Presented at : FACTA Symposium, Campinas, Brazil, April 1998
FACTA: Fundação APINCO de Ciência e Tecnologia Avícolas
Click Here to see the original version in Portuguese

Diagnosis, Management and Control of Avian Pneumovirus infection in broiler parent chickens.

Paul McMullin
POULTRY HEALTH SERVICES
Poultry Health Centre, Main Site Lane, Dalton,Thirsk, North Yorkshire, YO7 3JA U.K.
E.Mail PaulMcMullin@Poultry-Health.com

Summary:

Avian Pneumovirus is increasingly recognised as an important pathogen in many poultry-producing countries. This paper presents data collected during detailed investigation of 2 typical cases of a "Swollen Head Syndrome" in broiler parent chickens. One flock was 25 weeks old at onset and the other was 46 weeks old. Both cases initially presented as quite mild respiratory disease but rapidly progressed to severe neurological signs as a result of purulent inflammation of the skull bone sinuses. Intensive antibiotic therapy (both orally and by injection) seemed to be beneficial in limiting the losses. Only one of the two commercially available Elisa tests was capable of reliably detecting the response of these birds to Avian Pneumovirus virus. The implication of this experience for dealing with outbreaks and for the prevention of the disease are discussed.

Introduction:

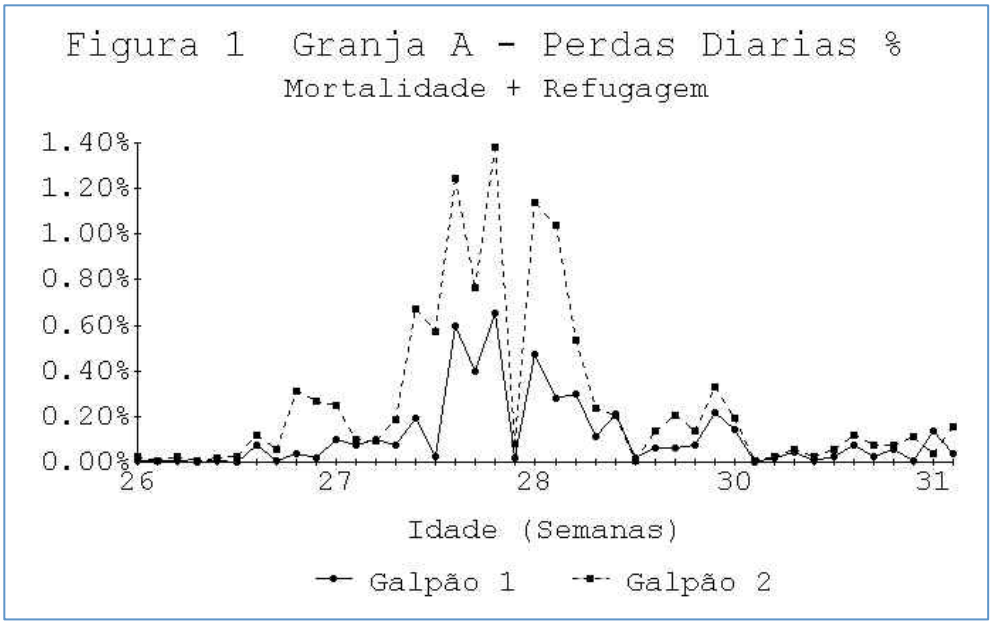
A new respiratory of poultry seems to have occurred first in turkeys (Buys, S.B. and du Preez,1980) and later in chickens (Morley and Thomson,1984) in South Africa. This disease was subsequently named Turkey Rhinotracheitis or TRT and has been seen also in Israel, France and Great Britain (Alexander,1993). In some other countries such as the USA, Canada and Germany *Bordetella avium* has been suspected to be the cause of similar condition in turkeys. The highly infectious nature of the disease prompted a search for a viral agent. The break-through came with the discovery that although the agent does not grow well in traditional embryo-inoculation tests, but that it could be demonstrated by its ciliostatic behaviour in tracheal ring organ cultures. This culminated in the description of a new viral agent (Wyeth et al, 1986). On electron microscopy samples of this agent were remarkably similar to those found associated with the disease in turkeys in South Africa (Buys & du Preez,1980). When the disease was first described in chickens in South Africa it had been postulated that it was caused by a mixed infection of a coronavirus and E.coli. (Morley and Thomson,1984). It is said to have existed in that country as long ago as 1971. They also reported that the "swollen heads" could be reproduced simply by scarifying the skin over the eye and inoculating a pure E.coli culture so it seems clear that not all cases of “Swollen

