

SEROLOGICAL MONITORING OF AVIAN PNEUMOVIRUS IN LITHUANIA

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ABSTRACT

Avian Pneumovirus is increasingly recognised as an important pathogen in many poultry producing countries. A total of 6 888 chicken sera collected from 2007 to 2008 on 21 farms were subjected to an enzyme – linked immunosorbent assay (ELISA) to test for antibodies against APV. Tests of blood sera show that from 66.99 % to 97.18 % of the tested samples contain antibodies against APV. In spite of the fact that broilers are not vaccinated against APV, part of bird blood sera was found positive. The immunity obtained by the breeder birds is of a different level.

KEY WORDS: avian pneumovirus, specific antibodies.

INTRODUCTION

Avian Pneumovirus (APV) is increasingly recognised as an important pathogen in many poultry – producing countries.

A new respiratory of poultry seems to have occurred first in turkeys (Buys, S.B. and du Preez, 1980) and later in chickens (Morley and Thomson, 1984) in South Africa. This disease was subsequently named Turkey Rhinotracheitis or TRT and has been seen also in Israel, France and Great Britain (Alexander, 1993). In 1985 TRT rapidly spread through the turkey industry in GB (Lister & Alexander, 1986) and at the same time a syndrome of broiler parent chickens characterised by respiratory sings and head swelling and neurological signs was described (O'Brien, 1985). The term Avian Rhinotracheitis (ART) has been applied to avian pneumovirus infection in turkeys and chickens. Serological evidence of APV is now available from many countries: United Kingdom, France, Spain, Germany, Italy, the Netherlands, Israel, Asia (Alexander D. J., 1997, Jones R. C., 1996, Cook, 2000).

APV is a viral respiratory disease agent. It is in the family *Paramyxoviridae*, order *Virinare*, and genus *Metapneumovirus*, composed of a single unsegmented stranded negative sense RNA (Pringle C. R., 1999). Electron –microscopy examination showed that an APV is pleomorphic fringed and usually roughly spherical in shape (Ganapathy K., 2007).

Infection is characterized by coughing, ocular and nasal discharge, tracheal rales and swelling of the infraorbital sinuses. In laying birds, there is a transient drop in egg production, along with mild respiratory tract illness (Jones R.C., 1996). Uncomplicated cases have low mortality (2 to 5 %), but APV infections accompanied by secondary infections (bacterial and/or viral) can result in up to 25 % mortality (Jones R.C., 1996). Silent infections are possible.

The virus replicates in the respiratory tract and the reproductive tract. It initially causes respiratory disease in infected birds and may also cause drops in egg production in layers and breeders. Infection in turkeys is commonly referred to as “Turkey Rhinotracheitis” and in chickens aMPV infection is commonly associated with the condition known as “Swollen Head Syndrome” (SHS).

Control is by improved biosecurity and vaccination with live and inactivated vaccines (Tarpey, 2007).

The virology laboratory at the Veterinary Institute of Lithuanian Veterinary academy has been testing bird blood sera for APV since the autumn of 2002.

Aim of investigation. Evaluation of APV seroepidemiologic situation on poultry farms in Lithuania.

MATERIAL AND METHODS

A total of 6 888 chicken sera collected from 2007 to 2008 at 21 farms were subjected to an enzyme – linked immunosorbent assay (ELISA) to test for antibodies against APV.

The FLOCKSCHEK APV antibody ELISA kits were used for serological tests of blood samples according to the producers recommendations. This assay is designed to measure the relative level of antibody to APV in chicken serum. Viral antigen is coated on 96-well plates. Upon incubation of the test sample in the coated well, antibody specific to APV forms a complex with the coated viral antigens. After washing away unbound material from the wells, a conjugate is added that 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 APV present in the test sample.

For the assay to be valid, the difference between the positive control mean and the negative control mean should be greater than 0.075. The negative control mean absorbance should be less than or equal 1.150. The presence or absence of antibody to APV is determined by relating the A (650) value of the unknown to the positive control mean. The positive control is standartized and represents significant antibody levels to APV in chicken serum. The relative level of antibody in the unknown is determined by calculating the sample to positive (S/P) ratio. An S/P (sample value related to positive control value) ratio was used for calculation of results.

S/P =	SAMPLE ABS – NEGATIVE CONTROL ABS
	POSITIVE CONTROL ABS – NEGATIVE CONTROL ABS

Serum samples with S/P ratios less than or equal to 0.2 should be considered negative. S/P ratios greater than 0.2 (titers greater than 396) should be considered positive and indicate vaccination or other exposure to APV.

