

**FIELD AND EXPERIMENTAL INFECTIONS WITH TURKEY HERPESVIRUS***Veld- en experimentele infecties met het kalkoenenherpesvirus***S. Van de Zande, H. Nauwynck, M. Pensaert**

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**ABSTRACT**

To determine the age at which a natural infection of turkey herpesvirus (HVT) occurs in turkeys, two studies were set up. A first study was performed on 6 Belgian turkey farms. On each farm, 20 turkeys were bled at two-week intervals, starting at the age of 7 days and ending at the age of 18 weeks, and the collected sera were examined for the presence of antibodies against HVT. Maternally derived antibodies, which were uniformly present at hatching, decreased during the first weeks of life and disappeared by the time the turkeys were five weeks old. An increase in anti-HVT antibodies was detected one week later. In a second study, 7-day old specific-pathogen free turkeys were inoculated intranasally with a Belgian HVT strain. Antibodies appeared 5 weeks after inoculation. From the serological data obtained in the two studies, it was concluded that a natural infection of HVT takes place shortly after hatching, within the first days of life.

**Key words:** Turkey herpesvirus - Serology - Epidemiology - Pathogenesis - Turkey

**SAMENVATTING**

Twee studies werden opgezet om het juiste tijdstip van een kalkoenenherpesvirus (HVT) infectie te bepalen bij kalkoenen. In de eerste studie, die werd uitgevoerd op 6 kalkoenedrijven, werd om de twee weken bloed verzameld van 20 kalkoenen vanaf een leeftijd van 7 dagen tot 18 weken. In de verzamelde sera werden antistoffen opgespoord tegenover HVT. Maternale antistoffen waren uniform aanwezig op de leeftijd van 1 week, daalden hierna en verdwenen op een leeftijd van 5 weken. Eén week later werd een seroconversie waargenomen. In een tweede studie werden 7 dagen oude specifiek-pathogeen vrije kalkoenen intranasaal geïnoculeerd met een Belgisch HVT-isolaat. Vijf weken na de inoculatie werden antistoffen gedetecteerd. Uit de serologische resultaten van beide studies kan besloten worden dat een natuurlijke infectie met HVT plaats vindt kort na het uitkippen, tijdens de eerste levensdagen.

**INTRODUCTION**

Turkeys are commonly infected in the field with turkey herpesvirus (HVT), a horizontally transmitted, non-pathogenic herpesvirus which shares some antigens with Marek's disease virus (Witter *et al.*, 1970; Witter and Solomon, 1971). The widespread presence of HVT has been demonstrated in Belgium, as well (Van de Zande *et al.*, 1997). Beside the fact that the infection is enzootic, relatively little information has been published to delineate the epidemiology of

HVT in its natural host. Witter and Solomon (1971) studied natural HVT infections in turkey flocks and found that HVT spreads rapidly (within 2-3 weeks) throughout the flock and persists in infected birds. However, the authors did not succeed in determining the exact time of exposure.

In the two present studies, this specific point in time has been determined. In an initial field study, the course of antibodies against HVT was followed in commercial turkey flocks. An additional experimental study was set up with a Belgian HVT isolate to al-

low interpretation of the serological data from the field, to obtain more information on the time of infection and to find indications of the origin of HVT in the field.

## MATERIALS AND METHODS

### Longitudinal study

Six Belgian turkey farms were randomly selected. Only two farms worked with an all-in all-out system. Blood was collected every two weeks from 20 identified turkeys, starting when they were 7 days old and ending when they were 18 weeks old (time of slaughter). Sera were tested for the presence of antibodies against HVT with the immunoperoxidase monolayer assay (IPMA), as described in detail previously (Van de Zande *et al.*, 1997). When the turkeys were 6 weeks old, a pool of buffy coat cells was collected from 10 of them on three of the six farms for isolation of HVT on secondary chicken embryo fibroblasts (CEF) using the technique described by Witter and Solomon (1971). The virus was identified by an indirect immunofluorescence (IIF) technique, using three different monoclonal antibodies, which differentiate the three different serotypes of avian herpesviruses (Lee *et al.*, 1982).

