Fowl typhoid and pullorum disease

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The terms describing serovars of Salmonella enterica subsp. enterica are presented as follows: Salmonella Enteritidis, S. Gallinarum, S. Pullorum.

Summary

Fowl typhoid (FT) and pullorum disease (PD) are septicaemic diseases, primarily of chickens and turkeys, caused by Gram negative bacteria, Salmonella Gallinarum and S. Pullorum, respectively. Clinical signs in chicks and poults include anorexia, diarrhoea, dehydration, weakness and high mortality. In mature fowl, FT and PD are manifested by decreased egg production, fertility, hatchability and anorexia, and increased mortality. Gross and microscopic lesions due to FT and PD in chicks and poults include hepatitis, splenitis, typhlitis, ophthalmitis, myocarditis, ventriculitis, pneumonia, synovitis, peritonitis and ophthalmitis. In mature fowl, lesions include oophoritis, salpingitis, orchitis, peritonitis and perihepatitis. Transovarian infection resulting in infection of the egg and subsequently the chick or poult is one of the most important modes of transmission of these two diseases. Salmonella Gallinarum and S. Pullorum can be isolated by use of selective and non-selective media. Salmonella Pullorum produces rapid decarboxylation of ornithine whereas S. Gallinarum does not, an important biochemical difference between the two bacteria. Both FT and PD can be detected serologically by use of a macroscopic tube agglutination test, rapid serum test, stained antigen whole blood test or microagglutination test. Both diseases can be controlled and eradicated by use of serological testing and elimination of positive birds. Vaccines may be used to control the disease and antibiotics for the treatment of FT and PD. Although FT and PD are widely distributed throughout the world, the diseases have been eradicated from commercial poultry in developed countries such as the United States of America, Canada and most countries of Western Europe. Both S. Gallinarum and S. Pullorum are highly adapted to the host species, and therefore are of little public health significance.

Keywords


Introduction

Fowl typhoid (FT) and pullorum disease (PD) are septicaemic bacterial diseases of primarily chickens and turkeys, although other birds, such as pheasants, quail, ducks, guinea-fowl and peafowl, are also susceptible. Fowl typhoid is caused by the bacterium Salmonella Gallinarum and PD is caused by S. Pullorum. Salmonella Gallinarum and S. Pullorum are highly host adapted and seldom cause significant problems in hosts other than chickens, turkeys and pheasants. Fowl typhoid was first recognised in 1888 by Klein (81) and PD in 1899 by Retger (109). Pullorum disease was called bacillary white diarrhoea before 1929. These two diseases seriously threatened the poultry industry in the early 1900s due to widespread outbreaks accompanied by high mortality. However, FT and PD have been eradicated from commercial poultry in the United States of America (USA) and the United Kingdom, primarily due to pullorum-typhoid programmes, namely: the National Poultry Improvement Plan (NPIP) and the Poultry Health Scheme, respectively. However, FT and PD...
are still common in many countries throughout the world. Details of various aspects of FT and PD can be found in reviews (29, 98, 107, 122, 131).

The diseases

Historically, FT was thought to be primarily a disease of growing and adult chickens and turkeys, whereas PD was primarily a disease of chicks and poults. In fact, both PD and FT are important and significant diseases of chicks and poults (64, 84, 91, 115, 156). However, growing and mature chickens and turkeys are probably more susceptible to FT than to PD.

Clinical signs

The clinical signs in chicks and poults due to FT and PD have been described previously (18, 19, 46, 47, 76, 92, 116, 156). These include morbund and dead birds in the incubator or shortly after hatching if the chicks and poults are hatched from infected eggs. The birds may manifest depression, somnolence, anorexia, huddling together, droopy wings, dehydration, laboured breathing, diarrhoea, ruffled feathers, weakness and adherence of faeces to the vent. In some situations, FT or PD may not be observed until five to ten days after hatching. The highest mortality usually occurs in birds of two to three weeks of age. Survivors may be greatly reduced in weight and poorly feathered, and may not mature into well developed laying or breeding birds. Flocks that have experienced a severe outbreak will have a higher percentage of carriers at maturity. Other signs, including blindness, swelling of the tibiotarsal joint and the humeral, radial and ulnar articulations may be observed.

In growing and mature fowl, clinical signs of FT and PD may not be apparent in some cases. Non-specific clinical signs, including a decline in feed consumption, a droopy appearance, or ruffled feathers and pale shrunken combs may be observed. Other signs, including decreased egg production, fertility and hatchability, may also be observed depending upon the severity of infection. Death can occur within four days of exposure but usually occurs after five to ten days. An increase in body temperature may occur as a result of PD and FT. Other prominent clinical signs include anorexia, diarrhoea, depression, dehydration and loss of weight.

Morbidity and mortality

Both morbidity and mortality can be highly variable due to various factors such as age of the bird, strain of the bird, nutritional status of the bird, flock management and concurrent infections. Mortality can range from 0% to 100%, especially in chicks and poults (64, 156). The greatest mortality is seen during the second week after hatching, with a rapid decline between the third and fourth week of age. Morbidity is generally higher than mortality. Birds hatched from an infected flock that are raised on the same premises exhibit lower morbidity and mortality than birds that are stressed by shipping. Economic losses due to PD and FT can be very high. This is manifested in the loss of birds, feed costs, veterinary costs, disposal of dead birds, etc. Although the exact figures are not available, the following is an example to illustrate the economic loss. Despite the eradication of PD in the USA, the effect of the disease was felt in 1990-1991 when a series of outbreaks occurred in a completely integrated broiler operation involving five States in the eastern USA (76, 116). The outbreaks eventually involved nineteen breeder flocks and more than 260 grower facilities. The outbreak was ultimately traced back to an infected grandparent male line breeding flock (76). Although the exact costs are not available, eradication of the grandparent line, parent flocks and growout birds, and the replacement of these birds entailed significant expense. Currently, the principal economic significance of FT and PD in developed nations is the cost of surveillance programmes.

