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Review On Current Status Of Pullorum Disease In Ethiopia

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Summary

Poultry represents important sources of food and income in the world in general and in Ethiopia in particular. However, many diseases and nutrition related disorders affect the poultry industry. Among this, Pullorum disease is widely distributed disease of fowl caused by salmonella pullorum. It is severe septicemia disease of domestic and wild fowl and remains an important disease for the poultry industry due to high morbidity and mortality. Disease can transmit both vertically and horizontally. The affected birds huddle under a heat source and are anorexic, depressed, dehydrated and have whitish fecal pasting around their vents. In mature birds pullorum disease is less severe or in apparent but characterized by decreased egg production, poor hatchability and fertility. Characteristic lesions include whitish nodes throughout the lungs and focal necrosis of liver and spleen. Tentative diagnosis mostly based on clinical signs, flock history, mortality and post mortem. Confirmatory diagnosis depends on the isolation of the organism and serological tests. Salmonella pullorum occasionally causes acute, self-limiting enteritis in people who eat massively contaminated food. Poultry meat is more popular in the consumer market due to easy digestibility and acceptance by the majority of people and it is also considered that poultry meat is still the primary cause of human food poisoning. Treatment of infected birds is required to decrease the rate of mortality and its spread in a flock but treated chicken remain carriers. Different salmonella serovars demonstrate varying levels of antimicrobial resistance. Successful control programs can be achieved by developing good hygiene and management together with routine serological tests and slaughter policy. Vaccination with effective vaccines to the layer or breeder flock is an important tool to control the pullorum disease. The disease is highly prevalent and cause huge economic losses due to high mortality, therefore, strict biosecurity measure should be conducted to prevent and control a disease.

Keywords: *Ethiopia, Poultry, Pullorum disease, Salmonellosis*

Introduction

Poultry is the largest livestock population in the world. They occupies a very crucial part of Ethiopian economy for being affordable, easily manageable and fast growing compared with other species of animals that provides people with animal protein. The total poultry population of Ethiopia is estimated as 57.01 million, which represents 60% of the total chicken population in East Africa (CSA, 2021). But the country, Ethiopia did not earn the expected production from its chicken flock population. From the factors that played an important role regarding this are poor husbandry practices, low productive breed of the birds and various viral and bacterial avian diseases (Netsanet *et al.*, 2012).

Chickens play an important role in the economy, supplying food, creating wealth through job creation for the booming population. The economic contribution of the poultry production sector is not still proportional to the huge chicken numbers, due to the presence of many productions, reproduction and infrastructural constraints. But, chickens are the backbone economy base of poor resource households in Ethiopia (Fikadu, 2021). Poultry played and still playing important social and cultural roles in the life of rural people for building social relationships with other villages. The contribution of smallholder poultry to the income and internal household position of women is also exist (Fenet and Alemayehu 2019).

The poultry production system in Ethiopia can be classified into three categories. Free-range chicken production system is practiced in most rural areas of the country and objectives of production are for household consumption and as source of additional income for the household. It covers 95-98% of the chicken production system of the country and it is not profitable since it is not market oriented. It contains small flock size (5-20) chickens per household) which are indigenous breed types mostly depend on locally available feed material as supplement with low health services and other management practices. Semi-intensive chicken production system is widely expanding in urban, peri-urban and rural areas of Ethiopia. The production is characterized with flock of 50–200 improved breeds per household kept in small night house with laying nest, feeder, vaccine and feed supplements. Intensive chicken production system is using more inputs (feeds and feeding, breed, health, housing and other inputs) than the above two chicken production systems. It is market oriented and the main objective of production is to get better profit. The number of chickens involved are relatively high (more than 200 chicken) (Fikadu, 2021). Chicken flocks are one of the most important sources of food and livelihood income in the world in general and in Ethiopia in particular (Alebachew and Mekonnen 2013).

Poultry production through introduction of exotic breeds was failed due to less adapted to local environment conditions, poor management; lack of input and output markets; and shortage of quality feeds, vaccines and veterinary inputs (Fikadu, 2021). Among bacterial disease salmonellosis particularly pullorum disease and fowl typhoid are the most common economically important poultry diseases (Endris, 2013). More than 2500 serotypes have been described mostly under the species only about 10% of these have been isolated from poultry (Bidhendi *et al.,* 2015). Among this pullorum disease is one of the commonest diseases of local poultry, which caused by salmonella pullorum species (*S. enterica subsp. enterica serovars pullorum*), which is a gram negative, non-motile, non-sporogenic and facultative anaerobic rod bacterium adapted to poultry. Including *Salmonella pullorum, Salmonella gallinarum*, *Salmonella enteritidis*, *Salmonella typhi* and *Salmonella dublin* are grouped under same sero-group D (Kabir, 2010). *Pullorum disease* is the most important global poultry diseases, cause a great economic loss due to high mortality rates which can reach up to 100%, decrease in production (eggs and chicks) and cost of medication both in humans and animals (Netsanet *et al.,* 2012). *Salmonellosis* is a common form of foodborne illness with major impact on human health and huge financial losses in poultry industry. Especially, increasing antimicrobial resistance in non-typhoid salmonella species has been a serious problem for public health worldwide (Niki *et al.,* 2017). *Salmonella* is one of the major causes of foodborne diseases worldwide (Zhao *et al.,* 2003). *Salmonella* infections are a worldwide major public health concern which is caused by non-typhoidal *S. enterica* serotypes (serotypes other than *S. typhi* and *S. paratyphi*) and is typically characterized by a self-limiting gastroenteritis syndrome (diarrhea, fever and abdominal pain), with an incubation period between 4 and 72 h and mortality being rare (Chen *et al.,* 2013). Poultry products are the major sources of human infection with non-typhoidal salmonella (Foley *et al.,* 2011).

