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Review

The perspective of immunoprophylaxis and selected immunological issues in the course of the turkey rhinotracheitis

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Abstract

This review article presents immunological issues in the course of the turkey rhinotracheitis (TRT) emphasizing local immunity mechanisms, both humoral and cell-mediated, in the upper respiratory system. Studies on the influence of the humoral immunity in the course of infection and vaccinations against TRT have revealed many times the absence of correlation between the titre of specific IgY anti-aMPV (avian Metapneumovirus) antibodies in the serum and in the upper respiratory washings and the immunity against the occurrence of the clinical form of the TRT. Considering the above, T cells are increasingly often regarded as the main factor involved in the upper respiratory immunity against the TRT. However, there have been just a few reports on the role of the T cells in the local immunity processes in the infection with aMPV in turkeys. Additionally, studies of the T-cell-associated immunity against the TRT have given ambiguous results.

Immunoprophylaxis issues against the aMPV infections are a significant part of the work where the authors confront current vaccination programmes against the perspectives of use of the future vaccines against the TRT. Future vaccines should face the following criteria: absence of the risk of immunosuppressive effect and reversion of vaccine strains virulence, ease-of-use combined with the possibility of administration of the vaccine to the large numbers of turkeys. The leading role in future vaccination programs for birds against the TRT is likely to be played by the *in ovo* technique and the recombinant vaccines. Great hopes are also linked with the development of subunit vaccines against the aMPV.

Key words: turkeys, rhinotracheitis, immunoprophylaxis, humoral and cell-mediated immunity

Introduction

Avian Metapneumovirus (aMPV) is a highly contangious pathogen which is responsible for infections of the upper respiratory tract, mainly in turkeys, but also in chickens. The disease caused by aMPV in turkeys is referred to as TRT (turkey rhinotracheitis). Infections with metapneumovirus cause great losses in the poultry industry, which stems mainly from a drop of birds weight, direct deaths, and a decrease in egg laying and immunosuppression, which increases birds' sensitivity to secondary infections with pathogens that

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would frequently be unable to cause a disease itself in its clinical form (e.g. *Bordatella avium*, *Pasteurella multocida*, *Mycoplasma gallisepticum*, *Chlamydophila psittaci*, *Ornithobacterium rhinotracheale*, *E. coli*) (Jirjis et al. 2004, Marien et al. 2005, Rubbenstroth and Rautenschlein 2010).

Metapneumoviruses, which used to be referred to as *avian pnemovirus* (APV) or *avian rhinotracheitis virus* (ART) are classified as a member of *Paramyxoviridae* family, genus *Metapneumovirus* (Rubbenstroth and Rautenschlein 2009). The genetic material of aMPV comprises single-strand RNA with the core part consisting of a helical nucleocapsid (Gough 2003). The virus RNA encodes 10 genes (inter alia, F, G, M, N) (Rubbenstroth and Rautenschlein 2010), whose expression products are involved in the TRT pathogenesis on one hand, but on the other, they enable identification of the aetiological factor by the RT-PCR (reverse-transcription polymerase chain reaction) techniques.

On the basis of numerous laboratory experiments (for example, ones using monoclonal and polyclonal antibodies, as well as molecular biology techniques), the viruses which cause TRT have been classified into 4 subtypes (depending on the genes structure) – A, B, C and D. Despite the differences in the antigen structure of different aMPV subtypes, cross-resistance has been shown to exist among the subtypes (to a different extent for each of them, however). The highest antigen similarity was shown (with monospecific antibodies) to exist between the A and B serotypes, whereas the C serotype was not equally neutralised with the same antibodies (Collins et al. 1993, Cook et al. 1993). It also cannot be ruled out that there are other subtypes of the TRT virus, but they have not yet been discovered or identified.

Metapneumovirus was first discovered in late 1970s in South Africa and now its range of occurrence covers the entire world, except Canada and Australia. The TRT in Poland was first noticed in 1992, with the number of severe cases in flocks of slaughter and breeding turkeys increasing in the second half of the year. As in the rest of Europe, A and B subtypes of the virus dominate in Poland (Pedersen et al. 2000, Rubbenstroth and Rautenschlein 2009, 2010).

