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# *Listeria monocytogenes* Infection in Poultry and its Public Health Importance with Special Reference to Food Borne Zoonoses

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**Abstract:** Listeriosis is a disease that causes septicemia or encephalitis in humans, animals and birds. Although, the disease is rare and sporadic in poultry but if occurs then causes septicemia or sometimes localized encephalitis. Occasionally, the disease is seen in young chicks and the causative agent, like in humans and animals, is *Listeria monocytogenes*. The organism is capable to infect almost all animals and poultry; however, outbreaks of listeriosis are infrequent in birds. It is widely distributed among avian species and chickens, turkeys, waterfowl (geese, ducks), game birds, pigeons, parrots, wood grouse, snowy owl, eagle, canaries, which appear to be the most commonly affected. Chickens are thought to be the carriers of *Listeria* and also the prime reservoirs for the infection and thus contaminate the litter and environment of the poultry production units. Listeriosis is often noticed along with other poultry diseases such as coccidiosis, infectious coryza, salmonellosis, campylobacteriosis and parasitic infections, signifying the opportunistic nature of the organism. Intestinal colonization of poultry and the presence of *L. monocytogenes* in feces represent a potential source of the organism for listeriosis in ruminants. Man gets infection from raw broiler meat due to *Listeria* contamination and unhygienic conditions of the processing area, rather than acquiring direct infection from birds. With the changing food habits of the people, the health consciousness is also increasing and since listeriosis has now been recognized as an emerging food borne zoonoses. Therefore, this review has been compiled to make aware the poultry producers and the consumers of poultry meat/products regarding the importance of the disease and its public health significance.

**Key words:** *Listeria*, public health, zoonoses, food borne pathogen, prevention and control

## INTRODUCTION

Food-borne zoonotic pathogens of poultry include *Salmonella*, *Campylobacter*, *Escherichia coli, Listeria*, *Arcobacter*, *Clostridium*, *Mycobacterium, Staphylococcus* and *Cryptosporidium*. Among these Listeria can cause severe and life-threatening complications (Patyal *et al*., 2011; Dhama *et al*., 2008; 2012; Ta *et al*., 2012; Kudirkiene *et al*., 2013; Asakura *et al*., 2013). Listeriosis is a disease that causes septicemia or encephalitis in humans, animals and birds (Wesley, 2007). In poultry, the disease occurs sporadically in the form of septicemia or localized encephalitis. Occasionally, the disease is seen in young chicks and the causative agent, like in humans and animals, is *Listeria monocytogenes*. The organism is capable to infect almost all animals and poultry; however, outbreaks of listeriosis are infrequent in birds. It is widely distributed among avian species and chickens, turkeys, waterfowl (geese, ducks), game birds, pigeons, parrots, wood grouse, snowy owl, eagle, canaries, which appear to be the most commonly affected (Gray, 1958; Dhama *et al*.,

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2009; Ivanovic *et al*., 2010; Shivaprasad *et al*., 2007). Chickens and turkeys are relatively resistant to experimental infection (Bailey *et al*., 1990). Chickens are thought to be the carriers of *Listeria* and also the prime reservoirs for the infection and thus contaminate the litter and environment of the poultry production units (Njagi *et al*., 2004). Listeriosis is often noticed along with other poultry diseases such as coccidiosis, infectious coryza, verotoxic *E. coli*, salmonellosis, campylobacteriosis and parasitic infections, signifying the opportunistic nature of the organism (Wesley, 1999; Uyttendaele *et al*., 1999; Adzitey *et al*., 2012; Cook *et al*., 2012). Intestinal colonization of poultry and the presence of *L. monocytogenes* in feces represent a potential source of the organism for listeriosis in ruminants (Cooper *et al*., 1992; Bailey *et al*., 1990). Even though, the organism infects poultry, human beings predominantly gets infection from raw broiler meat due to *Listeria* contamination and unhygienic conditions of the processing area, rather than acquiring direct infection from birds (Kosek-Paszkowska *et al*., 2005; Goh *et al*., 2012). With the change in food production, processing and distribution, changing food habits of the people towards ready-to-eat products, increased use of refrigeration for food preservation, increased interest in organic and natural products including free range birds, the health consciousness is also increasing and since listeriosis has now been recognized as an emerging food borne zoonosis (Farber and Peterkin, 1991; Low and Donachie, 1997; Kataria *et al*., 2005; Barbuddhe *et al*., 2008; Dhama *et al*., 2009; Dhama *et al*., 2011a; Milillo *et al*., 2012) with higher risk to newborns, pregnant women, old people and immune-compromised individuals. Therefore, this review has been compiled to make aware the poultry producers and the consumers of poultry meat/products regarding the importance of the disease and its public health significance.

