Respiratory diseases of turkeys – part one

by Hafez Mohamed Hafez, Institute of Poultry Diseases, Free University Berlin, Königsweg, 14163 Berlin, Germany.

Respiratory conditions are one of the most serious groups of diseases affecting turkeys and are continuing to cause heavy economic losses by increased mortality rates, increased medication costs, increased condemnation rates, drops in egg production, reduction of egg shell quality, and decreased hatchability.

Several pathogens are incriminated as the possible cause of respiratory diseases either alone (mono-causal) or in synergy with different other micro-organisms (multi-causal) or accompanied by non-infectious factors such as climatic conditions and management related problems (Table 1).

The severity of clinical signs, duration of the disease and mortality are extremely variable and are influenced by kind, virulence and the pathogenicity of the infectious agent as well as by many environmental factors such as poor management, inadequate ventilation, high stocking density, poor litter conditions, poor hygiene, high ammonia level, concurrent diseases and the type of secondary infection.

The diagnosis of respiratory disease is usually not a straightforward business. Beside the multifactorial nature of many infectious diseases there are also a lot of ill defined problems. Basically, the diagnosis consists of case history as well as management and environmental investigation. In addition, clinical investigations and post-mortem examination done on the farm is an important step toward disease diagnosis. However, clinical signs and necropsies are not the final step of the diagnosis. The final diagnosis can be reached by laboratory diagnosis.

The aim of this article is to explore current diseases and the type of secondary infection. The virus belongs to the family paramyxoviridae and genus metapneumovirus. Studies using APV virus isolates from different laboratories and countries have revealed close morphological, physio-chemical properties and antigenic relationships. However, results using cross neutralisation test indicate that the relationship between the BUT I 8544 and the German STG strains are closer than to a VCO3 strain isolated from turkeys in France.

Also, testing of serum samples originating from different countries in ELISA tests using different TRT isolates revealed that some serum samples obtained from France reacted only positive with a homologous antigen. On the other hand, testing serum originating from England and Germany reacted positive with both homologous and heterologous antigen. Juhasz and Easton (1994) divided the isolates into two subtypes A and B, on the base on sequences of the surface glycoprotein, G of five viruses. The UK isolates were judged to the type A and other isolates from Europe to the type B. Naylor el al. (1997) confirmed that outbreaks of respiratory diseases in turkeys in the UK during 1994-1995 were still associated with APV and that the virus was subtype B. In Germany both types A and B could be detected in both turkey and chicken isolates from 1987-1991. Also in Belgium both types were identified in farms with respiratory problems. In the USA the isolated AP viruses belonged to another subtype and designated as subtype C.

Recently non-A or non-B subtype APV was isolated in France from turkey flocks and the isolates seem to differ from subtype C also. The virus appears to be highly sensitive to different chemical disinfectants. Preparations of Lysoet-P A (disinfectant based on aldehyde, phenol and alcohol), VENNO-VET-1 (disinfectant based on different organic acids) and H2O2 were able to inactivate the virus at concentrations of 0.3% within 15 minutes.

The survival of avian pneumovirus (APV) in experimentally contaminated autoclaved and non-autoclaved turkey litter was studied at different temperatures (room temperature, 8°C, and -12°C). The results revealed the presence of APV RNA even after 90 days in the autoclaved litter samples kept at -12°C and at 8°C. The virus was isolated from the autoclaved litter kept at -12°C up to 60 days. From the non-autoclaved litter, viral RNA was detected up to 60 days and virus was isolated up to 14 days. The disease is spread by direct and indirect contact. Egg transmission is suspected.

Clinical signs are sneezing, nasal discharge, conjunctivitis, tracheal rales, sinusitis and sub-maxillary oedema. These signs have been seen in birds as young as 14 days old, but are more commonly observed in birds between 3-9 weeks of age. The morbidity approaches 100% within 24-48 hours.

The mortality rate is extremely variable from negligible to over 40%. In susceptible turkey breeder flocks, a drop in egg production that approaches 50%, or more for 2-4 weeks mostly accompanies clinical signs.