Pathology

Studies on gross and microscopic lesions of FT and PD have been sporadic. The earliest descriptions were made by Retger (110, 111). Since then, isolated cases have been reported in different species of birds involving various organs and at various ages, primarily in chickens and turkeys but also a few reports in pheasants, quail, ducks and guinea-fowl (16, 19, 28, 34, 41, 45, 46, 50, 54, 64, 65, 67, 68, 83, 91, 92, 104, 115, 116, 118, 121, 124, 130, 136, 157).

Gross lesions

In peracute cases of FT and PD, chicks may die in the early stages of brooding without exhibiting any gross lesions. In acute cases, enlarged and congested liver, spleen and kidneys can be seen (Figs 1 and 2). Livers may be enlarged and have white foci of necrosis (Fig. 3). Spleens may be enlarged and mottled white (Figs 2 and 4). Contents of the yolk sac may be coagulated, creamy or caseous. There may be fibrinous exudate in the pericardium, capsule of the liver and the peritoneum. In some birds, white nodules may be present in the epicardium and myocardium resembling tumours similar to those seen in Marek's disease (Figs 1, 5 and 6). Occasionally, these nodules in the heart may become sufficiently large to cause distortion in the shape of the heart (Fig. 7). This may lead to chronic passive congestion of the liver, resulting in ascites.

Similar small white nodules may be present in the pancreas, lung, muscle of the gizzard (Fig. 8), and occasionally in the wall of the caecum. The caecum may contain caseous cores in the lumen (Figs 2 and 9). Some birds may exhibit swollen joints (Fig. 10) containing creamy viscous fluid. Other changes include exudate in the anterior chamber of the eye, swollen foot pads or wing joints (46, 47, 76, 116, 156, 157).
Enlarged and congested liver with white nodules in the myocardium of an eighteen-day-old chick affected by pullorum disease

With permission from the American Association of Avian Pathologists

Fig. 1

Fig. 3
Liver with numerous foci of necrosis and inflammation from a chick affected by pullorum disease
Photo: courtesy Dr. L. Hanson, University of Illinois

Fig. 4
Spleen with white mottling from a chick affected by pullorum disease
Photo: courtesy Dr. R. P. Chin, University of California, Davis

Enlarged and congested spleen and liver with pale yellow cast in the lumen of caecum in a chick affected by pullorum disease

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Fig. 2

Fig. 5
Heart with small nodules in the myocardium of a twenty-one-day-old chick affected by pullorum disease
Fig. 6
Heart with prominent white nodules resembling tumours in the myocardium of a five-week-old chick affected by pullorum disease
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Fig. 8
Gizzard with yellow nodules of various sizes in the muscular wall extending from serosa of a six-week-old chick affected by pullorum disease
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Fig. 7
Misshapen heart due to multiple yellow nodules in the myocardium and chronic passive congestion of the liver in a six-week-old chick affected by pullorum disease
Note the thickened pericardium
With permission from the American Association of Avian Pathologists

Fig. 9
Caecum with multiple yellow casts in the lumen of a chick affected by pullorum disease
Photo: courtesy Dr P.P. Chin, University of California, Davis

Fig. 10
Severely enlarged tibiotarsal joints in the legs of a chicken affected by pullorum disease
Photo: courtesy Dr M. Peckham, Cornell University
In adult chickens, lesions may be minimal although the birds may be serologically positive or active reactors. Minimal lesions, such as small nodular or regressing ovarian follicles can be found in the ovaries of chickens. However, the lesions most frequently found in chronic carrier hens include a few misshapened and discoloured cystic ova among a few ovules of normal appearance (Figs 11, 12 and 13). These misshapen ova may be deformed, nodular, and may be attached to the ovary with a long pedunculated stalk which sometimes may become detached from the ovarian mass. The oviduct often contains caseous exudate in the lumen (Fig. 13). Ovary and oviduct dysfunction may lead to abdominal ovulation or oviduct impaction resulting in extensive peritonitis and adhesions of the abdominal viscera. Fibrinous peritonitis and perihepatitis, with or without the involvement of the reproductive tract, may be seen (Fig. 14). Ascites may also develop, especially in turkeys. Occasionally, the culture of salmonella from such lesions is difficult.

Other lesions may include increased pericardial fluid or fibrinous pericarditis, a mottled pancreas, and caseous granulomas in the lungs and air sacs (45). In the male, the testes may have white foci or nodules (54, 83). Lesions in turkeys are similar to chickens (67, 68), but ulceration may be observed in the small intestine as well as in the caecum. Although uncommon in chickens, this is a common finding in turkeys.

In guinea-fowl, lesions due to FT involving the respiratory tract are common. Lesions due to FT in ducklings and adult ducks are similar to those in chickens. In bobwhite quail (Colinus virginianus), enlarged spleens, grey necrotic foci in lungs and pale or discoloured livers were observed in PD (26). In young pheasants, yolk sac infection, pneumonia, hepatitis and typhlocolitis were the most common lesions; the gizzard was occasionally involved (104).

