Vertical transmission of fowl Adenovirus serotype 4 investigated in specified pathogen-free birds after experimental infection

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* Dedicated to Professor Dr. Gerhard Monreal, on behalf of his seventy-fifth birthday

Introduction

Fowl Adenoviruses (FAV) are the classical members of the genus *Aviadenovirus* belonging to the Adenoviridae (Benkö et al., 2000). One feature of all FAV serotypes is the vertical transmission of this pathogens, crucial for early infections of progenies. In combination with the horizontal transmission FAV infections are very widespread in poultry, especially in laying birds. In most cases more than one serotype could be isolated from a single flock (Yates et al., 1976; Cowen, et al., 1978a; Monreal, 1984).

Vertical transmission was first realised by Yates and Fry (1957) when chick embryo lethal orphan virus (FAV1) was isolated from chicken embryos inoculated with specimens not related to this pathogen. The same observation was already reported by Van Den Ende et al. (1949) who used embryonated chicken eggs for isolation of pathogens from mammals. In various reports FAV was also isolated from uninoculated eggs (Du Böse and Grumbels, 1959; Cook, 1968). Some evidence came also from cell cultures prepared from vertically infected embryos harbouring FAV. In addition, more and more viruses isolated in embryonic cell culture contained FAV and it was impossible to trace back the source of this contamination. But is was obvious that some of these agents had to come from the eggs instead from the material used for inoculation. The accidental isolation of FAV in several other cases underlined that FAV has the potential of vertical transmission. The aforementioned investigations resulted in the establishment of specified pathogen-free flocks.

In parallel to these *in vitro* investigations FAV infections were more and more regarded as primary pathogens involved in the induction of inclusion body hepatitis (McCarran, 1997). Consequently, some studies were performed in order to investigate the potential of vertical transmission, especially for some strains of serotype 8 reported from Australia and New Zealand (Reece et al., 1985; Safuddin and Wilks, 1991). With regard to strain variations, results received with a single serotype can not be taken as common rule for all FAV serotypes. Especially, if serotypes with new biological features arise. This happened in 1988 when a new disease condition, Hepatitis/Hydropericardium Syndrome or Angara Disease, was described in connection with an adenovirus infection (Shane and Jaffrey, 1997). The successful reproduction with plaque purified FAV strains from Ecuador and Pakistan and basic epidemiological data were essential to clarify the aetiology of the disease (Mazaheri et al., 1998; Hess et al., 1999). These investigations highlighted once again the variation among serotypes and even between FAV strains belonging to the same serotype.

The present investigation was performed in order to increase the knowledge about vertical transmission of FAV by creating basic data not yet available comparing virus excretion and antibody development in parent birds and progenies under experimental conditions.

Material and Methods

Virus

A plaque purified FAV4 virus (K31) was used to infect the hens. Propagation and purification of the virus was done as described recently (Mazaheri et al., 1998).

Animal experiment

Sixteen 33 week-old specified pathogen-free (SPF) parent birds (Valo, Lohmann Tierzucht GmbH, Cuxhaven, Germany) were kept in individual cages and were artificially inseminated. One week after the first artificial insemination the birds were infected orally with 1ml of K31, with a titre of $10^5$ TCID 50/ml. Prior to the infection blood samples were taken from the wing vein together with cloacal swabs and once again artificial insemination was performed. This procedure, bleeding, swabbing and artificial insemination was continued for 3 weeks post infection. Eggs were collected daily and all eggs collected during one week were incubated together. Collection of eggs started one week prior to infection and lasted until 4 weeks after infection. Eggs were candled every three days and dead embryos were investigated. Liver samples were taken for virus isolation. To keep the direct link between chicks and hen the eggs of each individual hen were hatched separately. From the hatched birds one was wing banded and continuously used for virus isolation. The birds of the same week were placed together in sepa-
rate cages resulting in 5 different groups (group 0—4) according to the time the eggs were laid. However, due to variability in laying performance, variation of fertility and some dead embryos, the number of the day-old progenies varied. All progenies were killed five weeks after hatch and parent birds were killed at 39 weeks of life.

Collection of samples
At 7, 14, 21 and 28 days post infection the birds were bled and cloacal swabs were taken for virus isolation. All birds killed at the end of the experiment were investigated by pathological investigation.

Laboratory investigations
Serology
Neutralization test was done in ninety six-well microtiter plate cultures of chicken embryo liver cells according to Monreal et al. (1980). Serum samples were diluted 1:2 following continuously double dilution up to 2048. Serum samples were incubated together with 100 TCID50 of K31 to determine the antibody level. Using this procedure the serum samples were investigated taken weekly from the parent birds and from the progenies at 1 and 35 days of life.