Large flock sized farms and farms that used antimicrobials were substantially associated with the occurrence of salmonella. Imprudent use of antimicrobials in the farms can favor the continual occurrence of antimicrobial resistant salmonella within the human and animal population (Betelhem *et al.,* 2020). The most commonly detected *Salmonella* serovars in chickens in each geographic area are also among the top serovars associated with human infections indicating the nature of salmonella to colonize the poultry farms (Foley *et al.,* 2011). *Salmonella* spp. has been detected in a range of foods and outbreaks have predominantly been associated with animal products such as eggs, poultry. At the time of slaughter, *Salmonella* infected poultry may have high numbers of organisms in their intestines as well as body surface and are therefore an important source of contamination in the poultry processing plants (Niki *et al.,* 2017).

Based on study conducted in Assosa and Bambasi towns of Benishangul-Gumuz region the prevalence of *Salmonella* spp. was 23.2% (Asmamaw and Gebrehiwot 2018). Knowledge on distribution of salmonella serovars in food animals and humans is useful to recognize the trends of salmonella epidemiology and to identify serovars that cluster over time and space. Now a day poultry industry is dramatically increasing in the world as well as in Ethiopia so that numerous business persons, university graduates and unemployed are engaged in this sector by opening poultry farm, however most of them have scarcity of information about impact of pullorum disease and this gap motivated me to write this manuscript.

Objectives

General objective

- To review on current status of pullorum disease in Ethiopia

Specific objectives

- To review on prevalence of poultry salmonellosis in Ethiopia
- To discuss on potential risk factors for the occurrence of poultry salmonellosis disease.

To highlight on drug resistance and possible prevention and control measures.

Literature Review

Poultry production in Ethiopia

Poultry production is one of Ethiopia's most important livestock subsectors. It is crucial in terms of creating job opportunities, improving family nutrition and empowering women. It is an appropriate business for low income households due to the small amount of land required and the low investment required to start and run the operation (FAO, 2019). Ethiopia's poultry sector can be classified into village or backyard, small-scale and commercial poultry production systems.

Poultry production is important for the economies of the majority of the country's regions (Fisseha *et al,* 2010). They are important sources of cash income for women, and some of the proceeds from the sale of family chickens are used to pay for children's school fees. It is also important to the society as a whole, especially during the holidays and the festive season they are consumed in every household (FAO, 2019).

About 78.85% of the Ethiopian poultry population consists of indigenous chickens, while the remaining 21.14% consists of exotic and hybrid breeds of chickens. The indigenous chickens of Ethiopia have various names and characterized based on their ecological or main habitat, depend on qualitative characteristics. The molecular characterizations of some Ethiopian indigenous chicken ecotype were conducted. The improvements of the productivity of indigenous chicken ecotype are mainly focused on the introduction of high yielding exotic chickens (Fikadu, 2021). The population is projected to be around 57.01 million, with indigenous, hybrid, and foreign breeds accounting for 88.19 percent, 6.45 percent, and 5.36 percent of the total poultry, respectively (CSA, 2021). Despite their importance, the production and reproduction performance of chickens in the country is under expectation owing to the gap between low productive indigenous and low adaptive ability of highly productive chicken breeds (Bayesa, 2021).

Etiology

Salmonellosis/ pullorum disease is caused by genus Salmonella of family *Enterobacteriaceae* that consists of more than 2500 serologically distinguishable variants (serovars). *Salmonella pullorum* and *Salmonella gallinarum* are especially adapted to poultry. They cause diseases known as pullorum disease (*S. pullorum*) and fowl typhoid (*S. gallinarum*). However, these two bacteria have been placed in a single species, *S. enterica sub spp. Enterica serovar pullorum gallinarum* of the family *Enterobacteriaceae*, which is highly host adapted. Salmonella are responsible for a variety of acute and chronic diseases in poultry. Although recent taxonomic refinements have indicated that all salmonellae associated with poultry are members of a single genetically defined species *S. enterica* the distinctions between the various serovars of salmonella are often epidemiologically significant (Jajere, 2019).

The two non-motile serovars, *S. pullorum* and *S. gallinarum*, which are generally host specific for avian species. Pullorum disease, caused by *S. pullorum*, is an acute systemic disease of chicks or poults. Fowl typhoid, caused by *S. gallinarum*, is an acute or chronic septicemic disease that most often affects mature birds. Pullorum disease and fowl typhoid in terms of history, clinical signs, epizootiology, lesions, and control and eradication procedures have many similarities. *Salmonella pullorum* species (*S. enterica subsp. enterica serovar pullorum*) is closely related with organism *Salmonella gallinarum* species (*S. enterica subsp. enterica serovar gallinarum*) and others like *S. enteritidis, S. typhi* and *S. dublin* and grouped under sero-group D. They classified under the family *Enterobacteriaceae* and genus salmonella (Kabir, 2010).

