CEVA HANDBOOK
OF POULTRY DISEASES

1 SALMONELLOSIS

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DEFINITION

Solmonelloses are acute or chronic intestinal infections in poultry characterized by septicaemic and degenerative necrobiotic lesions of the alimentary tract and the parenchymal organs.

HISTORY AND SYNONYMS

Salmonella bacteria are named after the American bacteriologist D. Salmon (1850–1914). In 1899 Dr. Leo F. Rettger isolated and identified an etiological agent from a case of pullorum disease and named it *Salmonella pullorum*. This discovery turned out to be of utmost importance for the development of the poultry industry (Bullis, 1977). Synonyms of Pullorum disease are white diarrhoea, and the now obsolete "bacillary white diarrhoea" and "fatal septicaemia of young Chickens". In 1929, the term Pullorum disease was approved and became internationally accepted. In 1909 Dr. Rettger and Stoneburn reported the transovarial transmission of the infection that was, in fact, the first report of such a route of infection transmission. The complete cycle of infection, along with evidence that infected chickens could be a permanent reservoir of infection and may transmit it via the eggs, was presented by Dr. Rettger and his collaborators in 1914. The agglutination test developed by Dr Jones introduced the diagnostic procedure for detection of the causative agent (Jones, 1913). Later, looking for other diagnostic tests, a rapid serum agglutination test (Runnells et al., 1927) and a test using whole blood (Bunyea et al., 1929) were proposed. The first did not find a broad application, and the second was based on using a live culture as an antigen that was capable of inducing infection. Later on, a whole-blood test using stained bacterial suspension (antigen) was proposed (Schaffer et al., 1931). According to early reports, this disease was considered of major significance for the poultry industry and outbreaks with mortality rates of over 85% were reported (Bullis, 1977). Subsequently, programmes for eradication and maintenance of Pullorum disease-free flocks were implemented.

Fowl typhoid in poultry is very closely related to Pullorum disease. It is caused by *S. Gallinarum* and is established in 1888 (Klein, 1889). During the next years, reports about the isolation of the bacterial agent came from different parts of the world. By that time, it was also called *Bacillus sanguinarum* (Moore, 1895). The name fowl typhoid was accepted in 1902 (Curtice, C. 1902).

CHARACTERISTICS AND CLASSIFICATION OF THE PATHOGEN

The *Salmonella* genus (Entero-bacteriaceae family) consists of over 2400 serologically different variants (serotypes). Avian salmonelloses can be classified in two groups. The first one includes infections (Pullorum disease and fowl typhoid) caused by the two non-motile serotypes *S. Pullorum* and *S. gallinarum*. The second group comprises infections caused by multiple motile *Salmonella* serotypes, most frequently *S. Enteritidis* and *S. Typhimurium*, isolates that are considered together as paratyphoid.

ETIOLOGY AND PATHOGENESIS OF FOWL TYPHOID AND PULLORUM DISEASE

S. pullorum and *S. gallinarum* are Gram negative facultative anaerobic rods, 0.5–2.5 µm in size. They do not form spores or capsules and are cultivated in standard nutrient media. It appears that *S. pullorum* grows more slowly than *S. gallinarum* and this is attributed to its lack of the capacity for oxidative assimilation of different amino acids (Stokes & Bayne, 1961). *S. pullorum* can be distinguished from *S. gallinarum* on the basis of cerain biochemical characteristics. According to some researchers, a decarboxylase test allows us to differentiate between both bacteria, with *S. pullorum* producing a positive result and *S. gallinarum* a negative response (Edwards, 1962; Costin et al., 1964). Both salmonellae possess the thermostable O-antigen but not the thermolabile H-antigen.

The pathogenicity of salmonellae is due to their endotoxin. Endotoxins of *S. gallinarum* are able to induce clinical signs within hours after intravenous injection to chickens (Smith et al., 1978). Formation of endotoxins, and thus entero toxigenicity in experimental conditions, is observed

8h after inoculation (Kokosharov, 2002; Kokosharov, 2004). In the view of the author, the state is related to apoptotic changes in blood cells and intestinal epithelial cells. The pathogenicity is best preserved in frozen or lyophilized state. Reports of a new toxin (haemolysin) have recently appeared (Agrawal et al., 2005). The entry of bacteria into the blood circulation after passing through the intestinal mucosa leads to generalized infection.