### Experimental study

Thirty-six specific-pathogen free turkey poults, free of maternally derived antibodies against HVT (Dr. Toquin, Ploufragan, France), were divided in two groups of 24 and 12 birds respectively. The turkeys were housed in two separate negative pressure isolators, which were placed in two different rooms. The poults had free access to food and water. Twenty-four poults were inoculated intranasally at 7 days of age with  $10^{4.6}$  plaque-forming units (PFU) of HVT obtained from one of the commercial turkey farms during the longitudinal study. The group of 12 poults were not inoculated. At 0, 3, 5, 8, 10, 14, 21, 28, 35, 42, 61 and 80 days post inoculation (dpi), two inoculated poults and one non-inoculated control poult were euthanatized. Samples from the thymus, spleen and bursa of Fabricius and buffy coat cells were collected for virus isolation. The tissue samples were treated as described by Witter *et al.* (1972). Briefly, each tissue was gently dispersed with two needles. The suspensions were centrifuged (200 x g; 10 minutes) twice after washing with phosphate buffered saline, and the supernatant was carefully removed. The concentration

of tissue cells or buffy coat cells was brought to  $10^6$ /ml. The number of infected cells was determined by cocultivating tenfold dilutions of tissue cells or buffy coat cells onto a monolayer of secondary CEF. The number of plaques was counted 7 days later. Serum was collected every week from the remaining turkeys and tested for HVT antibodies by the IPMA, as described previously (Van de Zande *et al.*, 1997).

## RESULTS

### Longitudinal study

Figure 1 presents the mean IPMA titres ( $\log_{10}$ ) of anti-HVT antibodies in 20 identified turkeys on six farms throughout the growing period. All turkeys on the 6 farms had maternally derived antibodies against HVT with a maximal level of between 1  $\log_{10}$  and 3  $\log_{10}$ . The maternally derived antibodies disappeared between the ages of 4 and 5 weeks on all farms. Seroconversion was observed at the age of 6 or 7 weeks. From the age of 11 weeks, antibody titres reached their maximal level of between 3  $\log_{10}$  and 4  $\log_{10}$  on all farms and this level was maintained throughout the growing period.

Based on the time of seroconversion in the three farms, it was decided to make attempts to isolate HVT in the remaining three farms at the age of 6 weeks. A herpesvirus was isolated from the buffy coat of turkeys from each farm. They were all identified as HVT.

### Experimental study

Table 1 shows the results of virus isolation from different lymphoid tissues. HVT was first isolated from the spleen, bursa of Fabricius and buffy coat of one of the two turkeys killed at 35 dpi. The amount of isolated HVT varied between 2.3  $\log_{10}$  and 4.2  $\log_{10}/10^7$  inoculated cells. From 42 dpi till the end of the experiment, HVT was isolated from the spleen, bursa of Fabricius, thymus and buffy coat cells. At 80 dpi, the spleen of one turkey became negative while virus was still present in the second turkey. No virus was isolated from the spleen, bursa of Fabricius, thymus or buffy coat cells of the non-inoculated control turkeys.

Antibodies against HVT were not found at the time of inoculation. They appeared at 35 dpi in 1 of the 9 remaining birds. At 56 dpi, all remaining turkeys had antibodies against HVT. The IPMA titre increased until the last birds were killed at 80 dpi. The maximal IPMA titre was 2.8  $\log_{10}$ .