Head Syndrome" are caused by the same agent or agents. In 1985 TRT rapidly spread through the turkey industry in GB (Lister & Alexander, 1986) and at the same time a syndrome of broiler parent chickens characterised by respiratory signs and head swelling and neurological signs was described (O'Brien, 1985). The term Avian Rhinotracheitis (ART) has been applied to avian pneumovirus infection in turkeys and chickens. Serological evidence of ART is now available from many countries (Cook, 1997).In the past year an avian pneumovirus has been isolated from turkeys in the US (Cook & Senne, 1998).

Diagnosis:

Clinical Signs: The first abnormality noted in broiler parents is usually a rapidly-spreading mild respiratory disease characterised by nasal exudate, inflamed eyes, with facial congestion and slight swelling in a small proportion of birds. These signs spread to birds in all air-spaces in each site over a period of 5-10 days. Flocks coming into lay undergo a check in the rise in egg production, the older flocks often suffered a drop in production. A couple of days after respiratory signs are first noted birds affected with neurological signs may begin to appear. In the early stages these birds sit for increasingly prolonged periods with eyes closed and neck retracted. More advanced cases show variety of "head-rolling" behaviours.

Mortality and Culling Records: In unvaccinated flocks culling and mortality peaks at 1-5% per week and falls off after about 2 weeks. Figure 1 below shows an example of the pattern of mortality found in one outbreak of TRT. Losses are expressed as a percentage of the birds placed. The dip on 1 day in the middle of the outbreak is simply a Sunday on which no culling was carried out.



Post-mortem Examinations: Gross Pathology: A summary of the findings for each group of birds examined in 2 outbreaks of the disease is presented in Table 1. During the early stages of the disease there are remarkably few internal lesions other than those in the head. The tracheas generally appeared normal. The early lesions of the head consist of sinusitis of the infra-orbital sinuses, rhinitis associated with a fairly clear nasal discharge and sub-cutaneous oedema around the eyes or over the top of the skull. Areas of pus in the sub-cutis are much more rare than in the same disease in broilers. When examining birds which have been culled some time previously by neck dislocation it can be very difficult to distinguish sub-cutaneous oedema from serum originating in the large blood clot in the neck. On cutting open the skull it is possible to identify areas of pus in areas of bone which should have a trabecular structure with air spaces. In the cases reported here the lesions were extensive resulting in fluid accumulation in the bone and much pus formation. These lesions were present in some birds very early on in an outbreak. While it was not possible to study the evolution of these lesions the impression was that they started in the bones on the floor of the cranium and tended to spread up around the ear canals on each side of the brain. One bird had a marked hump associated with osteitis in the frontal area. As the outbreak proceeds the sub-cutaneous swelling is less evident, the nasal exudate resolves into a layer of pus coating the internal surfaces of nasal passages (Fig. 4), and pus dominates the skull lesions which tend to be more dry in appearance. Wasting and dehydration may also be more evident (see Farm A, 27 weeks), and these severely affected birds will have ovarian regression and are out-of-lay.

TABLE 1 - A summary of post-mortem findings.

Farm	House	Age (wks)	Date	No.	Wt.(Kg)	SHS	SOS	SIN	NOD	OVR	DEH
A	C	25	28/3	5	1.85		2	1	2		

A	D	25	28/3	5	2.18	5	5	3				
A	C/D	27	15/4	9	1.43		9			9	5	
B	1/2	47	19.5	6	N.Avail.	6	6					

Diagnosis Codes
DEH = Dehydration
NOD = Not Diagnosed
OVR = Ovary in regression
SHS = Swollen Head Syndrome - Facial Cellulitis
SOS = Skull osteitis
SIN = Sinusitis

Histopathological Findings: Inflammatory cell debris associated with plugs of bacteria and sloughing of the surface epithelium is seen on the nasal turbinates. Extensive, mainly acute, inflammation may be found in the cancerous bone of the skull. Some lesions are purulent and contain clumps of gram-negative bacteria. Within the air-spaces in the skull the exudate may show some of the signs of a chronic inflammatory process.

