Etiology

‘Swollen head syndrome’ is another disease, which may be confused with infectious coryza. This syndrome is of unknown etiology. Broiler breeder flocks suffer from the disease with 2-3% drop in egg production. Some flocks (60%) show antibodies to turkey rhinotracheitis virus (Hafez H.M. and Lowren, U., 1990, *Vety Bulletin* 61: abstr 4120) *H. Av. paragallinarum* treated with potassium thiocyanate or hyaluronidase show haemagglutination of erythrocytes of chicken treated with formaldehyde (Yamaguchi, T., et al., 1989, *Avian Dis.*, 33: 511).

Host
The disease is mostly found in young adult chickens. In India, it is more common in areas of high altitude, like Bihar plateau, but may occur elsewhere especially in cold damp weather. It has been reported as an epidemic in Madhya Pradesh. Several species of birds may suffer from disease. In chicken birds of 14 weeks age or older suffer from disease. Incubation period is 1-3 days. Carrier birds exist and may start the disease (L.D. Swartz, 1997, UPA newsletter) particularly in intensive farming.
Epidemiology
Chronically infected or healthy carrier birds introduce the disease on a farm. It can also enter through clothing equipment and fomites. Spread of infection takes place by bird to bird contact, through aerosol coughed out into air or by contaminated water. Once the infection enters in a farm other clean batches are at a great risk of infection. The infected premises should be left vacant for 1 to 2 months after cleaning and disinfection.

Symptoms
The disease may occur in birds of any age but symptoms are prominent in adults. The acute form of the disease may pass off in about 10-12 days on an affected farm but the chronic form may exist for several weeks or months (4-12 weeks). There is foul smelling discharge from nostrils and eyes. Eyes are inflamed and may become closed. Wattles may be swollen. There may be difficulty in breathing. The face and wattles may be swollen. There may be sneezing and dyspnoea. Egg production may drop by 10-40 per cent but may be restored after 2 to 3 weeks. Mortality may be low but may be high in acute form, especially if complicated with other diseases like pox, CRD and infectious bronchitis. Mortality may be up to 50 per cent but usually it is around 20 per cent. Mortality varies according to virulence of the strain of organisms, but may go as high as 48 to 50%.

Pathogenesis
Hyaluronidase in the capsule is essential for virulence. On removal of hyaluronidase enzyme, virulence is lost. Lipopolysaccharide (LPS) is another toxic factor for chickens, which causes hydropericardium (Iritani, V. et al., 1981 Avian Dis.; 25: 29). The organisms resist phagocytosis by the presence of capsule. Ciliated epithelial cells are the main target cells for entry and colonisation.

Pathology
Face is swollen due to exudate in infraorbital sinuses (Fig. 1.1), which may become cheesy
later on. Conjunctivitis is severe. Nasal mucosa is also congested. Sometimes, there may be small pneumonic patches at the point of entry of bronchi into the lungs. The egg follicles may rupture due to oophoritis causing scattered yolk sticking to the air sacs and intestinal serosal surface. Mild epicarditis and tubulonephrosis may also occur.

**Histopathology**

In nasal passages, infraorbital sinuses and in trachea variable mucopurulent exudation are observed. There is almost complete deciliation and epithelial cells become swollen, vacuolated and dissociated with edematous separation from underlying propria. Marked mucous gland hyperplasia with mucous streaming is also evident. Fibrinopurulent cellulitis is observed in eyelids. Both dermis and subcutis are involved in inflammation but fibrin and heterophilic infiltration is greatest in subcutis. Later the exudate may convert into caseous mass surrounded by giant cells.

Air sacs show oedematous thickening, mesothelial hyperplasia, fibrin deposition and heterophilic infiltration in stroma. Sometimes fibrinopurulent exudate may accumulate on the surface of air sacs. Air sac lesions are seen in about 25% cases. (Droual, R. *et al.*, 1990, *Avian Dis.*, 34: 1009).

**Diagnosis**

Foetid odour of nasal exudate helps in diagnosis. For field diagnosis prepare smears from exudate in the eyes or nose and stain by Gram’s stain. On examination of smear, gram negative, bipolar filamentous or coccoid organisms may be found (Fig. 1.2).