The resistance of *Salmonella* organisms is relatively high. In soil and litter, they are preserved for more than 100 days, in the water – up to 200 days, and in dry condition – up to 7-8 years. They are rapidly inactivated by 2% formalin, 1–4% chlorinated lime or 5% phenol solution (Girginov & Iliev, 1984).

EPIDEMIOLOGY

Sick birds or reservoir hosts are sources of infection. Adult birds which carry the infection spread it through eggs, from which infected chickens are hatching. Some of them die during the first days following hatching, and survivors are reservoir hosts of the infection. Diseased birds, and reservoir birds to a lesser extent, shed the infection agent with faeces and contaminate the environment. The infection of healthy birds occurs via the faecal-oral route. Spread of infection by breakdown and eating of eggs from the floor or from the nests is also possible. In affected flocks, the number of eggs infected with *S. pullorum* or *S. gallinarum* may exceed 30%. Cannibalism is also a means for infection of healthy birds, as the etiological agent resides in internal organs and in the ovary in particular. Contaminated water, feed or litter, as well as the transfer of people and equipment between farms, are further sources of infection. Other vectors of infection include wild birds or fleas (Shivaprasad & Barrow, 2008).

Under natural conditions, chickens are susceptible to *S. pullorum* and *S. gallinarum*. The infection has also been observed in turkeys, pheasants, guinea fowl (Shivaprasad, 2000; Pennycott & Duncan, 1999).

There are reports of infected exotic birds (parrots, canaries) and ostriches. Waterfowl are relatively resistant. The susceptibility to *S. pullorum* infection is the highest during the first days after hatch. After the age of 5–6 weeks, morbidity and mortality from Pullorum disease is rarely seen. Revising the results from numerous experimental and field studies, Shivaprasad & Barrow, (2008) concluded that young birds (chickens and poults) are highly susceptible to *S. Pullorum* and *S. gallinarum,* while adult birds are mainly vulnerable to *S. gallinarum*. Infection of adult birds with fowl typhoid is usually encountered after an occult infection in reservoir hosts has become acute. This happens most commonly after challenge by factors resulting in reduced host resistance: high population density, extreme temperature variations, deficient feeding, poor hygiene etc.

Morbidity and mortality are very various and largely depend on the afore mentioned predisposing factors as well as on the species, age and gender of birds, the virulence of the causative agent, the exposure route etc.

CLINICAL SIGNS AND PATHOLOGY

Pullorum disease

Pullorum disease (PD) is an acute systemic disease in chickens and turkey poults, whereas adult birds are asymptomatic reservoir hosts.

Fig.1

The morbidity and mortality rates increase about the 7th to 10th day after hatching. Sick chickens appear somnolent, depressed and exhibit a retarded growth. The area around the vent is stained with diarrhoeic faeces.

Fig.2

In some chickens, the vent is stained by dried faeces with a characteristic chalkwhite colour due to the high content of urates («white diarrhoea»).

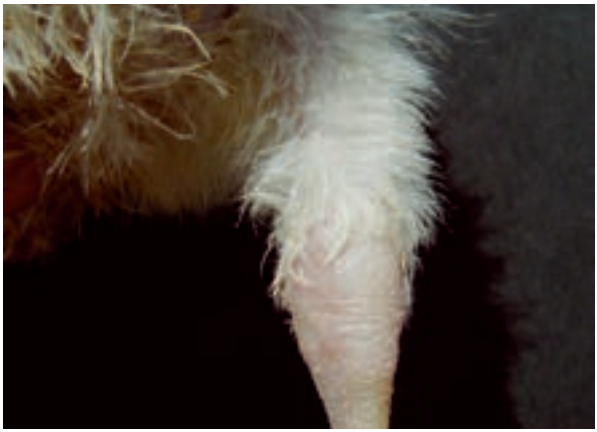

Fig.3
Tibiotarsal joint oedema is a possible accompanying sign of PD.

