Pathological Investigation on different forms of *E. coli* infections (colibacillosis) in broiler chicken and molecular confirmation

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Roll No.: 0121/05

Registration No.: 924

Session: January - June, 2021

A thesis submitted in the partial fulfillment of the requirements for the degree of Masters of Science in Pathology

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June, 2023

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June, 2023

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Acknowledgements

I would like to express my gratitude to Allah for enabling me to successfully complete my Master of Science (MS) dissertation in Veterinary Pathology at the Department of Pathology and Parasitology (DPP), Chattogram Veterinary and Animal Sciences University (CVASU). This accomplishment is a culmination of the support and guidance provided by various individuals, and I extend my heartfelt appreciation to all of them.

I am grateful to my supervisor **Professor Dr. Sharmin Chowdhury** and co-supervisor **Associate Professor Dr. Md. Shafiqul Islam**, Department of Pathology and Parasitology (DPP), Chattogram Veterinary and Animal Sciences University (CVASU). Their insightful guidance helped me a lot during the research work and writing the thesis paper, for which I would like to sincerely thank them.

I would like to thank **Professor Dr. Mohammad Mahbubur Rahman**, Department Head (previous), and **Professor Dr. Md. Abdul Alim**, Department Head (current), Professor **Dr. Md. Masuduzzaman**, **Professor Dr. Mohammad Alamgir Hossain**, **Professor Dr. AMAM Zonaed Siddiki**, Department of Pathology and Parasitology, CVASU, for providing technical support, laboratory facilities and valuable suggestions during this research work.

I wish to thank **DR. Subrata Paul** and **DR. Anamika Dhar** for assisting me during my research work regarding sample collection and tissue processing. I sincerely appreciate **DR. Muhyminul Islam**, **DR. Umme Aysha Habiba** for supporting me during the completion of my Master's degree. The author would like to acknowledge Advance Study and Research, CVASU for providing necessary research funds for this research work. The author humbly thanks to all the staffs of Department of Pathology and Parasitology of CVASU.

Finally, I would like to express my sincere gratitude to my husband **Sheikh Sayed Bin Rahman**, family members, friends and well-wishers for their support, encouragement, and blessings throughout my entire life.

The Author

June, 2023

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Abstract

Avian colibacillosis is a significant global infectious disease in birds of all ages, leading to substantial economic losses in poultry production. This disease is caused by *Escherichia coli* infection which is a highly prevalent bacterial pathogen in man and animals. Colibacillosis is a multifaceted syndrome distinguished by various organ abnormalities, including air sacculitis (61.67%), pericarditis (58.33%), perihepatitis (33.33%), yolk sac infection (35%), enteritis (31.67%). Research involving pathological examinations was conducted on spontaneous occurrences of avian colibacillosis to investigate the prevalence and pathological manifestations of *Escherichia coli* infection. After necropsy, we collected samples from lung, liver and spleen for bacterial isolation and subsequently for histopathological examinations. Following the bacterial isolation process, when we cultured the bacteria on agar, the outcomes revealed colonies with a distinct pink coloration on MacConkey agar and a distinctive metallic sheen on EMB agar. These characteristics led to the identification and consideration of *E. coli* in 45 cases. To confirm the presence of *E. coli*, PCR was conducted using the housekeeping gene primer (adk), resulting in the confirmation of *E. coli* in 37 suspected cases initially identified from agar cultures. Microscopic examinations revealed severe congestion (83.33%), hemorrhage (90%), focal area of necrosis (76.67%), prominent nuclear and cytoplasmic changes in hepatocytes with like fatty change (33.33%) in liver. In lung, edema (20%) and congestion (75%) were commonly observed almost all cases with thickening of alveolar septa. Hemorrhage and congestion (66.67%) also observed commonly in spleen along with depletion of lymphoid elements and multiple focal areas of necrosis (33.33%) in the primary follicles resulted in the formation of secondary germinal centers. Depletion of lymphocytes at times also accompanied by reticular cell proliferation (50%) in spleen. This study underscores the significant cellular-level changes associated with colibacillosis in commercially raised broiler chickens and suggests the need for further investigation to identify the specific serotypes of *E. coli* present in these chicken populations.

Key Words: postmortem, histopathology, lung, liver, spleen, congestion, hemorrhage, necrosis, bacterial culture, PCR, *E. coli*, broiler.

Chapter 1: Introduction

Since the start of the 21st century in Bangladesh, the poultry industry has emerged as a significant sector for generating quick profits, creating employment opportunities, and producing affordable animal protein (Saleque and Ansarey, 2020). The country's primary poultry species include chicken, duck, quail, pigeons, and turkey. Furthermore, chickens can be categorized into four primary types: Broiler, Layer, Sonali, and Local Indigenous.

Despite the growth, the poultry sector in Bangladesh faces various challenges and economic losses. Colibacillosis is a significant factor contributing to economic issues. losses in the global poultry industry caused by *E. coli* It is also a part of the normal intestinal flora in birds. (Zanella et al., 2000). Specific strain with characteristics associated with virulence, referred to as avian pathogenic *E. coli* (APEC) are capable of causing various diseases in poultry, including aerosacculitis, pericarditis, acute colisepticaemia,, omphalitis, yolk sac infection (Dozois et al., 2003; Vidotto, 1990; Gomis, 1997; Pourbakhsh et al., 1997; Dho-Moulin and Fairbrother, 1999). Colibacillosis can manifest in various forms depending on factors such as the bird's age, infection route, and virulence factors of the *E. coli* strain (Zanella et al., 2000).

Septicemia, a form of colibacillosis caused by *E. coli*, is characterized by sudden death in young broilers and rapid onset of symptoms such as depression, loss of appetite, and diarrhea. It can also lead to complications like pericarditis, perihepatitis, and peri-metritis in affected birds (Nakamura et al., 1985, Renu et al., 2012, Gangane et al., 2006, and Kumar et al., 2013). Omphalitis, also known as yolk sac infection or omphalophlebitis, is a common *E. coli* infection in newly hatched chicks. It occurs when bacteria or fungi enter the chick's body through the umbilical region. Signs of omphalitis in broilers include abdominal swelling, increased white blood cell count, and delayed absorption of the yolk sac.

Respiratory colibacillosis poses significant challenges to the poultry industry. It can manifest as chronic respiratory disease, swollen sinuses, or infectious coryza, making it difficult to differentiate from other respiratory infections like avian influenza (Barnes and Gross, 1990; Ewers et al., 2004). Clinical signs may include coughing, sneezing, nasal discharge, facial swelling, and conjunctivitis.

Several virulence factors contribute to the pathogenesis of colibacillosis. F1 (type 1) fimbriae enable adherence to the respiratory epithelial cell in the chicken's trachea and pharynx, while Temperature-sensitive hemagglutinin (Tsh) plays a role in colonizing air sacs.

The diagnosis relies on isolating and identifying *E. coli* from lesions for colibacillosis (Dziva and Stevens, 2008). It is crucial to prevent fecal contamination of samples. Caution should be exercised when isolating the infectious agent from the organs of decomposing birds, as *E. coli* easily migrates from the visceral organ to surrounding tissues in deceased birds (Lee, 2008). Another diagnostic method involves examining various lesions observed during post-mortem findings and further analyzing them under a microscope by preparing histopathological slides (Nolan et al., 2013)

Recognizing the specific damages caused by the disease is important for administering appropriate treatment. Acknowledging the importance of precise diagnosis and treatment based on pathological abnormalities, our emphasis in this study has been on investigating postmortem discoveries, molecular identification, and histopathological examinations related to colibacillosis. The research has specifically concentrated on enhancing diagnostic and treatment effectiveness through a comprehensive exploration of pathological and molecular aspects, aiming to improve the overall management of this disease.

Objectives:

- Examine the post mortem findings of *E. coli* affected chickens.
- Histopathological analysis of affected organs.
- Molecular identification of bacteria.

Chapter 2: Review of literature

Escherichia coli has been identified as the primary cause of colibacillosis, a disease with a global distribution and significant economic consequences, particularly in the broiler industry. The infection results in various health issues such as morbidity, lack of flock uniformity, reduced production, increased condemnations at slaughterhouses, and higher mortality rates (Barnes et al., 2003). Initially, the infection leads to septicemia, and in some cases, sudden death may occur. In other instances, localized inflammation in different organs can manifest, leading to conditions like pericarditis, perihepatitis, airsacculitis, salpingitis, omphalitis, and more (Dziva and Stevens, 2008). Given the substantial economic impact of avian colibacillosis, it is crucial to promptly and accurately diagnose the disease.

2.1: Broiler industry in Bangladesh

Since the start of the 21st century in Bangladesh, the poultry industry has emerged as a significant sector for generating quick profits, creating employment opportunities, and producing affordable animal protein (Saleque and Ansarey, 2020). The country's primary poultry species include chicken, duck, quail, pigeons, and turkey. Additionally, there are four main types of chickens: Broiler, Layer, Sonali, and Local indigenous.

As of the 2020-2021 production year, Bangladesh had a total of 365.8 million poultry, with 304.1 million being chickens (DLS, 2021). The country currently has more than 53,000 broiler farms and 18,000-layer farms operating at various scales. Sonali chickens constitute 28% of the overall chicken population in the country. Furthermore, approximately 6% of domestic chickens are raised in home gardens in rural areas. Sonali chickens are highly sought after and command higher prices due to their completely natural growth process (BBI, 2022).

In Bangladesh, the poultry industry has witnessed significant growth in recent years, providing employment opportunities to around 6.0-8.0 million people (Ahmed, 2019; OHPH., 2020; Saleque and Ansarey, 2020). The Department of Livestock Services (DLS) has registered 16 Grand Parent (GP) farms and 206 parent stock (PS) farms/hatcheries (BPICC, 2020; DLS, 2021). The country is also home to 113 poultry companies (BAB, 2020), 96 feed mills (BPICC, 2020), and 30 veterinary

pharmaceutical companies (BBI, 2022). In the 2020-2021 production year, Bangladesh produced 20,574.6 million eggs and 8.44 million metric tons of meat, surpassing the demand of 17,659.2 million eggs and 7.437 million metric tons of meat (DLS, 2021). This increase in production has led to a rise in per capita egg and meat consumption.

Despite the growth, the poultry sector in Bangladesh faces various challenges and economic losses. Lack of experience in poultry rearing, limited biosecurity and management system training, and farm security issues have been identified as major concerns (Rahman et al., 2021; Islam et al., 2014). Disease outbreaks and market price fluctuations have also led to farm failures and financial losses for poultry farmers (Islam et al., 2014).

The poultry industry in Bangladesh is further hindered by the emergence of diseases, escalating feed and medicine costs, and the global challenge of antimicrobial resistance (AMR). AMR not only increases production costs but also brings about diagnostic and treatment expenses, along with economic losses due to treatment failures (Mandal and Khan, 2017).

2. 2: Live Bird Market in Bangladesh

Poultry-based businesses have gained significant popularity and have become one of the leading sectors within the agribusiness industry in Bangladesh. In the 2018-2019 fiscal year, the livestock sector contributed 1.5% to the country's GDP, with a significant portion of this contribution coming from the poultry industry (BBS, 2018-19). In Bangladesh, it is common for people to purchase live birds from small stalls for poultry meat, and these stalls can be found in almost every region of the country. In larger

Once a batch of birds is sold, the shop owners clean up the stalls. However, there is often a lack of awareness about proper hygiene practices among the shop owners, resulting in the disposal of waste materials, which can potentially contaminate drain water and even groundwater. Consequently, when these live bird markets become contaminated with foodborne infections, they can pose a significant risk (Kabir et al., 2021).

2.3: Overview on colibacillosis

Avian colibacillosis, caused by *Escherichia coli,* is considered one of the primary causes of morbidity and mortality in poultry, either as a primary or secondary pathogen (Kabir, 2010). The gas exchange regions of the lung and air sacs are presumed to be the sites of entry into the bloodstream for *E. coli*, as they lack resident macrophages and are susceptible to bacterial invasion and colonization (Mellata et al., 2003). While colibacillosis can affect chickens of all age groups, broiler chickens between 4-6 weeks of age are particularly vulnerable and experience higher mortality rates (Leitner and Heller, 1992).

Colibacillosis is a complex syndrome characterized by multiple organ lesions such as air sacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis, or yolk sac infection. In specific avian species, *E. coli* has been associated with peculiar diseases. For example, in chickens, swollen head syndrome often results from a synergistic infection of turkey rhinotracheitis virus and E. coli (Stehling et al., 2003).

Colibacillosis, particularly the form known as colisepticemia, which involves respiratory tract infection followed by septicemia, is a leading cause of morbidity and mortality in poultry globally. It results in significant economic losses (Barnes and Gross, 1997; Ewers et al., 2003). While death is the typical outcome of colisepticemia, some birds may recover completely or with residual sequelae such as meningitis, panophthalmitis (swollen eye), osteoarthritis, synovitis, and coligranuloma (characterized by multiple granulomas in the liver, caecum, duodenum, and mesentery) (Barnes et al., 2003).

Colibacillosis is a significant problem in commercial poultry worldwide. It can lead to substantial losses, especially in cases of poor management or when birds are under stress, such as co-infections with chronic respiratory disease (CRD) or mycoplasma (Talha et al., 2001). *E. coli* primarily colonizes the lower intestine of warm-blooded animals and birds, causing gastroenteritis (Pelczar et al., 1986). With the expansion of poultry farming, colibacillosis has become a widespread issue in Bangladesh (Islam et al., 2003; Hossain et al., 2004).

Colibacillosis in broilers and layers in Bangladesh results in substantial economic losses due to increased morbidity, mortality, reduced production, and poor chick quality

(Islam et al., 2003; Rahman et al., 2003; Rahman et al., 2004; Hossain et al., 2004). Severe outbreaks can lead to mortality rates as high as 94% (Biswas et al., 2006; Haider et al., 2003; Roy et al., 2006).

2.4: *E. coli*

Escherichia coli is a type of Gram-negative bacterium, characterized by its oxidase-negative property and facultatively anaerobic nature. It appears as a straight, cylindrical rod, measuring 1.1-1.5 x 2.0-6.0, and belongs to the coliform group commonly found in the lower intestine of warm-blooded organisms (Tenaillon et al., 2010). The genus *Escherichia* diverged approximately 102 million years ago, aligning with the divergence of its hosts (Battistuzzi et al., 2004). While most *E. coli* strains are harmless, certain serotypes can lead to severe food poisoning in their hosts, occasionally causing product recalls due to food contamination (Vogt and Dippold, 2005). The non-pathogenic strains are part of the normal gut microbiota and can be beneficial to their hosts by producing vitamin K2 (Bentley and Meganathan, 1982).

E. coli is a normal resident of the avian intestinal flora, certain strains known as avian pathogenic *E. coli* can cause a range of diseases in poultry, resulting in substantial economic losses. (Barnes et al., 2008). Colibacillosis can affect various systems in poultry, including the respiratory, digestive, reproductive, and locomotor systems, as well as causing localized infections such as yolk sac infections, omphalitis, dermatitis, cellulitis, and panophthalmitis. These infections often occur as secondary infections, complicating primary diseases (Yogaratnam et al., 1995, Jakob et al., 1998, Georgopoulou et al., 2005).

O-specific antigens, which are components of lipopolysaccharides (LPSs) on the bacterial cell surface, are important markers used in the diagnosis of APEC strains. These antigens provide protection against immune system factors and play a role in immune evasion mechanisms. Serotyping is commonly used to determine the source of an outbreak and the pathogenicity of a strain, although it does not provide detailed information about the virulence characteristics of the bacteria (Mehat et al., 2021).

2.4.1: Mode of Transmission

Escherichia coli bacteria are commonly found in the intestinal tracts of most animals and are excreted in feces, often in substantial quantities (Mandal and Khan, 2017). New strains can enter poultry flocks through direct or indirect contact with other animals or their feces. Free-living birds, particularly ducks and passerine birds like European starlings, play a significant role in this transmission as they carry *E. coli* strains adapted to avian species. An example of a particularly potent strain, O86 APEC, (Vogt and Dippold, 2005) which has been reclassified as *E. albertii*, caused considerable mortality in wild finches in Britain but hasn't been detected in poultry.

In experiments involving laying hens, both their trachea, ceca (part of the digestive system), and oviduct (egg-producing organ) remained persistently colonized for at least 21 weeks after exposure to pathogenic *E. coli* (Mandal and Khan, 2017). Interestingly, although *E. coli* was present in the oviduct, it was not found on the eggshells.

The transmission of *E. coli* among poultry houses and farms can also involve darkling beetles, both in larval and adult stages. These beetles can become infected by consuming contaminated larvae or feces and subsequently transmit the bacteria. Houseflies also contribute to *E. coli* dissemination, with adult flies acting as mechanical vectors and their larvae developing digestive tract infections upon ingestion of bacteria. Infected houseflies can persist as reservoirs for virulent strains through their pupal and adult stages, enabling the transfer of antibiotic resistance and virulence genes.

2.4.2: An overview of *E. coli* bacteria in relation to poultry

Pathogenicity: APEC strains possess specific virulence factors that enable them to cause disease in poultry. These factors include fimbriae (such as type 1 fimbriae and P fimbriae) that aid in bacterial adherence to respiratory and internal organs, temperature-sensitive hemagglutinin (Tsh) that contributes to air sac colonization, and the aerobactin iron-sequestering system that facilitates growth in low iron environments (Dozois et al., 1994; Dho-Moulin and Fairbrother, 1999; Pourbakhsh et al., 1997).