Bacteriology: Samples were taken from affected bones of the birds submitted from Farm A. Samples were also taken from the nasal cavities of the 27 week submission. After removing skin and searing the surface the bones were aseptically opened with a large scalpel blade. Swabs or loops of material were taken and streaked on to Blood Agar and McConkey Agar. In most cases the sensitivity test was carried out by inoculating the complete surface of a Blood Agar plate using the swab before adding anti-bacterial impregnated discs. Where mixed cultures occurred then the sensitivity test was repeated on isolated colonies. *Escherichia coli* normally grows abundantly from the material taken from the purulent lesions in skull bone, usually in pure culture. *Staphylococcus albus* and *Proteus* sp. may also be present in samples taken from the nasal cavities.

Serology: Elisa tests for this infection were developed in a number of laboratories (Jones, et al., 1988, Cook et al. 1988, Chettle and Wyeth, 1988, Baxter-Jones,et al. 1989). Some of these tests were validated by comparison of results with those obtained in serum-neutralisation tests. With experimental infections of turkeys ELISA and serum neutralisation both reached high titres by 12 days post infection and titres remained high (log2 12-15) throughout the 89 days of observation .(Jones, et al., 1988). Antibodies to turkey rhinotracheitis virus have also been demonstrated in serum from commercially reared flocks of chickens (Cook et al. 1988, Wyeth, P.J. et al, 1987), though no particular association with disease was noted in one of these surveys. This suggested that chickens could become infected with a TRT-like agent without suffering serious disease. Subsequent use of the Elisa technique has, however illustrated some discrepancies as compared to indirect immunoflourescence (O'Loan, et al, 1990). Comparisons of the reactivity of Elisa tests based on antigens isolated in France and one test based on a GB isolate gave definite indications of antigenic variability (Eterradossi, N. et al, 1992). Generally speaking sera from GB reacted well with GB antigen, and poorly with French antigen, while sera from France showed the opposite effect. These authors considered that there was a possibility that these differences were due to different methods of antigen preparation. A commercially-available Elisa kit had been used in our

laboratory for over 2 years with apparently good results (Gerrard et al 1990). A range of serological tests have been carried out with a view to diagnosing the cause of these disease incidents. In addition to samples which are taken and stored routinely every 6 weeks, an attempt was made to provide extra information in each of the 2 incidents. At farm A 1 set of samples was taken from randomly selected birds at the beginning of the incident (26 weeks of age), and 2 weeks later a second sample was taken from birds which had been clinically affected and recovered (these were identifiable by the colour mark used to indicate antibiotic injected birds). At farm B one of the routine collections happened to be taken shortly after the beginning of the problem (47 weeks). One week later 20 affected birds were identified by numbered leg bands, blood sampled, injected with antibiotic and transferred to a "hospital" pen. Two weeks later (49 weeks of age) 19 of the identified birds had recovered and were re-sampled.

Despite regular monitoring using the rapid serum agglutination test neither of the flocks showed any reaction indicative of *Mycoplasma gallisepticum* or *M. synoviae* infection.