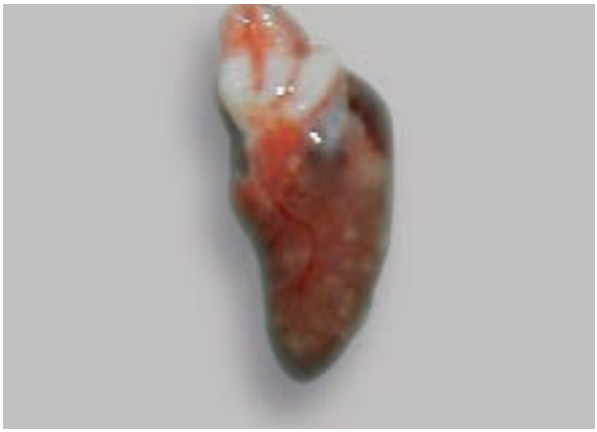

Fig.4
Typical for PD is the appearance of nodular inflammatory necrotic grey-white foci in the myocardium, varying in size from hardly visible with a naked eye to 2–3 mm in diameter or larger.

Fig.5

Similar nodular formations may be detected in the lungs, the liver, the gizzard wall, the intestines and the peritoneum. The mechanism of formation of nodules involves the appearance of necrotic foci as a result of the effects of the agent's endotoxin, through growth of lymphocytes and histiocytes.

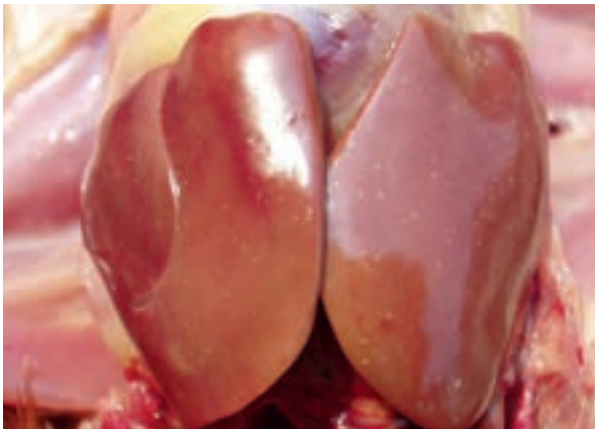

Fig.6

Sometimes, multiple grey-white miliary necroses could be detected in the liver, secondary to the agent's endotoxin effect.

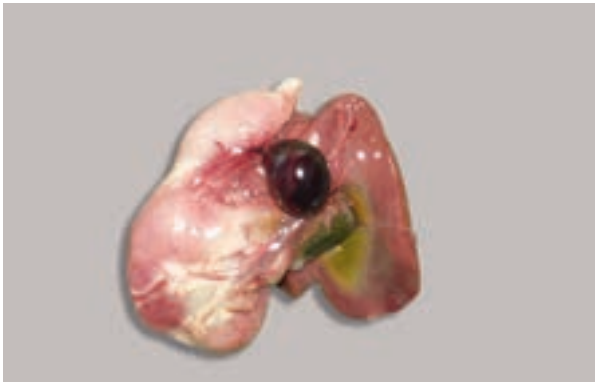

Fig.7
The enlarged and septicaemic spleen is also a common finding in chickens infected with *S. pullorum*.

Fowl typhoid

Fowl typhoid (FT) is an acute or chronic septicaemic disease that affects primarily adult hens and turkeys.

Fig.8
Acute fowl typhoid. A characteristic lesion for acute fowl typhoid in adult birds is the enlarged and bronze greenish tint of liver.

Fig.9

Acute fowl typhoid. In some instances, the enlarged liver is mottled with multiple miliarynecroses.

Fig.10

Acute fowl typhoid. In other cases, the size of liver necroses varies from miliary to spots with a diameter of 12 cm. Unlike Pullorum disease, fowl typhoid is lasting for months.

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Fig.11

Acute fowl typhoid. The spleen is 2-3 times bigger, sometimes with greyish-whitish nodules prominating on the surface, representing hyperplasic follicles.