## ETIOLOGY

The causative agent of disease is *Listeria monocytogenes*, which is gram-positive and saprophytic bacterium (Milillo *et al*., 2012) that tolerates a wide pH range and survives high and low temperatures, however, is found susceptible to pasteurization at 75°C for 10 sec. It is a small, facultative anaerobe, coccoid to bacillus-shaped, non-sporulating, motile bacteria that tends to form long filaments, particularly in older cultures; has flagella and shows characteristic tumbling motility at 22°C; can grow between 0 and 43°C with an optimal range of 30-37°C and a pH range of 4.5-9.6 (Junttila *et al*., 1988; Quinn *et al*., 1994; Huff *et al*., 2005; Sukhadeo and Trinad, 2009; Bhunia, 2008). There are 16 serotypes; of which serotypes 1 and 4 are mainly responsible for infection in most human and animals. The organism is widespread in nature and is found in soil, silage, sewage, surface water and in faeces from domestic/wild animals and birds (Fenlon, 1985; Quinn *et al*., 1994). Listeria, commonly found in temperate regions, can survive outside the body of host, under moist conditions for several years. *L. monocytogenes* occasionally causes disease in domestic animals/birds besides infecting many species of rodents and wild animals. It may also be recovered from the faeces of apparently normal domestic poultry. There is no evidence for egg transmission and the organism is not shed in the eggs of even highly inoculated laying hens (Malik and Vaidya, 2005).

## DISEASE IN BIRDS

There is no any pathognomonic sign of listeriosis in birds. However, young birds have chronic infection, while adult birds die suddenly with septicemia and occasionally show signs of meningoencephalitis. Young birds, being more susceptible to listeriosis may have mortality rate up to 40%. Disease incidences are influenced by factors such as immunosuppression and damp weather conditions, cold, moist litters etc. (Barnes, 2003; Kahn, 2005). The organism usually shed in all the secretions and excretions of the infected birds. Disease is transmitted through ingestion of contaminated feed, water, litter and soil. Infection can also follow inhalation or wound contamination. In birds, generally, the infection is subclinical and incubation period is not reported (Gray, 1958; Kurazono *et al*., 2003). Signs of infection, if seen, are suggestive of septicemia and may include depression and listlessness, emaciation, diarrhea and peracute/sudden death can occur at times (Akanbi *et al*., 2008). In the subacute and chronic forms, signs are related to encephalitis that includes spasms with stretching of neck and back followed by paralysis. Depression, incoordination, ataxia, torticollis, opisthotonous and other nervous signs are seen in the encephalitic form. The septicaemic form of listeriosis produces varied lesions, which includes fibrinous pericarditis, hydropericardium, petechial hemorrhages in proventriculus, heart and kidney, nephritis, oedema of the lungs, thickening of the airsac walls, splenic and liver enlargement, bile retention, necrotic areas in the liver and heart, enteritis and conjunctivitis (Cooper *et al*., 1992; Kahn, 2005; Kurazono *et al*., 2003). In acute form, lesions are mostly petechial hemorrhage on the serosa and generalized congestion. With the encephalitic form, no pronounced/visible gross lesions in brain are seen except

gliosis and satellitosis in the cerebellum and microabscesses in the midbrain and medulla. In some cases, Salpingitis may also occur in hens (Cooper, 1989; Kurazono *et al*., 2003).