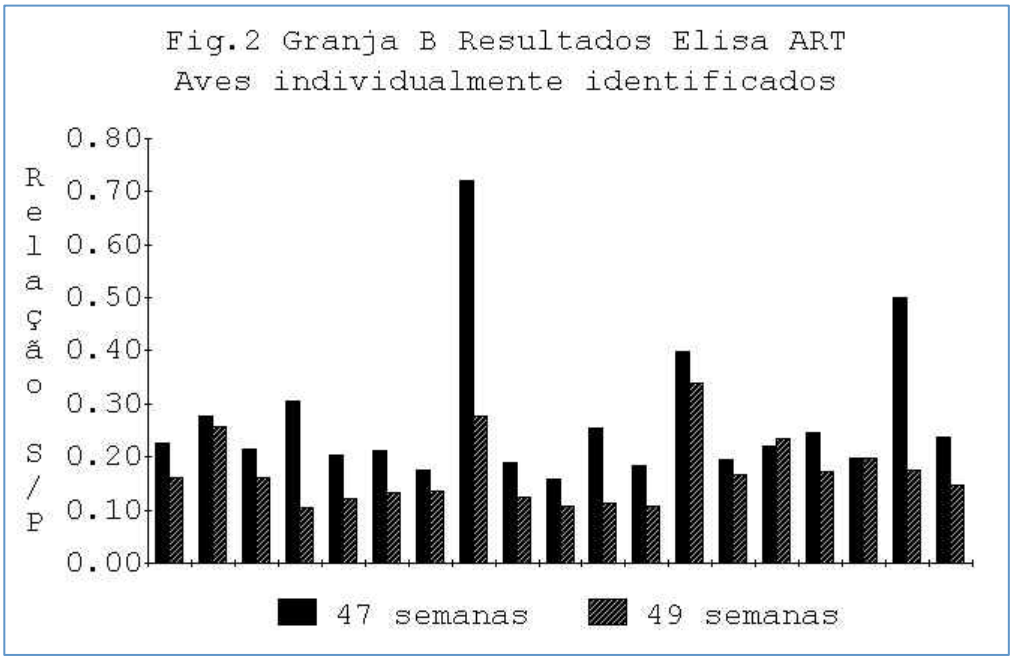
Since the clinical signs and pathology were strongly indicative of the aftermath of Avian Rhinotracheitis or Pneumovirus infection the serological tests for this infection are particularly interesting. Table 2 shows a summary of the results obtained with the available sera using our previously standard commercially-available Elisa test. As is normal practice in this laboratory the sera are grouped according to intensity of reaction, in the case of this test in bands of Serum-to-Positive ratios. This test kit has been very reliable in the sense that its internal controls worked well. The results obtained with these cases were far from convincing in favour of the hypothesis that ART was the cause of the problem. A large proportion of the samples tested were negative. The pattern of sero-conversion, where it occurred was also unexpected. In fact the convalescent samples tended to react less strongly than those taken earlier in the outbreak. This is best illustrated in Figure 2 which shows the S/P ratios of the individually identified birds from Farm B.

Shortly after these cases occurred a new Elisa test for Avian Rhinotracheitis became commercially available. This test is based is based on a competitive Elisa rather than the conventional method and the antigen used is derived from a chicken isolate (Svanova Biotech).

TABLE 2. Summary of serological results for Avian Rhinotracheitis (Pneumovirus)using kit A

Farm		Age				Titre Groups											
Code	House	(Wks)	Tstd.	Neg.	Susp.	2	3	4	5	6	7	8	9	10	11	12	Mean	St.Dev
A	1	23	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A	2	23	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A	1	26	20	18	0	0	1	0	0	0	1	0	0	0	0	0	0.5	1.6
A	2	26	20	17	2	1	0	0	0	0	0	0	0	0	0	0	0.2	0.5
A	1	28	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A	2	28	20	15	2	1	1	1	0	0	0	0	0	0	0	0	0.55	1.1
B	1	29	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0

B	2	29	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	3	29	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	1	34	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	2	34	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	3	34	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	1	47	15	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	2	47	15	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	3	47	15	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B		48	20	14	2	1	1	0	0	1	0	0	0	1	0	0	1.15	2.5
B		50	19	16	2	1	0	0	0	0	0	0	0	0	0	0	0.21	0.5



It was decided to submit a selection of samples to this new test. The results obtained are summarized in Table 3 below. Because of the different method used in this Elisa the titre groups are derived from bands according to Serum-to-Negative ratios. The samples tested using the new test were chosen with a view to demonstrating a rising titre from the acute to the convalescent stage of the disease process. At farm A all of the randomly collected samples tested at 26 weeks of age were already strongly positive, however the intensity of the reaction had increased when the convalescent birds were examined at 28 weeks of age. In other words these results are what we would expect if ART virus is a significant part of this problem. At Farm B the randomly-collected

samples from the beginning of the problem (47 weeks) already include many with a strong reaction. At that point the birds in house 3 were strongly positive, those in house 2 were moderately positive and those in house 1 were just beginning to sero-convert. Once again this matches the field observations with respect to the course of the disease across the site. The individually identified birds were already strongly positive at 47 weeks but showed an intensification of the reaction by 50 weeks.