Epidemiology

The epidemiology of salmonellosis is complex, which often makes the control of the disease difficult. The epidemiological patterns of infection and disease differ greatly between geographical areas depending on climate, population density, land use, farming practices, food harvesting and processing technologies and consumer habits. Additionally, because the biology of the serovars varies so greatly, it is impossible to avoid complexity when thinking about salmonellosis, salmonella infections or salmonella contamination. Salmonella spp. is one of the most important food borne pathogens and the third leading cause of human death among diarrheal diseases worldwide. The prevalence and diversity of salmonella enterica serovars in animal based foods (beef, pork, poultry, and seafood) throughout the five continents Africa, Americas North, Latin America, Asia, Europe, and Oceania (Ferrari *et al.,* 2019). The prevalence of salmonellosis in the different agro ecological areas has shown that this is due to the accessibility and waste disposal as well as hygienic practices of the society (Mengist *et al*., 2018). Poultry and the poultry environment serve as reservoirs for resistant and pathogenic salmonella. However, there was no evidence of transmission along the farm-to-fork continuum (Melissa *et al*., 2022).

Geographic distribution

Pullorum disease is widely distributed throughout the world but it has been eradicated from commercial poultry in many developed countries of Western Europe, the United States of America (USA), Canada, Australia and Japan (OIE, 2012). In many developing nations, including Latin America, the Middle East, the Indian subcontinent, Africa and perhaps other parts of the world's *Salmonella pullorum* infections are common and remain among the principal disease threats to poultry producers. In areas where they are absent from commercial chickens, Salmonella pullorum may still be present in backyard flocks and wild birds. In these areas, pullorum disease can also occur in intensively reared game birds including pheasants, partridges and guinea fowl (CFSPH, 2009). High prevalence has been reported from Thailand, Nigeria and other parts of Asia and Africa (Netsanet *et al.,* 2012).

Host range

Pullorum disease is host specific that affects mainly chicks under 3 weeks of age and the initial indication is usually excessive numbers of dead in shell chicks and death immediately after hatching, sometimes it may appear in adult birds. An infections can be found in many avian species including chickens, turkeys, quail, guinea fowl, pheasants, ducks, pigeons, sparrows, canaries, bullfinches and parrots; however, pullorum disease is uncommon except in chickens, turkeys and pheasants (Spickler *et al.,* 2010).

Morbidity and mortality

Pullorum disease is usually symptomatic only in young birds, but occasional outbreaks are reported in older animals. The morbidity and mortality varies by age, strain of bird, management, nutritional status, route and dose of exposure and other disease stresses like concurrent infections in the flock. PD in chicks can have up to 100 percent mortality, with the highest losses particularly in two to three week of age. Among chickens, lighter breeds such as leghorns are more resistant to pullorum disease than heavier breeds (Spickler *et* *al.,* 2010). Mortality is usually highest in chickens and poults and the morbidity rate is often significantly higher than the mortality rate and some birds recover (CFSPH, 2009).

Transmission

Salmonella can be spread by vertical transmission, via an egg-associated (trans-ovarian) transmission to progeny and horizontal transmission to other hosts including humans as well as various sources, including parent birds, feedstuffs, rodents, wild birds and other vehicles (Cui, 2013).

Vertical transmission

The vertical transmission may result when reproductive organs are infected with salmonella pullorum by direct contamination of the yolk, albumen, eggshell membranes or eggshells before ovi position (Cui, 2013). Localization of pathogen in the ovules before ovulation also probably establishes the chief mode of transmission due to this embryonic death and chick mortality are major problems (Haider *et al.,* 2014). Generally salmonella is having tendency to colonize in the lower part of the gut of chicken, cloaca, which can persist in both spleen and reproductive tract specially ovary and oviduct for a long period and infect eggs directly as with sexual maturation. Therefore, during process of egg formation there will be chance of infection on the pores of the egg. There are evidences that salmonella (pullorum*)* can become established in ova which results in vertical transmission of disease through yolk (Haider *et al.,* 2014).

Horizontal transmission

Chickens can get infected by salmonella via various vectors in their surrounding environment and then horizontally transmit it to others. These vehicles include feed and water, as well as wild animals. In one flock, salmonella can be transmitted via contaminated feces to individuals. Contaminated chickens become intestinal carriers, shedding the microorganism through their feces for long periods of time (Cui, 2013). Therefore feces, from infected birds are also an important source of bacteria for non-infected birds. Transmission may also occur within a flock as a result of cannibalism of infected birds, mating, egg eating and through wounds on the skin (Netsanet *et al.,* 2012). Contamination will also occur between flocks as contaminated transport vehicle, introduction of the contaminated birds or flocks and so on, may infect birds. Moreover, wild birds, mammals, rodents, insects etc. are generally regarded as the main reservoir for salmonella in the environment. Salmonella infected bird droppings contain salmonella that can contaminate the outer egg shells and may penetrate when crack the shell (Saha *et al.,* 2012).

Prevalence

Salmonella is prevalent in broilers, their feed and their environment and most of the isolates are resistant to most antimicrobials used in medical and poultry practices. This has significant implications for public health and antimicrobial resistance (Tesfaye *et al*., 2021). In Ethiopia, the current rates of mortality due to diseases from day old to adult chicken are estimated to be 20-50%. Among the diseases, pullorum disease is mentioned as it causes heavy economic loss which is similar to other African countries. The prevalence of Salmonella in exotic chicken indicates the potential importance of chickens as source of foodborne salmonellosis (Belege and Aragaw, 2020).