Fig.12

Acute fowl typhoid. Often, enteritis, especially of the anterior part of small intestine, sometimes with ulcerations, is present.

Fig.13

Acute fowl typhoid. More rarely, myocardial necroses due to Salmonella toxins are detected.

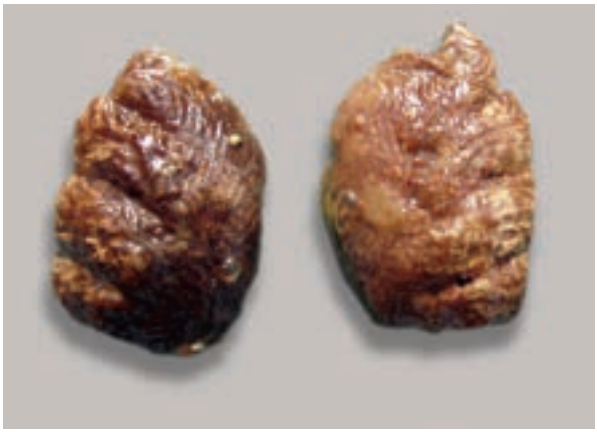

Fig.14

Acute fowl typhoid. The lungs acquire a characteristic brown colour. Necroses and even "sarcoma-like nodules" may be observed.

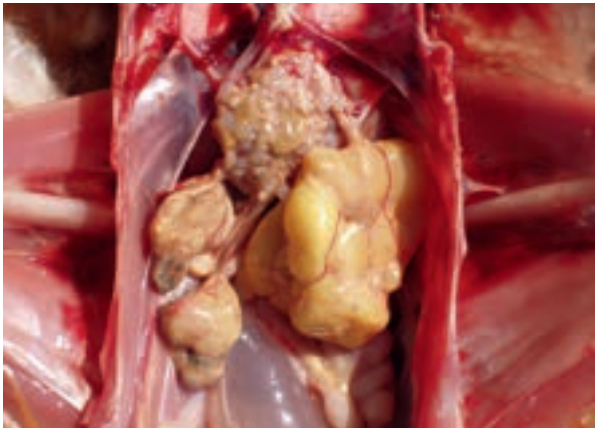

Fig.15

Chronic fowl typhoid. The lesions are primarily in the gonads. The ovaries are affected by inflammatory and degenerative changes.

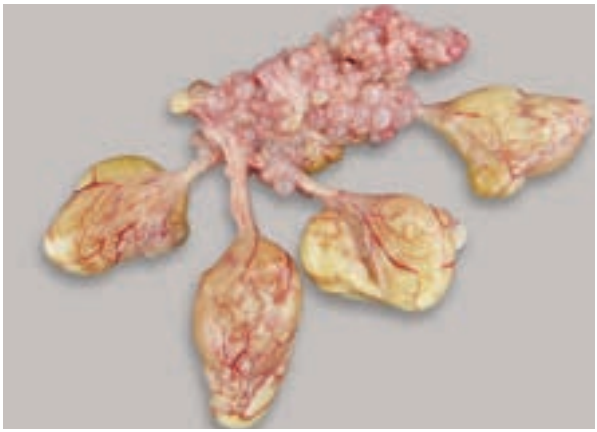

Fig.16

Chronic fowl typhoid. Frequently, affected follicles are deformed and appear like thick pendulating masses.

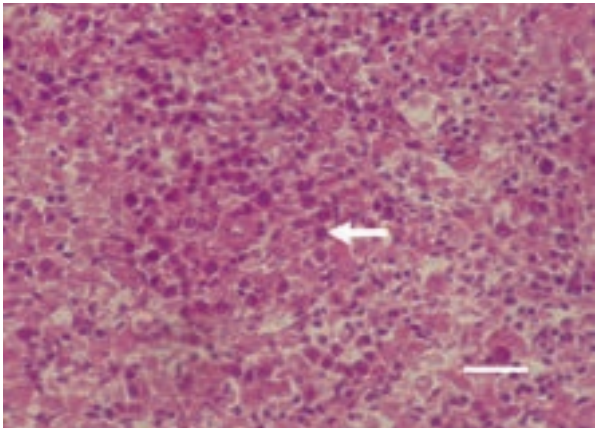

Fig.17

Pullorum disease. Perivascular proliferative inflammatory focus in the liver, consisting mainly of histiocytes and single epitheloid cells (arrow).
H/E, Bar = 25 µm.