Their recovery time are characterised by an increased number of thin and white shelled eggs as well as...
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in the number of unsettable eggs. This effect may be greater if infection occurs early in lay. The gross lesions include rhinitis, tracheitis and sinusitis. In many cases pericarditis airsacculitis with congestion of the lungs and fibrinous exudate in the pleural cavity have been observed.

Several reports showed synergism airsacculitis with congenital infections, especially E. coli, become dominant. Virus multiplication in tracheal organ cultures from chicken and turkey embryos results in a ciliostatic effect after several passages. The virus propagates in chicken embryos after yolk sac inoculation. Several blind passages are necessary to induce embryo mortality. The virus can also be isolated in different cell lines, such as monkey kidney cell line (VERO) or chicken embryo rough cell line (CER).

After several passages, a cytopathic effect with syncygium formation could be detected 5-6 days post infection. After initial isolation, the virus can be adapted to grow in chicken embryo fibroblasts, chicken kidney cells, BS-C-1 and QT-35-cell line.

Different serological tests have been used with the aim of detecting antibodies for diagnostic purposes. They include a serum neutralisation test (SN), indirect immunofluorescence (IIF), and the ELISA. However, the ELISA test is widely used since it has been developed in the tears and IgG in the tears and serum. Both primings gave complete protection against VS challenge three weeks later.

Protection appeared to be related to virus neutralising antibodies in the tears. This suggests that cell mediated immunity (CMI), either instead of, or as well as antibody responses, may be important in immunity to APV infections.

In contrast, chicks of the same age were only protected against VS challenge by VS priming. Priming with AS did not elicit virus specific antibodies.
in the tears or serum, nor virus neutralising antibodies. Results indicate that chickens and turkeys respond to different degrees to avian pneumoviruses. Cook et al. (1995) showed that pouls vaccinated with a type A avian strain had reduced clinical signs after challenge with a type B TRT-Virus. Eterradossi et al. (1995) have also reported that pouls that had been vaccinated with a type B vaginal strain did not show any respiratory signs when challenged with virulent type A or B viruses. In addition, Naylor et al. (1997) showed in single experiment in pouls inoculated with virulent strains of type A or B induced cross protection, although that protection was incomplete.

On the other hand, experimental studies have shown that TRT vaccines developed using either a subgroup A or B strain of APV are highly effective in controlling infections caused by the Colorado isolate. Because maternally derived antibodies do not interfere with vaccine take, there is no concern over the efficacy of TRT vaccines following early application.

Management has always been seen as a major factor in the effective control of APV infections and it seems likely that different management systems and probably different stocking densities in different areas all contribute to the different ways in which the live attenuated vaccines are used. However, when these vaccines are used correctly, their efficacy in controlling APV infection could be demonstrated.

Effective inactivated vaccines are available to protect laying and breeding turkeys against the effects of APV challenge on egg production. These vaccines can be monovalent ones or the TRT antigen may be combined with other antigens to provide multivalent vaccines. For optimum protection it is clear that a combination of live priming followed by injection of inactivated vaccine is required. However, in countries where live attenuated TRT vaccines are not licensed, benefit, in terms of protection against drops in egg production, is seen from the use of the inactivated vaccine alone.

Further efforts to develop a new generation of vaccines are in progress and showing different results.

### O. rhinotracheale (ORT)

Since December 1991 respiratory manifestations with different clinical courses have been observed in poultry flocks in different countries. Bacteriological examinations have resulted in isolation of slowly growing, pleomorphic Gram negative rods (PGNR). Initially, the bacterium was designated Puretella-like, Kingella-like, Taxon 28 or pleomorphic Gram negative rod (PGNR) before the name Ornithobacterium rhino-tracheale gen. nov. sp. nov. in the rRNA-superfamily was suggested. The infection has been recognised in many countries worldwide and incriminated as a possible additional causative agent in respiratory disease. Up to now, ORT has been isolated from chicken, chukar partridge, duck, goose, guinea fowl, gull, ostrich, partridge, pheasant, pigeon, quail, rook and turkey. It is an acute, highly contagious disease of chickens and turkeys.

The disease is spread horizontally by direct and indirect contact. Vertical transmission is suspected, since some recent research has isolated ORT at very low incidence from reproductive organs and hatching eggs, infective eggs and dead embryos.