**Histopathology or microscopic lesions**

In peracute cases, the only significant lesions may be vascular congestion in various organs, including the liver, spleen and kidney. In acute to subacute cases, typical lesions are acute necrosis of hepatocytes with fibrinous exudation and infiltration of a mixed population of inflammatory cells composed of heterophils, macrophages, lymphocytes and a few plasma cells (42, 136; H.L. Shivaprasad, unpublished findings). Occasionally, granuloma formations may be found within the liver, characterised by necrosis and accumulation of necrotic debris surrounded by multinucleated giant cells. Fibrinous exudate may be present on the capsule of the liver mixed with heterophils and mononuclear inflammatory cells. In chronic protracted cases, where white nodules appear in the heart, chronic passive congestion may occur in the liver, characterised by the degeneration of hepatocytes around the central veins and interstitial fibrosis. In acute stages, the spleen may have fibrinous exudation in the vascular sinuses.
Fig. 12
Ovary with numerous yellow nodular follicles from an adult chicken with pullorum disease

Fig. 13
Oviduct with distension due to exudate from an adult chicken affected by pullorum disease

Fig. 14
Yellow fibrinous exudate on the capsule of the liver and peritoneum of an adult chicken affected by pullorum disease

and in later stages, multifocal infiltration of mononuclear phagocyte system cells which gives the spleen a mottled white appearance on gross examination. Caeca may contain necrotic caseous debris within the lumen and necrosis of the mucosa with infiltration of heterophils into the lamina propria. In later stages, heterophils may be replaced by lymphocytes, macrophages and plasma cells, which may extend into the muscularis mucosa and muscular layers. Fibrinosuppurative and pyogranulomatous inflammation associated with large numbers of bacteria is a common finding in the yolk sac.

Fibrinosuppurative serositis is also a common finding characterised by exudate in the pericardium which extends into the epicardium and myocardium, in the peritoneal lining, on the serosa of the intestine, and on the pleura (136). However, the most characteristic lesions may be found in the heart and gizzard (H.L. Shivaprasad, unpublished findings). These lesions are characterised by locally extensive foci of myofibre necrosis with infiltration of heterophils mixed with few lymphocytes and plasma cells (Figs 15 and 16). In later stages, these cells may be replaced by large numbers of fairly uniform mononuclear cells of the histiocytic type with irregular vesicular nuclei and faintly staining foamy eosinophilic cytoplasm. These cells may be arranged in
solid sheets, forming nodules that often protrude from the epicardial surface. Such nodules, both grossly and microscopically, can be confused with certain tumours caused by Marek’s disease virus or possibly retroviruses. A similar process can also be seen in the gizzard and pancreas. Other microscopic lesions found in chicks include fibrinoheterophilic panophthalmitis, pneumonia characterised by infiltration of heterophils mixed with macrophages and plasma cells in the interstitium, and fibrinosuppurative inflammation of the synovium.

Microscopic lesions in adults include fibrinosuppurative to pyogranulomatous inflammation of ovarian follicles characterised by necrosis and fibrinosuppurative inflammation mixed with bacteria within the ovules to chronic pyogranulomatous inflammation (Fig. 17) (H.L. Shivaprasad, unpublished findings). In males, necrosis of the epithelial cells lining the seminiferous tubules may be seen, followed by fibrinosuppurative inflammation. Other changes include catarrhal bronchitis, enteritis, interstitial pneumonia and nephritis.

**Pathogenesis**

Little is known regarding the pathogenesis of FT and PD, probably due to the highly successful eradication programmes. Various salmonellae, including *S. Gallinarum* and *S. Pullorum*, were thought to survive and multiply in various organs due to an unknown control mechanism involving the reticuloendothelial system (15). This observation was confirmed when *S. Gallinarum* was found to be taken up by murine phagocytic cells in an *in vitro* experiment (103). Furthermore, *S. Pullorum* was shown to preferentially target the bursa of Fabricius prior to eliciting inflammation in the intestine of chicks (65). Infection with *S. Gallinarum* may also cause anaemia (39). The molecular factors of pathogenicity include plasmids responsible for virulence and virulence factors such as the virulence plasmid gene, invasion gene and fimbrial gene (1, 12, 13, 36, 41, 101, 114).

**Incidence and distribution**

Fowl typhoid and PD are widely distributed throughout the world. However, the diseases have been eradicated from commercial poultry in the USA and other developed countries including Canada, Australia, Japan and most countries of Western Europe. The last outbreak of PD in commercial poultry in the USA occurred from 1990 to 1991 (76). Similarly, an outbreak of FT occurred in commercial poultry in Denmark in 1992 (38). Fowl typhoid and PD are still common in many regions of the world, including Mexico, Central and South America, Africa and the Indian subcontinent (14, 21, 26, 35, 71, 72, 75, 87, 90, 92, 97, 118, 124, 125). However, the frequency of outbreaks in other areas, such as Eastern Europe, Russia, the People’s Republic of China, etc., is not well known.
China, South-East Asia and New Zealand is unknown. Fowl typhoid has not been reported in the USA since 1980 (4, 105).

Aetiology

Salmonella Gallinarum and S. Pullorum are both members of the family Enterobacteriaceae and are highly adapted to the host. The bacteria belong to serogroup D according to the Kauffmann-White scheme. The majority of strains of S. Gallinarum and S. Pullorum are very similar at a chromosomal level (99). Furthermore, S. Enteritidis, another member of serogroup Group D, is thought to be closely related to S. Gallinarum and S. Pullorum, based on multilocus enzyme electrophoresis (132). According to one study, the most recent common ancestor of S. Gallinarum and S. Pullorum was non-motile (85). Since diverging from this ancestor, the S. Pullorum lineage appears to have evolved more rapidly than the S. Gallinarum lineage (85).

