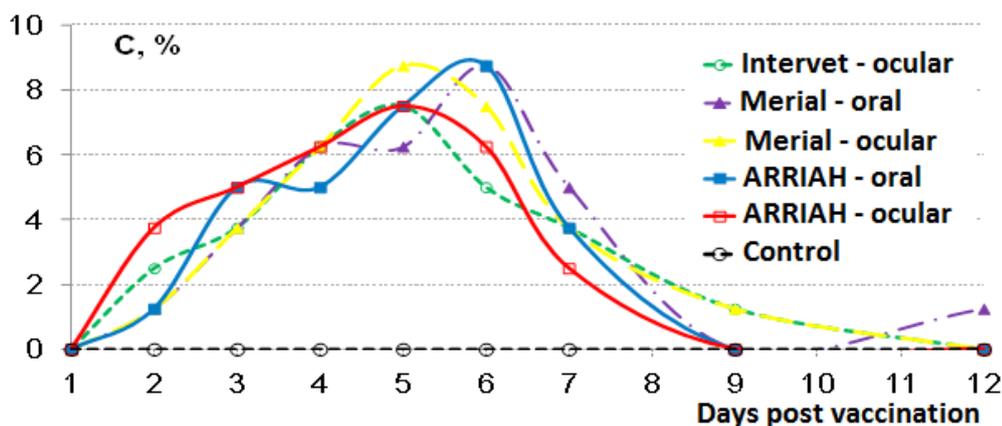


Fig. 2. Assessment of ciliostasis dynamics caused by vaccines of different producers depending on application route

ardless of application route. The greatest ciliostatic effect was observed on Day 3–7 (5–9% of ciliostasis). Ciliary activity was completely restored up to Day 9–12 post vaccination.

Serological monitoring of vaccinated birds. Humoral response before vaccination and on Days 7, 16, 19, 24, 28 post vaccination were assessed. Based on primary data, seroprotection level was calculated for each group (percentage of immune birds per group which developed specific antibodies in response to vaccination in protective concentration).

Test results (Fig. 3) allow judging that vaccines under study are highly immunogenic because they ensure seroprotection level of more than 80%. In test groups vaccinated with ARRIAH vaccines ocularly and orally the number of birds with protective antibody titres was 82 and 99% on Day 16, correspondingly. Intervet vaccine, applied ocularly, ensured 100% protection on Day 19. A strong humoral immune response to ocular vaccination against ILT using Merial vaccine was 89% on Day 16. Herewith the protective antibody level in chicks after oral vaccination using Merial vaccine developed a week later.

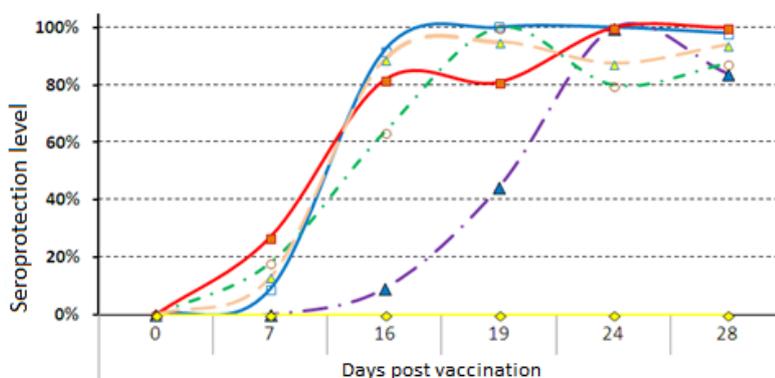
DISCUSSION

Under conditions of intensive management and high flock density circulating field viruses attenuate the population resistance and open the gates for secondary opportunistic pathogenic bacterial microflora.

ILTV pathogenic effect consists of reproduction in tracheal cilia which stop purifying inhaled air from extraneous matter. Normally, thanks to coordinated ciliary beating on tracheal mucosa, the particles with mucus are moved towards larynx and throat and are swallowed together with saliva into esophagus. At this stage the infectious process is likely to be completely resolved, if it is not complicated with high dustiness, gas contamination and bacterial microflora [3, 5, 7, 9].

During the past 20 years in the USA, Australia and other countries ILT has been considered an emergent problem in broiler flocks, because previously the outbreaks of moderate ILT were reported exclusively in egg-laying flocks. There was no need in specific ILT prevention on broiler farms [4, 8, 10]. Herewith it is necessary to take into account that oral vaccination of broilers is more convenient when compared to an ocular one.

Fig. 3. Serological monitoring after oral and ocular vaccination by different vaccines



	0	7	16	19	24	28
ARRIAH strain O, orally	0%	9%	92%	100%	100%	98%
Merial, orally	0%	0%	9%	44%	100%	84%
Intervet, ocularly	0%	18%	64%	100%	80%	88%
ARRIAH, strain O, ocularly	0%	27%	82%	81%	100%	100%
Merial, ocularly	0%	13%	89%	95%	88%	94%
Non-vaccinated controls	0%	0%	0%	0%	0%	0%

Previously the role of a “feral” vaccine ILT virus of embryonic origin was demonstrated in several outbreaks, occurred in broilers. ILT clinical manifestation was characterized by conjunctivitis, respiratory failure, high mortality and egg drop [4, 8, 10]. Comparison of cultural and embryo vaccines against ILT revealed a deficiency of an embryo strain, i.e. reversibility at passage 20 *in vivo* [6]. But a strict compliance with biosecurity rules on a poultry farm prevent transmission of a vaccine virus from vaccinated flocks to susceptible ones.

One eye serous conjunctivitis in 10–15% chicks in ocularly vaccinated groups was noted as a postvaccinal reaction during clinical observation.

Based on the results of the ciliostatic test, the cease in ciliary beating in 5–9% of epithelial area was identified, which is considered to be a moderate effect of vaccine strains regardless of the vaccination route. At the same time, as a rule, field isolates of different virulence cause ciliostasis in the whole surface of the tracheal mucosa [2, 3, 5, 7, 9].

When analyzing serological monitoring results, a sufficiently high immunogenicity of vaccines under study was established. The used seroprotection level, as opposed to seroconversion, imposes stricter requirements to vaccines, as it expresses the percentage of birds in vaccinated groups which developed protective levels of specific antibodies to a vaccine antigen. For ILT embryo vaccine, based on strain O (ARRIAH), a double value of positive-negative threshold of ELISA test-kit equal to 680 (2.832 lg) was taken [1].

Thus seroprotection level in test groups exceeded the minimal value (80%) on Day 16 post vaccination. But immunity level in poultry vaccinated with Merial vaccine, showed a week delay in immunity development. This is likely associated with the fact that the vaccination dose both for ocular and oral vaccination is the same: 500 EID₅₀.

The virus reproduction sites are a respiratory tract and a conjunctiva [11, 12]; when swallowing the vaccine with drinking water the significant part of the virus gets to an esophagus and does not induce the immunity. Only small part of the virus, which settled down on the palate of the mouth cavity is immunogenic after reproduction. Thus it suggests that immunity development is delayed due to a low inoculation dose, when the poultry is immunized orally.

For reference one inoculation ocular ARRIAH strain O vaccine dose contains at least 500 EID₅₀, and the oral dose is 4 times bigger than the ocular one, at least 2,000 EID₅₀; which ensures rapid development of a strong immunity.

CONCLUSION

The results of the study suggest that live vaccines authorized in the RF are safe and immunogenic. Herewith the domestic vaccine is highly competitive with the imported analogues in humoral response development dynamics and strength.

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