Clinical Manifestations: Avian colibacillosis is the term used to describe the disease caused by APEC. It encompasses various syndromes, including septicemia, respiratory infections, cellulitis, omphalitis, and yolk sac infections (Vidotto et al., 1990). The

severity and specific clinical signs depend on the strain's virulence factors, the site of infection, and the bird's age and immune status (Barnes and Gross, 1997; Ewers et al., 2003).

Economic Impact: Avian colibacillosis can result in significant morbidity and mortality in poultry, leading to economic losses in the form of reduced growth rates, decreased feed conversion efficiency, increased medication costs, and bird mortalities (Kabir et al., 2021). It is considered one of the major causes of morbidity and mortality in the global poultry industry.

Antibiotic Resistance: *E. coli* strains in poultry have exhibited increasing resistance to commonly used antibiotics, posing a challenge for disease control and treatment (Vogt and Dippold, 2005). This resistance can arise from both intrinsic and acquired factors, including the horizontal transfer of resistance genes.

Prevention and Control: Effective control and prevention strategies for avian colibacillosis include good management practices, biosecurity measures, vaccination programs, and responsible antibiotic use (Gomis et al., 1997; Pourbakhsh et al., 1997). Biosecurity protocols should focus on minimizing stress, providing proper ventilation, maintaining hygiene, and preventing contact with potential sources of infection (Dho-Moulin and Fairbrother, 1999).

2.4.3: Diseases caused by *Escherichia coli*

Avian pathogenic *E. coli* strains, also known as APEC (Dho-Moulin and Fairbrother, 1999), are associated with various diseases, particularly extraintestinal infections, causing significant losses in the avian industry (Gross, 1994). These diseases include inflammation of the oviduct, leading to reduced egg production and sporadic mortality in laying chickens and breeders. Additionally, APEC can cause salpingitis, which occurs when *E. coli* ascends from the cloaca to the oviduct, leading to peritonitis (Bisgaard et al., 1995).

Coli septicemia is a crucial disease caused by APEC strains and is thought to originate in the avian upper respiratory tract after primary infections by different viruses, such as Newcastle virus, Infectious Bronchitis virus, or Mycoplasma (Gross, 1994). These primary infections increase avian susceptibility to APEC strains due to cell damage in the upper respiratory tract and exposure to ammonia and contaminated dust in the

environment (Oyentude et al., 1978; Nagaraja et al., 1984). The respiratory infection caused by APEC strains, combined with viral infections, is considered the initial step in colisepticemia development in birds (Gross, 1994). Colisepticemia, also known as aero sac disease, mainly affects birds aged 2 to 12 weeks, with higher mortality rates in birds aged 4 to 9 weeks, sometimes reaching 20% (Dho-Moulin and Fairbrother, 1999). While death is the typical outcome of colisepticemia, some birds may recover, albeit with residual sequelae such as meningitis, swollen eye (panophthalmitis), osteoarthritis, synovitis, and coligranuloma (Hjarres's Diseases), characterized by multiple granulomas in the liver, cecum, duodenum, and mesentery (Bisgaard et al., 1995).

Some studies have shown a positive relationship between APEC and human extraintestinal pathogenic *E. coli* (ExPEC), particularly uropathogenic *E. coli* (UPEC) and newborn meningitis-causing *E. coli* (NMEC), suggesting that certain APEC strains may be potential zoonotic agents (Ewers et al., 2007; Moulin-Schouleur et al., 2007; Johnson et al., 2008).

2.5: Pathobiology of *E. coli*

2.5.1: Incidence and Distribution

Escherichia coli, commonly known as *E. coli*, have a wide distribution and can be found in the intestines of many animals, including humans (Broom and Kogut, 2018). They are beneficial in the lower intestinal tract, aiding in growth and inhibiting other harmful bacteria like Salmonella. While they are prevalent in most mammals and birds, healthy parrots might be an exception (Nakamura et al.,1985). *E. coli* is common in poultry intestines, particularly in young birds and those without an established normal flora. Different strains of *E. coli* colonize the cecal mucosa, and some strains persist over time while others are transient.

Around 10%-15% of coliforms in normal chickens could be potentially harmful serotypes. These intestinal strains might differ from those found in other parts of the same bird. *E. coli* in the intestines can harbor virulence factors and antimicrobial resistance. Pathogenic *E. coli* can be transmitted through eggs, leading to high chick mortality. Some strains that are resistant to antibiotics were passed from normal breeders to chicks, causing significant mortality (Nolan et al., 2015).

The main source of egg infection is fecal contamination on the egg surface, which can then penetrate the shell and membranes (Pourbakhsh et al., 1997). While coliform bacteria are present in litter and feces, *E. coli* makes up a small portion of the total bacterial population in litter. Environmental isolates of *E. coli* are distinct from those found in birds. Dust in poultry houses can contain *E. coli*, both inside and outside the houses. These bacteria can persist for a long time, especially in dry conditions (Vidotto et al., 1990; Dozois et al., 1994; Gomis et al., 1997; Dho-Moulin and Fairbrother, 1999).

Feed and feed ingredients are often contaminated with pathogenic coliforms, introducing new strains into flocks. Rodent droppings can also contain harmful coliforms, and mice can facilitate the transfer of genes between resistant and susceptible strains, especially when exposed to antibiotics. (Dho-Moulin and Fairbrother, 1999). Pathogenic serotypes can enter poultry flocks through contaminated well water, and the presence of *E. coli* in drinking water indicates possible fecal contamination and the potential presence of infectious agents transmitted through the fecal-oral route.

2.5.2: Natural and Experimental Hosts

Colibacillosis is a bacterial infection that affects many avian species, with commercial chicken, turkey, and duck flocks being the most commonly affected. Among them, broiler chickens and turkeys, in particular, are highly susceptible, and colibacillosis is considered the most prevalent infectious bacterial disease in these populations (Nakamura et al.,1985, Kumar et al., 2013). While it's most prevalent in commercial poultry, the disease also affects other avian species including quail, pheasants, pigeons, guinea fowl, waterfowl (such as ducks and geese), ostriches, emus, peacocks, and partridges. This is especially true when these birds are kept intensively in confined environments.

2.5.3: Age of host commonly affected

Colibacillosis can affect birds of all ages, but it is more commonly observed in young birds, and the disease tends to be more severe in this age group. This includes even developing embryos. While outbreaks can also happen in caged layers, and coliform salpingitis/peritonitis is a frequent cause of death in breeders. In older birds, colibacillosis often presents as a sudden and severe bloodstream infection (Zanella et al., 2000).

2.5.4: Host susceptibility factors

Host susceptibility and resistance factors play a significant role in the occurrence of colibacillosis, potentially even more so than bacterial virulence factors. Healthy birds with intact defenses are naturally resistant to *E. coli*, but infection can happen when protective barriers are compromised (e.g., wounds, weakened immune systems) or when birds are exposed to stressful conditions. Controlling colibacillosis relies on identifying and eliminating these predisposing causes (Vogt and Dippold, 2005).

Colibacillosis often overlaps with other diseases, making it challenging to pinpoint the impact of each agent. Various factors contribute to predisposition, such as infectious bronchitis virus (IBV) and hemorrhagic enteritis virus infections, exposure to ammonia, and interactions between different pathogens. (Vogt and Dippold, 2005). Stress can increase resistance to colibacillosis, possibly due to immune system development and defense mechanisms. Genetic lines of chickens and turkeys differ in susceptibility to *E. coli*, with rapid growth potentially reducing bacterial resistance. Selection for resistance is feasible, though heterosis may have a negligible effect (Zanella et al., 2000).

A study involving broiler lines and crosses showed substantial variation in mortality, lesion occurrence, and growth depression due to *E. coli* challenge. Similarly, different strains' responses to endotoxin were linked to changes in weight gain, bone breaking strength, and overall mortality. Physiological and behavioral reactions to endotoxin also varied among egg-laying strains (Vogt and Dippold, 2005).

2.5.5: Clinical symptoms

Clinical presentations of *E. coli* infections range from mild or unnoticed cases to severe manifestations just before death, with the type of disease caused by the bacterium influencing the severity of symptoms (Nakamura et al., 1985, Gangane et al., 2006, Renu et al., 2012, and Kumar et al., 2013).

Localized infections generally lead to milder signs compared to systemic diseases.Coliform cellulitis is usually only identified during processing, and lameness and slowed growth can occur due to skeletal issues arising from sepsis. Affected birds are often smaller than the rest of the flock and are found at the edges of the house, near walls, or under feeders/waterers (Nakamura et al., 1985). They might be subjected to pecking from other birds. Birds with joint or bone lesions in one leg exhibit a hopping

motion to relieve the affected leg, while those with both leg lesions might struggle to stand or walk. If the thoracolumbar spine is affected, birds arch their backs, sit on their hocks, and avoid putting weight on their feet, sometimes sitting back on their tails (Vogt and Dippold, 2005). Birds with chronic lameness have droppings caked around the vent and on abdominal feathers, and they show signs of anorexia and dehydration with green feces containing white to yellow urates (Gangane et al., 2006). Young birds with omphalitis and infected yolk sacs can experience difficulty walking due to abdominal distention affecting balance and weight distribution. Birds suffering from coli septicemia are often moribund or extremely lethargic. Reduced water intake is associated with a poor prognosis. Severely affected birds are unresponsive, sit hunched with their eyes closed, and may insert their beaks into the litter for support (Zanella et al., 2000). Dehydration is evident in dark, dry skin, particularly noticeable on shanks and feet. Young chicks with dehydration can display raised skin folds along the shanks and blackened toenails. Although not technically a clinical sign, outbreaks of colibacillosis are often indicated by a notable increase in flock mortality. Signs of predisposing or compounding factors often coincide with symptoms of *E. coli* infections (Gangane et al., 2006, Renu et al., 2012 and Kumar et al., 2013).

2.5.6: Risk factors of colibacillosis in poultry

Colibacillosis in poultry can be influenced by various risk factors. Some of the commonly identified risk factors include:

Environmental Contamination: Poor hygiene and sanitation practices in poultry farms can lead to the contamination of water sources, feed, and litter with pathogenic *E. coli,* increasing the risk of colibacillosis (Mellata, 2018).

Stress: Stressful conditions such as overcrowding, transportation, sudden changes in temperature, or inadequate ventilation can weaken the birds' immune system, making them more susceptible to *E. coli* infections (Johnson and Reid-Smith, 2010).

Nutritional Factors: Imbalanced or inadequate nutrition can compromise the bird's immune response, making them more vulnerable to *E. coli* infections (Broom and Kogut, 2018).

Co-infections: Concurrent infections with other pathogens, such as viruses or mycoplasmas, can increase the severity of colibacillosis in poultry (De Oliveira et al., 2018).

Age of Birds: Young birds are particularly susceptible to colibacillosis, especially during the first few weeks of life when their immune systems are not fully developed (Gross, 1994).

Bird Density: High stocking density can create stressful conditions and increase the chances of *E. coli* transmission within the flock (Broom and Kogut, 2018).

Biosecurity Measures: Poor biosecurity practices, such as the introduction of infected birds or contaminated equipment into the farm, can lead to disease outbreaks (Johnson and Reid-Smith, 2010).

Management Practices: Inadequate management practices, such as improper handling of eggs, poor brooding conditions, and unsanitary housing, can contribute to the spread of *E. coli* (Mellata, 2018).

It is essential to implement proper biosecurity measures, maintain good hygiene practices, and manage stress factors to reduce the risk of colibacillosis in poultry.

2.5.7: Status of virulence associated genes in *E. coli* in poultry of Bangladesh

Limited research has been conducted to assess the presence of virulence-associated genes in Bangladeshi broilers. Poultry intestines provide a conducive environment for *E. coli*, which can lead to colibacillosis in chickens when containing virulent factors. A recent study in Bangladesh found that the prevalence of *E. coli* virulent genes in poultry farms ranged from 75% to 100% (Saha et al., 2020). However, another study by Ivey et al. (2020) reported a lower prevalence of 36.36% in layer flocks in the same country. Additionally, Ahmed et al. (2020) aimed to identify virulent genes in commensal *E. coli* from broiler samples collected in Chattogram, Bangladesh. They identified 13 virulence genes in the 32 commensal *E. coli* genomes, with astA (EAST-1 heat-stable toxin) and iss (Increased Serum Survival) being the most common virulence determinants, present in 50% and 44% of isolates, respectively. A significant proportion of isolates (53%) carried multiple virulence genes, and seven of the 32 isolates had 4-6

virulence determinants, but none of the strains exhibited virulence gene combinations known to be characteristic of pathogenic subtypes.

2.5.8: Public health significance

While poultry has not been a major source of shigatoxin-producing *E. coli* (STEC) infections in humans, it is advisable to maintain vigilance due to the discovery of STEC, including *E. coli* O157:H7, in various bird species and poultry products. There is concern that poultry contaminated with avian pathogenic *E. coli* (APEC) could serve as a foodborne reservoir for extraintestinal pathogenic *E. coli* (ExPEC), which are responsible for human urinary tract infections, meningitis, and other extraintestinal diseases (Pourbakhsh et al., 1997). This concern arises from striking similarities observed in the genomic sequences, serogroups, virulence genotypes, phylogenetic types, plasmid content, antimicrobial resistance patterns, and disease-causing capabilities between certain APEC and human ExPEC strains. (Vogt and Dippold, 2005).

Furthermore, retail poultry meat has been found to harbor *E. coli* more akin to APEC and human ExPEC, as opposed to commensal *E. coli* found in bird feces during slaughter. Particularly, similarities are noted in the virulence plasmid content of these organisms. These plasmids, which contribute to the pathogenesis of diseases like colibacillosis, urinary tract infections, and meningitis, can also be transferred from *E.coli* to other human pathogens through conjugation (Dozois et al., 1994; Dho-Moulin and Fairbrother, 1999; Pourbakhsh et al., 1997).

Another concern is the presence of multidrug-resistance (MDR)-encoding islands within these virulence plasmids or their co-transfer with large MDR-encoding R plasmids (Dozois et al., 1994). These MDR islands or R plasmids confer resistance to various antibiotics and disinfectants. Although resistance to antibiotics used primarily in human medicine remains low among chicken *E. coli* isolates, the potential for gene transfer from APEC to human health-relevant organisms should not be overlooked.

Chapter 3: Materials and methods

3.1: Study duration and study population

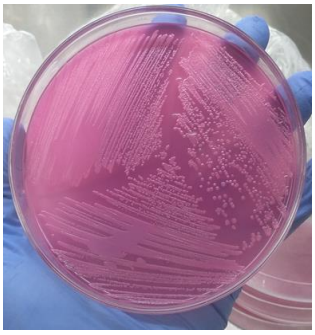
During the period from June 2022 to December 2022, total of 60 samples (Colibacillosis suspected) were collected from 150 diseased birds. Samples were obtained from the pathology lab within the Department of Pathology and Parasitology, where broiler chickens were brought for diagnostic purposes.

3.2: Sample collection and preservation

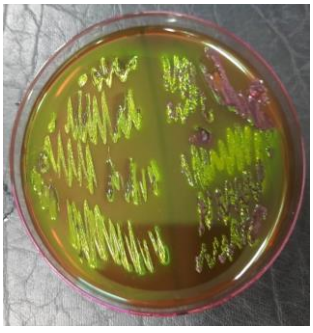
Tissue samples were taken from lung, liver and spleen immediately after necropsy for isolation of bacteria and store it in -20 degree C .Then a part of samples were preserved in 10% buffered formalin and Bouin's solution (10 Folds of the tissue size) in labeled plastic containers.

3.3: Isolation of *E. coli* from the collected samples

To isolate *Escherichia coli* from collected samples, a primary enrichment step was performed by inoculating the sample into a test tube containing buffer peptone water (BPW) and incubating it overnight at 37°C. For liver samples, after the primary enrichment, 2-3 grams of the sample were streaked on MacConkey agar medium and incubated at 37°C for 24 hours. Suspected *E. coli* colonies, identified by their bright pink color and large size on the MacConkey agar plate, were further streaked onto EMB agar plate and incubated at 37°C for 24 hours. The presence of a "green metallic sheen" colony morphology on the EMB agar was taken as indicative of *E. coli* growth. After that, isolate the bacteria in blood agar.



MacConkey agar



EMB agar

Blood agar

3.4: Molecular identification of *E. coli*

3.4.1: DNA extraction from bacteria

For the DNA extraction procedure from the isolated bacteria, the boiling method, as outlined by Englen and Kelley in 2000, was employed. In a nutshell, a loop containing new colonies, typically around 3-4 colonies, was selected from blood agar and transferred to 1.5 ml Eppendorf tubes containing 100µl of de-ionized water. The tubes were then vortexed to create a uniform cell suspension, and a ventilation hole was made on the lid of each tube to allow for pressure release during the boiling process. Subsequently, the tubes underwent high-temperature boiling at 99°C for 15 minutes using a heat block (Major Science Company). Immediately after boiling, the tubes were placed into an ice pack for 5 minutes to rapidly cool down. This method facilitated the breakdown of the bacterial cell wall, releasing DNA from the bacterial cells into the surrounding medium. Finally, the tubes with the cell suspension were centrifuged at 15000 rpm for 5 minutes. Subsequently, 50 µl of the supernatant, containing bacterial DNA from each tube, was collected in another sterile Eppendorf tube and stored at -20°C until further use.