## DIAGNOSIS

The disease can be diagnosed on the basis of history, clinical signs, post-mortem lesions and microscopical observation. Recognition of the infection is made by isolation and identification organism (Kahn, 2005). *Listeria* can be isolated easily from the clinical samples except the encephalitic form of disease in birds. (Cooper, 1989; OIE, 2006). However, being ubiquitous in nature and able to survive for long periods outside the body, it may be difficult to determine the source and spread of infection. *Listeria monocytogenes* need to be differentiated from other species of *Listeria*. Isolation of the bacteria can be attempted from clinical samples viz., faeces, blood, liver, heart, spleen, brain, CSF, meconium of newborns or foetus in abortion cases, vomitus, food stuffs/feed as the case may be (Gray and Killinger, 1966). It may be necessary to macerate material and then pre-incubate for several months at 4°C and to subculture at intervals before isolation (Quinn *et al*., 1994; Walker, 1999; Walker *et al*., 1990). Isolation of the organism by direct culture from the affected tissues is improved if the specimen is refrigerated, indicating its psychrophilic nature. Blood/tryptose agar or brain heart infusion medium is the best for isolation (Walker *et al*., 1990; Andrews, 2002). If, the isolation and culture of bacteria is not possible then, demonstration of antigen in fixed tissues from septicemic lesion can be done. Chicken embryos are infected readily and can be used for isolation by culturing via the allantoic cavity. The organism may be identified by demonstration in smear on Gram's staining, biochemical means (peroxide-anti-peroxide method), Immunofluorescence Test (IFT) or DNA analysis (AOAC, 2000; OIE, 2006; Dhama *et al*., 2009); Loop-mediated isothermal amplification (LAMP) (Tang *et al*., 2011). Pathogenicity testing of *Listeria* isolates should be done either by *in vitro* methods such as haemolysis on sheep blood agar, assay for PI-PLC (Phosphatidylinositol-specific phospholipase C) activity, CAMP test or by *in vivo* tests such as inoculation of mice (3 weeks old) through i/p route and inoculation of 10-day old chicken embryo through CAM (chorioallantoic membrane) route.

Serodiagnostic methods include serum agglutination test, Complement Fixation Test (CFT), Haemagglutination (HA) test, Haemagglutination Inhibition (HI) test, antibody precipitation test, growth inhibition test and Enzyme Linked Immuosorbent Assay (ELISA) (Capita *et al*., 2001; OIE, 2006; Dhama *et al*., 2009). The detection of antibodies against a haemolysin called listriolysin O (LLO) by plate or dot-ELISA has been reported to be useful for diagnosis of both septicaemic and abortion forms of listeriosis. Modified PI-PLC assay, Polymerase Chain Reaction (PCR) and multiplex PCR based on virulence-associated genes (*plc*A, *prf*A and *hly*A) of *Listeria* spp. have shown great promise as rapid and reliable diagnostic alternatives (Portnoy *et al*., 2002; Agersborg *et al*., 1997; Dhama *et al*., 2009).