Table 1: Overall prevalence of salmonella infection in poultry farms in different parts of Ethiopia.

Study area	Sample type	Total sample	Overall prevalence	Author and year of publication
Jimma	Swab	384	41.9%	Alebachew & Mekonnen, 2013
Mojo	Swab	205	15.2%	Fufa *et al*., 2016
Haromaya town	Fecal	150	5.3%	Jelalu *et al*., 2016
Bishoftu	Swab	189	26.5%	Tesfaye *et al*., 2021
South Ethiopia	Swab	270	16.7%	Reta *et al*., 2017
Jimma town	Swab	415	2.65%	Diriba *et al*., 2019
Adama & Mojo	Swab	384	2.9%	Betelhem *et al*., 2020
Kafa zone	Fecal	302	9.27%	Sultan *et al*., 2021
Assosa & Bambassi	Swab	384	23.2%	Asmamaw & Gebrehiwot, 2018
Addis Abeba	Swab	200	11.5%	Yasin, 2022

Table 2: Prevalence of pullorum disease in Ethiopia

Study area	Prevalence	Author and Year of publication
Mekelle area	32.8%	Netsanet *et al*., 2012
Jimma town	41.9% (PD and FT)	Alebachew and Mekonnen, 2013
Central part of Ethiopia	64.2%	Ashenafi *et al*., 2003
Haromaya and Dire Dawa	31.9% (PD and FT)	Tadele *et al*.,2014
Hawassa	8%(PD and FT)	Kassaye *et al*.,2010

Risk factors

Risk factors for Salmonella infections include a variety of foods. Meats such as chicken and pork can be contaminated. A variety of vegetables and sprouts may also have Salmonella. Lastly, a variety of processed foods such as chicken nuggets and pot pies may also contain these bacteria. Consumption of food purchased from places other than supermarkets was the main modifiable risk factor for salmonellosis (Who *et al.,* 2021). Other established risk factors include recent contact with a diseased patient or carrier, eating ice cream, flavored iced drinks, or food from street vendors, and raw fruit and vegetables grown in fields fertilized with sewage (Mogasale *et al*., 2014). Poor waste disposal and hygiene of workers in food handling and preparation activities would provide an obvious infection route. The clinical characteristics of salmonellosis in large animals vary depending on the various management systems used, the intensity of stocking, whether or not the animals are housed and the epidemiological characteristics of the different salmonella spp. (Kim *et al.,* 2014).

Animal risk factors

The important facts are chickens are natural hosts and highly adapted for these pathogens. Chickens also become sub clinically infected carriers and pass the infections to their embryos vertically through eggs and horizontally to other chicken by contamination (Tadele *et al.,* 2014). Depending on the age ability of infection to survive increases with age and mortality is greatest in newly hatched chicks. Therefore, sero prevalence increased with age of birds and there was high significance difference because *salmonella pullorum* antibody found more in adult poultry is that young chicks will die shortly after hatching and a clinical sign in pullorum disease usually seen in chicks younger than 3 weeks old that it is difficult to get the antibody on these chickens unless survive and becoming carriers (Netsanet *et al.,* 2012).

Environmental and management risk factors

Crowding, malnutrition and other stressful conditions as well as unsanitary surroundings can exacerbate mortality and a performance loss, especially in young birds (Kabir 2010). The presence of chicken manure and other moist organic materials facilitate the survival and growth of pathogen by providing the required nutrients and physical protection. The low prevalence in the intensive might be because in the commercial poultry farms there is routine vaccination program, good ventilation, proper spacing of poultry houses and again there is no mixing of breeds (species). But in backyard farming systems such activities may not be performed and traditionally they used some drugs which might not be the appropriate on its dosage and its quality. Then after the chickens develop resistance against the diseases and this makes them to be continuing their life as carriers (Netsanet *et al.,* 2012).

Pathogen risk factors

Salmonella are facultative intracellular organisms that can withstand the bactericidal effects of antibodies while living in the phagolysosome of macrophages. Salmonellas are more tolerant of different environmental factors than other members of their family. They multiply at temperatures between 8 °C and 45 °C, at water activities above 0.94, and in a potential of hydrogen (pH) range of 4-8. They are also able to multiply in an environment with a low level of or no oxygen (Eguale *et al.,* 2016).

Pathogenesis and clinical signs

Pathogenesis

The pathogenesis and pathogenicity of salmonella depend on the invasive properties and the ability of the bacteria to survive and multiply within the cells, particularly macrophages (Dalai *et al*., 2015). Salmonella is usually orally taken by hosts and first need to pass the acidic environment of the proventriculus so that it can move into the small intestine. Thus bacterial resistance against these acidic conditions plays an important role in infection. The bacteria infect and multiply within the cells of mononuclear phagocytic systems of the chicks and turkey. The principal site of multiplication of these bacteria is the digestive tract which may result in widespread contamination of the environment due to bacterial excretion through feces. *Salmonella pullorum* specially targets the bursa of fabricus prior to eliciting inflammation in the intestine of chicks (Teferi and Nejash 2016). Following invasion through the intestinal mucosa, cecal, tonsils and bursa of fabricus, the organisms are taken up by macrophages and ultimately enters into blood called bacteremia (Dalai *et al*., 2015). From blood, bacteria are seeded into cells and tissues of different organs such as liver, lung, spleen, kidney, different parts of reproductive tracts of hens and testes of male and other tissues producing pathological lesions. It is also confirmed that the bacteria invade ovary and egg follicles and this infection persists in ovary and egg follicles and transmits into laid eggs then to hatched chicks (Haider *et al.,* 2013).