The organisms are Gram negative, non-sporogenic, non-motile and facultatively anaerobic. The bacteria are slender rods measuring approximately 1.0 µm-2.5 µm in length and 0.3 µm-1.5 µm in width. Both S. Gallinarum and S. Pullorum are considered to be non-motile. However, motility and flagellation have recently been induced in S. Pullorum grown on solid media (33, 59, 70). Other workers were unable to induce motility in S. Pullorum when grown on Hektoen agar (32).

Both S. Gallinarum and S. Pullorum grow readily on beef agar or broth or other nutrient media. The bacteria are aerobic or facultatively anaerobic and grow best at 37°C. The organisms will grow in selective enrichment media including selenite F and tetraionate broths, and on differential plating media including MacConkey, bismuth sulphite and brilliant green (BG) agars. Salmonella Pullorum may occasionally fail to grow on certain selective media such as BG or Salmonella shigella agar, but grow satisfactorily on bismuth sulphite and MacConkey agars (31).

Colonies of S. Gallinarum and S. Pullorum appear as small, discrete, smooth, blue-grey or greyish-white, glistening colonies that are homogeneous and entire on meat extract or meat infusion agar (pH 7.0-pH 7.2). Most of the colonies remain small (1 mm or less), but isolated colonies may have a diameter of 3 mm to 4 mm or more. Occasionally, morphologically abnormal strains can be encountered. Inoculation of gelatin slants yields greyish-white surface growth with firm growth in the stab and no liquefaction. Growth in broth is turbid with a heavy flocculent sediment.

Both organisms can ferment arabinose, dextrose, galactose, mannitol, mannose, rhamnose and xylose to produce acid with or without gas production (23, 36, 140). Substances not fermented include lactose, sucrose and salicin. One important biochemical difference between the two organisms is that S. Gallinarum ferments dulcitol whereas S. Pullorum does not. In addition, S. Pullorum only occasionally ferments maltose. However, the major difference is that S. Pullorum produces rapid decarboxylation of ornithine, whereas S. Gallinarum does not. In addition, S. Gallinarum uses citrate, D - sorbitol, L - fucose, D - tartrate and cysteine hydrochloride gelatin (38). Some of these differences may be helpful in differentiating between the two organisms. However, variation in the characteristics of some strains can occasionally be observed, especially in regard to gas production.

Other techniques, including ribotyping and polymerase chain reaction, have been important tools to identify S. Gallinarum and S. Pullorum (37, 74, 143). These bacteria can be further typed for epidemiological studies by various techniques such as random amplification of polymorphic deoxyribonucleic acid (RAPD), plasmid profiling, phage typing and cloning of chromosomal fragments (3, 36, 37, 41, 78, 79, 141, 142, 155).

Antigenic variation

Both S. Gallinarum and S. Pullorum possess the O antigens 1, 9 and 12. Variation involving antigen 12 occurs in S. Pullorum, but no evidence exists for such variation in S. Gallinarum (43, 44, 149). The antigenic composition of S. Pullorum was shown to be 1, 9, 121, 122, 123; the variation involved minor somatic antigens 122 and 123. Standard strains of S. Pullorum contain a large amount of 123 and a very small amount of 122, but in variant strains the content of the two antigens is reversed. Intermediate strains are usually mixtures of 122 and 123 predominant colonies. However, DNA fingerprint analysis of these strains has raised some questions regarding such variation in the major somatic antigen 12 (155). Strains may also vary in content of the O-1 antigen. Tests to differentiate standard, intermediate and variant types of S. Pullorum have been described (147, 148, 150).

Like most pathogenic microorganisms, S. Gallinarum and probably S. Pullorum may lose virulence rapidly in artificial media; hence, cultures should be passaged serially in the natural host (the chicken) before testing the pathogenicity of such cultures. Pathogenicity of such cultures is best maintained in the lyophilised or frozen state. This may explain why various investigators have found variation in virulence among cultures of S. Gallinarum.

Epidemiology

Natural hosts

Chickens are the natural hosts for both S. Gallinarum and S. Pullorum. However, natural outbreaks of FT and PD have been described in turkeys, guinea-fowl, quail, pheasants, sparrows and parrots (107, 122, 131). In addition, outbreaks of FT have been described in ring-necked doves, ostriches and...
peafowl, and cases of PD in canaries and bullfinches. The susceptibility of ducks, geese and pigeons to S. Gallinarum varies, but all of these birds generally appear to be resistant. Significant differences also exist in susceptibility to PD among breeds of chicken (30, 120). White Leghorns appear to be more resistant than heavy breeds such as Rhode Island Red, New Hampshire, or crosses between the two (73). Differences in resistance of S. Gallinarum and S. Pullorum have also been shown in inbred lines of chickens (30). A greater percentage of females appear to remain as reactors, probably due to the sequestered nature of localized infection of the ovarian follicles.

Pullorum disease has been described as a naturally occurring or experimental infection in mammals such as chimpanzees, rabbits, guinea-pigs, chinchillas, pigs, kittens, foxes, dogs, swine, minks, cows and wild rats. Salmonella Gallinarum could be cultured for up to 121 days from the faeces of experimentally infected rats (9).

**Transmission**

Fowl typhoid and PD can be transmitted by a variety of means (16, 17, 55). The infected bird, such as a carrier or a reactor, is by far the most important means of perpetuation and spread of the bacteria. Birds may not only infect their own generation, but also succeeding generations, through egg transmission. Egg transmission may result from contamination of the ovum following ovulation or localization of the bacteria in the ova before ovulation (16, 17). Other modes of transmission include shell penetration, feed contamination, contact transmission either in the hatcher, brooder, cages or floor, cannibalism of infected birds, egg eating, and through wounds on the skin (69, 146, 151). Faeces from infected birds are an important source of contamination of other birds. Contaminated feed, water and litter can also be sources of S. Gallinarum and S. Pullorum. Attendants, feed dealers, bird buyers and visitors who move between farms and between bird houses may spread disease unless precautions are taken to disinfect footwear, hands and clothing. Similarly, trucks, crates and feed sacks may also be contaminated and can be the source of infection of birds. Wild birds, mammals, flies and insects may be important in mechanical spread of the organism.