3.4.2: Polymerase chain reaction (PCR) to test for the presence of *E. coli*

Polymerase chain reaction (PCR) assay was conducted for the final confirmation of the suspected isolates by conventional PCR using genus-specific primer Adk gene listed in Table 3.1. The detailed procedure that was followed is given below:

Table 3.1: Primer and oligonucleotide sequence used for the identification of *E.coli*:

Gene	Primer Sequence	Amplification (bp)	Reference
Adk	Adk F :5'-ATTCTGCTTGGCGCTCCGGG-3' Adk R: 5'-CCGTCAACTTTCGCGTATTT-3'	536bp	Wirth *et al*.,2006

PCR reactions were conducted with a final volume of 15 µl using 20 picomoles of each primer concentration.

Table 3.2: Contents of each reaction mixture of PCR assay

Serial No	Name of contents	Amounts
1	Thermo Scientific PCR Master Mix (2x)	12.5µl
2	20 pM Forward primer	1µl
3	20 pM Reverse primer	1µl
4	DNA Template	2µl
5	Nuclease Free Water	8.5µl
	Total	25µl

PCR was run on a thermocycler (Applied Biosystem, 2720 thermal cycler, Singapore) following the cycling conditions mentioned in Table 3.3

Table 3.3: Cycling conditions used during PCR

Serial No	Steps	Temperature and time
1	Initial denaturation	95°C for 2 minutes
2	Final denaturation (30 cycles)	95°C for 1 minutes
3	Annealing	54°C for 1 minutes
4	Initial extension	72°C for 2 minutes
5	Final extension	72°C for 5 minutes
6	Final holding	4°C

3.4.3: Visualization of PCR products by Agar Gel Electrophoresis

A 1.5% agarose gel (w/v) was utilized to visualize the PCR product. The following steps were undertaken in the procedure:

A conical flask was employed to thoroughly mix 0.75 grams of agarose powder with 50 ml of 1X TAE buffer. The mixture was then heated in a microwave oven until the agarose dissolved completely. The agarose mixture was allowed to cool to 50°C in a water bath, and a single drop of ethidium bromide was introduced. The gel casting tray was prepared by sealing the gel chamber ends with tape and placing the appropriate number of combs in the tray. The agarose-TAE buffer mixture was poured into the gel tray and left at room temperature for 20 minutes to solidify. Subsequently, the combs

were removed, and the gel was transferred to an electrophoresis tank containing 1X TAE buffer until it was fully submerged. A volume of 5 µl of the PCR product for a specific gene was loaded into a well in the gel. For comparison of the gene product's amplicon size, 3 µl of a 100bp plus DNA marker from Addbio INC, Korea was used. Electrophoresis was conducted at 110 volts and 80 mA for a duration of 40 minutes. Finally, the gel was examined using a gel documentation system known as the UVP UVsolo touch from Analytik Jena AG.

3.5: Histopathology slide preparation

3.5.1: Preparation of Sample

Grossly affected tissues were collected, identified, and preserved in Bouin's solution (10 Folds of the tissue size) in labeled plastic containers. The thickness of the tissue sample was 4-5 mm. Tissues were preserved for at least 7 days before processing.

3.5.2 Processing of tissue

Preserved tissues were processed following removal of fixative, dehydration, clearing, impregnation, and embedding.

Sample identification marks were made by a soft lead pencil and a garland (tissue string) of tissues was made considering the cut surface for sectioning. Then the tissue garlands were placed for an overnight wash in running tap water to remove the fixative. Dehydration was done by moving the tissues through ascending concentration of ethanol series (80% alcohol- two hours, 95% alcohol- two changes one hour each, 100% alcohol- three changes one hour each) for appropriate time to prevent shrinkage of cells. Clearing reagents should be miscible with the dehydrant and the paraffin. Xylene was used as a clearing reagent to replace alcohol (xylene- two changes one hour each, xylene- two hours. Impregnation of tissue by paraffin for complete removal of the clearing agent was done by three changes in paraffin bath (56-5^{8o}C), two hours each. The cooked tissues were kept overnight to rest. Embedding was done by placing the tissue in melted paraffin to make a block, which after solidification provided a firm medium for keeping all parts of the tissue intact when sections were cut.

3.5.3 Preparation of sections

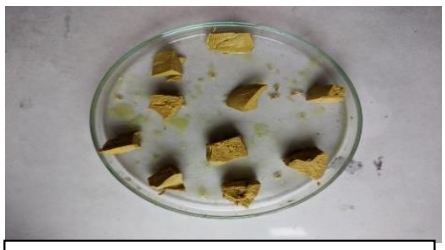
Tissue block embedded in paraffin was set in the rotary microtome machine and sections were cut at 3-5µm thickness until suitable ribbon was formed. The ribbon of tissue sections was placed in a warm water bath (55-58^{o}C) and allowed to spread. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. Sections were picked up on grease-free clear slides. Sections were the air-dried and placed on a rack.

3.5.4 Staining of tissue slides

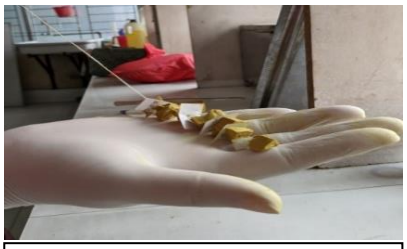
A regressive staining procedure was followed to stain the tissue slides. In the regressive staining technique, the sections were first overstained with a relatively neutral solution of hematoxylin. Then the excess stain was removed by using an acid alcohol solution. After that sections were neutralized with an alkaline solution (weak ammonia water) for better differentiation. Then the sections were counterstained with eosin followed by the removal of excess eosin by alcohol.

After staining and mounting cover slip the slides were air dried and then examined under microscope.

Histopathological work-flow

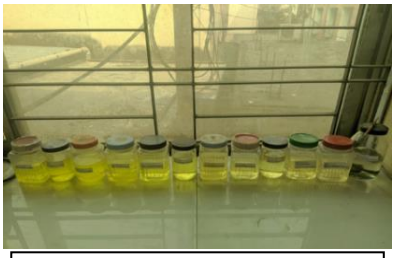


Cut sample into small pieces



Making garland

Overnight wash in running



Dehydration

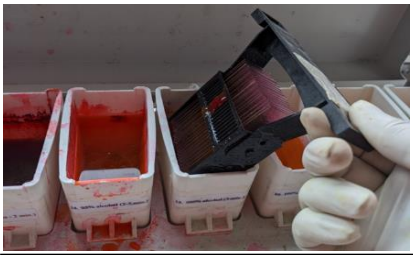
Impregnation of tissue by paraffin

Embedding

Embedded in paraffin block

Sectioning in rotary

Placing tissue section on glass slide



Staining of the tissue

Chapter 4: Result

4.1: Post Mortem Findings

4.1.2: Occurrence of different forms of colibacillosis in commercial broiler farms:

In the current study, total 60 death bird were assessed with *E. coli* infection based on necropsy and commonly found forms are pericarditis and perihepatitis (fig.: 2), airsacculitis, enteritis (fig.: 4 & 5), unabsorbed yolk sac (fig.: 7), omphalitis (figure 8) and sometimes we found mix forms (figure 9). Some of the observed findings included the presence of cloudy and thickened air sacs (fig.: 1), indicative of air sacculitis, a condition where the air sacs become inflamed. The liver capsule appeared congested and thickened (fig.: 2), and the lung was both congested and consolidated in certain chickens. Further findings encompassed a thickened pericardium (fig.: 2 & 9) and an enlarged spleen (fig.: 6), both demonstrating severe congestion. Collectively, these observations pointed towards the manifestation of the septicaemic form of colibacillosis, a condition where the *E. coli* bacteria have disseminated throughout the bloodstream. The graphical presentation below depicts the percentage occurrence of these types in our study.

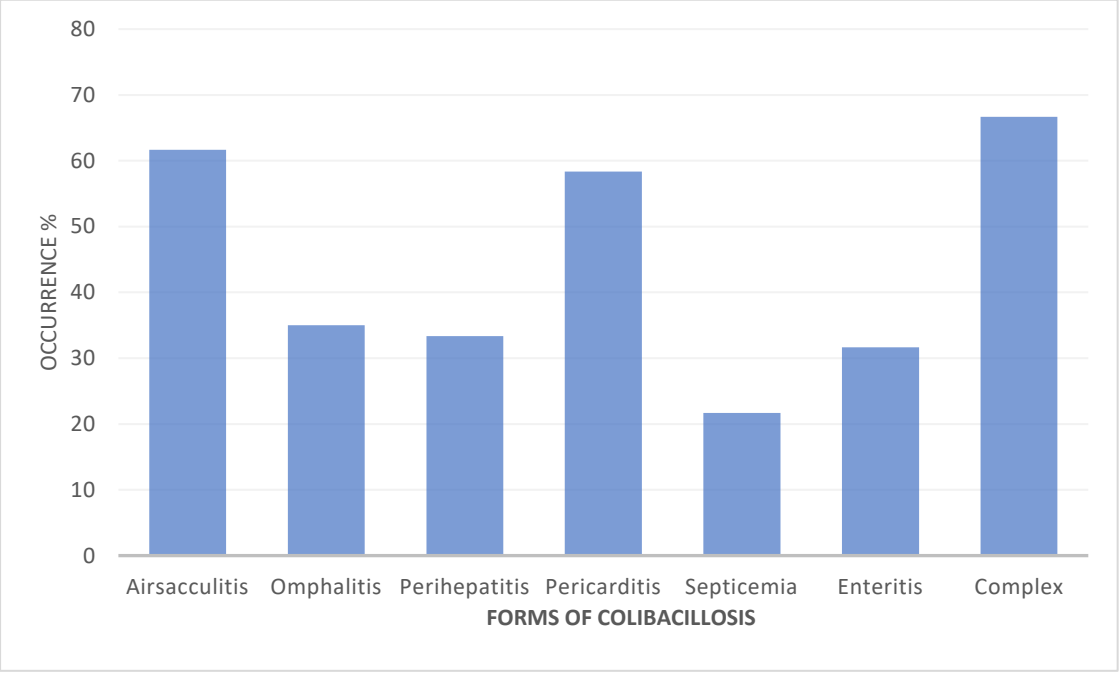


Figure 1: Occurrence percentage of different forms of colibacillosis in broiler chicken

In the analyzed sample, 37 instances of airsacculitis, 21 cases of omphalitis, 20 occurrences of perihepatitis, 35 cases of pericarditis, 13 instances of septicemia, and 19 cases of enteritis were identified. It is noteworthy that the majority of these cases manifested in conditions characterized by a mixed form. The prevalence of these varied pathological conditions underscores the complexity and multifaceted nature of the observed health issues within the studied population.

Table 4.1: Age wise percentage of *E.coli* Infections in broiler

Age (Week)	No. of broiler chicken	No. of confirmed affected bird	Percentage (%)
0 - ≤ 2	40	30	75 %
2 - ≤ 4	15	7	46.6 %
≥ 4	5	0	0 %

Age emerges as a crucial determinant in the susceptibility to this particular disease, with a notable concentration of bacterial infections occurring predominantly within the age range of 0 to 2 weeks in chickens. It is during this early developmental stage that the impact of the infection tends to be most severe, causing significant harm.

Gross lesions

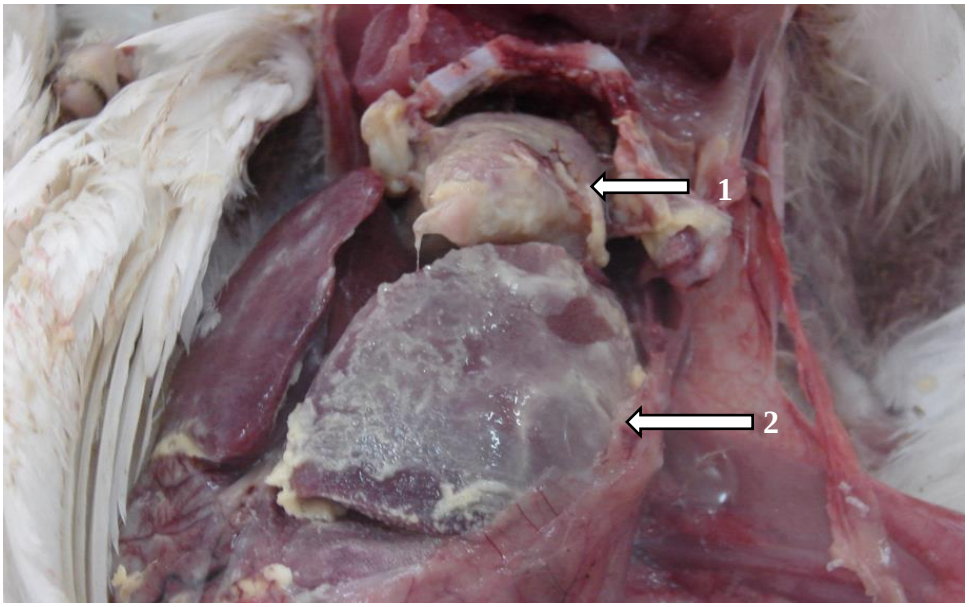


Figure 2: Fibrinous perihepatitis and pericardial sac with yellow fibrinous exudated adhering to heart due to infection