Clinical signs

Pullorum disease usually affects the first 2-3 weeks of age and occasionally occurs in adults (Rahman *et al*, 2016). In its acute form, a disease is almost exclusively a septicemic disease of young chickens. Clinical signs in chicks and poults include anorexia, diarrhea, dehydration, depression, huddling, ruffled feathers, weakness, pasting of the vent feathers, excessive numbers of dead in shell chicks and highest mortality after hatching occurs in 2-3 weeks of age. Feed and water intake dramatically reduced in infected group (OIE, 2012).

In mature bird's pullorum diseases is less severe or in apparent but laying hens are characterized by decreased egg production, poor hatchability and fertility. Trans-ovarian infection result an infection of the egg and hatched chicks or poults, which is one of the most important cause of embryonic death and chick mortality in poultry farm (Haider *et al*., 2014). Although clinical signs and post mortem findings of pullorum disease may be highly suggestive of the conditions, it is not sufficiently distinct from other causes of septicemia to be pathognomonic. It is therefore, necessary to confirm disease by isolation of the organisms. Serological tests can be used to establish the presence of the disease in a flock (OIE, 2012).

Drug resistance

Antimicrobial resistance is a bacteria's capacity to interfere with antimicrobial agents, hence reducing their fatal effect. Different salmonella serovars demonstrate varying levels of antimicrobial resistance. Antibiotic misuse in farm animals has dramatically contributed to the emergence and persistence of resistant strains. The global scenario has revealed a crisis in salmonella resistance since salmonella resistance to single antibiotics has been recorded and multiple drug resistance (MDR) has been reported worldwide (Raji, M.A.,

et al., 2021). In recent years, the antimicrobial resistance of salmonella spp. has increased due to the widespread use of antimicrobial agents in the food industry as veterinary medicine, growth promoting substances, prophylactics, and therapeutics. However, this undesirable trend has sparked a public concern since it is boosting not only the difficulty of treating salmonellosis but also the spread of antimicrobial resistance across the food chain supply, particularly among chicken and poultry products. Besides this, it has raised further concerns about the multidrug-resistant (MRD) strain. The MDR strains are those strains that are resistant to at least one microbial agent (Ricke and Calo 2015).

Salmonella isolated from litter, faces, and carcass found resistant to cefuroxime (45.2%), cefoxitin (1.9%), chloramphenicol (1.9%), nitrofurantoin (0.4%), pefloxacin (11.4%), and azithromycin (11%) (Melissa *et al*., 2022). From swab tested Salmonella isolates, 24 (92.3%) are intermediately or fully resistant to sulfisoxazole and streptomycin, 12 (46.2%) to cephalothin, while 11 (42.3%) are resistant to ampicillin, amoxicillin+clavulanic acid, kanamycin and chloramphenicol (Tadesse, 2018) and also 20 (80%) and 19 (76%) of Salmonella isolates from swab are resistant to streptomycin and tetracycline, respectively. There is also development of resistance to two or more antimicrobials Salmonella isolate from swab 19 (76%) (Betelhem *et al.,* 2020). Multidrug resistance (MDR) is observed among 3.8% of the salmonella isolates from fecal and all waste water, as well as 72.4% of carcass reinstate isolates are fully susceptible (Melissa *et al.,* 2022).

Salmonella isolates are all resistant to erythromycin and clindamycin. All isolates from swab are resistant to multiple antibiotics and sensitive to ciprofloxacin (100%) and chloramphenicol (87.5%) (Jelalu, K., *et al*., 2016). Multidrug resistance (MDR) to several drugs is common in *S. kentucky* and *S. saintpaul* (Tadesse, 2018). All isolates from fecal droppings were 100% resistant to oxytetracycline and ampicillin and among 28 isolated *S. enterica* species, 92.85% of them are showed multidrug resistance while (7.14%) of them showed extensively drug resistance. Out of this half of multidrug resistant isolates are resistant to 5–6 antimicrobials, while the remaining 7.14% of isolates showed resistance to 7 (seven) antimicrobials (Sultan *et al*., 2021). Detection of multidrug-resistant strains of salmonella in poultry farms suggests the need for detailed epidemiological and molecular studies to establish sources of acquisition of resistant salmonella strains (Betelhem *et al*., 2020).

Mechanisms of drug resistance in Salmonella

Antimicrobial resistance in Salmonella is mediated by several mechanisms that include drug inactivation, which is the most common cause of resistance. In this mechanism, antimicrobial agents are destroyed or inactivated through chemical modification using enzymes that catalyze reactions such as acetylation, phosphorylation, and adenylation. Salmonella can acquire antibiotic resistance by protecting the target site of the antibiotic, which can either be an enzyme or a specific cell structure. In addition, Salmonella can modify the antibiotic target site to avoid it from binding (Munita, 2016).

