INDIVIDUAL MONITORING IN FIELD CONDITIONS OF ELISA HAEMORRHAGIC ENTERITIS (HE) IN YOUNG TURKEY POULTS BORN TO VACCINATED BREEDERS

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Summary

Turkey adenovirosis or Haemorrhagic Enteritis (HE) is a viral disease caused by an adenovirus, the Haemorrhagic Enteritis Virus (HEV). HEV-infected turkeys may either die or recover from the disease. In field conditions, the implementation of a vaccination schedule based on a live HEV vaccine enables to control this form of the disease. A sub-acute form of the disease, i.e. spleen and liver enlargement with a mortality rate usually lower than 5%, observed between 7 and 9 weeks of age, has also been described in the field. In field conditions, turkeys are usually likely to suffer from HEV infection between 6 and 12 weeks of age. Turkey chicks are refractory to HEV infection before 3-4 weeks of age because of the presence of maternally derived antibodies which can be detected up to 6 weeks of age. In the absence of maternally derived antibodies, turkey chicks appear to be receptive to HEV, a type II adenovirus. Nevertheless, a refractory period from hatching to 13 days of age is classically described in chicks without maternally derived antibodies. Within this context, a study of the kinetics of HEV-specific ELISA antibody titres in standard turkey broilers was carried out from the first day of age (D1) to D35. The birds were vaccinated via drinking water with the live attenuated vaccine, Domermuth strain, on D28. The study was conducted in a poultry farm located in Western France. A group of 44 turkey chicks was separated from the main flock. HEV-specific ELISA antibodies were titrated individually between D2 and D34, with 9 repeats on each bird. Following the kinetic study, a serological follow-up of the turkeys belonging to the main flock was implemented on D57 and then on D78 in 20 randomly chosen birds. Mean titres (± standard error (SE), with a 5% alpha risk) showed a decrease in antibody titres during the kinetic study, from 13084±1094 on D2 to 321±161 on D27 and 132±70 on D31. The mean titre was lower than the positive threshold between D27 and D31. Mean titres increased after D37. The sample of 44 series of sera gave evidence of the great variability of titres on D2 (titres included between 3485 and 17984). The classification of titres into 4 different groups (<5000, 5000-10000, 10000-15000, and >15000) enabled a group-based analysis of kinetics and assessment of the advantage of interpretation of the serological results obtained on D2. On D57, the mean HEV-specific ELISA titre of 20 sera collected from randomly chosen turkeys was equal to 2540, with a minimum of 1109 and a maximum of 6317. The coefficient of variation of the series was equal to 53%. On D78, the mean HEV-specific ELISA titre of 20 sera collected from randomly chosen turkeys was equal to 15770, with a minimum of 7790 and a maximum of 20818. The coefficient of variation of this series was of 21%. The kinetics of maternally derived antibody decrease proved consistent with former field serological follow-ups performed in France in 1997-2001. Serological data collected in the field in Germany and during another HEV-specific ELISA serological study carried out by Bio Chêne Vert Laboratory, from data obtained in different production organisations located in different regions of France, were compared with the French field results. The kinetic study of maternally derived HEV-specific ELISA antibody decrease in broiler turkeys, in representative field conditions, clearly shows that the serological results obtained on D1 support the predictability of day of reaching threshold. D28, as a target date for vaccination, is the best compromise taking account of the individual variability of results, variability of breeder origins, and need of immune system stimulation against the risk of HEV circulation, which is quite frequent in field conditions.
Introduction

