# ANTIBODY RESPONSE IN CHICKS VACCINATED AGAINST INFECTIOUS BRONCHITIS VIRUS ANTIVIELU REAKCIJA CĀĻIEM, KAS VAKCINĒTI PRET INFEKCIOZĀ BRONHĪTA VĪRUSU

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#### ABSTRACT

Having tested 100 samples of blood sera from five broiler groups (one-day-old, 24, 30, 50 and 58 week old broilers) for IBV, it was found that vaccination provided adequate immunity from IBV. Mean antibody titers ranged from 3651 to 5021 ELISA titers on IDEXX IBV kits, the variation coefficient being from 48.2 to 88.9 %. **KEY WORDS:** infectious bronchitis virus, antibody, ELISA.

#### **INTRODUCTION**

Infectious bronchitis (IB) continues to be one of the most important diseases and causes of loss to the chicken industry. IB is prevalent in all countries with an intensive poultry industry. This infection is usually defined as an acute, contagious disease of chickens characterised primarily by respiratory signs. However, infections with the causative virus also lead to nephritis and egg production problems in laying hens (10, 13). IB is caused by infectious bronchitis virus (IBV), a single-stranded, positive-sense RNA virus member of the Coronaviridae family (2, 6, 14). It is difficult to diagnose due to the large number of subclinical forms and non-specific symptoms: i. e. respiratory signs and signs associated with superimposed infectious (1). For the control of infectious, broilers are vaccinated at 1-day of age with live attenuated vaccines. Breeders and egg layers are vaccinated at approximately 8weekly intervals with live attenuated vaccines and with inactivated vaccines after commencement of lay (8, 9). Vaccination programs against IB are based on the determination of IBV strains causing the disease in the field. More than 20 IBV serotypes have been recognized worldwide (2, 12). Generally, different serotypes do not cross-protect. Therefore, the serotype of the virus causing the disease must first determine so that the birds can be properly vaccinated (11).

Regular monitoring of sera from flocks for IB antibody titres may help to indicate the level of vaccine response. Because many chicken sera, especially from older birds, contain antibodies that are highly cross-reactive against antigenically unrelated strains, serodiagnosis of suspected disease outbreaks of IB cannot be used with a high degree of confidence (13, 14).

The aim of the present study was the evolution of antibody response in chicks vaccinated against infectious bronchitis virus.

#### **MATERIAL AND METHODS**

The blood sera were collected from poultry farm. In total 100 samples were tested. The serum samples were classified into five groups: one-day-old broilers, 24, 30, 50 and 58 week old broilers.

Diagnostic reagent kit manufactured by IDEXX firm was used in blood sera tests. FlockChek IBV is IDEXX's enzyme immunoassay for the detection of antibody to IBV in chicken serum. Viral antigen is coated on 96-well plates. Upon incubation of the test sample in the coated well, antibody specific to IBV forms a complex with the coated viral antigens. After washing away unbound material from the wells, a conjugate is added which binds to any attached chicken antibody in the wells. Unbound conjugate is washed away and enzyme substrate is added. Subsequent colour development is directly related to the amount of antibody to IBV present in the sample. The presence or absence of antibody to IBV is determined by relating the A (650) value of the unknown to the positive control mean. The positive control is standardized and represents significant antibody levels to IBV in chicken serum. The relative level of antibody in the unknown is determined by calculating the sample to positive [S/P] ratio. Serum samples with S/P ratios of less than or equal to 0,2 should be considered negative. S/P ratios greater than 0,2 (titer greater than 396) should be considered positive.

# RESULTS

Blood samples were taken from one-day-old, 24, 30, 50 and 58 week old chickens. 20 blood sera samples were collected from every group. All samples were tested for IBV antibodies using ELISA test according to the manufacturer's instructions. The analysis of the blood sera tested yielded the following data (Fig. 1).

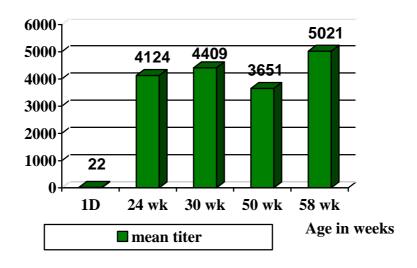


Figure 1. Antibody to IBV mean titer in serum.

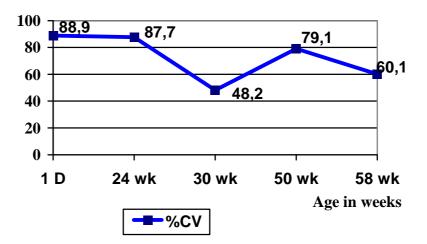


Figure 2. Distribution of %CV.

No antibodies were found in one-day-old chicks. During the  $24^{th}$  week the antibody titres ranged from 9444 to 13830 (groups 1-11), while those for the  $30^{th}$  week were from 10761 to 12611 (groups 2-8), the  $50^{th}$  week – 8994 to 13166 (groups 1-8) and the  $58^{th}$  week from 1843 to 14656 (groups 2-11).

The results obtained are presented in Fig. 2.

During the entire period of the experiment the CV% was rather high (from 60.1 to 88.1). Only the  $30^{\text{th}}$  week showed a decrease to 48%.