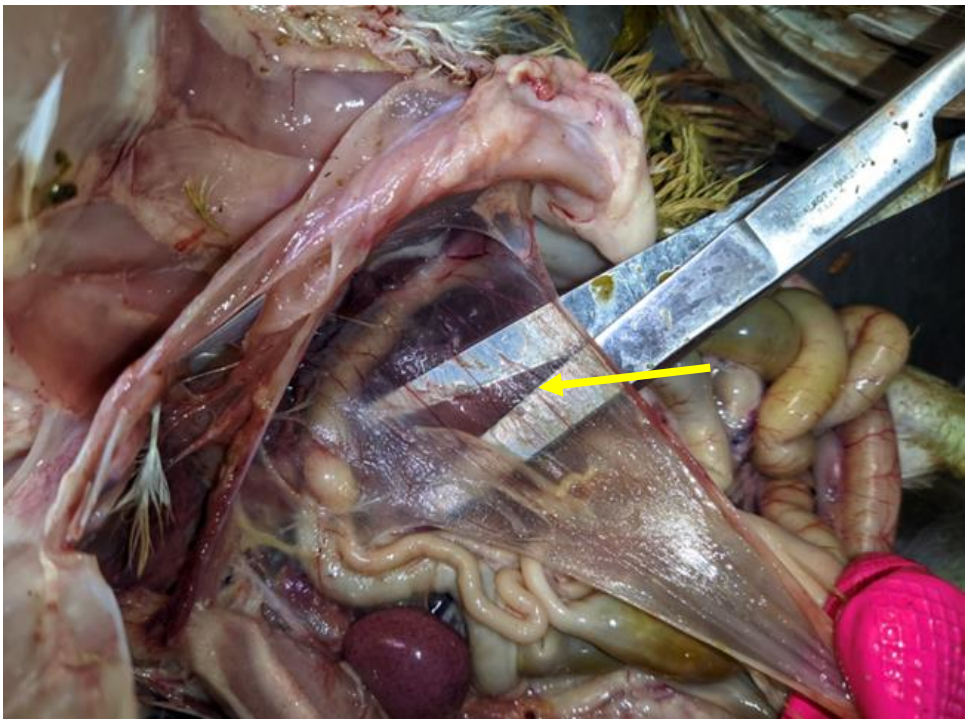


Figure 3: Thickening of air sac and proliferate blood vessel

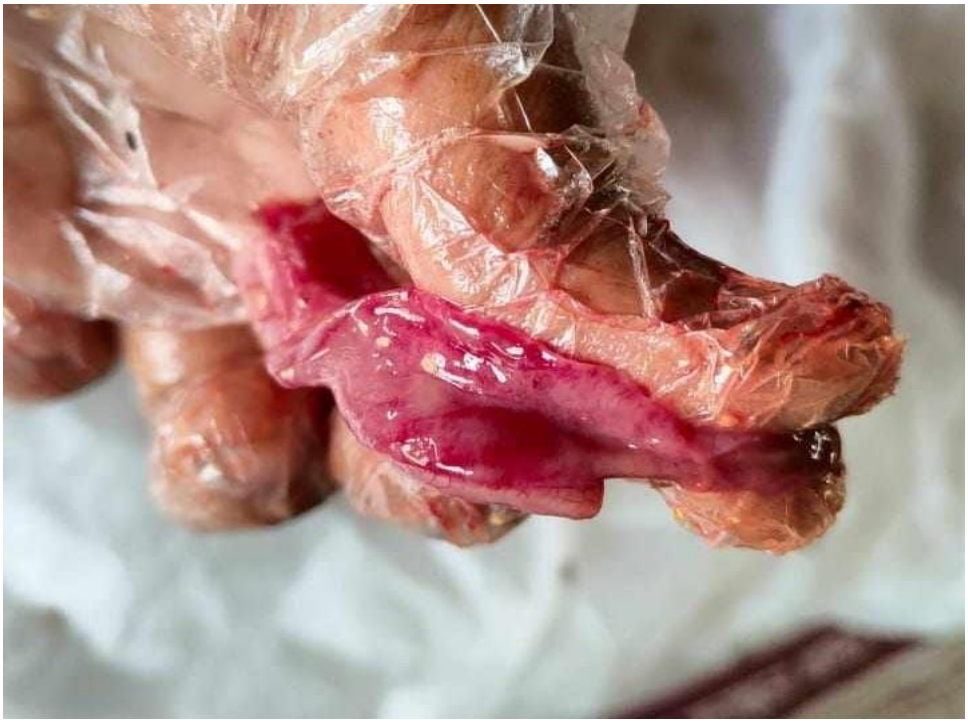


Figure 4: Hemorrhage in the duodenum and presence of mucus substance

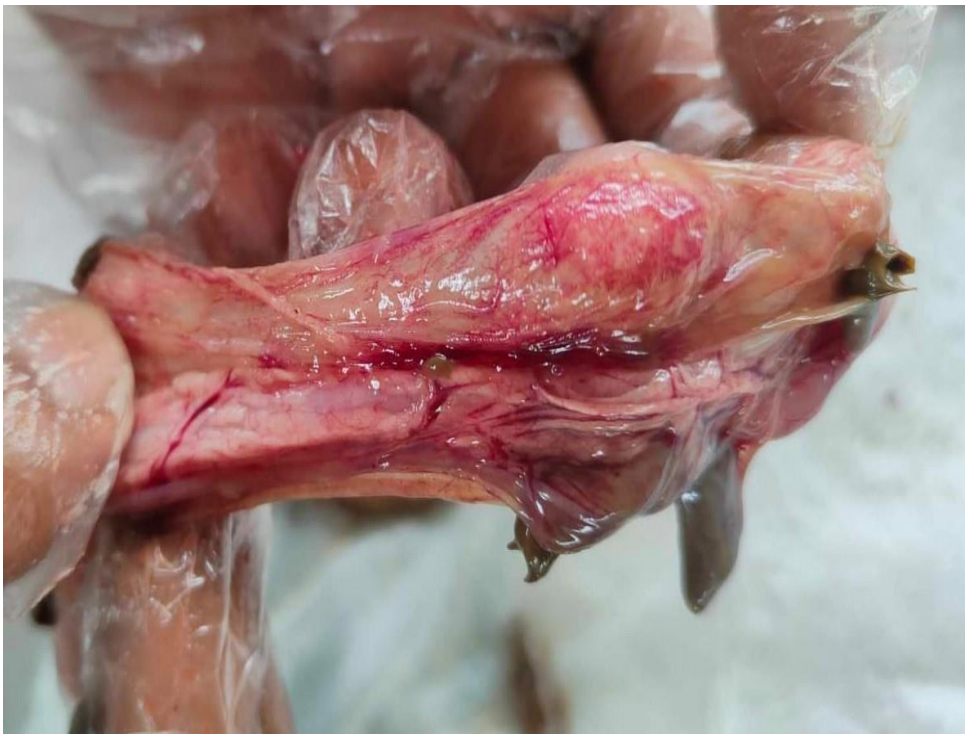


Figure 5: Showing enteritis in *E. coli* infected chicken

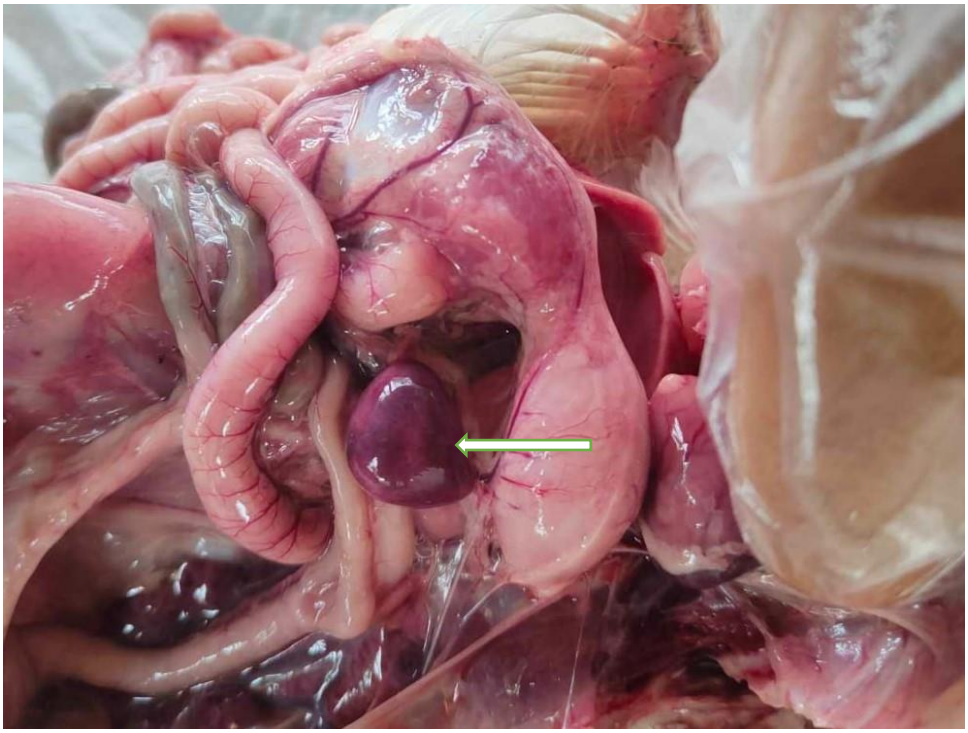


Figure 6: Showing Congestion of spleen in affected bird

:

Figure 7: Unabsorbed yolk sac in DOC (day old chick)

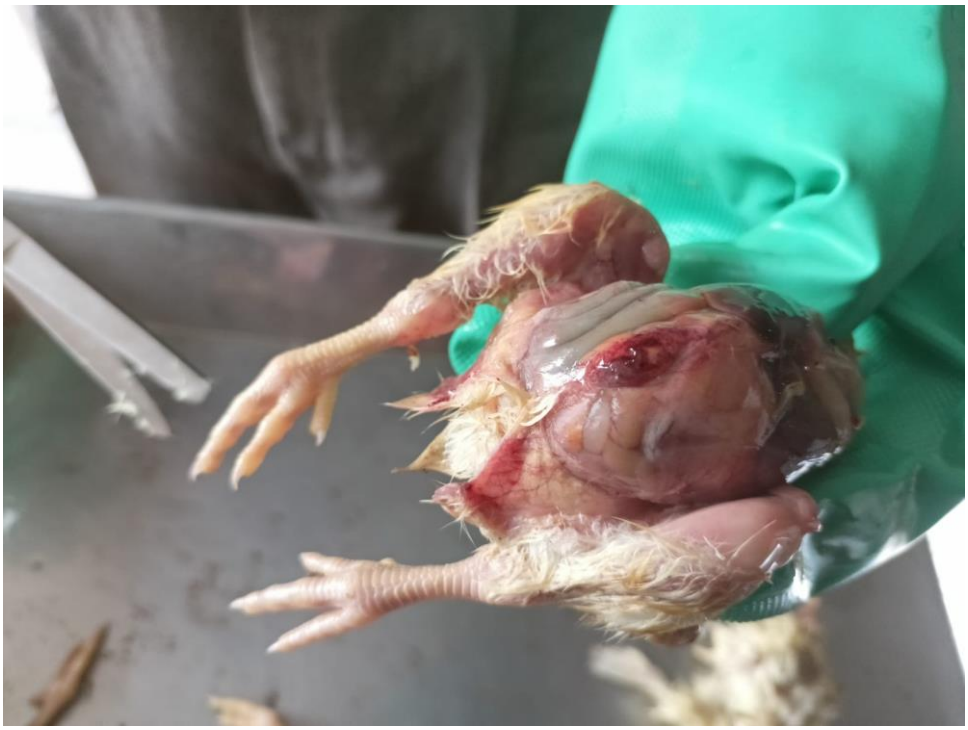


Figure 8: Omphalitis

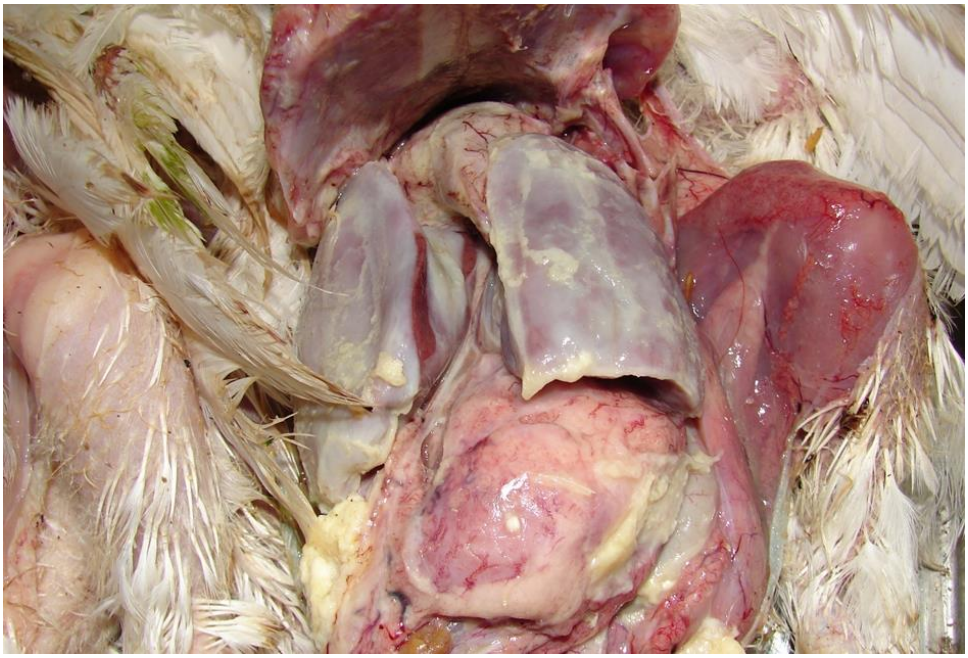


Figure 9: Septicemia

4.2: The colony characters of identified bacteria

The isolates were characterized using conventional bacteriology techniques. Identification was confirmed based on specific characteristics: the emergence of pink colored colonies on MacConkey agar (fig.: 10), and the presence of greenish colonies displaying a metallic sheen on EMB agar (fig.: 11) after an overnight incubation.

Figure 10: *E. coli* on MacConkey

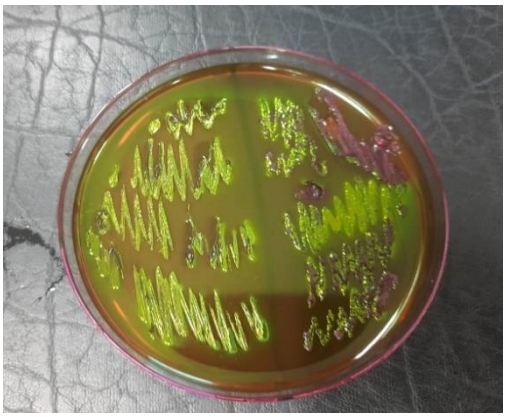


Figure 11: Metallic sheen of *E. coli* on EMB agar

4.2.1: Detection of *Escherichia coli* by PCR

Isolated sample from bacterial culture, 45 out of 60 *E. coli* strains were subjected to testing using housekeeping adk-f an adk-r primers and the resulting 536-bp amplicons were confirmed through 1% agarose gel electrophoresis. Similar outcomes were also documented by other researchers (Wirth et al., 2006), emphasizing the specificity of this 536-bp sequence to *E. coli.*

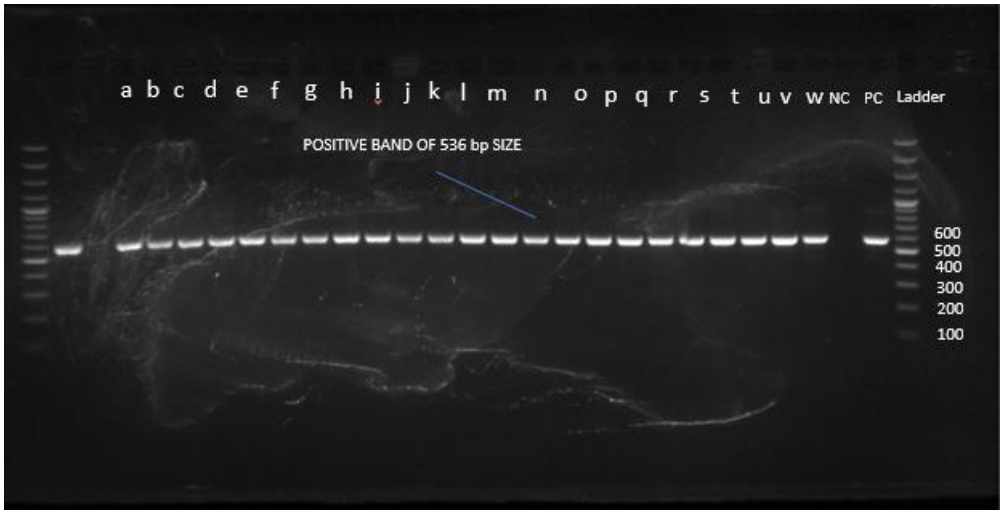


Figure 12: Electrophoresis on agarose gel showing the 536bp PCR product after amplified in field samples of locally isolated *E. coli*, NC- negative control, PC- positive control, (a-w) - Samples

Table 4.2: Percentage of positive sample in total

Number of collected sample	***E.coli* positive in agar**	**Percentage (In culture)**	**Number of confirmed positive in PCR**	**Percentage (In PCR)**
60	45	75%	37	61.67 %

4.1.2: Microscopic lesions

- **Liver:**

Histopathological evaluation of the liver disclosed the existence of widespread congestion within central veins and sinusoids (fig.: 13,14) along with focal instances of coagulative necrosis (fig.: 17, 18) and extensive cellular infiltrations (fig.: 19). Furthermore, dilated sinusoids accompanied by leukocytes were observed. We also observed cytoplasmic changes like fatty change (fig.: 26,27), more eosinophilic cytoplasm (fig.: 21, 22). In the nucleus we found karryolysis, karyorrhexis, pyknosis and loss of nucleus (fig.: 21) In early stages and mild cases of colibacillosis there was degeneration in the hepatocyte, individualization of hepatic cord along with distortion of hepatocyte (fig.: 31) hemorrhage and congested blood vessel in liver was generally observed in all age groups. In some cases, mild hyperplasia of kupffer cell was also seen (fig.: 30). There was also infiltration of numerous heterophils (fig.: 23, 24.29,32).

- **Lung**

Lungs normally foundcongestion and thickening of the space between alveoli (fig.: 33,34) and hemorrhages (fig.: 34). In some cases, in between hemorrhage and congestion (fig.: 36,37), there present of leucocyte and different mononuclear cells presence in the lung. Some cases, there present edematous fluid in the alveolar space(fig.: 34)

- **Spleen**

In certain instances, the spleen exhibited the presence of hemorrhage and congestion (fig.: 38, 44), along with the identification of thickened blood vessels (Fig.: 39). Additionally, the spleen displayed destruction of lymphocytes, depletion of lymphoid elements, and necrosis (Fig.:45). Necrosis was also observed in the germinal center (fig.: 41, 39). Furthermore, there was evidence of reticulo-endothelial cell proliferation (fig.: 42).