Turkey adenoviriosis or Haemorrhagic Enteritis (HE) is a viral disease caused by an adenovirus, the Haemorrhagic Enteritis Virus (HEV). This adenovirus, like that causing the marble spleen disease of pheasants, belongs to group II adenoviruses. The adenoviruses displaying a group antigen different from group II, i.e. Chicken Embryo Lethal Orphan (CELO) virus and other adenoviruses affecting the Gallus species – belong to group I. Virus incubation lasts 5-6 days approximately. Virus transmission is only horizontal, from bird to bird, and occurs by direct contact. The virus is secreted in droppings and can in theory persist several weeks in dead animals. In field conditions, the disease appears suddenly, when the acute form of the disease is involved, and lasts 6-10 days. Turkeys show signs of haemorrhagic enteritis (5-10% or more). Diseased turkeys may die or recover. At necropsic examination, the end part of the digestive tract of birds is bluish grey and contains dark coagulated blood. The duodenal mucosa is congestive and haemorrhagic, and the spleen enlarged. In field conditions, the implementation of a vaccination schedule based on a live HEV vaccine enables to control this form of the disease. A sub-acute form of the disease, i.e. spleen and liver enlargement with a mortality rate usually lower than 5%, observed between 7 and 9 weeks of age, has also been described in the field. Results of laboratory tests, HEV-specific ELISA serological and bacteriological tests (non-specific isolations of Escherichia coli, Pseudomonas sp., Pasteurella multocida, Ornithobacterium rhinotraceale, etc.) may conclude on the implication of type II adenovirus in this form of the disease. HEV immunosuppressive properties could be involved, although vaccination using the live attenuated vaccine via drinking water is largely but not systematically performed or sometimes performed without satisfactory know-how, hence detrimental to the quality of vaccine administration. Type II adenovirus isolation from spleens of turkeys suffering from the sub-acute form of HE, as shown by necropsic examination, is positive in 12% of organs, the Domermuth strain being isolated in all cases. It is impossible, based on the current knowledge of sub-acute HE, to establish a clear relationship between HEV infection and clinical signs (samples from DR J. Léorat and tests performed by Merial's laboratory, unpublished data). The microscopic examination of target organs of HEV-infected turkeys reveals the presence of reticulo-endothelial cells with intra-nuclear inclusions in the spleen, lymphocytic infiltrations in digestive tract and liver tissues, and lymphocyte depletion in the bursa of Fabricius. The immunodepressive potential of the viral agent causing HE in turkeys enhances secondary infections, mainly caused by E. coli (Pierson et al., 19961). From the immune system physiology viewpoint, T lymphocytes (cell-mediated immunity and immuno-modulation) and circulating monocytes (phagocyte activity of macrophages in tissues and immuno-modulation) are directly involved in interaction mechanisms between HEV and the host immune system. B lymphocytes (humoral-mediated immunity) appear to be the direct target of the HEV (Suresh et al., 19952, Rautenschlein et al., 19983). In field conditions, turkeys are usually likely to suffer from HEV infection between 6 and 12 weeks of age. Turkey chicks are refractory to HEV infection before 3-4 weeks of age because of the presence of maternally derived antibodies which can be detected up to 6 weeks of age (Fadly et al., 19894). In the absence of maternally derived antibodies, turkey chicks appear to be receptive to HEV, a type II adenovirus. Nevertheless, a refractory period from hatching to 13 days of age is classically described in chicks without maternally derived antibodies (Fadly et al., 19825). Within this context, a study of the kinetics of HEV-specific ELISA antibody titres in standard turkey broilers was carried out from the first day of age (D1) to D35. The birds were vaccinated via drinking water with the live attenuated vaccine, Domermuth strain, on D28.