### DISCUSSION

Serology is used for diagnostic purposes, for health and epidemiological monitoring and for monitoring vaccinations. The most popular technique under field the conditions is the indirect ELISA test since it can detect antibodies. The popularity of the ELISA method under field conditions is due to its simplicity, speed, low cost and the fact that a large number of samples can be examined at the same time (1). Commercial kits for ELISAs are available – these are based on several different strategies for the detection of IBV antibodies. Usually, such test have been evaluated and validated by the manufacturer, and it is therefore important that the instruction specified for their use be followed carefully.

No antibodies against IBV were found in the blood sera of one-day-old chicks. This could be explained as follows: the antibody mediated immunity in young birds (less than 1 week old) cannot be adequately measured with ELISA's. This is because with early vaccination, locally produced immunity (or cellular immunity) in the trachea and Harderian glands play an important role. Local cellular immunity, in general, does not produce circulating antibodies in the blood, which are measured by ELISA's. This means that young birds can have little or no FlockChek titers, but are in fact protected by local immunity (5, 6). Blood sera tests of 24 and 30-week-old birds when their immunity became mainly antibody mediated showed a substantially increased antibody count, the mean titre being 4124 and 4409 respectively. However, during the 24<sup>th</sup> week of age the CV was 87.7%. This indicates an improper administration of vaccines. The coefficient of variation (%CV) tells how variable a mean titer response of a flock is. The lower the %CV, the more uniform distribution of the titers and the better the vaccination. A flock with higher than 60% CV indicates that there are birds with little or no response, in addition to birds with a high or adequate response to vaccination (4). During the 50<sup>th</sup> and 58<sup>th</sup> weeks the %CV slightly decreased but remained rather high at 79.1% and 60.1% respectively.

In Europe, titers expected at the processing plant from flocks without IBV problems run about 500-1000 ELISA titers on IDEXX IBV kits. But in some areas, titers expected for IBV at the processing plant can be between 4000 and 5000. These high titers are related to a good vaccine immune response and the interaction with residual IBV on the farm (4).

## CONCLUSION

The analysis results of blood sera prove that poultry farms have chosen the appropriate vaccines and optimal vaccination time.

#### REFERENCES

- 1. Borne P.-M., Comte S. Vaccines and vaccination in poultry production. Ceva sante animale, 2004. 137 p.
- Cavanagh D., Nagi S. Infectious bronchitis. In: Diseases of poultry, 11<sup>th</sup> ed.Y. M. Saif, H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougald and D. E. Swayne, eds. Iowa State Inoversity press, Ames, IA. 2003. P. 101-119.

- 3. De Wit J. J., Mekkes D. R., Kouwenhoven B., Verheijden J. H. M. Sensitivity and specificity of serological tests for infectious bronchitis virus antibodies in broilers. Avian Pathology. 1997. Vol. 26. P. 105-118.
- 4. FlockChek Infectious Bronchitis Disease Antibody Test Kit. Animal Health Updates. August 2003 Edition. <u>www.idexx.com/production/livestockpoultrynews</u>.
- 5. FlockChek Poultry Production Guide. Version 2.20. 1997. P. 5-11.
- Gelb J. J. and Jackwood M. W. Infectious bronchitis. In: A laboratory manual for the isolation and identification of avian pathogens, 4<sup>th</sup> ed. D. E. Swayne, J. R. Glisson, M. W. Jackwood, J. E. Pearson, and W. M. Reed, eds. American Association of Avian pathologist, Kennett Square, PA. 1998. P. 169-174.
- 7. Gelb J., Nix W. A., Gellman S. D. Infectious bronchitis virus antibodies in tears and their relationship to immunity. Avian Diseases. 1998. Vol. 42. P. 364-374.
- 8. Glisson J. R. and Kleven S. H. Poultry vaccines. In: Peters A. R. (Ed.) Vaccines for Veterinary Applications, 1993. P. 165-198.
- 9. Ignjatovic J. and Galli L. Immune responses to structural proteins of avian infectious bronchitis virus. Avian Pathology. 1995. Vol. 24. P. 313-332.
- 10. Ignjatovic J. and Sapats S. Avian infectious bronchitis virus. Revue scientifique et technique de l office international des epizootines. 2000. Vol. 19. P. 493-508.
- 11. Jackwood M. W., Hilt D. A., Lee Ch. W., Kwon H. M., Callison S. A., Moore K. M., Moscoso H., Sellers H., Thayer S. Data from 11 years of molecular typing infectious bronchitis virus field isolates. Avian Diseases. 2005. Vol. 49. P. 614-618.
- 12. Lee C W., Hilt D. A., Jackwood M. W. Redesign of primer and application of the reverse transcriptase polymerase chain reaction and restriction fragment length polymorphism test to the DE072 strain of infectious bronchitis virus. Avian Diseases. 2000. Vol. 44. P. 650-654.
- 13. Manual of standards for diagnostic tests and vaccines. Office International des Epizooties, World Organisation for Animal Health, Paris. 2000. <u>www.oie.int</u>
- 14. Wit J. J. D. Detection of infectious bronchitis virus. Avian Pathology. 2000. Vol. 29. P. 71-93.