Both S. Gallinarum and S. Pullorum may survive for several years in a favourable environment, but are less resistant than paratyphoid salmonellae to heat, chemicals and adverse environmental factors (107, 131). For example, S. Gallinarum was killed within 10 min at 60°C, within a few minutes by direct exposure to sunlight, within 3 min by 1:1,000 phenol, 1:20,000 dichloride of mercury or 1% potassium permanganate, and in 1 min by 2% formalin (107). Agar cultures may rapidly lose pathogenic character. Salmonella Gallinarum was found to retain viability for up to forty-three days, subject to daily freezing and thawing (100). Organisms survived more than 148 days at -20°C, even though they were accidentally thawed twice. Salmonella Gallinarum can survive in the faeces from infected chickens for up to 10.9 days when kept in a range house, and for two days less in the open (128). Compounds containing phenol were the most effective disinfectants for control of S. Gallinarum in the field, followed by quaternary ammonium compounds and iodophores (20).

**Diagnosis**

A definitive diagnosis of FT and PD requires the isolation and identification of S. Gallinarum and S. Pullorum, respectively. However, a tentative diagnosis can be made, based on the flock history, clinical signs, mortality and lesions. Positive serological findings can also be of great value in detecting infection; however, negative results should not be considered adequate for a definitive diagnosis, because of the delay of three to ten or more days in appearance of agglutinating antibodies following infection. In addition, cross-reactions with other salmonellae, such as S. Enteritidis, should be considered when interpreting serological results (52, 123, 145). A brief overview of diagnostic techniques will be given here. More detailed information is provided elsewhere (98).

**Isolation of Salmonella Gallinarum and Salmonella Pullorum**

Since FT and PD are systemic diseases, especially in young chicks and pouls, these bacteria can be isolated from most body tissues. The liver, spleen, yolk sac and caeca are most commonly involved in FT and PD, and are the preferred organs for culture. Lesions may also occur in the heart, gizzard, pancreas and lungs, which are also suitable specimens for isolation. If lesions are present in the reproductive organs of mature birds, the ovarian follicles, oviduct and testes can be cultured. Other sites, such as peritoneum, synovium and the interior of the eye can also be cultured. Beef extract or infusion broth or tryptose agar in tubes or Petri dishes are all satisfactory for primary isolation. Enrichment broths or selective media may also be used if tissues are decomposed.

Birds with chronic FT or PD that are detected by serological tests may or may not have gross lesions. In such instances, thorough culturing of the internal organs is necessary. A detailed outline for examination of such specimens can be found in the NPIP manual used in the USA (7). This procedure may be summarised briefly as follows: grossly, normal or diseased internal organs including heart, liver, gall bladder, spleen, kidney, pancreas, testes/ovary and oviduct should be cultured directly onveal infusion (VI) and BG agar plates and incubated for 48 h at 37°C. In addition, portions of internal organs should be pooled, ground, or blended in ten times their volume of VI broth; 10 ml aliquots of the suspension are transferred to 100 ml of both VI and tetraionate BG (TGB) broth and incubated for 24 h at 37°C.
The broths are then plated on VI and BG agar, incubated and examined after 24 h and 48 h. If contamination with proteus or pseudomonas is a problem, BG sulphapyridine agar plates can be used.

The digestive tract should be cultured using individual cotton swabs for the upper, middle and lower intestinal tract, including both the caecum and the rectum/cloacal area. The swab should be deposited in 10 ml TBG broth, incubated and plated as previously described for the internal organs. In addition, portions of the gut should be pooled, ground, or blended, in ten times their volume of TBG broth. A quantity of the suspension from the digestive tract (10 ml) is transferred to 100 ml of TBG broth and incubated at 42°C or 37°C for 24 h. The higher incubation temperatures for TBG broth reduce populations of competitive contaminants common in gut tissue.

Suspect colonies are transferred to triple sugar-iron (TSI) agar and lysine-iron (LI) agar and incubated at 37°C for 24 h. Cultures revealing typical reactions of salmonellae or arizonae on TSI or LI agar slants should be identified by appropriate biochemical or other tests. All salmonella cultures should be serologically typed. Use of nonselective media demands careful aseptic techniques but has the advantage of greater dependability in securing isolation of S. Gallinarum and S. Pullorum. Also, other bacteria capable of producing cross-reactions with pullorum typhoid antigen may be more dependable demonstrated. Polymerase chain reaction has also been used to detect isolates of S. Gallinarum (143).

Identification of cultures

Colonies of S. Gallinarum may appear smooth, blue-grey, moist, circular and entire after 24 h of incubation. Colonies of S. Pullorum are small, smooth and translucent on nutrient media. Careful initial culture of tissues on nonselective media should usually result in pure cultures. If pure cultures are not obtained, or if an enriched medium has been used, transfer of individual colonies to TSI agar slants for preliminary differentiation is often advantageous. Salmonella Gallinarum and S. Pullorum produce a red slant with a yellow butt that shows delayed blackening from H2S production. Reactions listed in Table I, which can be determined within 24 h, provide identification of a number of other common pathogens and allow differentiation between the two organisms.