Another mechanism of resistance in Salmonella is through the reduction in its membrane permeability, thus preventing drug entry (Ugboko and De, 2014). The alteration occurs when membrane proteins undergo changes through new genetic information that alters the membrane transport system pores and thus prevents the passage of antibiotics. Salmonella has also developed the ability to pump out a drug after it has gained entry into the system, using efflux pumps. This mechanism is relatively nonspecific and can pump out many different drugs, including fluoroquinolones, ß- lactams and carbapenems (Munita, 2016). Many of these efflux pumps are encoded by genes within mobile elements such as plasmids. In Salmonella as well as in other bacteria, resistance to antibiotics may be mediated to a lesser extent by chromosomal mechanisms based on mutations in genes that code for either the target of the drug or the membrane transport system that controls the uptake of the drug. For example, chromosomal-mediated quinolone resistance may result from selective pressure on the bacterial population due to the uncontrolled use of the drug (Ugboko and De, 2014).

Diagnosis

Clinical diagnosis

Tentative diagnosis mostly based on clinical signs, flock history, mortality and postmortem. Most common clinical signs of pullorum disease in affected birds are huddling under a heat source and are anorexia, depression and have whitish fecal pasting around their vents. The mortality rate is high up to 100% occurs in birds of 2-3 weeks of age. Characteristic lesions include unabsorbed yolk, whitish nodes throughout the lungs and focal necrosis of liver and spleen (Kasech, 2015). Laboratory confirmatory diagnosis depends on the isolation of the organism while serological tests are satisfactory for establishing the presence and estimating the prevalence of infection within a flock (OIE, 2012).

Cultural isolation and identification

Salmonella is gram negative, facultative anaerobes, non-spore forming, and roads. This organism isolated from blood, crop, liver, spleen, heart, lungs, cecum, yolk and reproductive organ of hen after infection. They will grow on most standard nonselective media, as well as on selective media including MacConkey, brilliant green and xylose lysine deoxycholate agars (Haider *et al*., 2012). *S. pullorum* and *S. gallinarum* grow well in pure culture on non-selective media, but selective and enrichment media have been described that contain substances to inhibit the growth of extraneous organisms. *Salmonella pullorum* may grow slowly and produce very small colonies on selective media so incubation of plates for 48 hours is recommended. The efficiency of recovery of Salmonella varies according to circumstances and experience in the use of a medium is an important but unquantifiable factor. Some complex media may have an inhibitory effect on these organisms, so that it is advisable to use both non-selective and selective media for isolation from tissues. Non-inhibitory media include nutrient agar and blood agar, on which colonies are seen to be smooth, translucent, slightly raised, and about 1–2 mm in diameter. *Salmonella gallinarum* grows more rapidly than *S. pullorum* and produces larger colonies with a distinctive smell resembling that of seminal fluid on most media (OIE, 2018).

Growth morphological characteristics of salmonella on selective media

MacConkey agar

MacConkey agar is inhibitory to non-enteric organisms; it differentiates lactose fermenters (pink colonies) from non-lactose fermenters (colorless colonies). NaCl is omitted to limit the spread of proteus colonies. Salmonella colonies are smooth and colorless. *Salmonella Pullorum* produces smaller colonies than other salmonellae. MacConkey is the agar of choice for direct plating from tissues (OIE, 2018).

Xylose lysine deoxycholate agar

This agar is inhibitory to non-enteric organisms. Salmonella Pullorum grows sparsely as small red translucent colonies. S. Gallinarum colonies are small, dome-shaped, and may have a central black spot due to H2S production, but this reaction may be delayed or variable (OIE, 2018).

Brilliant green agar

Brilliant green agar (BGA) is inhibitory to coliforms and most Proteus strains; useful for distinguishing enteric organism colonies. Salmonellae form low, convex, pale red, translucent colonies of 1–3 mm in diameter, similar to Citrobacter. Proteus forms pin-point colonies, Pseudomonas aeruginosa appears as small red colonies, and lactose fermenters are green. Salmonella Pullorum produces smaller more pale colonies than other salmonellae. BGA is the agar of choice following enrichment, but low numbers of Salmonella can be easily overgrown by competing organisms when BGA is used for fecal and environmental samples (OIE, 2018).

Biochemical tests

Salmonella species do not ferment lactose and sucrose but fermenting dextrose and mannitol produced both acid and gas which is corresponded with the findings of others. *Salmonella pullorum* produce both acid and gas whereas *Salmonella gallinarum* produce only acid and all of the isolates were indole negative, methyl red positive and VP test negative which are special biochemical characters for salmonella spp. that previously suggested by a number of scientists (Hasan *et al.,* 2010).

Serological test

Serology can be used to detect infected flocks and estimate the prevalence of infection within a flock. Serological tests include the slide agglutination test (SAT), rapid whole blood agglutination test, rapid serum agglutination test, tube agglutination and ELISA (Spickler *et al*., 2010). The principle of the agglutination test is based on the presence of corpuscular antigen (such as bacteria), which is complex, by specific antibodies forming an antigen antibody network. This results in visible clumping of the antigen. By gravity, these clumps are deposited on the bottom of micro titer cup clearing the formerly turbid supernatant (Almaz, 2006).

The SAT test was performed according to the procedure described by OIE (OIE, 2012), with crystal violet stained standard *Salmonella pullorum* antigen. *S. pullorum* antigens of 30 µl and chicken sera of 30 µl were placed side by side with a micropipette on ceramic tiles (plate) and mixed thoroughly by stirring with tooth pick followed by rocking. The results were observed within two minutes. In positive cases agglutination or precipitation reactions were observed where as in case of negative there is no agglutination reaction (Netsanet *et al.,* 2012).