For laboratory diagnosis, swabs of cotton or stelile bacteriological loop may be taken from infraorbital sinus and trachea, eyes, lungs and air sacs and sent immediately for cultural examination to the nearest laboratory. Blood agar plate may be inoculated at 37°C. For 24 to 48 hours under 5% CO2 or candle jar. Translucent colonies of 1 mm or less diameter develop. Haemagglutination test can be done with the serum of the recovered bird up to one year. Immunoperoxidase test is also useful (Nakamura, K. *et al.* 1995, *Vety Record*, 132: 557). Polymerase chain reaction (PCR) is confirmatory. For PCR the nasal exudate can be used directly.

For diagnosis, haemagglutination inhibition (HI) and dott-blotting tests are reliable. To produce suitable antigens for these tests chicken meat infusion (CMI) broth and agar are suitable. The dot-blotting test can be used to serotype large number of strains (Yamaguchi, T., 1990, *Avian Dis.*, 34: 52 and Yamaguchi, T. *et al.*, 1991, *Avian Dis.*, 35: 965).

One of the field tests is to inoculate the nasal secretions of a suspected sick bird into the nostrils of a susceptible, healthy bird. The disease should develop in the test bird in 24-48 hrs.
Treatment

Sulphachloropyridazine and trimethoprim (1:5) is a combination of choice. It is easily soluble in drinking water (Cosumix Plus, a Novartis product). Sulphadiazine and Trimethoprim, Sulphathiazole and Trimethoprim, Sulphadimidine, Ampicillin, Tiamulin, Mycomulin (Concept), Diamatilin (Novartis) Erythromycin, Gentamicin, Enrofloxacin, Pefloxacin, Flumeqin are other effective drugs for mass treatment of poultry. Amoxycillin can also be given in drinking water as ‘pulse dosing’ for 3-5 days. Against the gram-negative organisms, sulphachloropyridazine is reported to be 3 times more potent than sulphadiazine and sulphamethazole. Sulphachloropyridazine is also effective against gram-positive organisms.

For prevention of entry of infection specially in threatened outbreaks, any of the several water sanitizers given in separate chapters can be given in drinking water. According to Takahashi et al. (1990, J. Japan Vet. Med. Assoc., 43: 187) *A. paragallinarum* show highest susceptibility to ofloxacin.

Prevention

All in and all out programme is better for preventing the disease. Mixing birds of different ages or species helps the disease onset. Other preventive measures are:

1. If the disease affects a layer flock, it is better to start with fresh stock since carrier birds (symptomless) do exist even after recovery.
2. The causal bacteria are fragile. They are killed in 2 min at 55 °C, in 6 hrs. at room temperature and few days in winter temperature.
3. Cleanliness and good management can prevent the disease. The cleaned and disinfected premises may be left vacant for 30-60 days before reuse or used 1 week after disinfection.
4. Keep the density of the birds to minimum.
5. Vaccines are available in India. Some people have used killed haemophilus, isolated from the flock, as vaccine by mixing with adjuvant and injecting intramuscularly. A vaccine (killed) prepared by Ventri Biologicals can be given at 8 weeks of age for prophylaxis (see under chapter on vaccines). Trivalent vaccine in oil adjuvant is the best vaccine. It is given at the age of 10-17 weeks. Immunogenicity of serovar B is different from that of A and C. (Yamaguchi, T. et al., 1991, Avian Dis., 35: 965).

A new tetravalent vaccine against types A, B, C and variant type B was used as oil adjuvant vaccine at 8 and 16 weeks age which gave good protection in Netherland (Jacobs, A.A.C. et al., 2003, Avian Path., 32: 205). Two vaccinations, subcutaneously and repeated after 1 to 1 ½ months are adequate.

Key Points for Diagnosis

1. Sudden drop in egg production.
2. Nasal and ocular discharge.
3. Swelling of face and sinus just in front of the eyes.