Additional differentiation tests described in the section entitled 'Aetiology' may be necessary to identify isolates that produce atypical reactions (chiefly fermentation of maltose or no gas production). Decarboxylation of ornithine by S. Pullorum is the single most dependable test for differentiating maltose-fermenting S. Pullorum strains from S. Gallinarum.

### Table I: Biochemical reactions used to differentiate between Salmonella Gallinarum and Salmonella Pullorum

<table>
<thead>
<tr>
<th>Reactant or property</th>
<th>Salmonella Gallinarum</th>
<th>Salmonella Pullorum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>Fermented with no gas</td>
<td>Fermented with gas</td>
</tr>
<tr>
<td>Lactose</td>
<td>Not fermented</td>
<td>Not fermented</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Not fermented</td>
<td>Not fermented</td>
</tr>
<tr>
<td>Mannitol</td>
<td>Fermented with no gas</td>
<td>Fermented with gas</td>
</tr>
<tr>
<td>Maltose</td>
<td>Fermented with no gas</td>
<td>Usually not fermented</td>
</tr>
<tr>
<td>Dulcitol</td>
<td>Fermented with no gas</td>
<td>Not fermented</td>
</tr>
<tr>
<td>Orotate</td>
<td>Not fermented</td>
<td>Fermented</td>
</tr>
<tr>
<td>Indole</td>
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<tr>
<td>Urea</td>
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<td>Not hydrolysed</td>
</tr>
<tr>
<td>Motility</td>
<td>Non-motile</td>
<td>Non-motile</td>
</tr>
<tr>
<td>Agglutination</td>
<td>Positive with group D</td>
<td>Positive with group D</td>
</tr>
</tbody>
</table>

Serology

Various serological tests have been used to detect FT and PD. These include the macroscopic tube agglutination (TA) test, rapid serum (RS) test, stained antigen whole blood (WB) test, and microagglutination (MA) test using tetrazolium-stained antigens (51, 77, 113, 119, 139, 152). In some countries, such as the USA, the standard procedure for detecting breeding flocks chronically infected with S. Gallinarum and S. Pullorum is to use the standard strains of S. Pullorum (1, 9, 12) for tube and serum plate antigens and both standard (1, 9, 12) and variant (1, 9, 12) strains of S. Pullorum for the polyvalent rapid WB plate antigens. These antigens will detect flocks infected with either S. Gallinarum or S. Pullorum. However, these antigens will cross react with the sera from birds infected with other salmonellae, most notably S. Enteritidis (51, 123, 145). In Japan, the antigen used for the WB test is prepared from cultures grown in a continuous-flow, broth-culture system in which it is necessary to mix sublots to secure desired agglutination (138).

Enzyme-linked immunosorbent assays (ELISA) for detecting S. Gallinarum and S. Pullorum antibodies have been developed by using lipopolysaccharides from these salmonellae as antigens (14, 93, 94). This technique can be used for screening large numbers of blood samples. As with other techniques, the ELISA may show positive reactions to other salmonellae, especially those belonging to group D. Salmonella Pullorum can be detected serologically using S. Enteritidis flagella as an antigen in an ELISA (53). Recently, a dot immunobinding assay (DIA) was compared with a serum TA test for detecting serological responses in vaccinated and unvaccinated chickens following a challenge with virulence plasmid-cured S. Gallinarum (89). The DIA was found to be very sensitive and detected antibodies to high...
titres in birds challenged with *S. Gallinarum*. This assay also
detected in high titres antibodies to *S. Gallinarum* in
vaccinated and unchallenged birds which failed to react by TA
(89).

**Public health implications**

Since *S. Gallinarum* and *S. Pullorum* are highly adapted to the
host, the diseases are of little public health significance.
Occasional PD in humans has been reported following
ingestion of contaminated food containing massive numbers
of *S. Pullorum* (95, 108). The symptoms are characterised by
a rapid onset of acute enteritis followed by prompt recovery
without treatment. Experimental reproduction of
salmonellosis using four strains of *S. Pullorum* in humans and
large numbers (billions) of bacteria produced only transient
illness followed by prompt recovery (88). *Salmonella*
Gallinarum is rarely isolated from humans and is of little
public health significance. According to a report of the
Centers for Disease Control and Prevention, Atlanta, USA,
eight *S. Gallinarum* isolates and eighteen *S. Pullorum* isolates
have been detected out of a total of 458,081 salmonella
isolates from humans between 1982 and 1992 (5). The
presence or absence of symptoms in the individuals from
which the salmonellae were isolated was not recorded.

**Prevention and methods of control**

Fowl typhoid and PD are excellent examples of diseases that
have decreased in prevalence in some of the advanced
countries or have been eradicated by application of basic
management procedures or eradication programmes. One of
the basic requirements is to establish breeding flocks free of
*S. Gallinarum* and *S. Pullorum*, and to hatch and rear progeny
under conditions that will preclude direct or indirect contact
with infected chickens or turkeys. Since egg transmission
plays an important role in the spread of these two diseases,
only eggs from flocks known to be free of FT and PD should
be introduced into hatcheries. Chickens and turkeys are the
primary hosts of *S. Gallinarum* and *S. Pullorum*, and free
flying birds and other fowl are not principal reservoirs of
infection. Thus, eradication of these diseases from chicken
and turkey breeding flocks is a fundamental step towards
eradication of FT and PD in commercial poultry flocks.