Virus isolation
Cloacal swabs were placed in 2 ml of tissue culture medium and passed through a 0.2 µm filter. From the filtrate, 0.5 ml were inoculated onto chicken embryo liver (CEL) cells prepared from 11 day-old chicken embryos. In case no cytopathic effect was present 3 days post infection, the cells were frozen and thawed three times followed by sonication. After that 0.5ml were transferred onto new cells, a procedure done for up to three times. The livers of dead embryos were homogenized (10% suspensions) in phosphate buffered saline and clarified by centrifugation at 1000 g for 15 min. The supernatant was passed through a 0.2 µm filter and processed in the same way as the cloacal swabs. The cell cultures were prepared according to standard protocols (Bauer and Monreal, 1986; Monreal et al., 1980).

Results
Clinical symptoms in the parent birds
No clinical symptoms were noticed in the parent birds after infection with K31. There was no influence on the laying performance. Each bird laid 6 eggs on average per week prior to and after infection.

Pathological investigation of dead embryos
Dead embryos were investigated over the experimental period of 5 weeks during which eggs were collected. A total of 9 embryos died, traced back to 5 different parent birds. The majority of the embryos (5) originated from the eggs laid in the second week p.i.. The other four dead embryos were noticed from the eggs set to hatch in the following week (3 weeks p.i.). Four embryos died when they were 6 days old and further four embryos died at the age of 8 days. One embryo died on day 18 of incubation. Livers were collected from all the embryos which died 8 or 18 days after setting the eggs.

Clinical symptoms in the progenies
A total of 5 groups of progenies was assigned each consisting of 16 (2 groups), 12, 10 or 13 birds, respectively. None of the birds showed any clinical signs and no bird died during the whole experiment (Table 1).

Serological response of the parent birds
The serological response of the parent birds was determined weekly. The results are summarised in Table 2. No antibodies were detected before infection and one week p.i.. A total of 13 birds showed a weak antibody response in the 2nd week p.i. All birds seroconverted at three weeks p.i. and the level of antibodies varied from 1:32 to 1:1024.

Serological response in the progenies
The results of the serological response in the progenies is also given in Table 2. Antibodies against FAV4 were only detected in the birds which hatched from eggs collected in the 3rd or 4th week after infection of the parent birds. The maximum antibody level achieved at the first day of life was 1:512 and at the age of 35 days 1:64, at which time the majority of birds turned out to be negative.

Virus isolation from the parent birds and progenies
Overall no virus excretion was noticed prior to infection from the parent birds and the respective progenies (Table 3). None of the infected parent birds excreted virus until 2 weeks p.i. Whereas all parent birds excreted virus 2 and 4 weeks p.i. no virus excretion was noticed in the third week p.i.

No adenovirus could be isolated from the livers of the embryos which died during incubation. Nine out of twelve progenies (group 2) hatched from eggs collected 2 weeks after infection of the parent birds excreted virus with the meconium at day-old. However, no further spread of virus

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<th>Table 1. Experimental design of the five different groups of progenies</th>
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<td>Experimentelle Zusammensetzung der fünf verschiedenen Gruppen von Nachkommen</td>
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<td>a group sizes varied according to variation in laying performance and hatched chicks</td>
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<td>b group 0 should be regarded as control group</td>
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occurred in these birds until they were 4 weeks-old. From none of the day-old progenies (group 3) virus could be isolated, even though all cloacal swabs were positive at later stages. Virus isolation was positive from 4 out of 13 birds hatched from the eggs collected 4 weeks p.i. However, all of the 5 tested birds excreted virus at 7 days of life.

**Discussion**

Even though vertical transmission of Fowl Adenoviruses (FAV) is a known fact there is no study in which SPF-birds were infected with a single serotype and seroconversion together with viral excretion were monitored in parent birds and progenies over several weeks in parallel. The present study was set up to achieve more knowledge about this issue.

Once again the intermittent excretion of fowl adenovirus could be demonstrated. Concurrent with virus excretion of the parent birds meconium samples of the progenies turned out to be positive, a clear indication for the vertical transmission of the virus. Vertical transmission lasted for the whole time period of the trial, different to the investigations reported for FAV1 (DAWSON et al., 1981). The finding that the parent birds excrete live virus up to 4 weeks post infection, in the presence of neutralising antibodies and concurrent with some positive meconium samples of the progenies, is also contrary to the results reported by COWEN et al. (1978b). In these investigations the vertical transmission stopped with the detection of neutralising antibodies. It could well be that strain specific variations are the reason for this differences underlining once again the heterogeneity of FAV serotypes.

Vertical transmission in the presence of neutralizing antibodies was also reported for FAV8 viruses (SAIFUDDIN and WILKS, 1991). The highest load of viral antigen detected by ELISA was noticed in the egg yolk but no attempts were made to isolate virus from these eggs. In the present investigations no adenovirus could be isolated from the embryos which died during incubation. REECE et al. (1985) reported also the failure to isolate virus from embryonated eggs after infection of parent birds. In their investigations the persistence of FAV8 for up to 8 weeks was reported in the caecal tonsils of the parent birds.