2. **ORNITHOBACTERIUM RHINOTRACHEALE INFECTIONS**

*Ornithobacterium rhinotracheale* is a bacterium which produces mild disease such as sneezing, poor growth and even pneumonia, air sacculus and heavy mortality. At times sudden deaths in young birds due to encephalitis and meningitis. The disease is worldwide. Sometimes
lameness is seen in turkeys and chickens. (Van Empel P. and Hafez, H. 1999, Avian Pathol, 28: 217-227). Hosts—Chickens, turkeys, pigeons, ducks, geese, guinea, foul, quails etc. Chickens and turkeys up to 8 weeks of age are more susceptible. The disease may affect more than one bird species. Eight weeks age is not the cut off age since older birds may also be affected.

**Cause**

*Ornithobacterium rhinotracheale* is a Gram negative, rod shaped or pleomorphic, 0.6 to 5 μm long bacterium. It is slow growing, cultivable on 5-10% sheep blood agar under 5-10% CO₂ for 48 hours or more. It is better to add 5 μg/ml of gentamicin and polymixin because most of the *O. rhinotracheale* strains are resistant to these antibiotics. At the same time these antibiotics may inhibit fast growing contaminants such as *Escheria coli* etc. Colonies are 1-5 mm in diameter and grey to grey white in colour. To prevent the contaminants particularly *E. coli* to overgrow and making isolation difficult. 5 μg of gentamicin and polymixin per ml of sheep blood agar is added before incubation. The organisms can also grow on MacConkeys agar. There are 18 serotypes out of which serotype A is most important in chickens.

**Symptoms and Lesions**

There are slight respiratory symptoms of sneezing poor growth and mortality. At postmortem, lesions of air sacculitis and pneumonia are observed. Infection sometimes may be asymptomatic, causing mortality (sudden) due to encephalitis and meningitis. There may be drop in egg production and their quality. Wild birds may be reservoirs of *O. rhinotracheale*.

Some respiratory viruses and *chlamydophilia* and *Bordetella avium* bacteria may also help *O. rhinotracheale* infection as a secondary infection. Stress, poor ventilation may also precipitate infection. Role of mycotoxins is also questionable.

Mortality may go up to 20% in few days time.

Lesions particularly in broilers include air sacculitis with yoghert like, white exudate along with lots of fibrin, foamy exudate mainly in the abdominal air sacs. Lungs show pneumonic patches. Sometimes arthritis, osteomyelitis is seen in cases of lameness. Pneumonia and mortality is higher in turkeys.

### 3. GALLIBACTERIUM INFECTIONS

*Gallibacterium anatis*, a bacteria, may cause sudden mortality and septicaemia or purulent (pus forming) salpingitis, oophoritis and even peritonitis. *G. anatis* has too biovars which are:

(a) **G. anatis haemolytica**: It produces β haemolysis of 1-2 mm diameter on blood agar.

(b) **G. anatis biovar anatis**: This biovar produces mild disease (respiratory; in ducks and geese (non haemolytic)).

*G. anatis* is a Gram negative, nonmotile, rod shaped or pleomorphic organism. Sometimes they are in pairs since it belongs to family Pasteurellaceae (Bojesen A.M. et al, 2007 Syst. Appl. Microbiol. 30: 119-127). The bacteria produce semi transparent colonies of 1.2 mm diameter in 24 hours at 37°C.

**Hosts**

It affects domestic poultry and also wild birds. Among domestic species ducks, geese, turkeys
and pheasants are important. The disease has been reported from Europe, America, Australia, Asia and Africa.

**Epidemiology**


**Symptoms**

The main symptoms are diarrhoea and pasty vent along with drop in egg production at peak of laying. The birds are unthrifty, dull. Sometimes the disease takes acute septicaemic condition with sudden mortality in healthy layer’s.

Complication with *E. coli* may result in purulent peritonitis. Immunosuppression may also complicate the disease. Virulence also differs from strain to strain.

**Pathology**

At postmortem purulent salpingitis and oophoritis are mainly seen in classical disease. Purulent peritonitis may be seen occasionally. Lesions of septicaemia may be seen in septicaemic form.

**Diagnosis**

*G. anatis* are found normally in upper respiratory and lower reproductive organs hence culturing the organisms from ovary, salpinx peritoneum blood or spleen is important.

Culture is possible on enriched blood agar, producing colonies as described above.

**Hints for confirmed diagnosis**

(a) haemolysis around 1-2 mm, shiny, translucent colonies grown at 37°C.