After infiltrating into the body, aMPV replicates in the epithelial cells of the upper respiratory tract, leading to their exfoliation and deciliation, which results in damage to the whole mucosa. The virus may infiltrate into the blood, causing temporary viraemia. It has been shown that replication of aMPV in young turkeys is restricted to the upper respiratory tract and the virus has been isolated from swabs taken from the nasal conchae up to 14 days after experimental infection, with a higher percentage of positive results for birds infected with the A subtype (5 out of 6 tested birds after infection) of the virus as compared to infection with the B subtype (2 birds out of 6 tested) (Liman and Rautenschlein 2007), which indicates higher replication capability of A subtype aMPV in the mucosa of the upper respiratory tract (as compared to the B subtype). Virus replication outside the respiratory tract in female breeding turkeys takes place in the reproductive system, from where aMPV was isolated up to 9 days after experimental infection (Jones et al. 1988).

TRT virus causes an immunosuppressive effect, which results from reduced activity of immune cells, but also from damaging the respiratory mucosa, which is the natural defensive barrier of the body (Rubbenstroth and Rautenschlein 2009). Numerous studies have confirmed the immunosuppressive effect of aMPV in turkeys, which manifests itself, inter alia, in weakened mitotic reaction of T cells and uneven post-vaccination immunity of birds immunised against HEV (*Haemorrhagic enteritis virus*) (Chary et al. 2002a,b).

Immunoprophylaxis against the TRT

The vaccination schedule in flocks of turkeys intended for slaughter and reproduction provides multiple vaccinations with the usage of live attenuated and inactivated vaccines (in reproductive flocks). The first vaccination (live attenuated vaccine) is made in one-day-old poults in a growing facility or earlier, in a hatchery, by the aerosol method (macromolecular spray). Subsequent vaccinations (in week 3 and 9-10 of their lives), also with the use of live vaccines, are made by giving it to birds with drinking water or in aerosol. Additionally, in the flocks of turkeys intended for reproduction, there are immunoprophylaxis procedures done with inactivated vaccines, usually twice before the egg-laying period as intramuscular or subcutaneous injections (Koncicki 2005, Koncicki 2006).

The aim of the immunoprophylaxis procedures using inactivated vaccines is to induce a booster effect and to create a high titre of IgY antibodies, which are passed to the eggs and then to the poults by layers. The aim of the antibodies is to protect the young birds in the early period of their rearing (about 3 weeks) against infection with pathogens their parents had contact with, either in a natural or artificial (vaccination) way. However, it has been shown in many experiments that the titre of specific anti-aMPV IgY antibodies in the birds serum does not correlate with immunity of the respiratory mucosa against the TRT virus. The fact necessitates vaccinations in newly-hatched poults as early as on the first day of their lives, which aims at providing the maximum pro-

tection of their upper respiratory tract against infection with aMPV. However, despite early implementation of specific vaccination programmes in poults, sometimes post-vaccination immunity is breached by aMPV. This results from antigenic variability of the avian metapneumovirus on one hand, and on the other, it is indicative of a deficit in the immunoprophylaxis programmes which are associated with incomplete knowledge of the mechanisms of post-vaccination immunity against the TRT virus, including the effect of maternal immunity, which has not been properly studied.

The specific TRT prophylaxis programmes described above also have other disadvantages. Using inactivated vaccines in injection is very costly and labour-consuming and – due to the route of administration of the vaccine virus, such vaccination is rather ineffective in developing local resistance in the upper respiratory tract, which is a gateway through which aMPV enter the body. On the other hand, vaccines based on live attenuated viruses are presumed to retain their immunosuppressive properties. Additionally, the virulence of these vaccine viruses may be reversed (Catelli et al. 2006), especially in flocks where vaccinated birds live alongside those which have not been subjected to specific immunoprophylaxis against the TRT virus.

Considering the above, it seems necessary to improve the current immunoprophylaxis programmes. Future vaccines should face the following criteria: absence of the risk of immunosuppressive effect and reversion of vaccine strains virulence, ease-of-use combined with the possibility of administration of the vaccine to the large numbers of turkeys (Liman et al. 2007). The leading role in future vaccination programs for birds against the TRT is likely to be played by the *in ovo* technique and the recombinant vaccines. Great hopes are also linked with the development of subunit vaccines against the aMPV.