RESULTS AND DISCUSSION

Commercial ELISA kits are regularly used to detect serum APV antibodies, and the results need to be carefully analysed and interpreted. Commercial ELISA kits had been used about 6 years in our laboratory. According to Heckert R.A. et al. (1994) the ELISA is 98,7% sensitive and 99.5% specific and is capable of detecting serological responses as early as 11 days after chickens had been experimentally exposed to APV.

The serum samples were classified into 3 groups: one-day-old broilers, euthanized 39-48-day-old broilers and breeder birds.

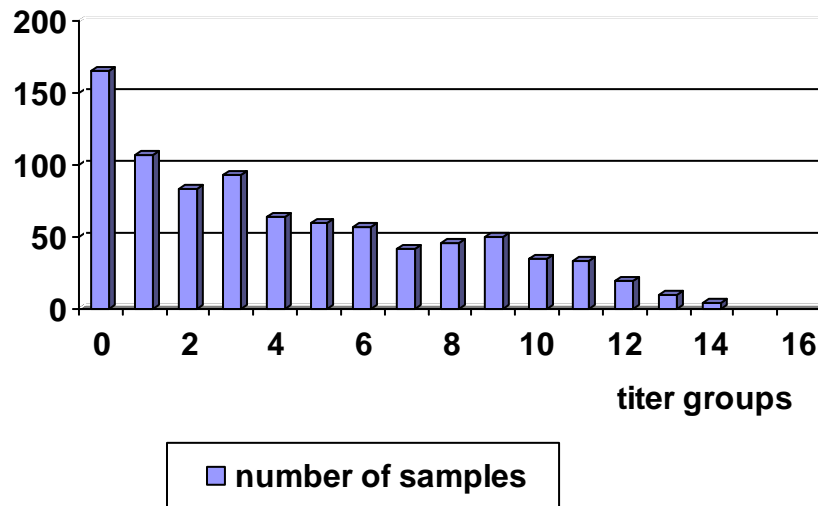


Figure 1. Antibody titers at the age of one day.

The test results of one-day-old chicken blood sera (Fig. 1) show that 81.06 % of the tested samples contained APV antibodies, while 18.94% were negative. In total were tested 871 samples. The mean titre (6527) was unevenly distributed (from group 1 to 14) which is evident in the variation coefficient (115.1%).

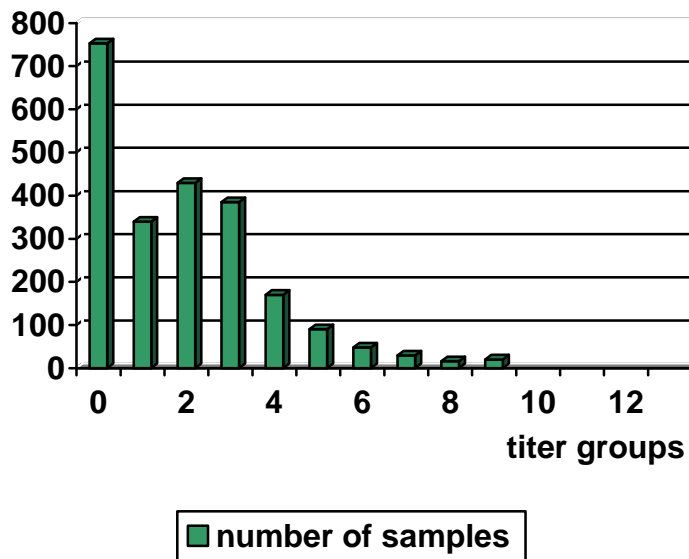


Figure 2. Antibody titers at the age of 39-48 days.

The test results of blood sera taken from the euthanized 39-49-day-old broilers (Fig. 2) show that 33.01% of the tested samples contained no APV antibodies while 66.99% were positive. The mean titre was 1888 and the variation coefficient was 126.3%. In spite of the fact that broilers are not vaccinated against APV, 1528 of euthanized bird blood sera were found positive. As no evident clinical symptoms were present, it can be concluded that a weakly virulent APV strain circulate in the farm causing the formation of antibodies.

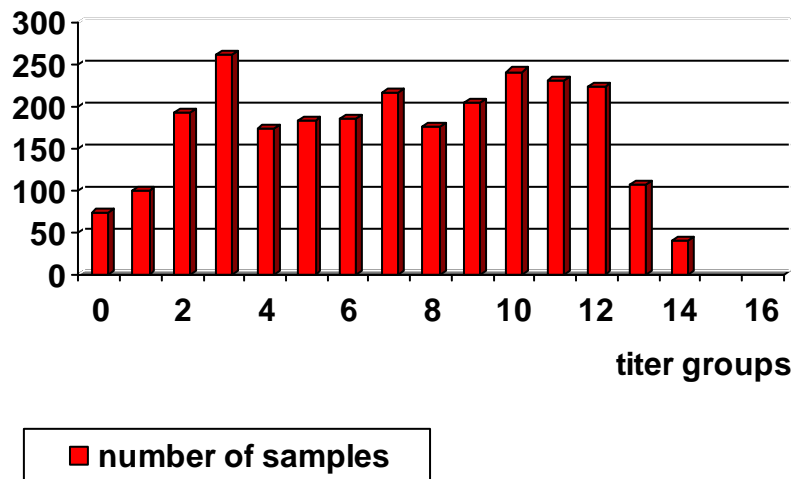


Figure 3. Antibody titers at the breed broilers.