TABLE 3.Summary of serological results for Avian Rhinotracheitis (Pneumovirus) using kit B

Farm		Age				Titre Groups											
Code	House	(Wks)	Tstd.	Neg.	Susp.	2	3	4	5	6	7	8	9	10	11	12	Mean	St.D.
A	1	26	8	0	0	0	0	1	1	3	0	2	1	0	0	0	6.5	1.6
A	2	26	8	0	0	0	1	1	0	2	2	0	2	0	0	0	6.38	2
A	1	28	8	0	0	0	0	0	0	0	1	1	3	2	1	0	9.13	1.2
A	2	28	8	0	0	0	0	0	0	0	0	1	1	5	1	0	9.75	0.8
B	1	47	7	3	4	0	0	0	0	0	0	0	0	0	0	0	0.57	0.5
B	2	47	7	1	2	0	2	0	1	0	0	1	0	0	0	0	3.0	2.6
B	3	47	7	0	0	0	0	0	0	1	2	1	3	0	0	0	7.86	1.1
B		48	20	0	0	0	0	0	0	0	1	4	5	9	1	0	9.25	1.0
B		50	19	0	0	0	0	0	0	0	0	0	3	9	7	0	10.2	0.7

On both farms the antibody response as measured by the new test closely matched what we would expect if ART virus was a cause of this problem. Possible reasons for the discrepancy between the 2 tests are discussed below.

The results obtained in these cases do seem to bear a striking similarity with those reported by Eterradossi and others (1992). Certainly it is clear that the sort of sero-conversion demonstrated in a number of cases in broiler parents in the mid-80's (Wyeth et al, 1987) was not demonstrable in these cases using the conventional test. We are obtaining similar discrepancies in results when examining sera from broiler flocks which appear to have undergone Avian Pneumovirus challenge. There is no suggestion that the lack of sensitivity of this test reported here is caused by any manufacturing problem. Both the control sera supplied with the kit and internal laboratory sera continued to react normally. It would appear that the virus currently involved in field challenge has a degree of change in antigen structure, the antibody to which was being detected by this kit. The sera were also tested for antibodies to Newcastle Disease virus. Titres were high before the incidents (as we would expect in oil-vaccinated birds) and showed no tendency to a rise in titre co-incident with the clinical problem. Further tests suggested that IB challenge may have been involved in the clinical problem at Farm A but not at Farm B. This may partly explain why the problem was so severe at Farm A and dragged on over a longer period.

Virology: Gough and Collins (1989) examined the antigenic relationships of three turkey rhinotracheitis viruses from different laboratories. They compared strains by electron microscopy, polypeptide analysis, double immunodiffusion and cross neutralisation tests. These strains were considered to be antigenically similar. These authors proposed that the TRT virus belongs to the Pneumovirus genus of the family paramyxoviridae. More recently a number of authors have demonstrated substantial antigenic variation, and serotypes A and B have been designated. Tracheas were collected from each of the birds examined during field post-mortem examination at farm B at 47 weeks of age. Examination of this pool of tracheas by polymerase chain reaction was positive for the presence of Avian Rhinotracheitis Virus. Preliminary analysis of the products of this examination suggests that this isolate is substantially different from the initial field isolates of ART virus made in the late 1980's (D. Cavanagh, unpublished) and has been designated "subgroup B". It has recently been suggested (Cook & Senne, 1998) that the pneumovirus recently isolated from turkeys in the US does not fall into either the A or B sub-group.