Since the first report in 1982 on the possibility of using the *in ovo* technique in protection from infectious diseases in poultry (Sharma and Burmester 1982), this technique has become an increasingly popular subject of the scientific research into the possibilities of immunoprophylaxis with respect to various viral diseases. Currently, the technique is successfully used in protecting chickens from the negative effects of infections with the field strains of Marek's disease virus. Research into the possibilities of using the *in ovo* vaccination technique has also been conducted for the TRT.

Worthington et al. (2003) examined the effect of attenuated TRT virus subtype A (aMPV/A) administered by the *in ovo* technique on day 24 of incubation of turkey embryos and found the vaccine to affect the development of immunity against the virulent strains of aMPV. The birds showed specific immunity to experimental infection by the eye-drop application of the virulent aMPV up to week 14 after hatching, with the immunity developing earlier as compared to that developed after spray vaccination of the one-day-old birds (Worthington et al. 2003, Tarpey and Huggins 2007). The researchers also reported on the harmlessness of *in ovo* vaccination to the hatching rate even when the recommended vaccine dose was exceeded tenfold. They also noticed the absence of any negative effect of maternally derived antibodies (MA+ groups) on the development of post-vaccination immunity. As it has turned out, *in ovo* administration of a reduced dose of the vaccine virus to MA+ embryos resulted in the immunity against infection with virulent TRT virus of 77% of birds hatched from the eggs (Worthington et al. 2003).

Similar studies have been conducted with B and C subtypes of aMPV virus (aMPV/B, aMPV/C) (Tarpey and Huggins 2007, Cha et al. 2011). It has been found that introducing attenuated C subtype of aMPV virus (aMPV/C) to amniotic fluid of turkey eggs, free of maternally derived anti-aMPV/C antibodies, on day 24 of their incubation does not affect the hatching rate, despite minor lymphocytic infiltration in the upper respiratory mucosa. Additionally, the virus showed higher ability to penetrate into the respiratory tract and was isolated from the upper respiratory tract and from the lungs. Such aMPV penetration does not take place after vaccination on the first day after hatching. In order to determine the effectiveness of *in ovo* vaccination, birds were infected experimentally in week 3 of their lives with the virulent aMPV subtype C, administered to the conjunctival sac. Compared to the control groups (not vaccinated), much lower index of histopathological changes and higher resistance to the virus were observed in the upper respiratory tract of the birds which were in contact with the attenuated virus during the embryonic period, which was reflected in a lower number of viral RNA copies isolated from the upper respiratory swabs determined by the qRT-PCR (quantitative RT-PCR) (Cha et al. 2011).

Despite incomplete maturity of the respiratory tract and the immune system in turkey embryos on day 24 of incubation, which may be indicated by the fact of isolation of the aMPV from the lungs and lack of production of the specific anti-aMPV/C IgA (Cha et al. 2011) in birds which were vaccinated by the *in ovo* technique, this technique may be claimed to have a reducing effect on sensitivity of turkey chicks to infection with the field strains of aMPV at the early stages of rearing. Considering the above and the high environmental pressure of the virus in the poultry breeding facilities, one may speculate that the method

of *in ovo* vaccination, as being less stressful for the young birds, may in future become the main tool in the immunoprophylaxis against the TRT.

New opportunities in the TRT immunoprophylaxis should be provided by the recombinant vaccines, based on genes which encode fusion protein (F) of the aMPV. Qingzhong et al. (1994) examined a recombinant vaccine based on the fowlpox virus, which encoded aMPV protein F and found it to be effective and that this vaccine reduced the sensitivity of the birds to the experimental infection with the virulent TRT virus 2 weeks after second vaccination, in both superconjunctivally and intrnasally challenged groups of birds. The course of the disease in the immunised turkeys was milder after inoculation and the birds' upper respiratory tracts were more resistant to the aMPV, which reduced the capability of the virus replication in the nasal and tracheal mucosal membrane (Qingzhong et al. 1994). These findings are similar to those of the study by Kapczyński and Sellers (2003), who also showed higher immunogenicity of protein F of the TRT virus as compared with the recombinant vaccines based on the genes which encoded protein N of the aMPV. Despite the positive results of the studies above, such vaccines have disadvantages, such as high cost of production and the necessity of parenteral administration (intramuscular injection), which may limit their more widespread application.