Histopathological slides of liver

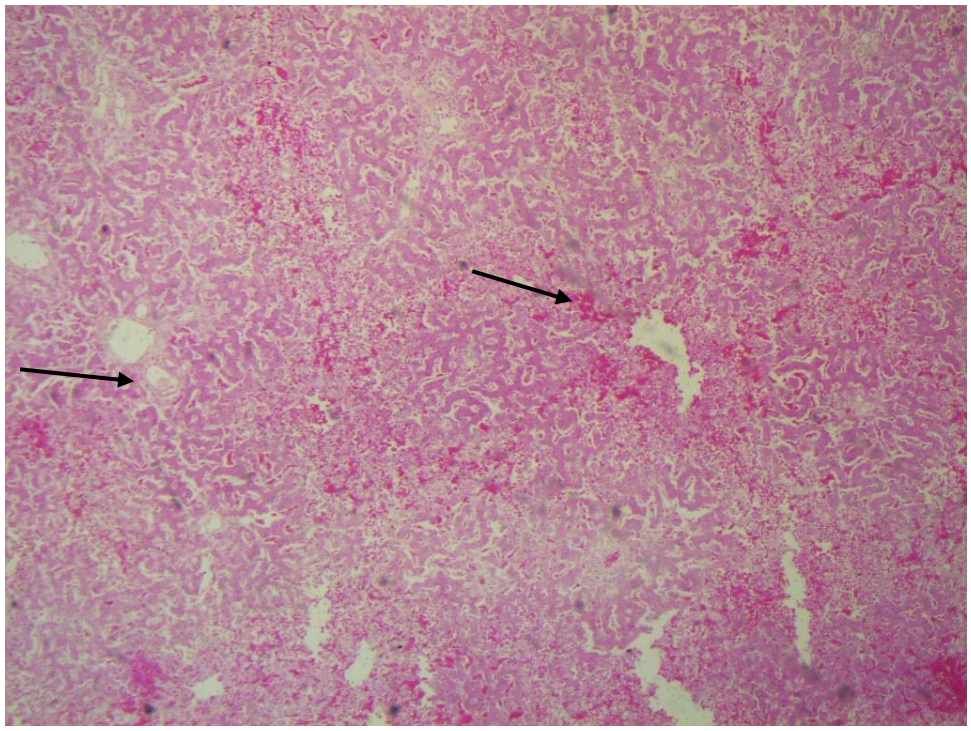


Figure 13 vascular congestion & hemorrhage

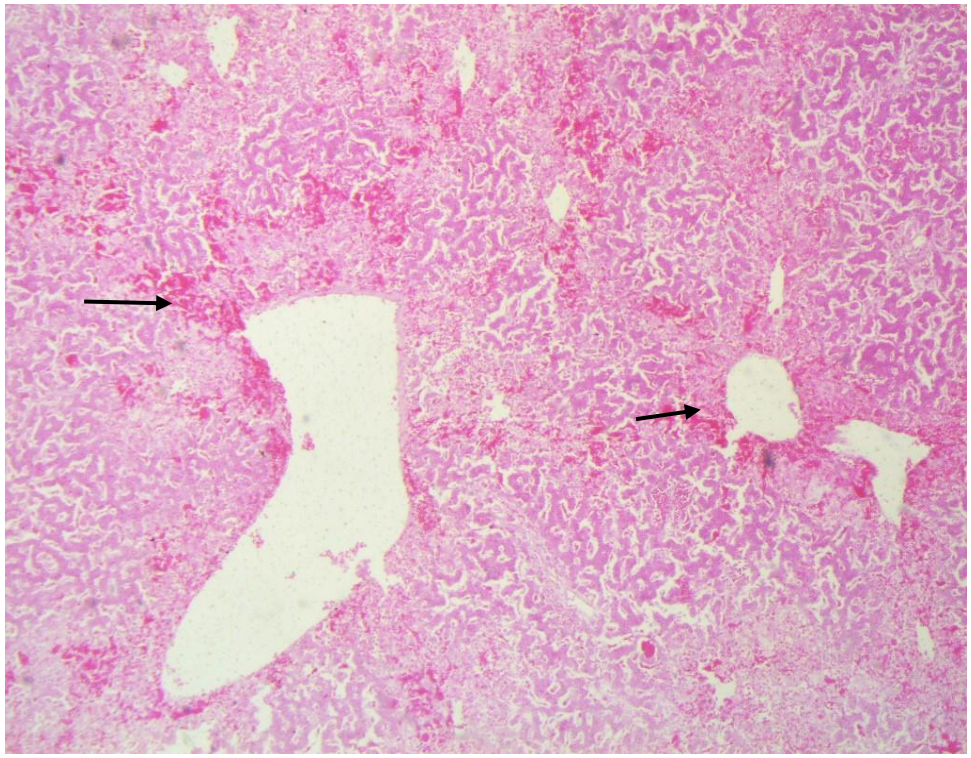


Figure 14: Congestion & hemorrhage surrounding the central vein

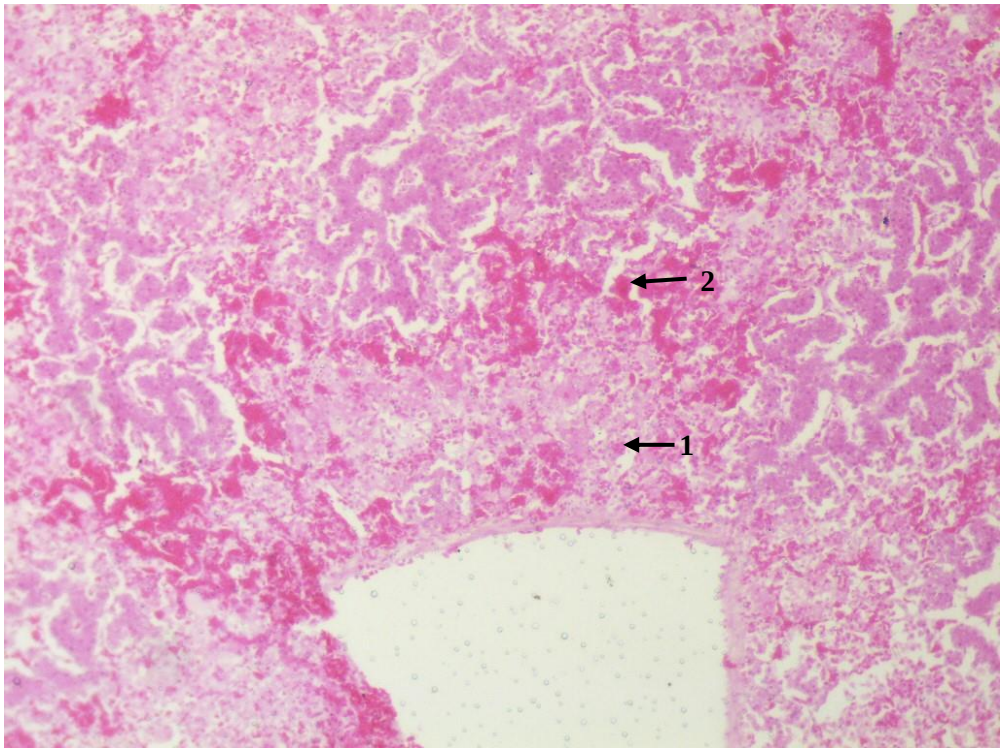


Figure 15: Necrosis around the central vein (1) & hemorrhage in the surrounding area (2)

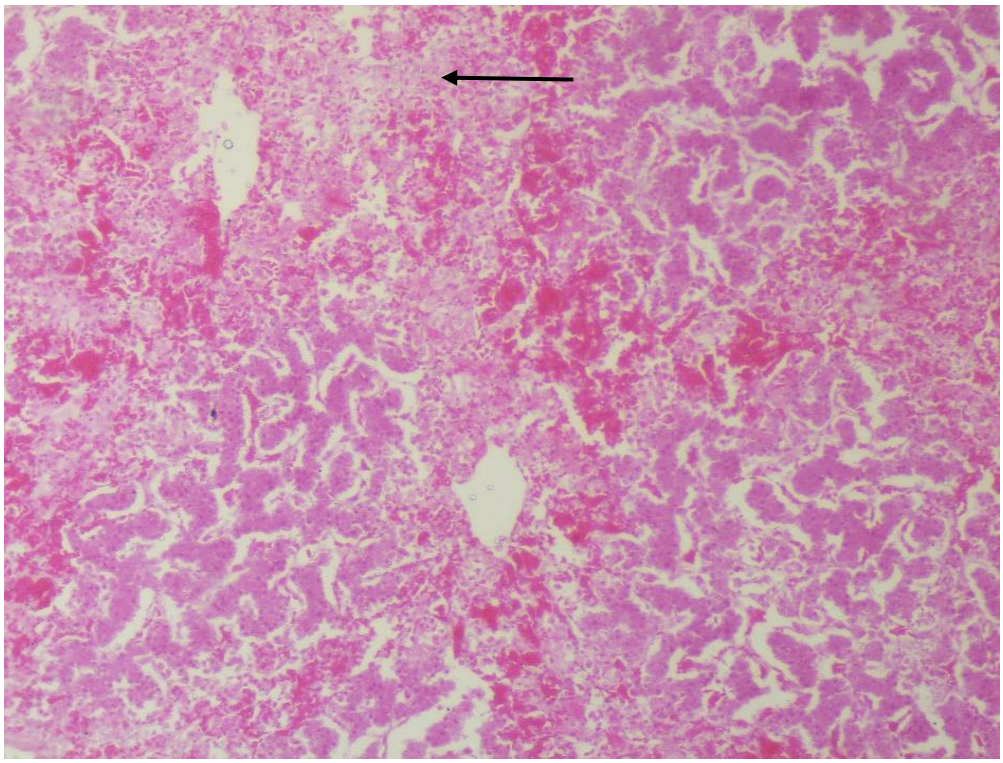


Figure 16: Hemorrhage and congestion, loss of architectural details due to necrosis