(b) Pleomorphic, rods, or bipolar, Gram negative morphology, in the exudates.

(c) Agglutination test, ELISA test, polymerase chain reaction (PCR) with a specific antiserum or probe.

In Denmark latex agglutination test and enzyme linked immunosorbent assey (ELISA) are being developed (Bojensen, *et al.*, 2008, *Poultry Diseases* 6th Edn., p. 162).

**Control**

1. Immunosuppression may flare up the disease because *G. anatis* is an opportunistic pathogen.

2. Drug resistance is a problem with this organism. Narrow spectrum antibiotics generally are useful such as penicillin, sulmamethoxazole, trimethoprim etc.

3. Lowering of egg production must be watched to suspect and investigate the disease.

4. High level of farm biosecurity is essential.

5. Commercial vaccine is not available but an autovaccine prepared from the affected flock may be attempted by an expert poultry pathologist.
4. AVIAN MYCOPLASMA INFECTIONS

Out of three genuses of Mycoplasma namely, Mycoplasma, Ureaplasma and Achoplasma only Mycoplasma genus is significantly Pathogenic for chickens. The class Mollicutes should include all the above three GENERA. Mollicutes differ from conventional bacteria by having no cell wall and having a thin cell membrane.

There are 23 avian mycoplasma species. Out of these Mycoplasma gallisepticum, Mycoplasma synoviae, Mycoplasma meleagridis and Mycoplasma iowae are associated with poultry diseases.

5. MYCOPLASMA GALLISEPTICUM

Mycoplasma gallisepticum produces following poultry diseases.

(i) Chronic Respiratory Disease in chickens
(ii) Sinusitis in Turkeys

Epidemiology

Infection spreads in a flock through air by coughing and sneezing in to in-contact birds. Extent of infection differs from strain of the myeoplasma, density of birds, dust, ammonia gas or mycotoxins in feed. M. gallisepticum attach to the respiratory tract epithelium by lipoprotein structures on their surface (Ley, D.H. 2003, Diseases of Poultry, 11th Edn. Iowa State University Press, Ames, 1A. pp. 722-744). Infection may also pass in to chicken and turkey eggs and kill embryos.

Chronic Respiratory Disease (CRD) (Avian Respiratory Mycoplasmosis)

Etiology

The organisms may be isolated as round colonies with a central nipple, as described in the section of laboratory methods. The organisms grow at pH 7.8 and at 37-38 °C in a medium containing 10-14% porcine serum, which should be heat inactivated. Phenol red and dextrose in the medium indicate growth by change of colour of medium from red to orange in 5-10 days. 2, 3, 5 tetrazolium chloride also indicates growth by change in colour. M. gallisepticum differ in virulence from strain to strain (Ley, D.H. 2003, Diseases of Poultry, Diseases of Poultry, Iowa State University Press, Ames, 1A. p. 722-744).

Host

The disease commonly affects 4 to 10 weeks old birds especially the broilers and turkeys. It may occasionally affect older birds as a complicated disease caused by another bacteria Escherichia coli and sometimes respiratory viruses. The disease may also to spread in ducks, geese, guinea fowl, quails, pigeons etc. M. gallisepticum can escape immune system and persist in host for long time.

Symptoms

The disease starts with sneezing, coughing, respiratory distress or gargling sounds during respiration. Unlike other respiratory diseases, it spreads relatively slowly to other birds. Eyes may show frothy exudate and conjunctivitis. Sometimes, when very chronic, the symptoms
may hardly be seen. There may also be reduction in egg production to as much as 50%, 7-14 days post-infection (Evans, R.D. et al., 1992, *Avian Dis.*, 36: 956).

Other copathogens such as mild newcastle disease virus, 1B virus influenza 4, LT virus, adenovirus reovirus and even vaccines may precipitate CRD.

**Pathology**

The most important pathological lesion is *cloudy appearance of one or more air sacs*. Usually, the cases become complicated and show cheesy material in the air sacs. Trachea and conjunctiva may be congested and there may be pericarditis and fibrinous covering on liver in cases complicated with *E. coli*. Histopathologically, the most important finding is lymphoid follicle formation in the mucosa of the trachea and air sacs, and tubulo-alveolar elongation of tracheal glands. *Presence of lymphoid follicles in air sac membranes, walls of air vesicles, tracheal and respiratory bronchiolar mucosa are considered pathognomonic* (Mahajan, S.K., 1976, Ph.D. thesis, submitted to Haryana Agric. University, Hisar, India).