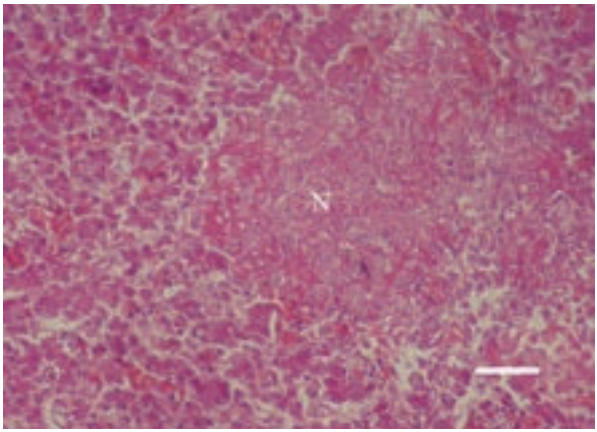

Fig.18

Fowl typhoid. A reactive fibrinoid necrosis (N) in the liver. H/E, Bar = 40 µm.

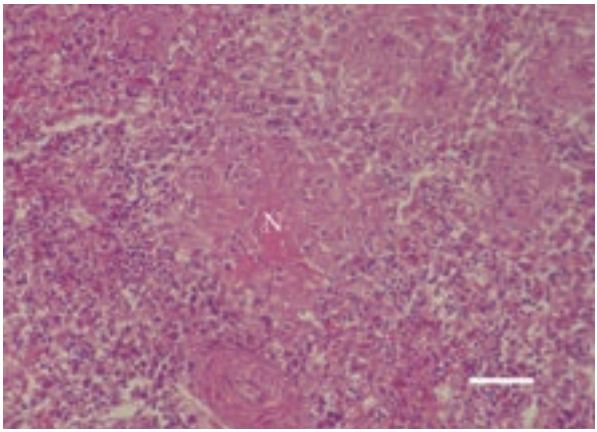

Fig.19

Fowl typhoid. Periarteriolar fibrinoid necrosis (N) in the spleen. Marked cell reaction in the periphery of the necrotic foci involving lymphocytes, histiocytes and single granulocytes. H/E, Bar = 50 µm.

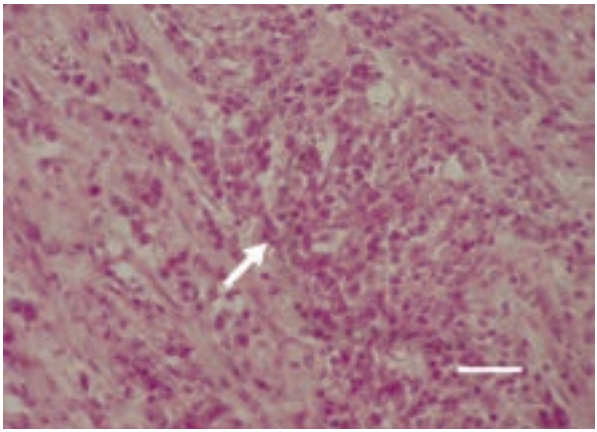

Fig.20

Fowl typhoid, hen. Mononuclear inflammatory cell proliferate in the myocardium (arrow). H/E, Bar = 30 µm.

DIAGNOSIS

A tentative diagnosis can be made on the basis of the history of the problem and the flock, the development of the disease, clinical signs and lesions. Initial diagnosis is confirmed after isolation and identification of the etiological agent. Microbial agents can be isolated from most internal organs, because PD and FT are systemic infections. Routine microbiological techniques for differentiation of both bacteria (*S. pullorum/S. gallinarum*) are based on the aforementioned biochemical properties. At present, molecular methods are being developed in order to distinguish between both serovars. Duplex PCR primers are available, targeted at genetic markers allowing for the identification of *S. Pullorum* and *S. gallinarum* (Barrow & Freiras Neto, 2011).