## PUBLIC HEALTH IMPORTANCE AND FOOD-BORNE ZOONOSIS

The organism is important because of its ability to cause human infections following contact with infected birds or consumption of contaminated poultry or poultry products, especially those that are pre-cooked and ready to eat. Human listeriosis is a worldwide phenomenon, causing several food borne outbreaks, especially in the developed and under developed countries (Schlech, 2000; Schlech *et al*., 1983; OIE, 2006). The disease ranks second only to salmonellosis in food poisoning and the infection has serious implications in very young children (neonates) and immunocompromised individuals, where the mortality is even higher (30-40%), making it a serious public health hazard (Barbuddhe *et al*., 2008). Milk and animal/poultry meat are considered as major sources of infection (Goh *et al*., 2012). Direct contact with animals/birds is of little importance in transmission except in case of highly susceptible persons. Person-to-person spread, though recognized, is uncommon. Consumption of poorly cooked meat and contaminated ready-to-eat poultry products have been responsible for the disease (Dhama *et al*., 2011a-c). As *Listeria* has the capability to grow even at low temperatures, refrigeration always won't ensure protection. This clearly highlights the importance of cooking the food materials to high temperatures. In humans, the incubation period varies from 1 day to 3 weeks. The disease condition manifests as meningitis or encephalitis characterized by high temperature, stiffness of neck, ataxia, tremors, seizures and fluctuating consciousness. Headache, vomiting, fever, malaise, pneumonia and conjunctivitis have also been observed (Rocourt and Bille, 1997; Slutsker and Schuchat, 1999). In personnel, who work at poultry processing plants, conjunctivitis has been linked to handling of apparently normal but Listeria-infected chickens. The onset is sudden and death may follow within 24-48 h. The infection may also cause abortion and stillbirth in pregnant woman and also gets transmitted to the neonates (Rocourt and Bille, 1997; Swaminathan, 2001).

Transmission of food-borne avian diseases primarily occurs via food-chain by fecal-oral route. Contamination of food materials can also occur by unhygienic food handling practices, contaminated water and by flies and insects (Dhama *et al*., 2011a).

## PREVENTION AND CONTROL

In recent years, *Listeria* has been established as an important food-borne pathogen and has become a major concern to the food industry and health authorities. The disease can be prevented by identification and elimination of the possible sources of infection; and by practicing a high standard of hygiene and sound management practices in the poultry farm and also in the poultry processing zones (Cutter and Henning, 2003; Swaminathan, 2001; Wesley, 1999; Rossi *et al*., 2008). Apart from that strict hygienic and sanitation procedures along with culling or isolation of affected birds should be adopted to control the disease. The use of antibiotics in feed been reported for prophylaxis of listeriosis in poultry. Once entering the meat processing facility, *Listeria* becomes resident and may be able to survive non-stringent sanitation procedures (LaBudde, 1999; Tompkin *et al*., 1992; Slutsker and Schuchat, 1999). The unusual growth and survival properties of *L. monocytogenes* and its ability to adhere to various surfaces, contributes to the complexity and difficulty of eliminating this organism. Hence, proper sanitation and disinfection of processing plants along with discarding the infected birds at entry level are essential (Kosek-Paszkowska *et al*., 2005; Malik and Vaidya, 2005). The presence of the organism in cooked food usually indicates inadequate cooking or cross contamination after cooking (Barbuddhe *et al*., 2008). Methods like controlling the pH, water activity, use of preservatives, restricted shelf lives etc. may help in overcoming the problem of contamination. The incidence of *Listeria* has been found higher in frozen meat than in fresh meat since the frozen meat and meat products are more liable to be contaminated during their preparation and storage (Cutter and Henning, 2003; Tompkin *et al*., 1992). The disease can be prevented in human beings by avoiding consumption of *Listeria* contaminated food-stuffs. In addition the increase in the spread of antimicrobial resistance among bacterial pathogens is also increasing public health concern.

At the level of consumers, the identification of high-risk foods and the education of high-risk individuals are to be given due consideration (Rebagliati *et al*., 2009). The immunocompromised persons, pregnant women and old people are at high risk of infection (Rappaport *et al*., 1960; WHO Working Group, 1988). *Listeria* is often resistant to most commonly used antibiotics but it will respond to high levels of tetracyclines, which are efficacious in both acute and subacute form of the disease. Chortetracycline is probably the ant-microbial drug of choice. Treatment of the chronic form is usually unsuccessful. So far no vaccination is available. With the generalized use of antibiotics in poultry feed for growth promotion, the cases of listeriosis in poultry have decreased to few only (Low and Donachie, 1997; Gray and Killinger, 1966; Barnes, 2003). Individuals should be advised of the need to follow the general guidelines as well as strictly following additional precautions detailed below.

