Respiratory diseases of turkeys – part one

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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 some currently important diseases, which are incriminated in respiratory diseases in turkeys, their diagnosis and control approaches.

Ocular and nasal discharge caused by turkey rhinotracheitis (TRT).

Avian pneumovirus (APV)

APV also known as turkey rhinotracheitis (TRT) was first described in South Africa in the late 1970s. Some years later, TRT was reported in Europe, Israel, South America, Middle and Far East as well as USA.

Table 1. Some possible causes of respiratory disease of turkeys.

NON-INFECTIOUS	INFECTIOUS
Management	**Viral agents**
Litter quality	TRT, ND, Influenza A, PMV3, Pox
Stocking density	
Ventilation rate	**Bacterial agents**
Temperature	ORT, P. multocida, mycoplasma,
High ammonia level	C. psittaci, E. coli, Bordetella avium
High dust concentration	
Feed	**Mycotic agents**
High dust content	Aspergillus fumigatus
Vitamin A deficiency	**Parasites**
	Syngamus, Cryptosporidium

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 1 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 and at higher rates with the 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 et 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 Lysovet-PA (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 0.5% 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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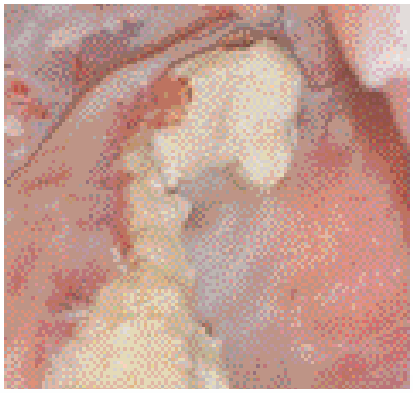

Airsacculitis.

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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 between TRT and Bordetella avium, Mycoplasma gallisepticum as well as E. coli co-infections. Serological surveillance for antibodies to other avian pathogens indicated that in turkey flocks after natural exposure to TRT infection there was a significant increase in TRT antibody levels which was usually accompanied with an increase in the number of positive sera to avian adenovirus and increase in antibody level against Ornithobacterium rhinotracheale and to Chlamydia psittaci. Diagnosis of pneumovirus 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 laboratory methods. Immunofluorescence and immunperoxidase tests can be applied to detect viral antigen in tissue sections or cell cultures and currently PCR are widely used in many laboratories for detection and typing.

Recommended tissues for virus isolation are sinuses and nasal exudate, larynx, trachea and lungs from birds in the early stage of the disease. It is important to collect the samples as early as possible after infection.

Isolation of the virus is less successful from birds showing severe signs, since secondary bacterial 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 syncyium 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 many laboratories and is available commercially as kits.

This method has a low cost advantage and provides more rapid results in comparison to the neutralisation test.

Prophylaxis and control

Drug therapy directed to secondary bacterial infections using different antibiotics revealed different results.

However, therapy alone is of little value, unless it is accompanied with improvements in all aspects of management; especially the ventilation, stocking density, litter condition and general hygiene.

Vaccination

Several live TRT and inactivated vaccines based on subtype A or B are currently licensed in many countries worldwide. In general, application of the live vaccines in turkeys appears to give good protection with low antibody responses.

The vaccines are able to protect the birds against clinical signs and sharply reduce the economic losses. The level of antibodies is poorly correlated with protection.

According to Khehra and Jones (1999) following priming with virulent strain (VS) or attenuated strain (AS) preparations of avian pneumovirus, two-week old turkey poults elicited virus specific IgA 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.

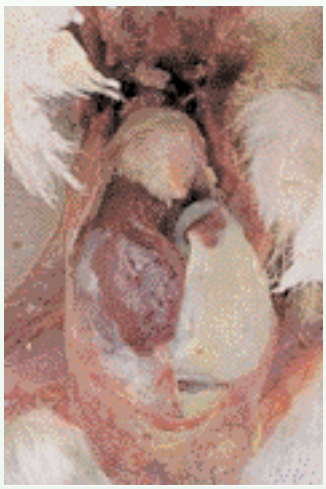
In contrast, chicks of the same age were only protected against VS challenge by VS priming. Priming with AS did not elicit virus specific antibodies

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 poults vaccinated with a type A vaccinal strain had reduced clinical signs after challenge with a type B TRT-Virus.

Eterradossi et al. (1995) have also reported that poults that had been vaccinated with a type B vaccinal 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 poults 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



Pericarditis plus perihepatitis.

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 as Pasteurella-like, Kingella-like, Taxon 28 or pleomorphic Gram negative rod (PGNR) before the name Ornithobacterium rhinotracheale 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, infertile eggs and dead embryos.

Currently 18 serotypes designated A to R seem to exist. Neither the origin nor the serotype of the O. rhinotracheale 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.

Purulent pneumonia – O. rhinotracheale.

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. rhinotracheale isolates are resistant to the enrofloxacin antibiotic, whereas in France, Belgium and Israel most isolates are sensitive.

In Canada pure ORT could be isolated from enrofloxacin 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.

Malik et al. (2003) examined in vitro antibiotic resistance profiles of 125 isolates of ORT strains isolated from turkeys in Minnesota during 1996-2002. A majority of isolates was sensitive to clindamycin, erythromycin, spectinomycin, and ampicillin.

Resistance against sulfachloropyridiazine 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, enrofloxacin and oxytetracycline was variable. However, consistent higher minimal inhibitory concentrations values were obtained for gentamicin, fosfomycin, trimethoprim, sulfamethazine, sulfamerazine, sulfaquinoxaline and sulfachloropyridazine.

Under field conditions water medication using amoxicillin at a dose level of 250ppm 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 disinfected houses especially in areas with intensive poultry production as well as in multiple age farms.

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 bacterins 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. ■