Material and methods

A kinetic study of HEV-specific ELISA antibody titres was carried out in a poultry farm located in Western France (Brittany region), in an area of intensive poultry production (North of the French ‘department’ of Morbihan). A group of approximately 40 turkey chicks (44 exactly after count on D2) was separated from the main flock. They were individually identified with numbers (from #1 to #44). Vaccination via drinking water was carried out at 28 days of age on the entire flock including the study birds. The vaccine
used for mass administration was Merial's live attenuated vaccine, Domermuth strain, batch #L70626. Vaccination was implemented by adding the vaccine suspension to drinking water. Forty-four (44) blood samples were collected 9 times from the identified study birds. Each series of blood samples was collected according to the following schedule: D2, D8, D19, D21, D24, D27, D31. Following the kinetic study, a serological follow-up in turkeys belonging to the main flock was also implemented (collection of blood samples from 20 randomly chosen birds on D57 then on D78). Blood samples were then centrifuged and sera analysed by Bio Chêne Vert laboratory (ZI de Bellevue 2, F-35221 Châteaubourg Cedex). The ELISA (Enzyme Linked ImmunoSorbent Assay) technique was the relevant method used to detect anti-HEV antibodies (Haemorrhagic enteritis virus antibody test kit, Symbiotics Corporation6, formerly KPL) both in turkey chicks (maternally derived antibodies) and in vaccinated turkeys for vaccine intake monitoring purposes. The ELISA test was also intended for the diagnosis of HEV infection in field conditions (monitoring of field HEV virus circulation). Antibody titration in sera to test for type II adenovirus (HEV) infection in turkeys is an indirect ELISA technique based on a classical in vitro antigen-antibody reaction. Results were expressed as individual antibody titres. Optical density (OD) was measured at a wavelength of 405-410 nm. Sp = (sample OD – mean OD of control samples) / (mean OD of positive samples – mean OD of control samples). Titres were calculated according to the formula suggested by the ELISA kit supplier6 i.e. \[ \log_{10} \text{titre} = (1.464 \log_{10} \text{Sp}) + 3.197. \] The coefficient of variation was calculated by taking account of the titres obtained in the series of results obtained on D57 and D78 (sera collected from randomly chosen birds in the main flock). The positive threshold was considered equal to 147 as per laboratory standards. A kinetic study of individually monitored HEV-specific ELISA antibody titres was based on these data. Individual titres were calculated for all the series of samples coming from the 44 duly identified 44 turkeys. Individual results were obtained from D2 to D34, with nine repeats on each bird. The objective of the study was to determine reference kinetics to support the technical approach of vaccination of turkeys against HE.

Results

Figure #1 below illustrates all the HEV-specific ELISA titres obtained on D2, D8, D19, D21, D24, D27, D31, D34 and D37 (series of individual titres per day of serological follow-up). Titre heterogeneity is high. The lowest titre on D2 was equal to 3485 (bird #25) and the highest was equal to 17984 (bird #26.) The individual follow-up confirmed the great variability of results. Three out of 44 (3/44) titres (7%) were lower than 5000, 4/44 (9%) included between 5000 and 10000, 19/44 (43%) between 10000 and 15000, and 18/44 (41%) greater than 15000.

![Figure #1: Daily serological follow-up of series of individual titres.](image)

Mean titres (± 5E with a 5% alpha risk) showed a decrease in antibody levels throughout the kinetic study, from 13084±1094 on D2 to 321 ± 161 on D27 and 132±70 on D31.
The mean titre was lower than the positive threshold between D27 and D31. Mean titres increased after D37 (see Figure #2 below). This reference kinetic study could be used to determine a vaccination schedule for the control of HE in turkeys. The sample of 44 series of serums revealed great variability on D2 (titres included between 3485 and 17984.)

![Mean titres](image)

**Figure #2:** individual mean titres per day of serological follow-up.

The classification of titres into 4 different groups (<5000, 5000-10000, 10000-15000, and >15000) enabled a group-based analysis of kinetics and assessment of interest of interpretation of the serological results obtained on D2 (very low, low, medium or high titres). For very low titres (3/44, <5000), a mean titre of 0 was obtained on D21 (see Figure #3 below). For low titres (4/44, 5000-10000), a mean titre of 0 was obtained on D24 (see Figure #4 below). For medium titres (19/44, 10000-15000), the mean titre obtained was equal to 122±111 (above threshold) and 59±41 on D27 and D31, respectively (see Figure #5 below). For high titres (18/44, >15000), the mean titre was equal to 260±89 and 35±24 on D31 and D35, respectively (see Figure #6 below).

![Titres < 5000](image)

**Figure #3:** Titres <5000 (very low).
The mean HEV-specific ELISA titre of 20 sera collected from randomly chosen turkeys on D57 was equal to 2540, with a minimum of 1109 and a maximum of 6317, with a coefficient of variation of 53%. On D78, the mean HEV-specific ELISA titre of 20 sera collected from randomly chosen turkeys was equal to 15770, with a minimum of 7790 and a maximum of 20818, with a coefficient of variation of 21%.