**Diagnosis**

1. The lesions of air sacs at postmortem examination are very important.
2. When the symptoms and postmortem examination indicate suspicion of CRD, then do rapid plate agglutination test as below. Many birds should be examined in a suspected flock.

   For this test, coloured antigen can be prepared or obtained from manufacturers, in small vials. For this test, about 0.1 ml (one big drop) of coloured antigen is placed on a clean glass slide and mixed with one loopful (bacteriological loop) of blood of a live, diseased bird (blood is taken from wing vein). The blood and antigen are mixed by rotating the slide. If floccules (fine clumps) of blue colour develop within 3 minutes after mixing, the test is considered positive. If the temperature is cold, then finer clumps develop.

   **RSA (Rapid Serum Agglutination Test):** This is a modified test. For this the avian test serum is heated at 56° C for 30 minutes in water bath. The serum is diluted 1:5 or more and the test is done at room temperature. Examination is done at 2 minutes. If there is clamping of antigen then other tests are carried out. RSA test cannot be used to test egg yolk antibodies.
4. Haemagglutination inhibition (HI) test may be done as for Ranikhet disease.
5. An allergic, intradermal test by inoculation of protein antigen, intradermally produces oedematous swelling within a few or 12 to 24 hrs in infected bird. Details of preparation of antigen and method of the test have been given under the section of laboratory methods. The test was compared and found better than agar gel precipitation test (Verma, S.K. et al., 1980, *Indian J. Poult. Sci.*, 15: 90).
6. Fluorescent antibody technique: Using a specific conjugate against *M. gallisepticum* the organisms can be demonstrated in smears, sections or blood of infected birds examined under the fluorescence microscope.
7. Isolated colonies of *M. gallisepticum* can be confirmed by: (a) capacity to agglutinate chicken or turkey erythrocytes; (b) biochemical properties to ferment maltose and glucose
but not dulcitol, lactose or salicin; (c) FAT using specific tagged antiserum (Corstvet, R.E. and Wadler, W.W., 1966, Am. J. Vet. Res., 27: 1703); (d) growth inhibition test by observing inhibition of growth by discs impregnated with specific antisera against various Mycoplasma species.

National Poultry Improvement Plan of USA has approved rapid serum plate (RSP), HI and enzyme linked immunosorbent assay (ELISA) for detection of mycoplasmal antibodies (Cummins D.R. et al., 1990, Avian Dis., 34: 36).

Dot immunobinding assay has been used by Cummins et al. (1990, loc-cit). For this, antigen is bound to nitrocellulose plates which can be stored for a long time.

**Prevention**

If *M. gallisepticum* are killed in the hatching eggs then the flock can be maintained free from the disease. This is because one out of about 50 eggs laid by an infected hen contains these organisms and after hatching the infected hatched chicks spread infection to others.

**Killing *M. gallisepticum* in hatching eggs**

(i) Eggs are warmed at 37.8 °C for 2-3 hrs by keeping them in egg incubator. After this, the eggs are dipped in a watery solution of .04 to 0.1 per cent tylosin tartarate or erythromycin or gentamycin, the temperature of which should be 1.7 to 4.4 °C (refrigerator temperature outside freezing chamber). Eggs are dipped in the solution for 10 to 30 min. This method causes expansion of egg contents by heating from 37 °C to 38 °C (incubator temperature) and dipping in cold antibiotic solution causes shrinkage and entry of the solution, sufficient to kill the organisms (Dhawale, A., 1999, Poultry International, 38 (11): 97-98).

(ii) *M. gallisepticum* in eggs can also be killed by heat treatment, which kills the organisms but not the chick embryo. The eggs at room temperature (25-26 °C) are kept in an incubator having temperature of 46 °C, for a period of 12 to 14 hrs. Then these eggs are incubated for normal hatching in incubator. This method has a disadvantage of lowering the hatchability by 8 to 12 per cent.