Ganapathy K. (2007) maintains that a positive detection reflects exposure to the APV, a negative results does not rule out that the birds have not been exposed. This is because APV-infected chickens may not necessarily produce humoral antibodies, or antibodies may not have been at a detectable level at the time of the sampling. For an optimum detection of APV antibodies, it is best to use ELISA plates that are coated with a homologous antigen.

The test results of the blood sera from breed birds are presented in Figure 3. The mean antibody titre was 12801, CV – 75%. 97.18 % of the samples were positive and 2.82% was negative. Despite the fact that all breed birds were vaccinated against APV, however 74 blood sera samples were negative. The high CV indicates that the immunity obtained by the birds is of a different level. According to the FlockChek recommendations only a lower than 40% CV proves that the vaccination is effective and acquired immunity is even.

The immune status of a flock is best assessed by monitoring and recording antibody titers in representative samples as a function of time. The resulting flock profiles allow an assessment of the distribution of antibody titers and an analysis of changes in titer over time. Serological screening of blood samples can provide early detection so that other control measures can be instituted.

In Europe, vaccination is helpful for controlling APV. Vaccination programmes to protect against APV vary according to many factors (vaccination frequency, choice of vaccines and virus strains, methods). Generally, a single live vaccination in broilers and one or two live vaccinations followed by one inactivated vaccine in layers and breeders are sufficient to provide protection against APV clinical signs and loss of egg production/quality (Ganapathy K., 2007).

To control APV spread, biosecurity procedures must be a priority. Effective communication and cooperation among poultry growers couple with integrated poultry company management is essential. If APV positive flocks are identified, isolate younger birds from older flocks. Wild bird control is important, as free-living birds are suspected in carrying the virus.

CONCLUSIONS

APV seroepidemiologic situation have showed, that in spite of the fact that broilers are not vaccinated against APV, part of bird blood sera were found positive. It can be concluded that a weakly virulent APV strain circulate in the farm causing the formation of antibodies.

The immunity obtained by the breeder birds is of a different level.

REFERENCES:

1. Alexander D.J. Newcastle disease and other *Paramyxoviridae* infections. In B.W. Barnes, H.J. Beard, C.W. McDougald and L. Saif, Jr. (ed.). Diseases of poultry, 10th ed. Iowa State University Press, Ames. 1997. 541-569.
2. Alexander, D. J. Pneumoviruses (Turkey Rhinotracheitis and Swollen Head Syndrome of Chickens) in Virus Infections of Birds, J.B. McFerran and M. S. McNulty eds. Elsevier Science Publishers B. V. 1993.
3. Buys, S. B. and du Preez, J. H. A preliminary report of the isolation of a virus causing sinusitis in turkeys in South Africa. *Turkeys* June 36 & 46. 1980.
4. Cook J.K. Avian pneumovirus infections of turkeys and chickens. *Veterinary Journal*. 2000. 160: 118-125.
5. Ganapathy K. Avian metapneumovirus. *World Poultry*. 2007. 23: 35-37.
6. Jones R.C. Avian pneumovirus infection: questions still unanswered. *Avian Pathology*. 1996, Vol. 25. P. 639-648.
7. Lister, S. A., and Alexander, D. J. Turkey Rhinotracheitis – a review. *Veterinary Bulletin*. 1986. 56: 637-663.
8. O'Brien J. D. P. Swollen head Syndrome in broiler breeders. *Veterinary Record*. 1985. 117: 619-620.
9. Pringle C.R. Virus taxonomy – 1999. The universal system of virus taxonomy, updated to include the new proposals ratified by the International Committee on Taxonomy of Viruses during 1998. *Archives of Virology*. 1999. 144: 421-429.
10. Tarpey, I. & Huggins, M.B. Onset of immunity following in ovo delivery of avian metapneumovirus vaccines. *Veterinary Microbiology*. 2007. 124: 134-139.
11. Heckert R.A., Myers D.J., Afshar A., Riva J. Development and evolution of enzyme-linked immunosorbent assay for the detection of antibodies to avian pneumovirus. *Avian Diseases*. 1994. 38: 694-700.