Differential diagnosis

The differentiation of disease symptoms and lesions produced by *Salmonella pullorum* (Pullorum disease) and *Salmonella gallinarum* (Fowl typhoid) are clearly indistinguishable. These bacteria are also very similar in terms of antigenic and biochemical properties. However, Fowl typhoid is mainly affects mature birds (OIE, 2012). White nodules in internal organs can be confused with Marek's disease or hepatic lesions caused by *Yersinia pseudotuberculosis* (Spickler *et al*., 2010). Respiratory tract lesions should be differentiated from Aspergillosis and joint lesions with synovitis and bursitis caused by other bacteria or viruses (Netsanet *et al*. 2012).

Necropsy findings

The gross lesions in young birds usually include unabsorbed yolk sacs and classic gray nodules in the liver, spleen, lungs, heart, gizzard, intestine and cheesy material in ceca, swollen and congested spleen and enlarged kidney. The gross lesions become reduce gradually after three weeks of infection. Small lesions in the liver and spleen of pullorum infected birds may show a 'white spot' appearance that is not seen with gallinarum; however, this lesion is not pathognomonic (OIE, 2012). Adult carriers may develop misshapen or shrunken ovaries with atrophic, regressing ovarian follicles. The affected ova contents of the yolk sac may be coagulated, creamy or caseous material enclosed in a thickened capsule. These degenerative ovarian follicles were closely attached to the ovary and they were pedunculated and sometimes detached from the ovarian mass. In a cock white foci in the testis are observed after two weeks infection and size of the infected testes are reduced compared to the normal testes (Haider *et al*., 2013).

Treatment

No treatment is likely to effect complete elimination of carrier from infected birds. Treatment of infected birds is required decrease the rate of mortality and its spread in a flock. To combat the dehydration loss due to diarrhea fluid and electrolyte therapy is the first line of treatment for the affected birds. Antibiotics therapy either in intramuscular or oral route are recommended for reducing the infection. Antimicrobial therapy using antibiotics should be done after conducting antimicrobial susceptibility test as R-plasmids are common in coding for multiple resistance in salmonellae spp. (Dalai *et al*., 2015), because it shows resistance against many antimicrobials; tetracycline, aminoglycosides and sulpha drugs. Amoxicillin, potentiated sulfonamide, tetracycline, fluoroquinolone are a drug of choice for the treatment of salmonellosis (Tadele *et al*., 2012).

Table 3: Traditional medicinal plants used for treating salmonellosis in chickens

Local name	Scientific name	plant parts used	Application method
Nim	Azadirachta indica	Leaf	Fresh leaf chopped and added to drinking water
Shiferaw	Moringa stenopetala	Leaf	chopped fresh leaf mixed drinking water and then given
Lemon	Citrus lemon	Fruit	Extracted juice mixed with little water and then given
Nech beharizaf	Eucalyptus globulus	Leaf	Chopped fresh leaves mixed water and given as drinking water
Manderin	Citrus reticulata	Fruits	Extracted juice mixed with little water and then given

Source: (Shishay, 2019).

Prevention and control

Management strategies

The principal management procedures should include strict biosecurity measures. Proper biosecurity measures will prevent the spread of infective material from house to house and from farm to farm. Successful control programs can be achieved by developing good hygiene and management together with routine serological tests and slaughter policy. Repeated testing and removal of carriers can sometimes eliminate the infection from a flock. More often, the entire flock is depopulated and the premises are cleaned and disinfected before restocking (Teferi and Nejash, 2016).

The eradication of pullorum disease requires the establishment of infection-free breeding flocks. Poultry should be purchased from certified infection-free stock or tested before adding them to a flock. Since egg transmission plays an important role in the spread of disease, only eggs from flocks known to be free of PD should be introduced into hatcheries. They should be hatched and reared in clean conditions where they cannot contact infected birds, dead birds and potentially infected surface water, or other sources of organisms (Kabir, 2010).

Houses should be designed to exclude rodents and free-flying birds. The premises and mechanical carriers like footwear, human clothing, hatchery disciplines, equipment's, litters, incubators, trucks and processing plants should be cleaned and disinfected regularly for biosecurity (Teferi and Nejash, 2016). Compounds that contain phenol are the most effective disinfectants under field conditions, but quaternary ammonium compounds and iodophores may be used. However, control of these diseases is complicated by vertical transmission since hens become sub clinically infected carriers and pass the infections to their embryos in the egg (Spickler *et al.,* 2010).

Vaccination

Pullorum vaccines are used in chickens in some countries where the disease is endemic. Vaccination can reduce clinical disease and mortality (Spickler *et al.,* 2010). For prevention and control of the PD and other Salmonella infections, vaccination with effective vaccines to the layer or breeder flock is the most important tool. Both live attenuated and killed vaccines have many benefits and both vaccines are considered as potential to control the host specific salmonella in poultry by reducing the mortality and fecal shedding to the environment.

Economic importance

Salmonella infection is one of the most important global poultry diseases in avian species because of its huge economic impact, worldwide distribution and difficulty posed in the control of the disease (Kabir, 2010). *Salmonella pullorum* is the main cause of considerable economic importance in the poultry industry, particularly in developing countries with a poultry industry. The pathogen not only can cause high mortality rates among young chicks but also persists for a long period in the spleen and the reproductive tract, leading to the infection of eggs or progeny. A great economic loss due to the disease is that it causes high mortality rates which can reach up to 100%, decrease in production (eggs and chicks), condemnation of affected carcass and cost of medication both in humans and animals. Direct health costs such as hospitalization, consulting a physician and laboratory testing as well as the costs of lost labor in relation to a case of salmonellosis are estimated as part of a multidisciplinary task (Netsanet *et al*., 2012). Eradication of the carrier parent flocks and grow out chicken and there placement by new chicken also cause significant economic loss. Exportable eggs and meat of chickens must be free from salmonella, which cause great effect on currency of one country getting from the sector. It remains a serious economic problem to livestock in countries where measures of control are not efficient or in those where the climatic conditions are favor for spread of these microorganisms (Hossain *et al.,* 2011).