**Management practices**

Management practices broadly designed to prevent the
introduction of infectious agents should be applied to prevent
introduction of FT or PD. Carriers must be eliminated on a
regular basis. Some of the specific practices include the
following:

- chicks and poults should be obtained from sources free of
  FT and PD
- typhoid-free and pullorum-free stock should not be mixed
  with other poultry or confined with birds not known to be
  free of FT and PD
- chicks and poults should be placed in an environment that
  can be cleaned and sanitised to eliminate any residual
  salmonellae from previous flocks
- in order to minimise the introduction of *S. Gallinarum* and
  *S. Pullorum* through feed ingredients, chicks and poults
  should receive pelleted, crumbled feed. Feed ingredients
  must be free of salmonella
- a sound biosecurity programme must be in place so that
  the introduction of salmonellae from outside sources is
  minimised.

Houses should be designed to exclude rodents and free-flying
birds. Insect control, the use of clean or chlorinated water,
and the use of thoroughly disinfected footwear and clothing
are essential for biosecurity. Measures must be implemented
to prevent exposure of birds to contaminated poultry
equipment, handling crates and trucks. Dead birds should be
disposed of properly.

**Elimination of carriers**

One of the most important methods of controlling FT and PD
is by serological monitoring of flocks (see the section entitled
'Serology'). Tube agglutination, RS, WB and MA tests are all
effective in detecting carriers, but the MA test offers an
economic advantage. In the USA, the WB test is not accepted
for testing turkeys as the test was determined to be unreliable.
Testing of birds by several methods at three to five week
intervals is essential to detect reactors which then can be
removed. A single test may miss reactors for several reasons,
as follows:

- serum agglutinin titres in infected birds may be too low to
  produce significant agglutination at the usual dilutions of 1:25
  or 1:50
- a delay of at least several days occurs between infection
  and the development of agglutinins
- following the removal of the reactors, environmental
  contamination may serve as a source of infection for other
  birds at a later date.

In the USA, testing for NPIP (7) accreditation is allowed after
chickens and turkeys reach approximately sixteen weeks of
age.

An ELISA test which can be economical for screening large
numbers of flocks and birds is also available for screening of
flocks for FT and PD (14, 93, 94).
Infection must also be confirmed by careful bacteriological examination of one or more serological reactors. The antigen which is used for detecting S. Gallinarum and S. Pullorum is known to cross-react with other bacteria, resulting in false positives (51, 123, 145). These bacteria could be other types of salmonella such as S. Enteritidis or S. enterica subsp. arizonae, or other bacteria such as Staphylococcus epidermidis, Escherichia coli, and others (49, 145). Non-Gallinarum and non-Pullorum reactors may range from a few birds in a flock to as many as 30% to 40%. Thorough bacteriological examination of representative reactors is often the only dependable method for determining the infection status of a flock. This is also probably the only method of distinguishing between infections by S. Gallinarum and S. Pullorum.

If an attempt is made to free a flock of infection, retesting of the infected flock should be performed at two to four week intervals until two consecutive negative tests of the entire flock are secured at an interval of twenty-one days or more. In the majority of cases, infection can be eliminated from the flock through testing at short intervals and removal of infected birds. Two or three retests are often sufficient to detect all infected birds. However, infection occasionally continues to spread within a flock, and the disease cannot be eliminated by repeated testing and removal.

Criteria of eradication

The essentials of an eradication programme for an area or a country may include the following:

a) fowl typhoid and PD must be reportable diseases
b) infected flocks must be placed under quarantine and infected flocks marketed under supervision
c) all reports of FT and PD must be investigated by an authorised state or federal agency or other officials
d) importation regulations must require shipments of poultry and hatching eggs to be from sources considered free of FT and PD
e) regulations must require poultry at public exhibitions to be from flocks free of FT and PD
f) total participation must be required of poultry breeding flocks and hatcheries in a typhoid pullorum control programme, such as NPIP in the USA or an equivalent in other countries.

Although advanced countries have limited the presence and spread of FT and PD in commercial flocks, these diseases are still present in small backyard flocks (6, 45, 104, 139, 144; H.L. Shivaprasad, unpublished findings). However, the usual separation of commercial and non-commercial poultry has been effective in preventing transmission of S. Gallinarum and S. Pullorum between these populations. The infected backyard flocks still continue to pose a threat to commercial flocks. Therefore, continued testing of commercial breeding flocks is necessary to rapidly detect any spread of FT and PD from non-commercial poultry.

Treatment

Every effort should be made to eradicate FT and PD, and treatment should be the last option. Various sulphonamides, followed by nitrofurans and several other antibiotics have been found to be effective in reducing mortality from FT and PD. However, no drug or combination of drugs have been found to be capable of eliminating infection from treated flocks. Sulphonamides that have been used in treatment of FT and PD include sulphadiazine, sulphamerazine, sulphathiaole, sulphamethazine and sulphaquinoxaline (2, 24, 96). However, most studies have indicated that despite treatment, a significant number of infected birds can remain among the survivors and become carriers (24, 106, 112). Various other antibiotics can be used for controlling and treating FT and PD, including furaltodone, furazolidone, chloramphenicol, biomycin, apramycin, gentamicin and chlorotetracycline (8, 22, 27, 48, 56, 57, 107, 127, 135, 137, 153, 154). Resistance to some of these antibiotics has been reported (63, 80, 117, 129, 133, 134).

Care must be taken to follow the directions given by the manufacturer in regard to the route of administration, dosage, duration of treatment, and the withdrawal period for each antibiotic before use. Some details regarding these aspects have been described in reviews (107, 123, 131). Furazolidone treatment may interfere with antibody production and should be avoided six weeks prior to testing (66). A choice of antibiotics available for use varies by country.