Bacteriophage therapy is also being used to control *Listeria* in poultry meat products so as to check and eliminate human infection (Leverentz *et al*., 2003; Carlton *et al*., 2005; Kim *et al*., 2008; Bigot *et al*., 2011). Phage treatment during processing and packaging is has been studied to check *L. monocytogenes* in raw and ready to eat meat and poultry products (Soni *et al*., 2010). Probiotics have also been suggested to play role in inhibiting *Listeria monocytogenes* (Dhama *et al*., 2011b). These novel anti-listerial agents need much to be explored for their practical applicability to control listeriosis and its food borne zoonoses in humans.

## WAYS TO MINIMIZE INFECTION IN HUMANS

Reduce the risk of cross contamination by keeping uncooked meat away from other food substances. Wash the hands, knives and cutting boards after handling uncooked meat. Moist heat (121°C for a minimum of 15 min) or dry heat (160-170°C for 1 h) can kill the organism present in utensils. Thorough and proper cooking of raw meat has to be practiced (Bremer *et al*., 2002; Rebagliati *et al*., 2009). Food should be properly stored and follow good kitchen hygiene practices. Packed and frozen meat products have to be further heated in accordance with manufacturer's instructions. Disinfectants like 1% sodium hypochlorite, 70% ethanol or glutaraldehyde should be used in processing units to inactivate the organism. Disease can be satisfactorily prevented by wearing protective clothing while handling infected birds or their tissues. Limiting listeriosis requires implementation of effective food safety control measures and ensuring that these control strategies are consistently met (Oyarzabal, 2006; Adzitey and Huda, 2010).

The most appropriate strategies for its control in foods are Good Manufacturing Practices (GMP), good hygiene and sanitation in operating procedures and Hazard Analysis Critical Control Point (HACCP) programs. These procedures will help in minimizing the

environmental contamination by this organism and prevent cross-contamination in processing and packaging units as well as retail counters. There should be appropriate time and temperature controls throughout the entire distribution and storage period of packed meat/meat products. Post-packaging treatments are to be implemented to destroy *L. monocytogenes* in food products (USFDA, 2001). *L. monocytogenes* can also colonize various inert surfaces and can form biofilms on food-processing surfaces (Roberts and Wiedman, 2003). Science-based education and risk communication strategies aimed at susceptible populations and focused on high-risk foods should be delivered through health care providers or other credible sources of information. High-risk individuals should be provided with guidance on safe and healthy eating practices, with specific information on high-risk foods that they should avoid. Since, this organism will have the ability to grow at low temperature, so thorough cooking, prevention of cross-contamination and short-term refrigerated storage of cooked perishable foods are some of the steps to avoid the disease (Oyarzabal, 2006; Gillespie *et al*., 2006; Dhama *et al*., 2009).

## CONCLUSION AND FUTURE PERSPECTIVES

*L. monocytogenes* is ubiquitous, opportunistic and a very important food-borne pathogen that continues to pose worries to the food industry and health authorities. Their infection is severe in high risk individuals. The main source of infection is through the consumption of contaminated food. Ready-to-eat foods, meat and meat products and milk and milk product are the major source of outbreaks and most research has concentrated in this area. Their ability to survive in refrigeration and wide environmental conditions increases the plight of achieving zero or minimal tolerant of *L. monocytogens* in foods. To control this zoonotic pathogen, accurate techniques for the diagnosis like isolation and then identification of *Listeria* is very crucial. To reduce its incidence, clean and hygienic rearing of birds at farms, their processing, packaging at plants and then marketing at retail outlets are very important. Although, the direct transmission of Listeria from birds to man is rare but the there is need to further elucidate the factors involved in its transmission from poultry to human beings. This underlines the fact that *L. monocytogenes* is not only important for public health but also has a socio-economic importance upon the production of food and on food businesses worldwide, including those involved in international trade.

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