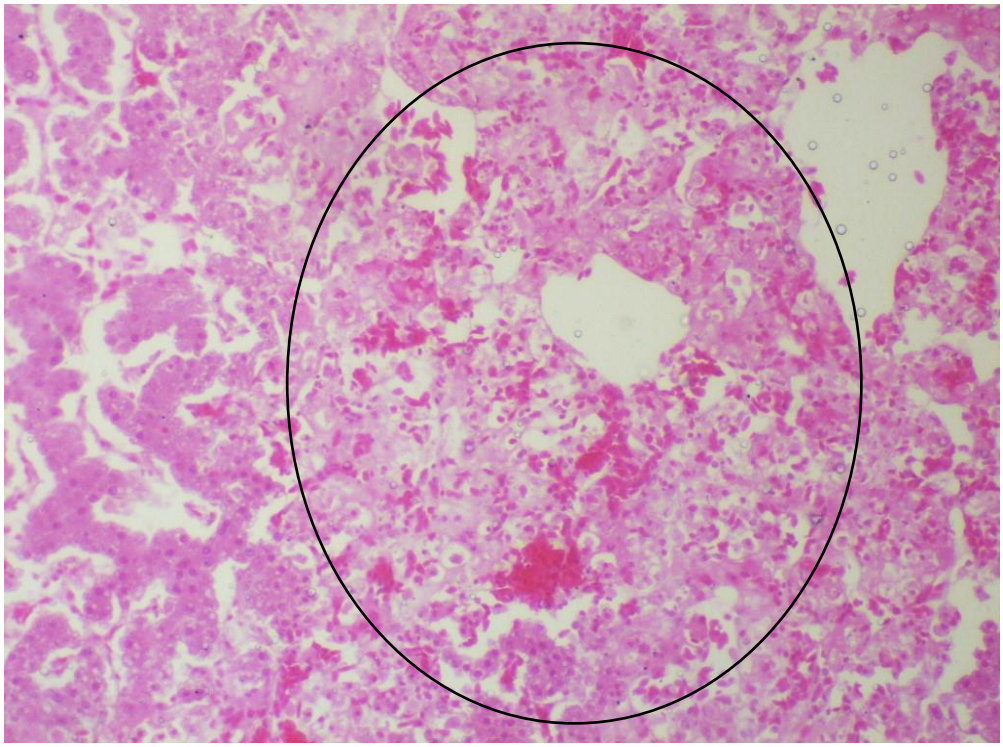


Figure 17: Necrosis around the central vein & hemorrhage

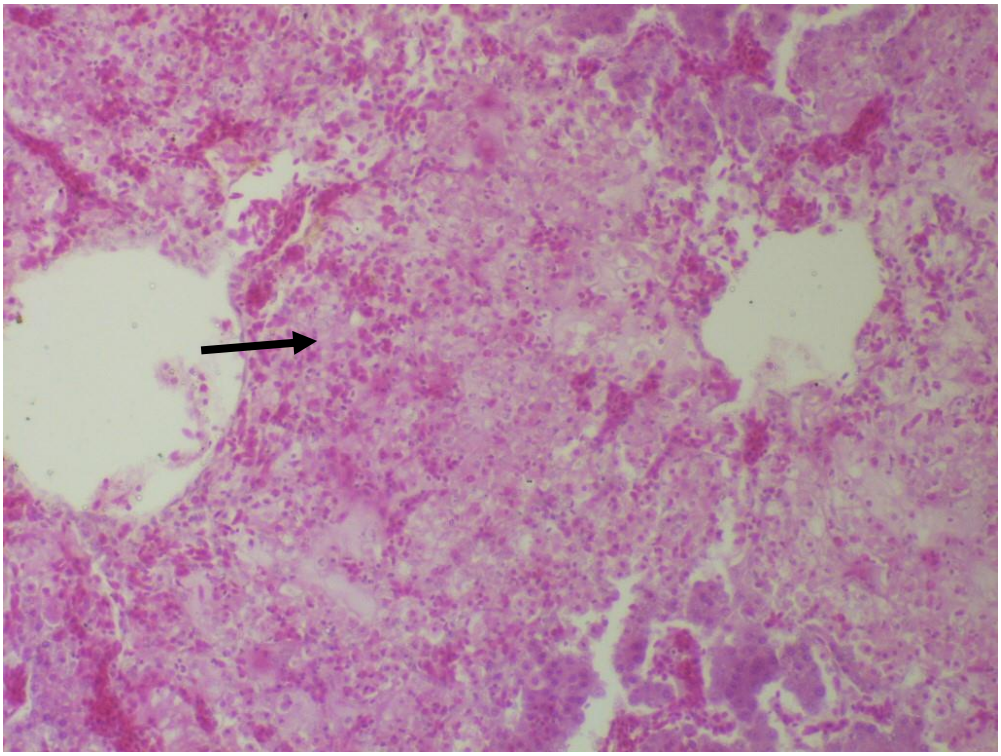


Figure 18*:* Loss of architectural structure due to necrosis

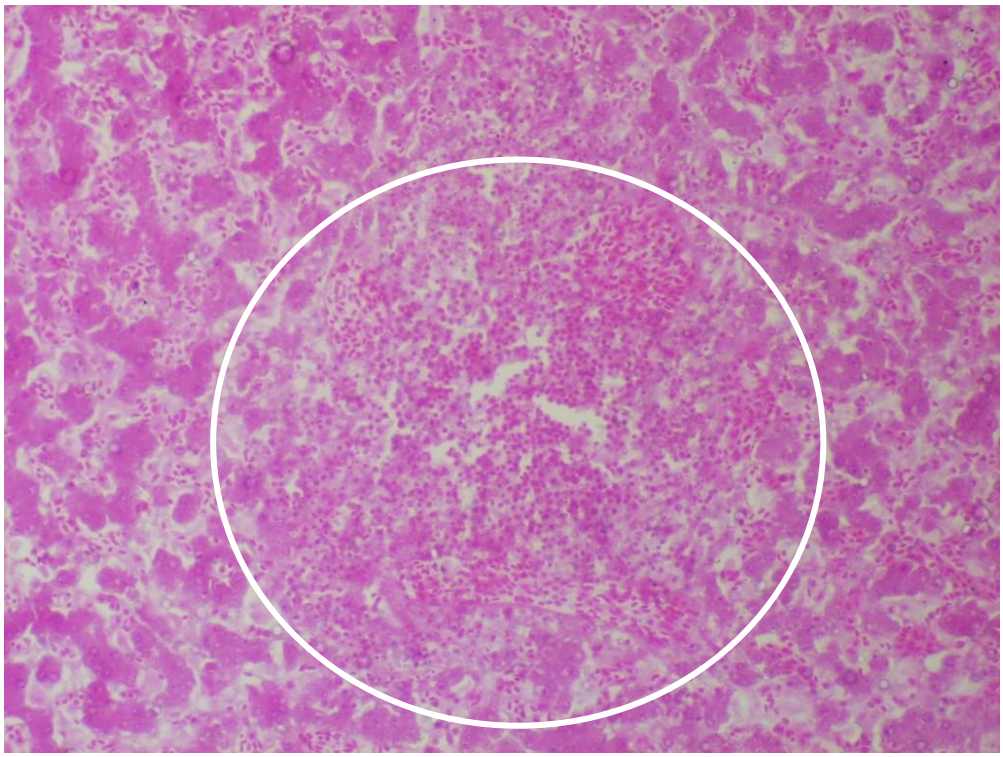


Figure 19: Reactive cells accumulation among hepatic cords

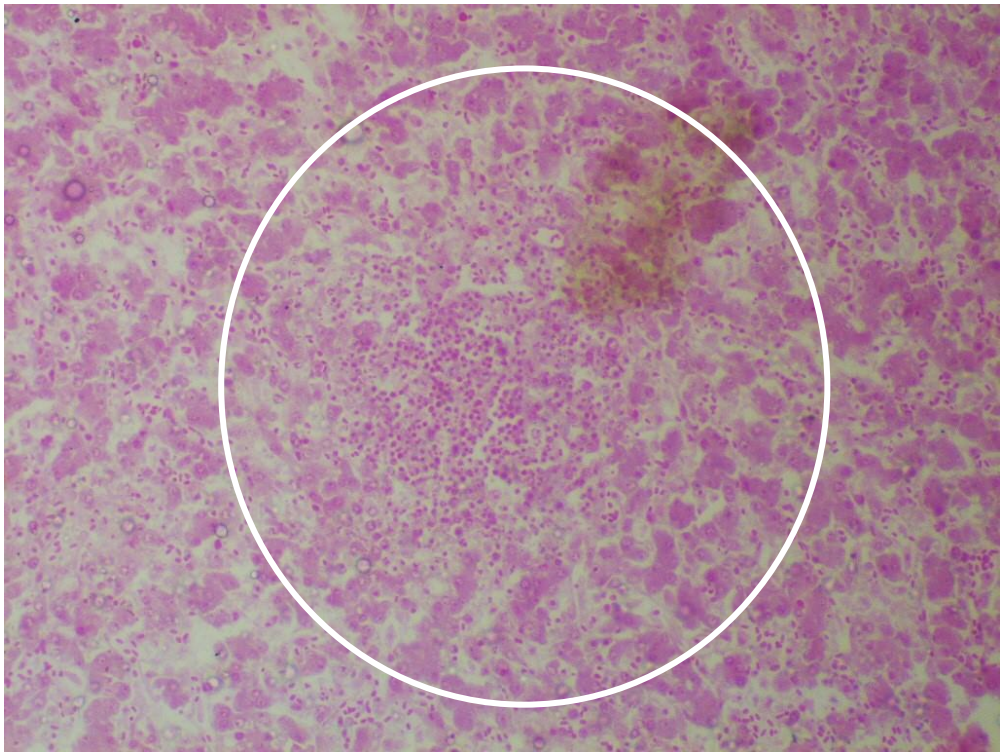


Figure 20: Necrosis with loss of cellular and architectural details

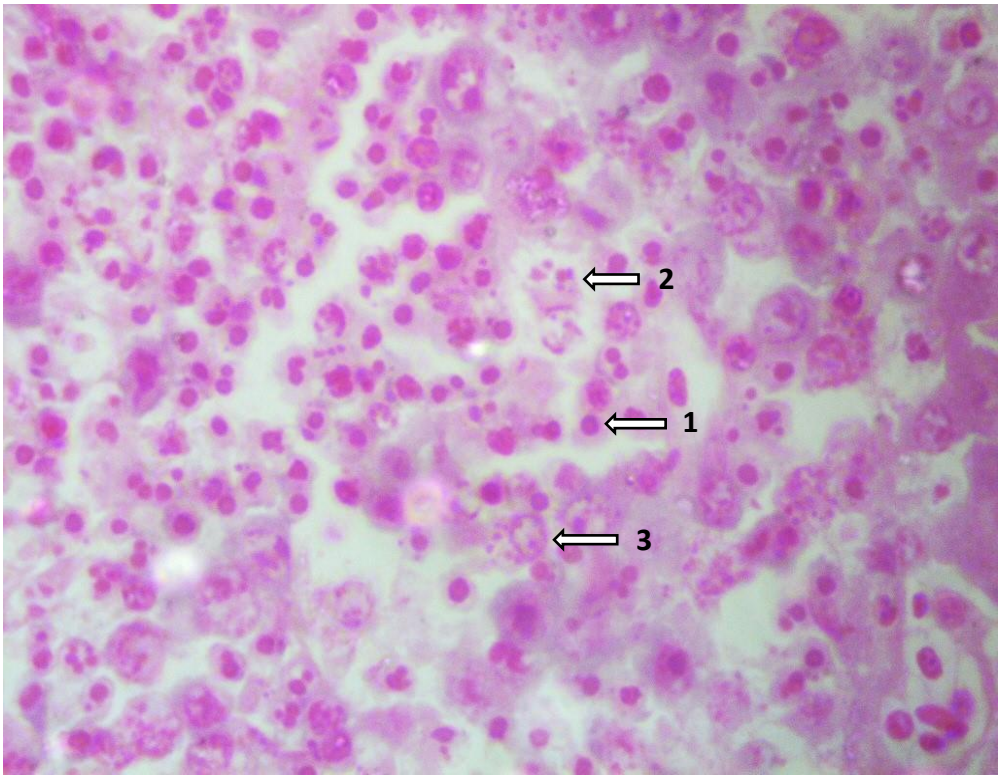


Figure 21: Changes in the nucleus and cytoplasm. (1)- pyknosis (2)- karyorrhexis (3)- karyolysis