In an effort to meet the expectations towards modern vaccines, Liman et al. (2007) made an attempt to develop a new generation vaccine based on the genes which encoded protein F and on the protein F itself, administered oculonasally. The vaccine was prepared by embedding the genetic material (F-protein encoding gene), and the protein in the microparticle carrier – polymer PLGA (poly D,L-lactide-*co*-glycolide acid). The results have shown that it is safe to use this kind of vaccine, and that simultaneous stimulation of both local and systemic cellular and humoral immunity takes place in the turkeys following such vaccination. Following experimental infection the researchers observed a considerable increase in the $CD4^+$ lymphocytes subpopulation, both in the Harderian gland and in the spleen, as compared to that found in the control groups (not vaccinated). At the same time, the aMPV was less frequently isolated from the nasal conchae swabs in the vaccinated turkeys from day 9 after experimental infection as compared to the control groups, but more positive results were recorded in groups immunised with the PLGA-based vaccine as compared with the birds which were immunised with the conventional vaccine based on the live attenuated TRT virus (Liman et al. 2007). It turns out that using a PLGA-based vaccine results in developing partial immunity against the infection (42% reduction of the intensity of clinical signs following experimental infection) and speeds up the process of recovery in turkeys, but it is not as effective as current live vaccines (100% reduction of clinical signs) (Liman et al. 2007). Considering the above and the costs associated with preparation of such a vaccine, its clinical application is questionable, although further studies aimed at developing a safe and effective vaccine against the TRT virus may be expected.

Selected immunological issues in the course of the TRT

The current vaccination programmes applied in reproduction flocks aim to induce a booster effect, with the resulting production of the high titre of IgY antibodies in laying hens. The immunoglobulins they produce are subsequently transferred to the eggs and then they protect the hatching chicks from the pathogens from the environment on the first days of their lives. Moreover, serological monitoring, carried out with the use of ELISA tests, which for example enables confirming the presence of the aMPV infection in a flock, allows detection of new infections as well as an assessment of the effectiveness of immunoprophylaxis against the TRT, is based on detection of the specific immunoglobulins Y in birds' blood serum. However, studies of the influence of humoral immunity on the course of infection and vaccinations against TRT have indicated many times the absence of correlation between the titre of specific IgY anti-aMPV antibodies in the serum and in the upper respiratory washings, and the immunity against the occurrence of the clinical form of the TRT. Jones et al. (1992) demonstrated that turkeys following chemical suppression of the B lymphocytes by intravenous injection of cyclophosphamide on the first days of their lives, which resulted in the absence of the anti-aMPV antibodies following vaccination against the TRT, did not show the lack of immunity against the infection. Turkeys have shown full resistance against the virulent virus 21 days following vaccination (Jones et al. 1992). The results of these studies are similar to the findings of Rubbenstroth and Rautenschlein (2009), who made intravenous injections of the anti-aMPV IgY to turkeys and found no difference in the course of infection with the virulent strains of the TRT virus between them and turkeys which were not subjected to passive immunisation.

Considering the above, detection of a high titre of anti-aMPV IgY antibodies in birds' blood serum cannot be regarded as a clear sign of the development of post-vaccination resistance to the infection with field

strains of the TRT virus. Moreover, there have been numerous reports on varying sensitivity of the commercial ELISA kits, which stems from differences in the antigen structure of the viruses used for plates coating in the tests and the viruses occurring in the birds' breeding environments (Eterradossi et al. 1995, Mekkes and Wit 1998).

On the other hand, however, Jones et al. (1992) compared changes in non-vaccinated birds following administration of cyclophosphamide and in those without chemical immunosuppression and found the course of the disease to be much more severe after experimental infection in birds deprived of humoral immunity, which indicates that immunoglobulins can reduce the effects of infection with a virulent virus, although they themselves cannot provide the resistance against the aMPV infection.