Currently 18 serotypes designated A to R seem to exist. Neither the origin nor the serotype of the O. rhinotracheal strains have an effect on the pathogenicity. Most of the chicken isolates belong to the serotype A.

The turkey isolates are more heterogeneous and belong to serotype A, B and D. There are many reports showing synergism between ORT and Newcastle disease, turkey rhinotracheitis, infectious bronchitis, Bordetella avium, Escherichia coli as well as Chlamyd-ophila psittaci.

Diagnosis of ORT on the basis of clinical features and pathological lesions is often difficult since they may be confused with other infectious conditions. Proof of infection, therefore, must be confirmed by isolation and identification of the causative agent. Further possibilities for the detection of ORT are immuno-histochemical staining as well as polymerase chain reaction. Serological examinations for detection of antibodies can be carried out using slide agglutination test, DOT Immunobinding assay or ELISA tests. The serotype specificity of the ELISA depends on the method of antigen extraction used for coating the ELISA plates.

Examination of serum samples collected from commercial flocks in all three systems showed similar results on flock bases using these ELISA tests with only some minor variations on sample bases.

Because titres decline rapidly after peaking, serum samples for flock screening should be taken frequently. The advantage of the serological tests over bacteriological examination is that antibodies persist for several weeks after infection and the bacterial shedding is short.

However, ORT excretion and antibody response may also be affected by a number of factors such as antibiotic therapy and vaccination. The influence of antibiotic therapy on the serological response to ORT remains unclear.

Popp and Hafez (2002) carried out investigations to determine the effect of drug therapy using amoxicillin on the antibody kinetics after experimental infection. The results showed that immediate treatment did not influence the antibody response. While the treatment starting at seventh day post infection resulted in lower antibody response compared to infected control.

### Treatment and control

The treatment of ORT infections is very difficult because different strains have variable susceptibilities to antibiotics. ORT acquires resistance against antibiotics easily.

The sensitivity pattern depends on the source of the strain and the routinely used drugs in an area. It should be emphasised that for successful treatment an investigation of the sensitivity pattern of the isolated strain is necessary.

In Germany and the Netherlands most O. rhinotracheales isolates are resistant to the enrofloxacine antibiotic, whereas in France, Belgium and Israel most isolates are sensitive.

In Canada pure ORT could be isolated from enrofloxacine treated birds. Van Veen (2003) tested strains originating from field cases of diseased broiler flocks from the Netherlands that were isolated in the period 1996-1999. The sensitivity of ORT strains was significantly decreased over the years.


Resistance against sulfachloropyridazine decreased from 1996 to 2002, but an increase in resistance was seen against gentamicin, ampicillin, trimethoprim sulfa, and tetracycline. The resistance against penicillin remained constant from year to year.

Soriano et al. (2003) determined the minimal inhibitory concentrations of 10 antimicrobial drugs for Mexican isolates and found a marked resistance trend.

The susceptibility of ORT to amoxicillin, enrofloxacine and oxytetracycline was variable. However, consistent higher minimal inhibitory concentrations values were obtained for gentamicin, fosfomyacin, trimethoprim, sulfamethazine, sulfameterazine, sulfadiazine, sulfanilaxe and sulfachloropyridazine.

Under field conditions water medication using amoxicillin at a dose level of 250 ppm for 3-7 days gives satisfactory results. ORT is highly sensitive to chemical disinfectants. However, ORT is endemic and can affect every restocking even in previously cleaned and disininfected houses especially in areas with intensive poultry production as well as in multiple age flocks. Failure to clean and disinfect properly after an infected flock has left, can cause infection of the neighbour flocks and the causative agent continuously cycling from house to house.

Several attempts to combat the infection using vaccines were carried out with different results. In the field, vaccinations with autogenous inactivated oil-adjuvant vaccines were proven to be successful in reducing the outbreaks of ORT.

Field trials using monovalent or trivalent bacteria in meat turkey flock resulted in induction of antibodies for short duration.

The mortality rates as well as the condemnation rates were, however, higher in the unvaccinated group compared to the vaccinated groups. Live vaccination is also feasible, but up to now no non-virulent strains of ORT have been found.

A temperature sensitive mutant of ORT has some protective properties, but more tests are needed to evaluate the efficacy and safety of this strain.