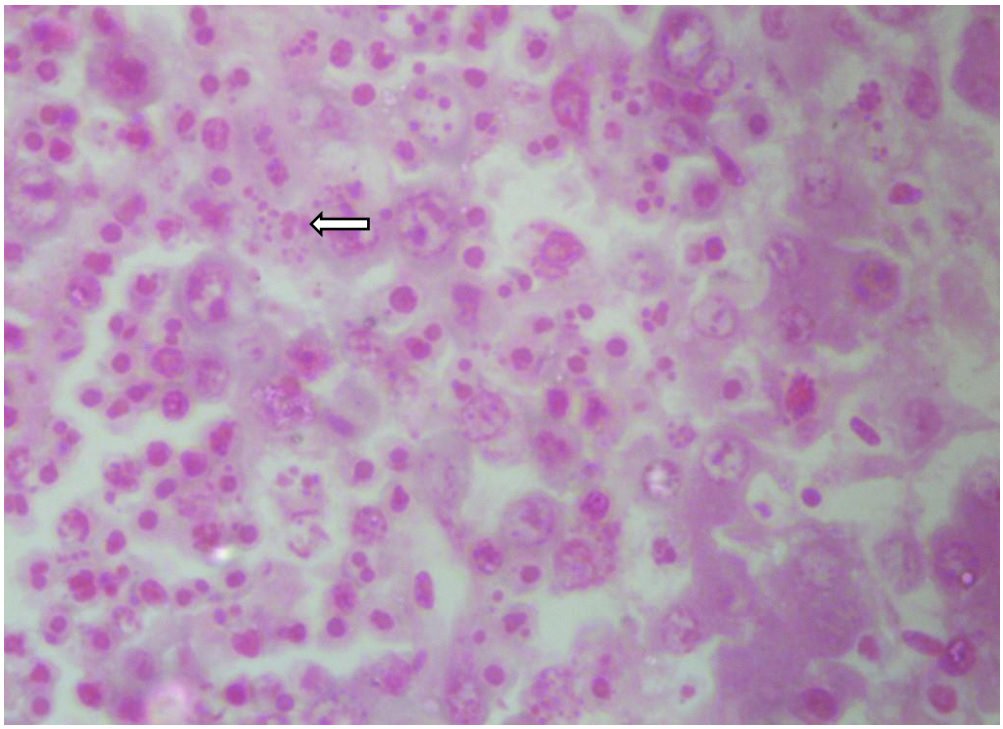


Figure 22: Destruction of hepatic cell due to necrosis

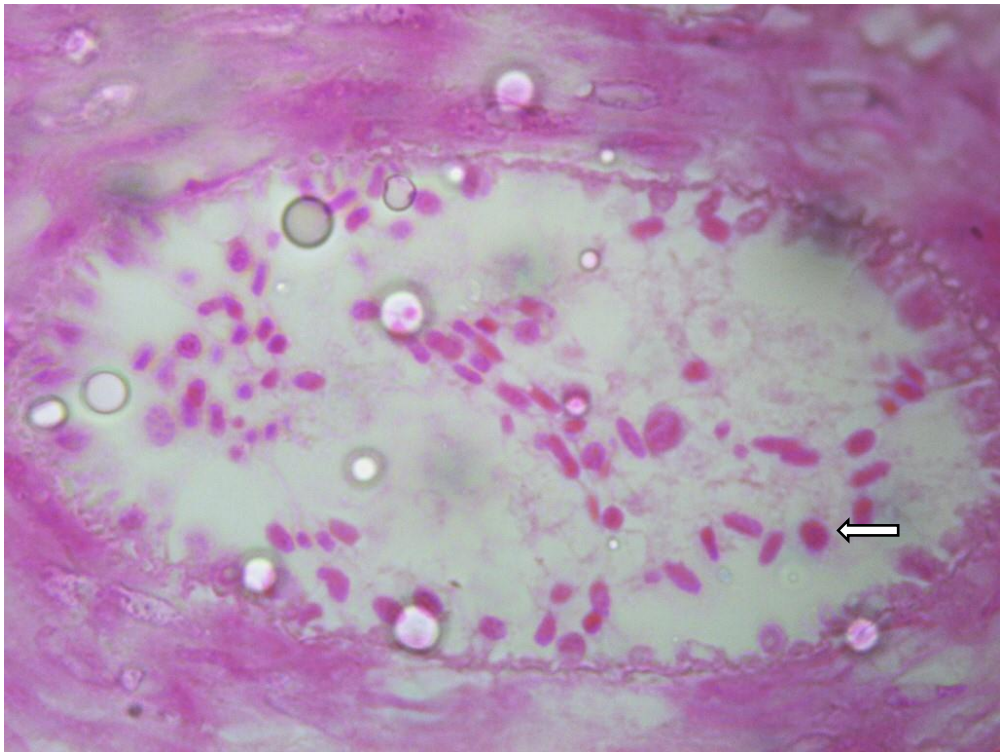


Figure 23: Lymphocytic infiltration within central artery

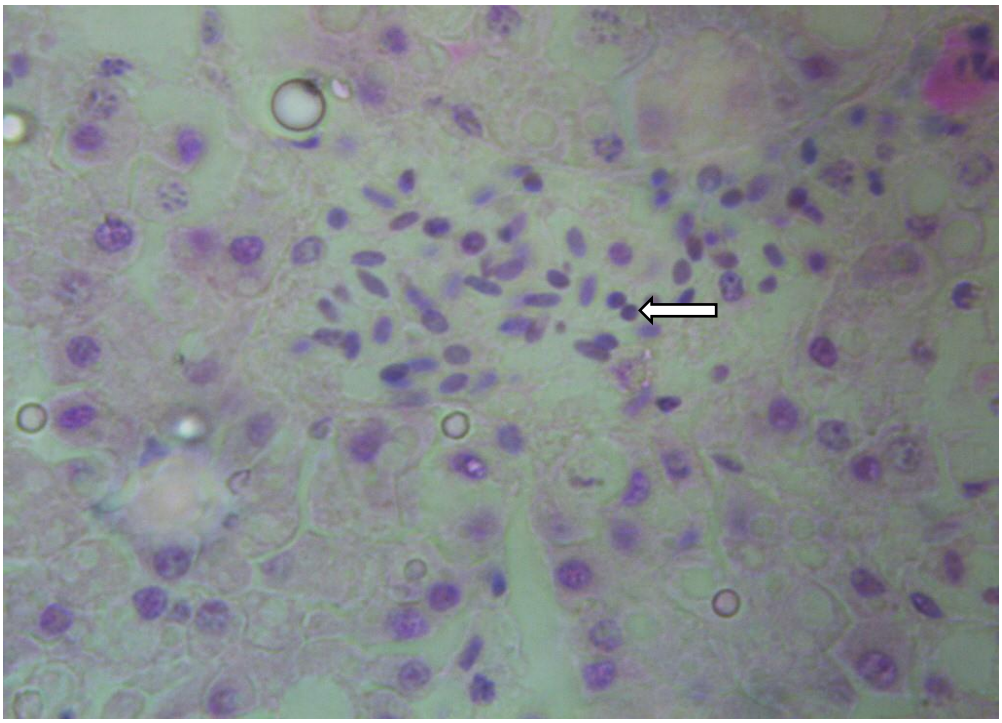


Figure 24: Cellular infiltration of reactive cells

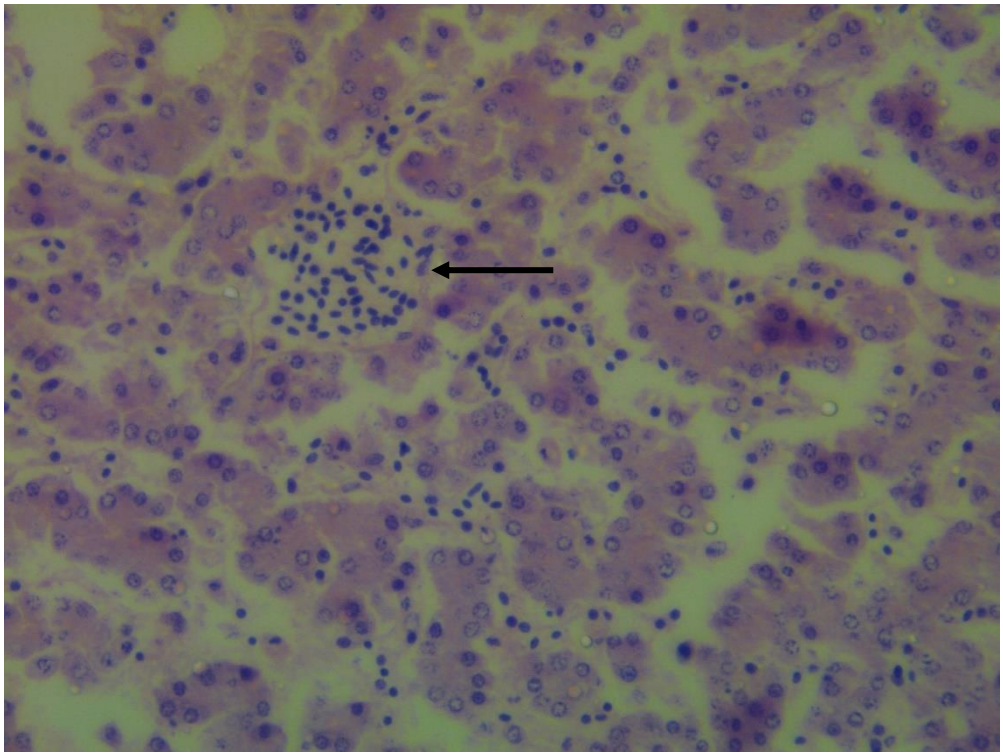


Figure 25: Lymphatic infiltration in sinusoid space

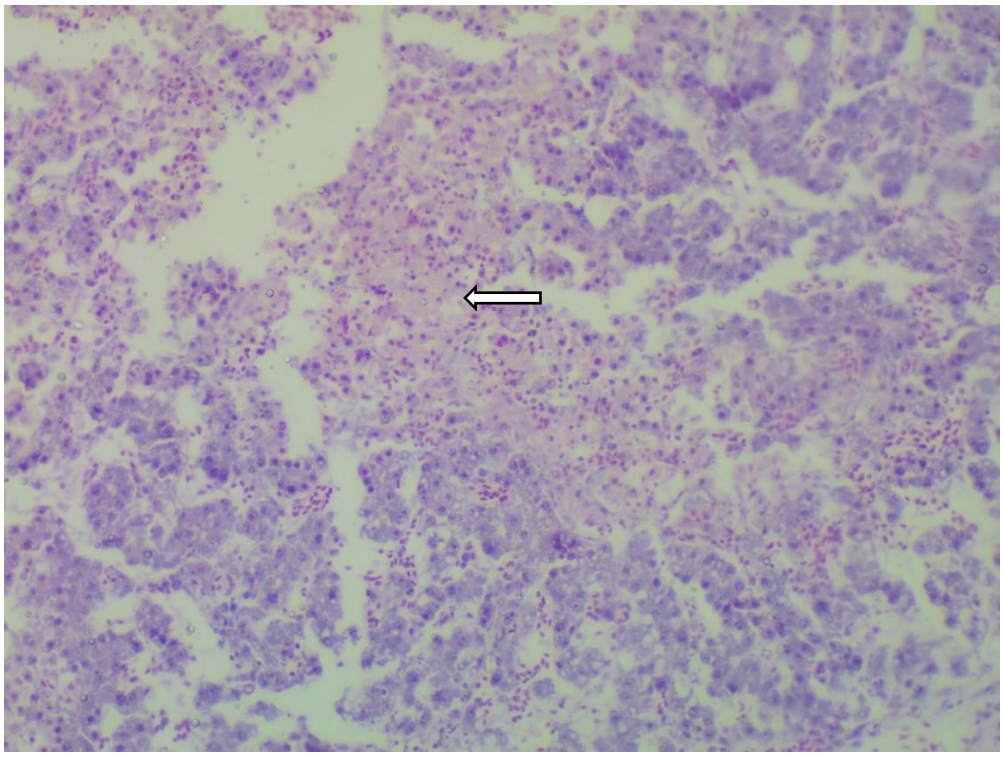


Figure 26: Loss to architectural details in hepatic cord

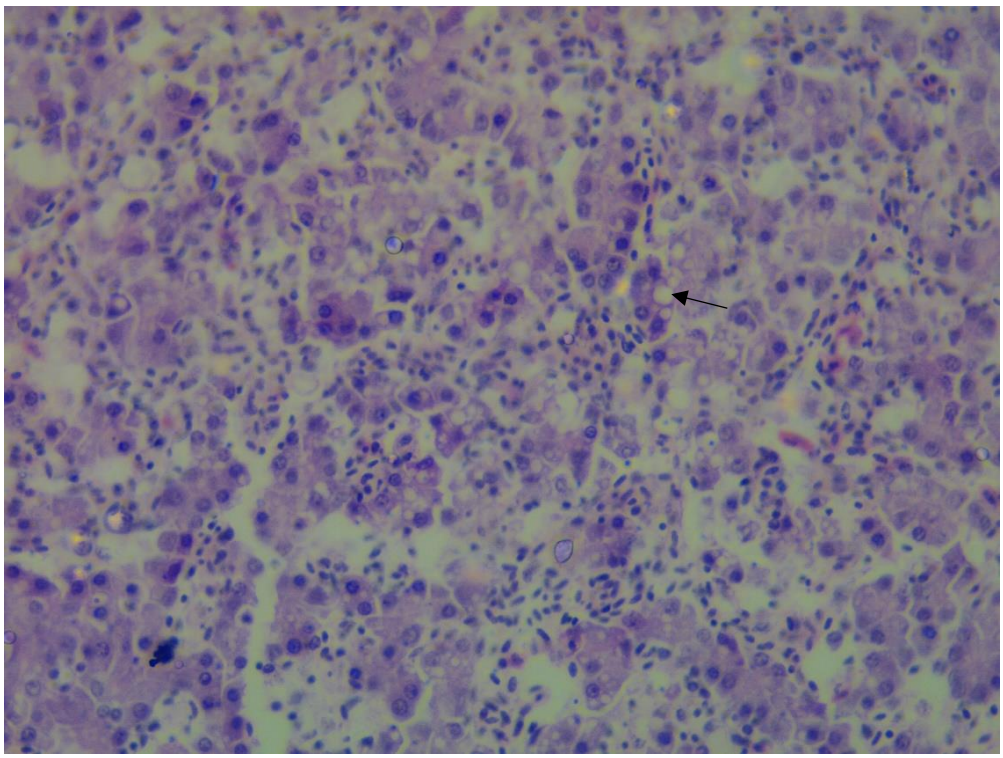


Figure 27: Fatty change in liver

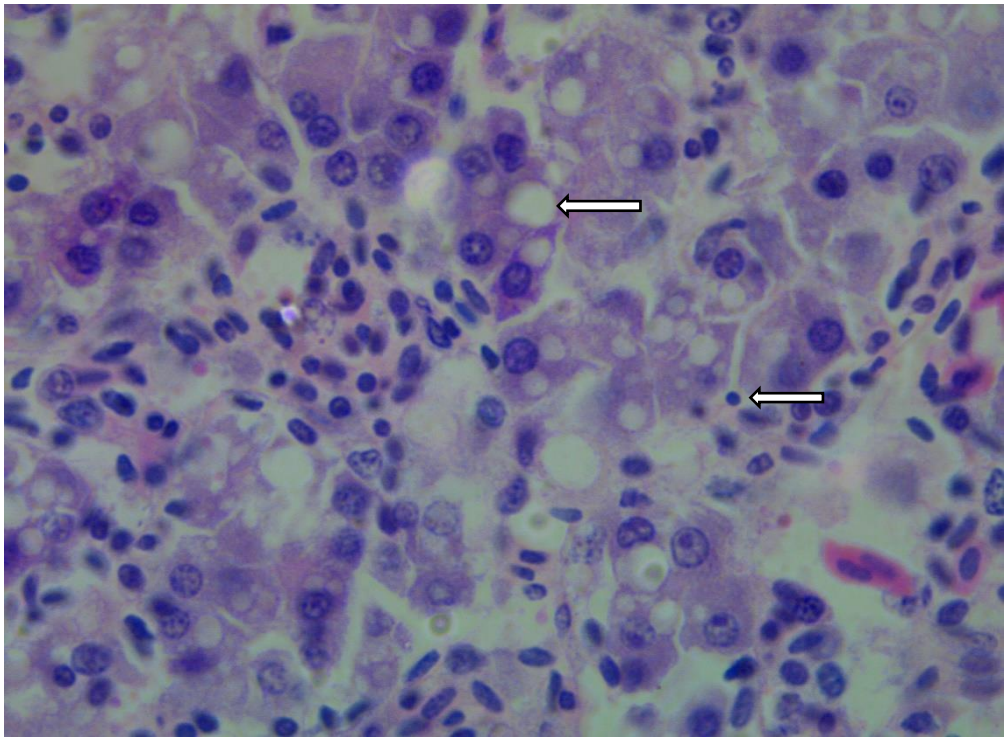


Figure 28: Fatty change in liver and presence of lymphocyte

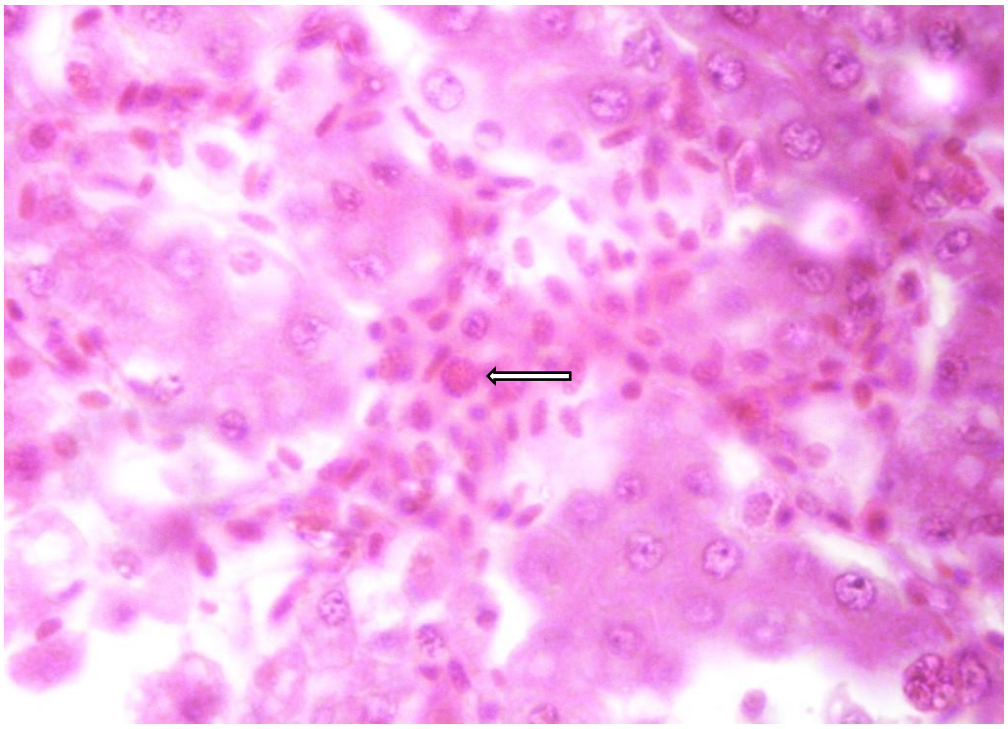


Figure 29: Presence of heterophil

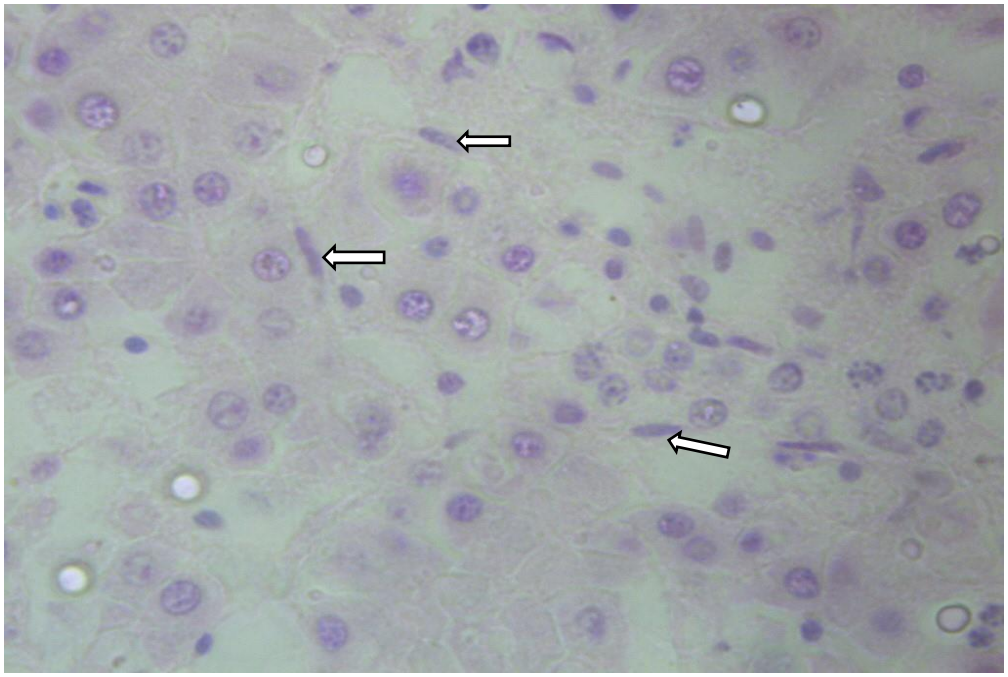


Figure 30: Kupffer cell hyperplasia

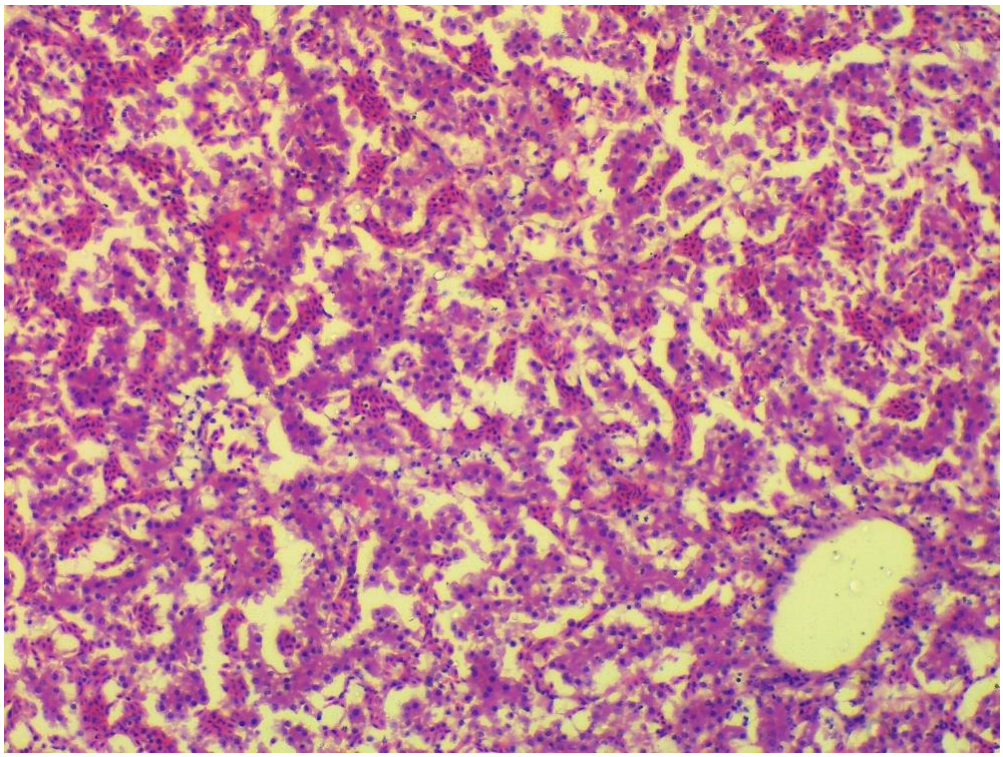


Figure 31: Individualization of hepatic cord

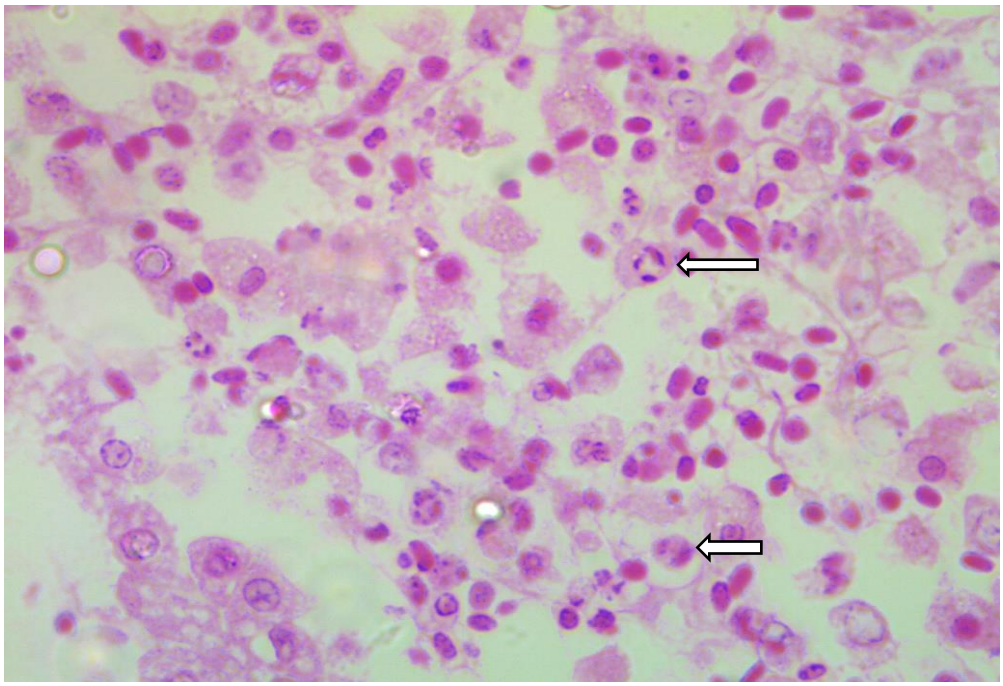


Figure 32: Presence of heterophil and macrophages

Histopathological slides of lung

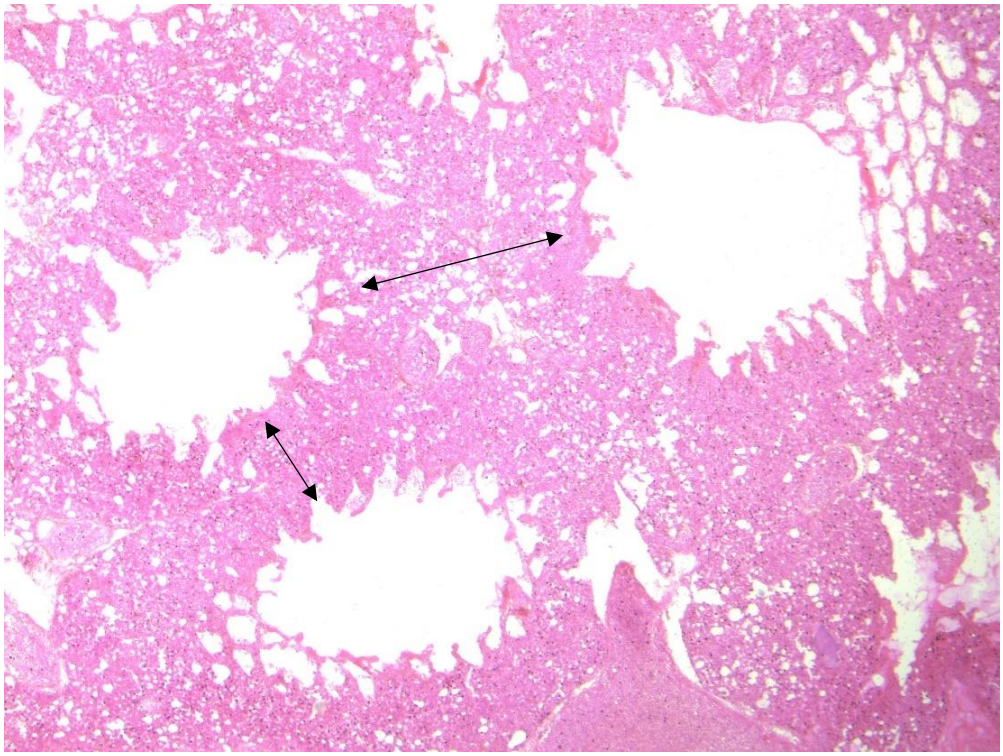


Figure 33: Thickning of alveolar septa of affected lung

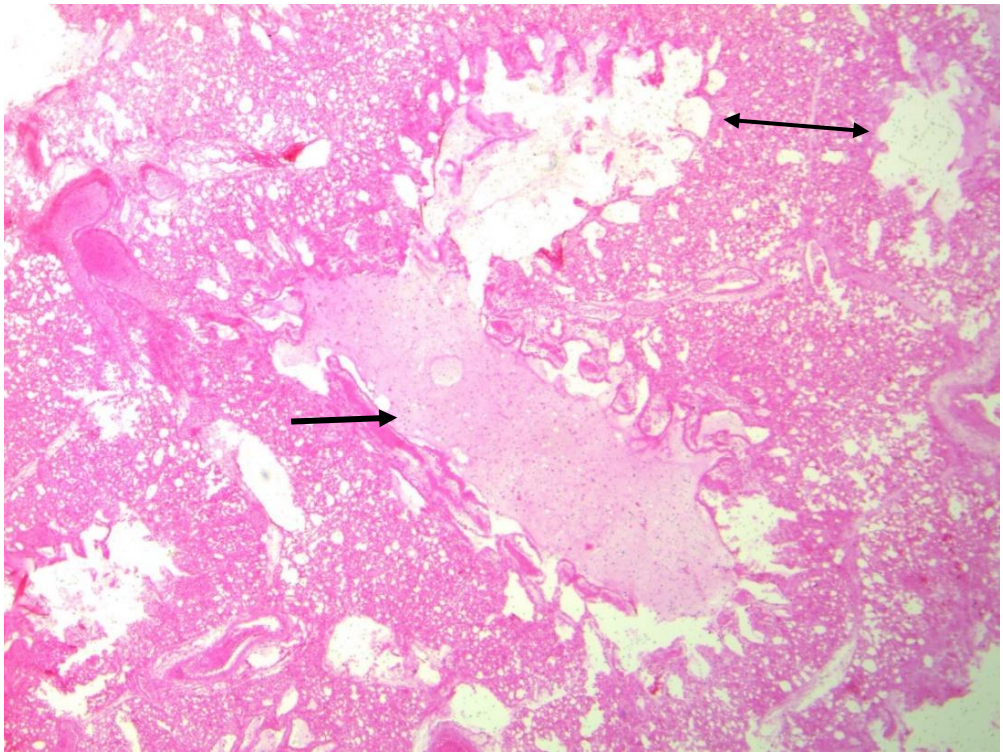


Figure 34: Thickning of alveolar septa , presence of exudates in the alveolar space