DIFFERENTIAL DIAGNOSIS

PD and FT should be differentiated mainly from *E. coli* toxaemic and septicaemic states, *P. multocida* infection (septicaemic and some local forms, particularly arthritis) and other salmonelloses. If sarcoma-like nodules are present in the viscera in PD, Marek's disease and aspergillosis should be considered.

ETIOLOGY AND PATHOGENESIS OF PARATYPHOID INFECTIONS

Fowl paratyphoid is an acute or chronic disease in domestic fowl and many other avian or mammalian species, caused by some motile *Salmonella* serotypes that are not host-specific. The aetiological agents include about 10-15 *Salmonella* serotypes and the most common isolates are *S. Enteritidis* and *S. Typhimurium*. Other serotypes such as *Salmonella* Paratyphi B var Java, *S. Agona* and *S. Heidelberg* are now seen more frequently as a cause of food poisoning from chickens.

They are short Gram negative rods 1–4 µm in length. The motility in most bacteria is realized by 8–12 peritrichous flagella. They do not form spores or capsules and grow under aerobic and anaerobic conditions. Salmonellae possess a thermostable O antigen connected to the body and a thermolabile H-antigen related to flagella. A protocol for serological systematics of salmonellae on the basis of reactions with O- and H-antigens is developed (Ewing, 1986).

The resistance of organisms in the environment, especially when dry, is rather high. In such state, during the hot seasons *S. Typhimurium* can survive up to 3 months in the soil, and in dry avian faeces for up to 2 years (Girginov & Iliev, 1984). According to the same authors, it could be preserved for 17–24 days in hatcheries and up to 3–6 months in carcasses.

At 80°C, salmonellae are destroyed after 10 min, and at 65°C after 1-2 hours. Formalin at 1% kills them after 1-2 min, and 5% chlorinated lime after 5 min. Chemical disinfectants (phenols and quaternary ammonia compounds) are widely used for that purpose.

Most fowl paratyphoid organisms contain an endotoxin, responsible for their pathogenic effects. The enterotoxic activity of salmonellae cause filling of the intestinal lumen with fluid, whereas a thermostable cytotoxin provokes breakdown of intestinal epithelial cells (Koupal & Deibel, 1975; Kopanic et al., 1994).

EPIDEMIOLOGY

The primary sources of infection are diseased birds and birds having survived the infection – reservoir hosts and shedding birds. Chickens hatching from the eggs of infected birds are diseased, and also exhibit a higher embryonic mortality.

Animal protein feed ingredients (meat and bone meal, fish meal) may be infected with salmonellae. Other important reservoir hosts are rodents (rats and mice) and wild birds that could come into contact with farm flocks.

Evidence for vertical transmission has been found in ducklings, turkey poults and chickens (Girginov & Iliev, 1984). A side from this route of transmission, under suitable conditions the salmonellae may penetrate through eggshells after just 6 min at 37°C (Williams et al., 1968). Newly hatched chicks are most vulnerable, and susceptibility decreases with age. The mortality of natural paratyphoid infection reaches a peak at 3–7 days of age. In adult birds, morbidity and mortality rates are insignificant. (Gast, 2008). An increased susceptibility is observed in newly hatched ducklings and goslings and at a lesser extent – in chickens and pigeons. Factors contributing to the spread of paratyphoid infections are excessive stocking density, overheating, deficient feeding, poor hygiene in the hatchery and premises etc.

Fig.21

The highest morbidity and death rates are usually observed during the first 2 weeks after hatching. The chickens are drowsy, with eyes closed, ruffled feathers and grouped near the sources of heat.

Fig.22

Haemorrhagic fibrinoustyphlitis is a possible finding.

Fig.23

Diarrhoea, dehydration and pasted down appearance around the vent are observed. Pathoanatomically, marked catarrhal haemorrhagic enteritis is observed. Often the caeca are filled with gelatinous, fibrinous, cheese-like exudate. This is a finding, characteristic for salmonellosis, but it is not specific for any of serotypes. The inflammatory fibrinous exudate in caeca often forms casts with the shape of mucosal folds.