Differential Diagnosis: A wide range of pathogens can cause signs of respiratory disease in chickens - Newcastle disease (of varying degrees of pathogenicity), Avian Influenza, Infectious Bronchitis, Avian Pneumovirus or Rhinotracheitis Virus, Infectious Laryngotracheitis, Fowl Pox, *Haemophilus paragallinarum, Mycoplasma gallisepticum, M.synoviae*, and *Pasteurella multocida*. *E.coli* is frequently involved as a secondary infection and certain other bacteria such as *Bordetella avium* (previously *Alcaligenes faecalis*) and *Ornithobacterium rhinotracheale* have also been reported to exacerbate or cause respiratory disease. *M.g.* and *M.s.* may be excluded on the basis of the serological findings. Lack of a rising titre for other viruses in serological tests and negative results in appropriate virological tests and PCR's. Infectious laryngotracheitis, Fowl Pox, Fowl Cholera and Infectious Coryza may usually be excluded on the basis of the gross pathology.

Treatment and Advice: Antibiotic medication is recommended for outbreaks showing a bacterial component. This may be administered in feed or drinking water. Long-acting injectable formulations may be beneficial, especially if administered to birds showing early neurological signs. Further advice should include the culling of severely affected birds and special attention to the ventilation in order to maximise bird comfort. Based on the experience of these outbreaks the following course of action would seem to be optimal:

- 1. Confirm diagnosis, bacteriology on infected skull bones, sensitivity tests.
- 2. Review management with a view to eliminating any obvious problems, concentrating on achieving
- optimal ventilation both during the day and through the night.
- 3. Consider setting up a temporary hospital pen with easy access to feed and water.
- 4. Initiate soluble antibiotic medication in accordance with sensitivity.
- 5. Cull severely affected birds and inject moderately affected birds with a long-acting formulation with a similar mode of action to the soluble product in use.
- 6. Continue medication by the in-feed route for a further 2 weeks.

This approach will probably work equally well regardless of which predisposing viral infection is involved.

Control : Since these and similar cases our objective has been to implement a programme of immunisation capable of adequately protecting the birds from the effects of the primary viral pathogen. Both a number of different live and one inactivated ART vaccines have become available. Recent experience suggests that either 2 applications of live vaccine in rear (e.g. 10 and 19 weeks) or a live dose followed by inactivated vaccine given at the same time as the conventional "triple" vaccine, are capable of inducing a satisfactory immune response. No cases similar to these have yet been seen in flocks vaccinated to either of these protocols. Data generated using an artificial challenge model (J.Cook Pers.Comm) suggest that the live + inactivated programme is more effective in limiting the adverse effects of this virus on egg production. Consideration must be given to the range of challenge viruses occurring. Some companies use a B-type live priming vaccine and follow this with the A-type inactivated vaccine in an attempt to achieve immunity against the range of possible challenge viruses. Consideration should also be given to ways of improving the management of the flocks, especially with respect to ventilation. Lower average temperatures (within limits) are likely to be better than inadequate ventilation. If the weather is very dry and litter is dusty then misting with water may be beneficial. Bio-security measures should be reviewed even though the usual barriers could possibly be circumvented by air-borne transmission.

Conclusions: Infection with a strain of Avian Pneumovirus (TRT/ART virus) seems to be an important precipitating factor leading to the occurrence of "Swollen Head Syndrome" in broiler parent chickens. In the 2 outbreaks reported here only the females were affected. There was some reduction in egg production and in hatchability associated with the disease outbreaks, though shell quality did not seem to be markedly affected. The main cause of mortality and culling was severe purulent inflammation affecting the skull bones. Chronic inflammatory changes in the nasal passages suggests that this is the most likely route by which the E.coli infections reach the skull bones. There were marked differences in the abilities of 2 commercially-available Elisa tests to detect the serological response to ART virus in these cases.