One question which has to be addressed is the failure of the used FAV4 isolate (K31) to induce Hepatitis/Hydropericardium Syndrome (HHS). TORO et al. (2001) demonstrated the effect of vertically induced HHS using a pathogenic FAV isolate from Chile. However, these authors used light brown layers which are described to be highly susceptible, indicating a possible influence of the breed to develop HHS (TORO et al., 1999).

The intention of the present study was to imitate under experimental conditions the field situation in which horizontal infection of parent birds will result in vertical infection of progenies. Most likely, vertical transmission is not the critical point to induce HHS, especially in SPF birds. SAIFUDDIN and WILKS (1991) stated in their work that virus introduction into a flock could well happen through vertical transmission but other factors may contribute to the maintaining and spreading of infection.

Following vertical transmission, the horizontal spread in the flock is the main source of infection as virus is excreted to a high extent with the faeces (MONREAL et al., 1979). Critical for this could be the time period between vertical virus transmission and seroconversion of the parent birds followed by transmission of maternal antibodies.
In the present investigation this gap lasted for only one week, between 2 and 3 weeks post infection of the parent birds. However, even during this small time period no obvious clinical signs of the progenies were noticed. In our recent studies we showed that the viral dose and way of administration is crucial to reproduce the disease in SPF birds (MAZAHERI et al., 1998). It could be that the amount of virus transmitted vertically was too low to induce clinical signs. Virus excretion in broilers peaked between 4 and 6 weeks of age, most likely based on the decrease of maternal antibodies at that time (McFERRAN, 1981). Concurrent with this report Hepatitis/Hydropericardium is described in 3–5 week-old broiler flocks (SHANE and JAFFERY, 1997).

The breed of birds and route of inoculation is important as already mentioned above. In our recent investigations with SPF chicks we had to choose high doses and intramuscular injection to induce mortality in birds older than one week of age, whereas one-day-old chicks died already after oral application. However, most likely birds in the field are much more susceptible for the disease condition.

After developing neutralizing antibodies in the parent birds the progenies are obviously protected by maternal antibodies. FADLY and WINTERFIELD (1973) reported that chickens derived from breeders infected with FAV could not be challenged successfully with the same strain until 3 weeks of age, based on the presence of maternal antibodies. The authors used a 1:10 dilution of a egg derived non-titrated virus, involved in Inclusion Body Hepatitis. However, TORO et al. (2002) reported that maternal antibodies are not sufficient to protect chicks completely after intramuscular challenge with highly pathogenic FAV4 isolate at 15 days of age. It is obvious that experimental conditions are difficult to set up, imitating the field situation. As FAV antibodies are widespread in commercial birds the main priority in this study was the negative FAV antibody status of parent birds and the usage of SPF birds. In this context, the present investigations gain new insights into the spread of FAV4 from the parent birds to progenies.

Summary

Vertical transmission is reported as an important feature of fowl adenovirus (FAV) to spread from parent birds to progenies. In the present investigation specified pathogen-free artificially inseminated hens were orally infected at peak of lay with FAV4 strain K31 to investigate the effect on general health and to follow up antibody levels and virus excretion in parent birds and progenies. No influence on the health status of the parent birds and the progenies could be noticed. Although some embryos died during incubation no adenovirus could be isolated from the livers of those embryos. Seroconversion in the parent birds was noticed 2 weeks post infection and the level of antibodies increased until the end of the experiment at which time all birds showed an antibody response. Maternal antibodies were noticed in those progenies hatched from eggs collected as early as 3 weeks post infection of the parent birds. At this time all of the progenies had maternal antibodies whereas the majority of these birds had no more antibodies at the age of 5 weeks, when the experiment was terminated. Virus excretion was noticed in the parent birds 2 weeks after infection and turned out to be intermittent, as no virus excretion was found 1 week later but again 4 weeks post infection. Virus excretion in day-old progenies was noticed concurrently with virus excretion in parent birds and all of the progenies got infected during life time, except those birds hatched from eggs collected 1 week post infection of the parent birds. Most importantly, vertical transmission of viable virus was demonstrated in the presence of neutralizing antibodies of parent birds, such underlining the importance of this transmission route for FAV.

Keywords

Fowl Adenovirus serotype 4, Vertical transmission, virus detection, antibody development

Zusammenfassung

Vertikale Übertragung des Hühneradenovirus Serotyp 4, untersucht in Spezifizierten-Pathogen-Freien Tieren nach experimenteller Infektion


Stichworte

Hühneradenovirus Serotyp 4, Vertikale Übertragung, Virusnachweis, Antikörperbildung

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