**Killing *M. gallisepticum* in hatched chicks**

Alternatively tylosin can be given to newly hatched chicks by mixing in their drinking water in concentration of 0.05 per cent on 1st day of hatching. Similar treatment is repeated on 4th or 5th day after hatching.

**Isolation hatching and rearing**

This method of control is useful on small hatching and rearing farms. For this, eggs are hatched in groups of 50 to 80. Since approximately one out of 50 eggs is likely to have *M. gallisepticum*, many of the batches of chicks may not have them at all. These chicks are reared in isolation (in batches of 50 to 80) and tested by serum agglutination. The reactors are removed.

**Use of Tiamutin for prevention of CRD**

Tiamutin has been introduced in India and other countries for prevention and treatment of CRD and coryza. The drug is specific in its action against *M. gallisepticum*. It is also useful against *Staphylococci*, *Streptococci*, *Haemophilus* and obligate anaerobes, being a bacteriostatic. Tiamutin has been recommended for prevention as well as treatment of CRD and coryza. Following is the dose schedule of tiamutin recommended by its manufacturer.
1st dosing schedule for prevention in 1000 birds per day up to 6th week

(i) Initial dosing
5 g daily in 12 to 15 litres of drinking water on 4th, 5th and 6th days.

(ii) Booster dosing
27.5 g in 50 to 60 litres of drinking water on 21st day.

(iii) Both the doses require a total of 42.5 g of tiamutin for 1000 broilers.

2nd (alternative) dosing schedule for 1000 broiler chicks kept up to 7 to 8 weeks

(i) Initial dosing
5 g daily in 10 to 12 litres of drinking water on 2nd, 3rd and 4th days.

(ii) 1st booster dosing
20 g in 40 to 50 litres of water on 18th day.

(iii) 2nd booster dosing
35 g in 70 to 80 litres of water on 32nd day.

The preventive treatments have been claimed to result in 7 per cent better feed conversion, 10 per cent better weight gain and reduction in mortality by about 15 per cent.

Treatment
Whenever the disease is observed it indicates poor feeding, management or presence of fungal toxins in feed or other forms of stress. The causal organisms are found in many of the healthy poultry and wild birds and they precipitate the disease under the influence of above mentioned factors. When disease occurs, 0.05 to 0.1 per cent tylosin in drinking water can be given for 3 to 5 days. Oxytetracycline (oxysteclin or terramycin) or chlortetracycline can also be given at the rate of 200 g per tonne of feed. Streptomycin, erythromycin, spiramycin etc. are the other antibiotics which can be effective (For details, see therapeutic index).

Tiamutin 45 per cent soluble granules should be administered in concentration of 0.025 per cent for 3 consecutive days (in drinking water). The drugs like monensin, naracin or salinomycin are incompatible with tiamutin. Treatment may also be given for E. coli the common complicating organisms in case of CRD. Other coccidiostats and drugs can be administered with tiamutin. Chlortetracycline at 100 ppm and neomycin at 50 ppm in feed for 4 to 5 days have given better therapeutic effect in broilers inoculated with M. gallisepticum and E. coli at 16 days of age (Lu, C.F.; 1989, Taiwan J. Vety. Med. & A.H. 54: 61). Enrofloxacin at the dose rate of 50 ppm gave better protection than tylosin and tiamutin.

Vaccines
Vaccinal F strain of Mycoplasma gallisepticum a unique strain has been shown to contain strongly immunogenic epitopes. It is expected that F strain vaccine may become available commercially in future. Recently, oil emulsified M. gallisepticum vaccine has become available in U.S.A. and Japan (Kelven, S.H., et al., 1984, Isr. J. Med. Sci., 20: 989). Chicken immunised with M. gallisepticum bacterium with carrageenan (ICGN) as adjuvant were resistant to arisacculitis on challenge test (Elfaki, M.G. et al., 1992, Vaccine, 10: 655). During the last few years new vaccine strains “6/85 and ‘ts11’ have been evolved which are better than F strain (Dhawale, A., 1999: 38 (11); 96-98)‘. ts 11 vaccine is given as eyedrop and 6/85 as aerosol but ts 11 appears to be better because it can be used with virus vaccines of respiratory tract.