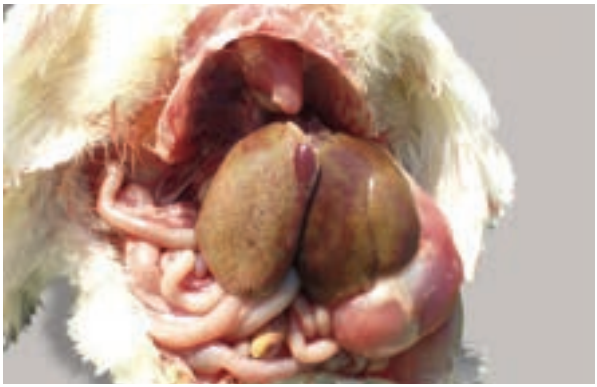

Fig.24

In some cases, necrotic foci are found in the liver.

DIAGNOSIS

Paratyphoid infections may be tentatively diagnosed on the basis of epidemiological and clinical data and lesions, but the diagnosis is only confirmed after isolation and identification of the etiological agent.

Dead chicken carcasses or embryos are suitable for laboratory examination. For detection of salmonellae, standard culture methods are employed. For detection of infection in live birds, serological test are most appropriate: i.e. agglutination tests and ELISA. The same methods are used to detect antibodies in egg yolks.

Paratyphoid infections should be differentiated from *E. coli* septicaemia, pullorum disease and *Pseudomonas aeruginosa* infection in chickens during the first week after hatch.

MANAGEMENT, PREVENTION AND CONTROL OF SALMONELLOSES

The treatment inhibits but does not eradicate the infection. The appropriate treatment minimizes the death rate until the birds develop immunity.

Measures for control on the spread of salmonelloses include mainly a thorough investigation of breeder flocks, breeder eggs (usually, a sample of 10–20 eggs) and hatcheries. Monitoring by periodic examination of samples taken from the environment (faeces, litter, dust) is also important. This allows for monitoring of the penetration of salmonellae in buildings through the personnel, disease vectors, equipment and other sources (Gast, 2008).

Control of salmonelloses mainly takes the form of serological testing and eradication of reservoir hosts. This approach is possible where the levels of infection are low and eradication is possible. The utilization of vaccines is considered appropriate in case of high infection levels or when eradication is not a viable alternative, for instance when environmental control is not possible (Barrow &

Freitas Neto, 2011). Discussing the alternatives for immune protection against salmonellosis, Barrow (2007) outlines several criteria an ideal vaccine should meet:

a) efficient protection against systemic and mucosal infection;

b) safety for animal and human health;

c) efficacy in reducing intestinal colonization and, consequently, reduced environmental contamination and egg infection;

d) cost efficiency. The use of live and inactivated vaccines dates back several decades. The first efficient live attenuated vaccines contained the 9R and 9S strains (Smith, 1956).

Although 9S was believed to be more protective, one of the advantages of 9R is that is also protects against *S. Enteritidis* (Barrow et al., 1991; Feberwee et al., 2001). The 9R vaccines were widely used, but a certain residual virulence remained in newly hatched chickens which persisted for several weeks (Barrow et al., 1991).

Gast (2008) reports a renewed interest in alternative vaccines (bacterins) in recent years inrespect to *S. Enteritidis*. There are reports of a considerable reduction in the spread of *S. Enteritidis* isolates from faeces, internal organs and eggs after subcutaneous or intramuscular application of adjuvant vaccine to layer hens (Gast et al., 1997; Clifton-Hadley et al., 2002). A reliable protection against infection of reproductive organs and egg contamination after immunization of layer hens with purified *S. Enteritidis* fimbriae is also reported (De Buck, 2005). Some vaccines containing inactivated *S. Enteritidis* and *S. Typhimurium* cultures ensure stable cross-protection against *S. Gallinarum* as well, by reason of the identical antigenic structure of the somatic O-antigen.

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