REFERENCES

1. Alexander, D.J. Pneumoviruses (Turkey Rhinotracheitis and Swollen Head Syndrome of Chickens) in Virus Infections of Birds, J.B.McFerran and M.S.McNulty eds. Elsevier Science Publishers B.V. 1993.
2. Baxter-Jones, C., Grant, M., Jones, R.C. and Wilding, G.P. A comparison of three methods for detecting antibodies to turkey rhinotracheitis virus. Avian Pathology 18:91-98, 1989.
3. Bisgaard, M. and Mutters, R. A new facultatively anaerobic Gram-negative fermentative rod obtained from different pathological lesions in poultry and tentatively designated taxon 14. Avian Pathology 15:117-127, 1986.
4. Buys, S.B. and du Preez, J.H. A preliminary report of the isolation of a virus causing sinusitis in turkeys in South Africa. Turkeys June 36 & 46 (1980).
5. Buys, S.B., du Preez, J.H. and Els, H.J. Swollen Head Syndrome in Chickens. A preliminary report on the isolation of a possible aetiological agent. J. S. Afric. Vet. Assoc., 60:221-222 (1989)
6. Cook, J.K.A. Respiratory disease in chickends, including the significance of avian pneumovirus. Simposio Facta (1997).
7. Cook, J.K.A. and D.A. Senne Turkey Rhinotracheitis - relationship between different virus sub-

groups. 1st International Symposium on Turkey Diseases Berlin 19th-21st February (1998)
8. Chettle, N.J. and Wyeth, P.J. The use of an Elisa test to detect antibody to turkey rhinotracheitis. Br. Vet. J. 144:282-287 (1988)
9. Cook, J.K.A., Dolby, C.A., Southee, D.J. and Mockett, A.P.A. Demonstration of antibodies to turkey rhinotracheitis virus in serum from commercially reared flocks of chickens. Avian Pathology 17:403-410, (1988).
10. Eterradossi, N., Toquin, D., Guittet, M. and Bennejean, G. Discrepancies in turkey rhinotracheitis ELISA results using different antigens. Vet.Rec. 131:563-564, 1992.
11. Gerrard, C., Whitworth, A., Chettle, N. and Wyeth, P. Avian rhinotracheitis diagnostic kit [letter]. Vet.Rec. 126:342, 1990.
12. Gough, R.E. and Collins, M.S. Antigenic relationships of three turkey rhinotracheitis viruses. Avian Pathology 18:227-238, 1989.
13. Jones, R.C., Baxter-Jones, C., Savage, C.E., Kelly, and Wilding, G.P. Experimental infection of chickens with a ciliostatic agent isolated from turkeys with rhiontracheitis. Vet. Rec. 120:301-302 (1987)
14. Jones, R.C., Williams, R.A., Baxter-Jones, C., Savage, C.E. and Wilding, G.P. Experimental infection of laying turkeys with rhinotracheitis virus: distribution of virus in the tissues and serological response. Avian Pathology 17:841-850, 1988.
15. Jones, R.C., Naylor, C.J., Bradbury, J.M., Savage, C.E., Worthington, K. and Williams, R.A. Isolation of a turkey rhinotracheitis-like virus from broiler breeder chickens in England. Vet.Rec. 129:509-510, 1991.
16. Lister, S.A., and Alexander, D.J. Turkey Rhinotracheitsi - A review. Vet. Bull. 56:637-663, 1986).
17. Morley, A.J. and Thomson, D.K. Swollen-head syndrome in broiler chickens. Avian Diseases 28:238-243, 1984.
18. O'Brien J.D.P. Swollen Head Syndrome in broiler breeders. Vet.Rec. 117:619-620 1985.
19. O'Loan, C.J., Allan, G.M., McNair, J., Mackie,D.P., and McNulty, M.S., TRT virus serology: discrepancy between Elisa and indirect immunoflourescence. Avian Pathology 19:173-180 (1990)
20. Picault, J.P., Giraud, P., Drouin, P., Guittet, M., Bennejean, G., Lamande, I., Toquin, D., and Gueguen, C. Isolation of a TRT-like virus from chickens with swollen head syndrome. Vet.Rec. 121:135, 1987.
21. Wyeth, P.J., Gough, R.E., Chettle, N.J. and Eddy, R. Preliminary observations on a virus associated with turkey rhinotracheitis. Vet.Rec. 119:139, 1986
22. Wyeth, P.J., Chettle, N.J., Gough, R.E. and Collins, M.S., Antibodies to TRT in chickens with swollen head syndrome. Vet.Rec. 120:286-287, 1987