Discussion

The kinetics of the decrease of maternally derived antibodies is consistent with former serological results obtained in the field in France, as shown by the 1997-2001 serological results (see Figure #7 below). On average, minimum levels were observed between D28 and D42 (decrease of maternally derived antibodies, then increase due to vaccine intake and later to field virus circulation).
Field serological data obtained in Germany were compared with French field results, focusing on the serological follow-up of weeks 4 and 5. Low levels of antibodies were detected in one flock on week 4 of age (130±17 in 20 samples) and in three further flocks on week 5 of age (75±37 in 20 samples, 110±48 in 10 samples, 200±56 in 10 samples). Antibody titres were in the expected range for the age, between four and five weeks of age.

Another HEV-specific ELISA serological study carried out in the field was performed by Bio Chêne Vert laboratory. A total of 25 flocks of turkey broilers, coming from different production organisations from different regions of France and born to different breeder flocks, were included in the study. Successive groups were included into the study for some of the selected buildings. All the flocks were included because of their clinical status, which included the hypothesis of HEV infection. Blood samples were collected on average at 1.48 days of age. The flocks in which blood sampling was performed after 3 days of age were removed from the selection. Only turkeys from one flock were blood sampled on D3 (study flock #11). In those flocks in which blood sampling was carried out after 3 days of age, the coefficient of variation proved much higher. Such an observation is made when the amount of blood samples is low as regards the amount of birds belonging to the flock (lower than 10 in a flock of 7000-9000 turkeys on average). Because of the lack of reliability of results, all such series of samples were withdrawn from the study as well. The average number of blood samples per series was 12. Twenty-five (25) different flocks were included in the study, from February 2003 to April 2004 (14 months). The mean minimum and maximum titres were equal to 9482 and 18983, respectively, and the mean titre was equal to 15549.

Antibody titres on D2 (which can be extrapolated to day-old birds) are predictable values which can fit the physiological decrease of maternally derived antibodies in standard birds reared in usual field conditions. The greater they are (e.g. greater than 15000), the later the disappearance of maternally derived antibodies which have an in vivo neutralising effect upon virus strains (Fadly et al., 1989). In such conditions, the mean titre is equal to 260±89 on D31. This titre is close to the proposed threshold of 147 according to laboratory references and was expected before performing the study. When titres are very low (<10000), a nil titre (equal to 0) is obtained between D21 and D24. The target date for live HE vaccination is D28. This is the best compromise whatever the level of maternally derived antibodies, when no serological data are available on D1. Post-vaccination seroconversion is demonstrated by a mean titre of 2540 with a
HEV circulation in current French field conditions is frequently observed, even in breeders. Such general field observations are confirmed by the serological results obtained on D78. The mean titres obtained on D78 increase to 15770 with a coefficient of variation of 21%, sign of a high level of homogeneity of titres. Sub-acute clinical signs of haemorrhagic enteritis (enlarged spleen and liver) and concomitant infection due to O2 K1 *E. coli* have been observed on D57 (communication from Dr J. Léorat.) HEV-specific ELISA seroconversion can be linked to HEV circulation. Implication of HEV in the sub-acute form of the turkey haemorrhagic enteritis with only signs of spleen and liver enlargement should be further investigated using other laboratory tools. The purpose of individual monitoring on a representative sample (n > 30) of animals is to have a clearer idea of individual variability. However, the origin of turkeys (one or several flocks of breeders) is never entirely secured and clearly identified. Up to now, vaccination against HEV is the only guarantee against all the forms of disease caused by the virus.

**Conclusion**

The kinetic study of maternally derived HEV-specific ELISA antibody decrease in broiler turkeys, conducted in representative field conditions, clearly shows that the serological results obtained on D1 support the predictability of the day of reaching threshold. The target date for vaccination (D28) is the best compromise, taking account of the individual variability of results, variability of breeder origins, and need of immune system stimulation against the risk of HEV circulation, quite frequent in field conditions.

**References**