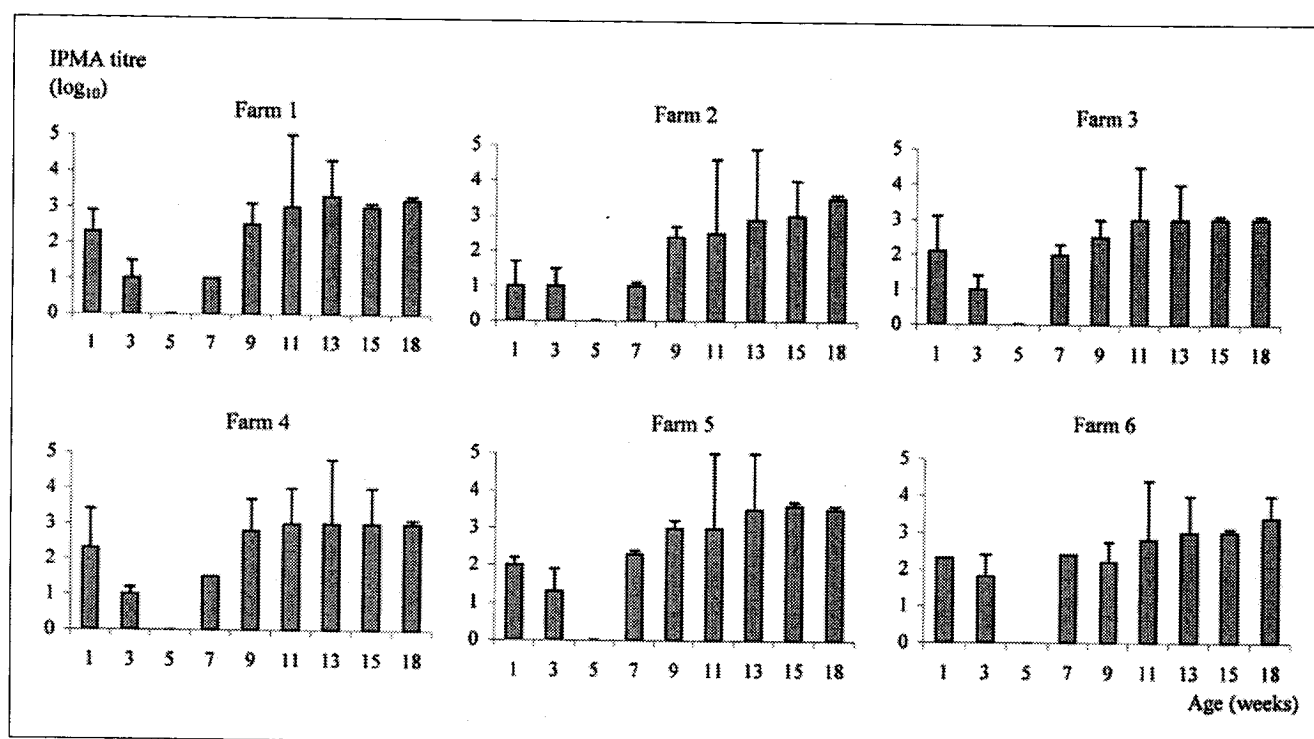


Fig. 1. Course of IPMA titres of antibodies against turkey herpesvirus in 20 identified turkeys from 6 farms throughout the growing period.

## DISCUSSION

To examine the time of exposure to HVT in commercial turkey flocks, two studies were performed. In the longitudinal field study, it was demonstrated that after the disappearance of maternal antibodies at 4 to 5 weeks of age, seroconversion against HVT always occurs within 1 to 2 weeks. This is in agreement with the findings of Witter and Solomon (1971). In their study, maternal antibodies fell below detectable levels at the age of 2 weeks and the development of an active immunity was detected from the age of 6 weeks. However, with the serological data found in the present longitudinal study and in the study of Witter and Solomon (1971), it is impossible to determine the exact time of exposure to HVT. For this reason, an experimental inoculation with a Belgian HVT isolate was set up in the present study. It was clearly demonstrated that after intranasal inoculation, turkeys develop antibodies starting from 35 dpi. This means that the poults become infected during their first days of life. Since maternally derived antibodies against HVT were present in all turkey poults, it is clear that they do not protect against HVT infection. The results imply that HVT infections in the field occur shortly after hatching, either in the hatcheries or on the farms. Congenital transmission of HVT can be excluded, since virus isolation from embryos of infected breed-

ers gave negative results (Prem *et al.*, 1972). In the hatchery, the virus could possibly come from the HVT vaccine that is generally used against Marek's disease in both chickens and turkeys at the time of hatching. However, it is unlikely that the vaccine virus spreads upon vaccination because the vaccine is given intramuscularly. On the farm, it could be that the poults are infected by persistently infected older animals that are present when 1-day-old poults arrive on the farm. The occurrence of airborne transmission has already been demonstrated by Witter and Solomon (1971). Another source of infectious HVT could have been the environment, which had been contaminated with HVT by the previous flock and not adequately cleaned and disinfected afterwards. For Marek's disease virus, another poultry alphaherpesvirus, it has been demonstrated that the virus can survive for years in the absence of birds in insufficiently washed and fumigated poultry stables.

Several HVT pathogenesis studies have already been performed. Witter *et al.* (1972) found that turkeys become viremic 8 days after intra-abdominal inoculation. After Fabricant *et al.* (1981) infected 3-week-old turkeys intra-abdominally, the virus was isolated from the spleen at 3 dpi and neutralizing antibodies were found at 14 dpi. The attempts made in our study to isolate the virus from lymphoid

tissues and to demonstrate antibodies in turkeys gave quite different results. The time between inoculation and first isolation of HVT from the blood and the appearance of antibodies was 4 weeks. This difference may be due to the intranasal method of inoculation. Where the virus is hidden between the time of inoculation and the time of first isolation is unknown. The most probable explanation is that the virus is present in a latent state in a restricted number of cells (this number being below our detection limit). To our knowledge, this is the first experiment described in which HVT has been communicated by the intranasal route, which is most close to the natural route (Witter and Solomon, 1971).

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