Vaccination

A number of investigators have evaluated killed and modified live bacteria for FT. Studies on the use of 9R strain as live oral or injectable vaccine with or without oil adjuvants have reported variable results (60, 61, 62, 102, 126). Similarly, outer membrane proteins from S. Gallinarum have been reported to offer better protection than the 9R live vaccine in terms of clearance of the pathogenic strain from internal organs (25, 27). More recently, immunisation against FT by the use of mutant strains of S. Gallinarum and the virulence-plasmid-cured derivative of S. Gallinarum appear to be promising in protecting birds challenged with S. Gallinarum (10, 11, 58, 158). Since FT and PD have been eradicated from commercial flocks in many developed countries, the incentive for the production of vaccines is small.

Though not a vaccine, the administration to young broiler chickens of S. Enteritidis-induced immune lymphokines significantly reduced the horizontal transmission of
S. Gallinarum (82, 86). Competitive exclusion has also been attempted to control FT, using S. Enteritidis or S. Gallinarum (40).

**International trade implications**

Export of poultry and poultry products should be made only from flocks known to be serologically negative for FT and PD.

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**Typhose et pullorose aviaires**

H.L. Shivaprasad

**Résumé**

La typhose et la pullorose aviaires sont des maladies septicémiques touchant essentiellement les poules et les dindes et dues à des bactéries ne prenant pas la coloration de Gram, *Salmonella* Gallinarum et *S. Pullorum*, respectivement. Les signes cliniques chez les poussins et les dindonneaux sont, entre autres, l’inappétence, la diarrhée, la déshydratation et l’anémie ; ils s’accompagnent d’une forte mortalité. Chez les poules adultes, la typhose et la pullorose se manifestent par une chute de ponte, une baisse de la fertilité et un trouble de l’éclosion, ainsi que par de l’anorexie et une mortalité accrue. Les lésions macroscopiques et microscopiques observées chez les poussins et les dindonneaux atteints de typhose et de pullorose comprennent, notamment, une hépatite, une splénite, une typhlité, une anémie, une myocardite, une ventriculite, une pneumonie, une synovite, une péritonite et une ophtalmie. Chez les volailles adultes, on observe des lésions telles que des ovarites, des salpingites, des orchites, des péritonites et des péri-hépatites. L’infection transovarienne, qui se traduit par la contamination de l’œuf et donc du poussin ou du dindonneau, est l’un des principaux modes de transmission de ces deux maladies. On peut isoler *S. Gallinarum* et *S. Pullorum* par des moyens sélectifs ou non sélectifs. *Salmonella Pullorum* produit une décarboxylation rapide de l’ornithine, contrairement à *S. Gallinarum* ; c’est une différence biochimique importante entre les deux bactéries. Des tests sérologiques permettent de déceler la typhose et la pullorose, notamment la réaction de macroagglutination en tube, l’agglutination rapide, le test de coloration de l’antigène en sang entier ou la micro-agglutination. Les examens sérologiques et l’élimination des volailles ayant réagi positivement permettent de prévenir et d’éradiquer ces deux maladies. Les vaccins sont indiqués pour lutter contre la typhose et la pullorose et les antibiotiques pour traiter ces deux maladies. Largement répandues dans le monde, la typhose et la pullorose ont, cependant, été éradiquées des élevages avicoles commerciaux dans des pays développés comme les États-Unis d’Amérique, le Canada et la plupart des pays d’Europe occidentale. *Salmonella Gallinarum* et *S. Pullorum* sont étroitement adaptées à leurs hôtes et n’ont pas d’incidence significative en santé publique.

**Mots-clés**

Tifosis aviar y pulorosis

H.L. Shivaprasad

Resumen
La tifosis y la pulorosis son enfermedades septicémicas causadas por sendas bacterias gramnegativas (*Salmonella Gallinarum* y *S. Pullorum*, respectivamente) que afectan sobre todo a pollos y pavos. Entre los signos clínicos de esas infecciones en pollitos y pavipollos cabe citar anorexia, diarrea, deshidratación, decaimiento y elevada mortalidad. En aves maduras las principales manifestaciones clínicas son, además de la anorexia y el aumento de la mortalidad, la pérdida de fertilidad y el descenso de las tasas de producción huevera y de eclosión. Las lesiones macro y microscópicas que ambas enfermedades provocan en pollitos y pavipollos son: hepatitis, esplenitis, tiflitis, onfalitis, miocarditis, ventriculitis, neumonia, sinovitis, peritonitis y oftalmitis. Las aves maduras, por su parte, pueden presentar ooforitis, salpingitis, orquitis, peritonitis y perihepatitis. La infección transovárica, que provoca la infección de los huevos y después de los polluelos, es uno de los principales mecanismos de transmisión de estas dos enfermedades. Para aislar *S. Gallinarum* y *S. Pullorum* en cultivo pueden utilizarse medios selectivos o no selectivos. Entre *S. Pullorum* y *S. Gallinarum* hay una importante diferencia bioquímica: la primera induce una rápida descarboxilación de la ornitina y la segunda no. Tanto la tifosis aviar como la pulorosis pueden detectarse por medios serológicos, utilizando pruebas de macroaglutinación en tubo, de aglutinación rápida, de tinción antigénica en muestras de sangre entera o de microaglutinación. Aplicando pruebas serológicas y sacrificando después a los ejemplares positivos es posible luchar contra ambas enfermedades hasta llegar a erradicarlas. También pueden utilizarse vacunas para controlar la propagación de ambas infecciones, y antibióticos para tratarlas. Pese a la amplia distribución (a escala mundial) de la tifosis aviar y la pulorosis, países industrializados como los Estados Unidos de América, Canadá y muchos países de Europa Occidental han conseguido erradicarlas en las poblaciones de granjas avícolas. *S. Gallinarum* y *S. Pullorum* están muy adaptadas a sus especies huéspedes, por lo que revisten escasa importancia en términos de salud pública.

Palabras clave

References