Public health importance

Poultry meat is more popular in the consumer market due to easy digestibility and acceptance by the majority of people and it is also considered that poultry meat is still the primary cause of human food poisoning (Dalai *et al.,* 2015). Salmonella is the bacterial agent most frequently involved in cases of food borne disease all over the world. The agent is normally transmitted to humans by means of foods of animal origin, such as meat and eggs. *Salmonella typhimurium* and *salmonella enteritidis* is the most common agent of the food borne disease in humans. PD has also public health significance. The diseases can spread via meat and eggs. *Salmonella pullorum* occasionally causes acute, self-limiting enteritis in people who eat massively contaminated food (Haider *et al*., 2013).

Salmonella infections in humans can range from a self-limited gastroenteritis usually associated with non-typhoidal Salmonella (NTS) to typhoidal fever with complications such as a fatal intestinal perforation (OIÉ, 2019). Outbreaks of Salmonellosis have been reported for decades, but within the past 25years the disease has increased in incidence in many continents. The disease appears to be most prevalent in areas of intensive animal husbandry (OIÉ, 2019). The incubation period in people is variable but is usually between 12 and 36 hours. Salmonella invade epithelial cells in the ileum and proliferate in the lamina propria and profuse, watery diarrhoea results. Some isolates produce a heat-labile enterotoxin, which initiates diarrhea. Sequelae include post-enteritis reactive arthritis and reiter's syndrome and systemic infection can result. Individuals can develop carrier status of up to 6 months in duration (Buncic, 2006)

Despite mounting concerns about other pathogens in recent years, salmonella remains among the leading causes of foodborne disease throughout the world. According to the Centers for Disease Control and Prevention (CDC, 2022), Salmonellae are responsible for at least 1.4 million illnesses, 15,000 hospitalizations and 400 deaths each year in the United State of America. Salmonella outbreaks can have particularly severe consequences for highly vulnerable populations in facilities such as day care centers and nursing homes. Poultry products are frequently identified as important sources of salmonellae that cause human illness. An estimated 182,060 Americans became infected with *Salmonella enteritidis* during 2000 after consuming contaminated eggs (Schroeder *et al*., 2005).

The CDC regularly reports Salmonella outbreaks that are associated with poultry and poultry products (CDC, 2005). These food products are generally recognized as a primary source of salmonellosis. Poultry and eggs are considered one of the most important reservoirs from which Salmonella is passed through the food chain and ultimately transmitted to humans. With increasing consumption of poultry and poultry products, the number of salmonellosis associated with poultry continues to be a public health issue in the US. Transmission of Salmonella to humans typically occurs when ingesting foods that are directly contaminated by animal feces or cross contaminated by other sources (Modaressi *et al*., 2010). Depending on the host species, age and pathogenicity of the microorganism and its adaptation to the host, Salmonella may cause severe disease, or go unnoticed and remain in the host for months or years. In this case, the host will be a reservoir of the bacteria for susceptible animals. The most common symptoms include diarrhea, abdominal pain, vomit and nausea, and may occur together with prostration, muscle pain, drowsiness and fever. Although symptoms generally disappear after 5 days, the microorganisms may be excreted in the feces for many weeks. Children, mainly those younger than 1 year of age, elderly and immune-compromised patients are much more susceptible to the disease, and may present more severe infections, such as sepsis, which may lead to death (Pinto et al., 2004).

Conclusion And Recommendations

Pullorum disease is widely distributed disease of fowl caused by *Salmonella pullorum*. It is an important disease for the poultry industry due to high morbidity and mortality mostly in young chicks up to 2 to 3 weeks of age. Chickens are natural hosts and highly adapted for these pathogens. It has been eradicated from commercial poultry in many developed countries of Western Europe, USA, Canada, Australia and Japan but it is prevalent in countries like Latin America, the Middle East, the Indian subcontinent, Africa and perhaps other parts of the world's. The disease can transmit both vertically (trans-ovarian) and horizontally through different contamination. Despite mounting concerns about other pathogens in recent years, salmonella remains among the leading causes of foodborne disease throughout the world. Antibacterial treatment helps to reduce mortality; however, a pathogen resistance against many antimicrobials and treated chicken remain carriers. It also cause huge economic losses due to high mortality and difficulty posed in the control of the disease. Salmonella infections in humans can range from a self-limited gastroenteritis usually associated with non-typhoidal Salmonella to typhoidal fever with complications such as a fatal intestinal perforation.

Therefore, based on the above conclusions the following recommendations are forwarded:

- Poultry and hatchery eggs should be purchased from certified infection-free stock.
- Principal management and strict biosecurity measure should be conducted to prevent introduction of disease.
- Using antibiotic therapy should be done after conducting antibiotic susceptibility test.
- Effective vaccine should be produced for disease serotype.

- Farmers should be advised and educated on the use of salmonella free parents

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