The main function in the humoral immunity of the macroorganisms mucosal membranes is performed by the secretive immunoglobulin A (sIgA), which covers the mucosal membrane surface, causing what is called "immune exclusion" by inhibition of the absorption of soluble antigens as well as by blocking adhesion sites and microorganism invasion into the epithelium (Snoeck et al. 2006). Little is known about the role of sIgA in the local immunity of the upper airways against the TRT virus. Cha et al. (2007) reported about an increasing subpopulation of the B cells with the IgA^+ isotype in the upper respiratory mucosa in turkeys on day 7 after exposing them to the TRT virus, with a concurrent increase in the IgA secretion in the nasal discharge. The results may indicate a certain contribution of sIgA to immunity of the respiratory tract to the TRT. However, as described above, anti-aMPV antibodies do not play a major role in the resistance to infection with virulent strains of the TRT virus. In the light of the current knowledge, it may seem that specific antibodies (including sIgA) have the main aim in reducing the replicating ability of the virus in the upper respiratory tract, which relieves the clinical signs of the TRT and speeds the birds' recovery.

Considering the above, it is the T cells that are increasingly often regarded as the main factor involved in the upper respiratory immunity against the TRT. However, there have been just a few reports on the role of the T cells in the local immunity processes in the infection with aMPV in turkeys. Additionally, studies of the T-cell-associated immunity against the TRT have given ambiguous results. Liman and Rautenschlein (2007) observed a considerable increase in the percentage of the $CD4^+$ subpopulation of T cells in the Harderian gland, with concurrent increase in the (interferon gamma) expression in turkeys, both those vaccinated and otherwise, on days 7-14 after experimental infection, whereas the percentage of the $CD8^+$ subpopulation did not change. The researchers have pointed out to a brief increase in the percentage of the $CD4^+$ T cells as the main cause of a short period of the post-vaccination protection against infection with the virulent TRT viruse and to a low contribution of humoral immunity to resistance against the aMPV (Liman and Rautenschlein 2007). These findings may indicate that T cells (mainly $CD4^+$) contribute to the development of the immunity against the TRT virus. Additionally it turns out that chemical suppression of the T cells by intravenous administration of cyclosporin A negatively affects the course of the TRT in turkeys, mainly during recovery. It has been shown that clinical signs in turkeys after inoculation with the virulent aMPV are much more severe and persist for a longer period after cyclosporine A induced immunosuppression. At the same time, the histopathological changes observed in the tracheal sections (e.g. heterophil infiltrations, loss of cilia and exfoliation of epithelial cells) were observed for a longer time after experimental infection as compared to the control group (Rubbenstroth et al. 2010). The results show the considerable contribution of the T cells to the immunity against aMPV and that they are involved in the processes of cleaning and regeneration in the upper airways in turkeys in the course of infection with the TRT *Metapneumovirus*. Moreover, it turns out that both Th1 and Th2 subpopulations of the T helper cells are involved in post-vaccination immune processes in the upper respiratory tract, which has been shown based on an increase in the expression of genes which encoded (known as antivirus cytokine, produced, for example, by the Th1 cells) and interleukine 10 (which plays a regulatory role in intensifying inflammation processes and tissues damage, caused by viruses, produced, for example, by the Th2 cells) following turkey vaccination against the TRT (Cha et al. 2011).

On the other hand, the study conducted by Rubbenstroth and Rautenschlein (2010) has shown that chemical suppression of the T cells does not negatively affect the development of post-vaccination immunity. After being vaccinated, birds showed full resistance against experimental infection, which calls into question the correlation between the T cells and resistance to the TRT infection. The researchers have shown that the long-lasting replication of the vaccine virus takes place in the upper airways of the birds, which, they claim, triggered another defensive mechanism, probably linked to the innate immunity (Rubbenstroth and Rautenschlein 2010).

It can be claimed in conclusion that the problem of immunisation of turkeys against the TRT and consequent immunity of the birds against infection with

the field virus is a complex one and requires further studies. These issues are very important from a practical point of view because cases of overcoming post-vaccine immunity in turkeys immunised against the aMPV are frequent and they result in huge economic losses to the breeders.

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