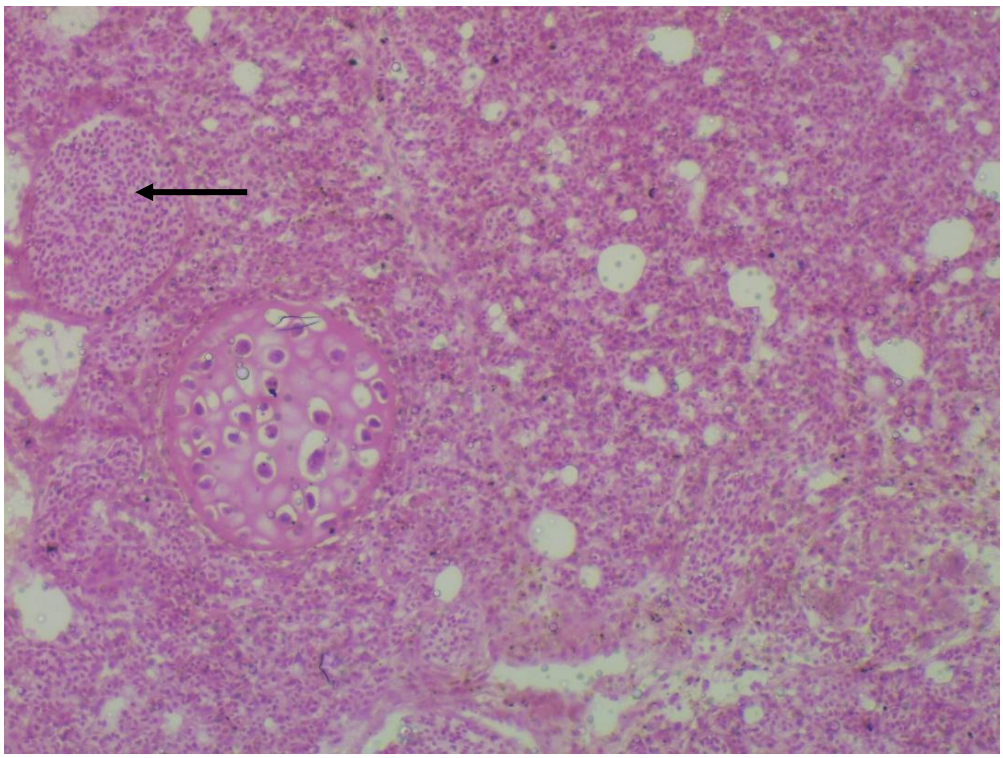


Figure 35: Congestion in the affceted lung

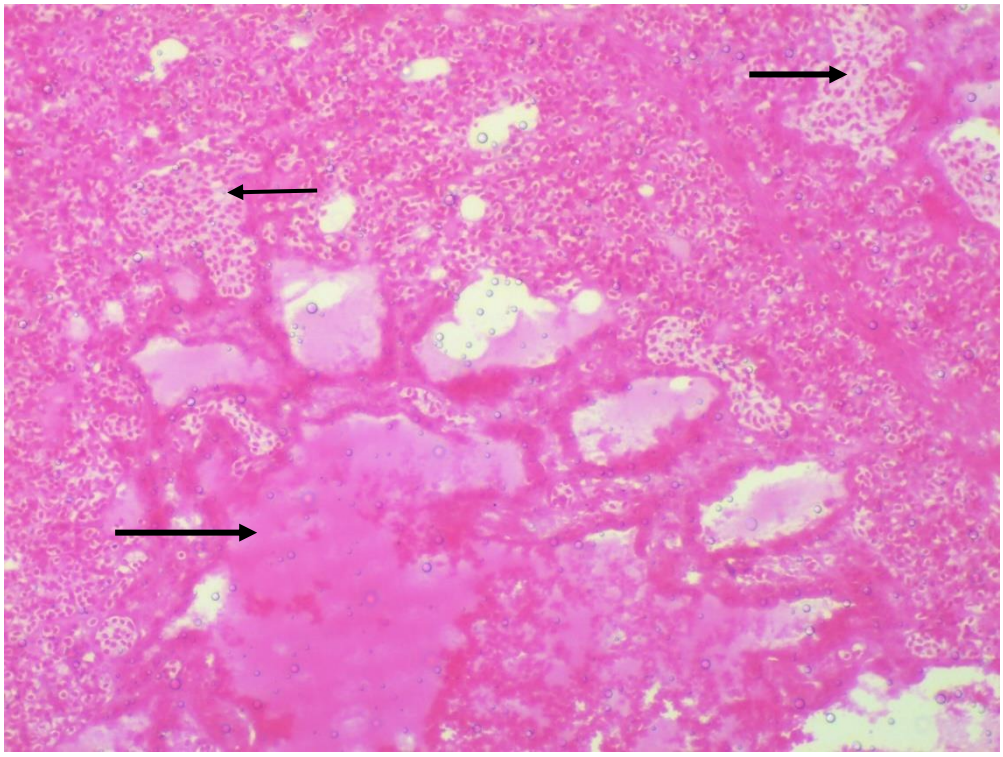


Figure 36: Congestion, hemorrhage & edema in the affected lung

Histopathological slides of Spleen

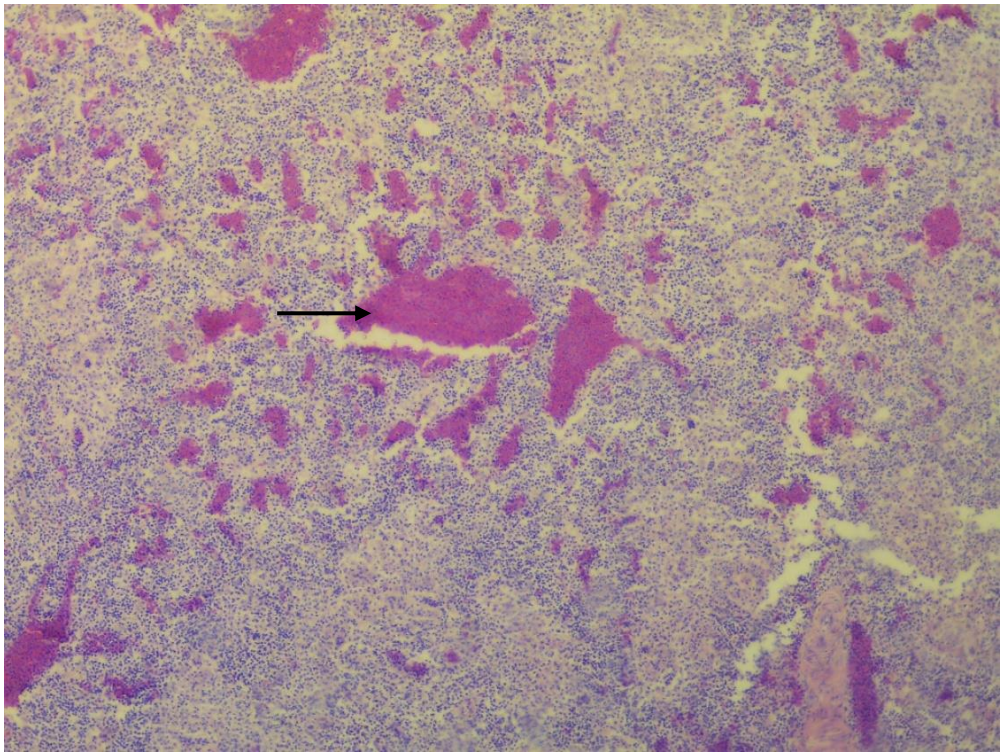


Figure 37: Hemorrhage and congestion in spleen

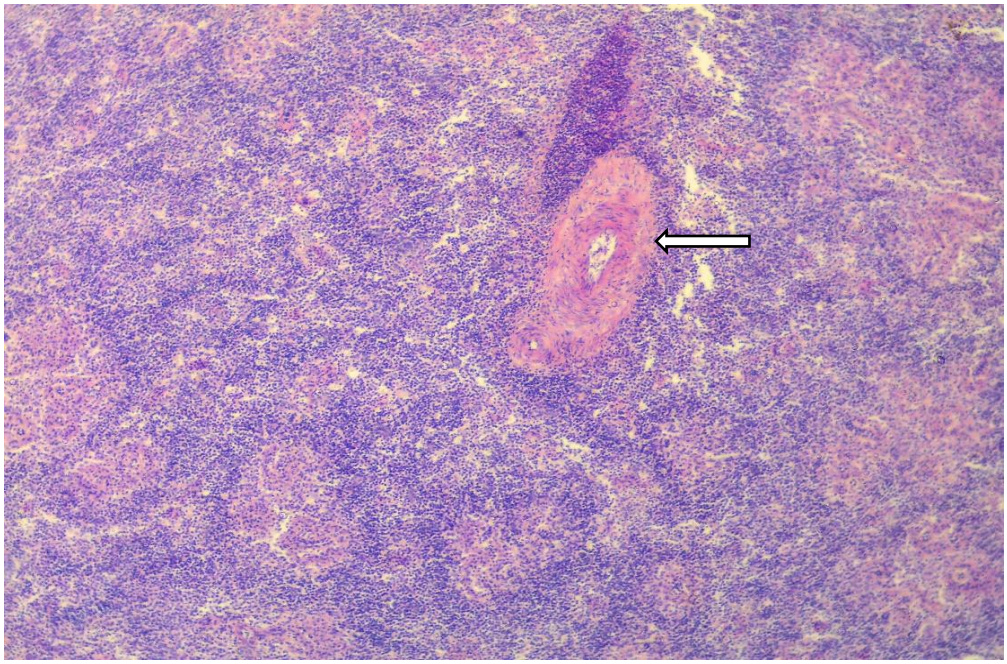


Figure 38: Thickening of artery and fibrous tissue proliferation

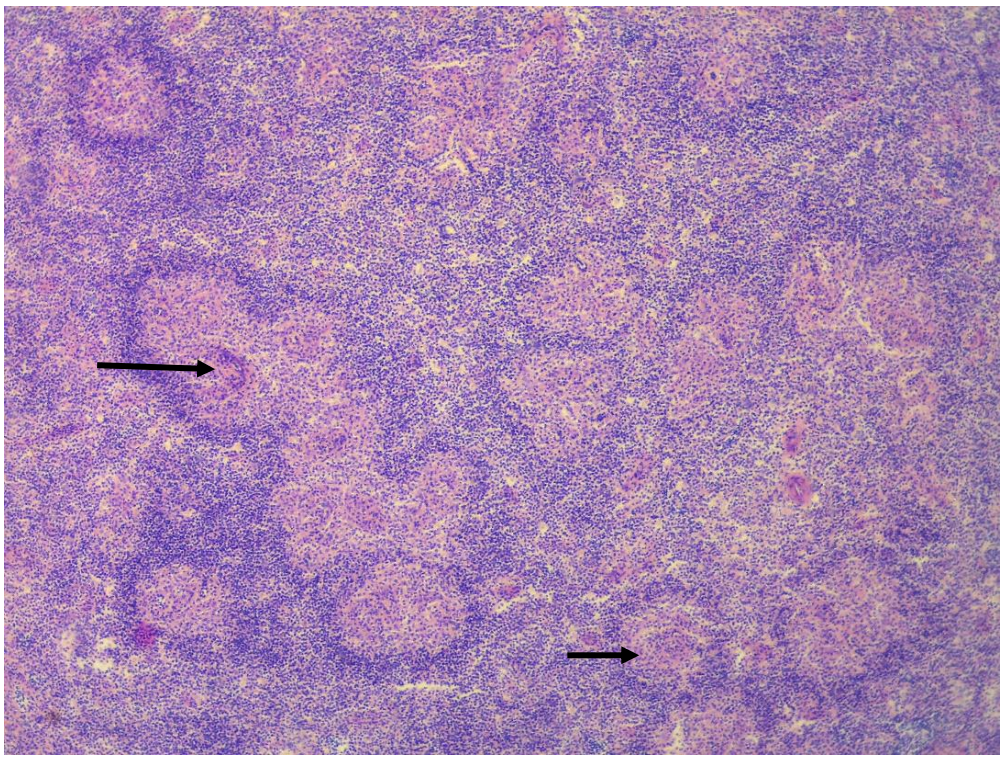


Figure 39: Destruction in white pulp in affected spleen

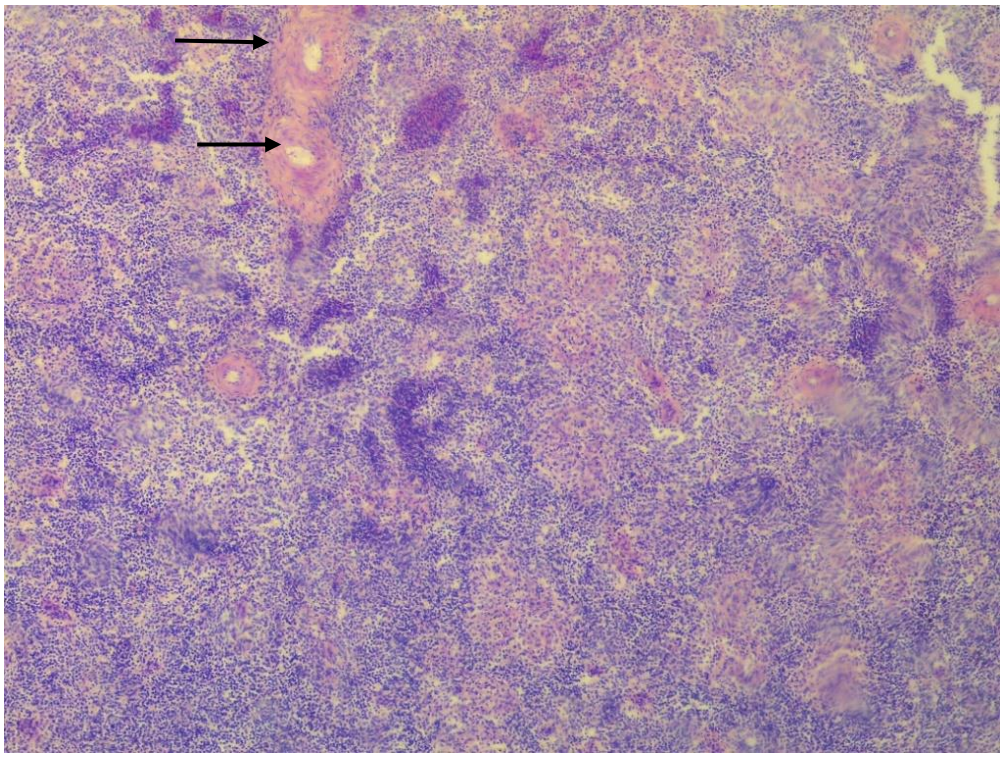


Figure 40: Spleen become smaller in size and artery become closer to one another

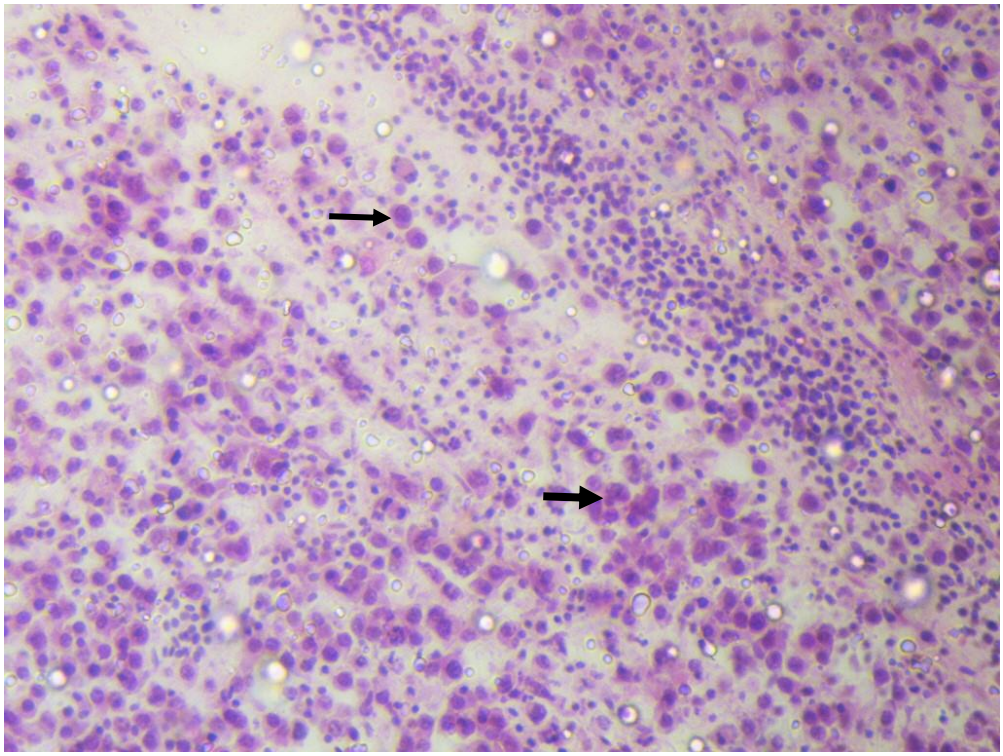


Figure 41: Proliferation of RE cells

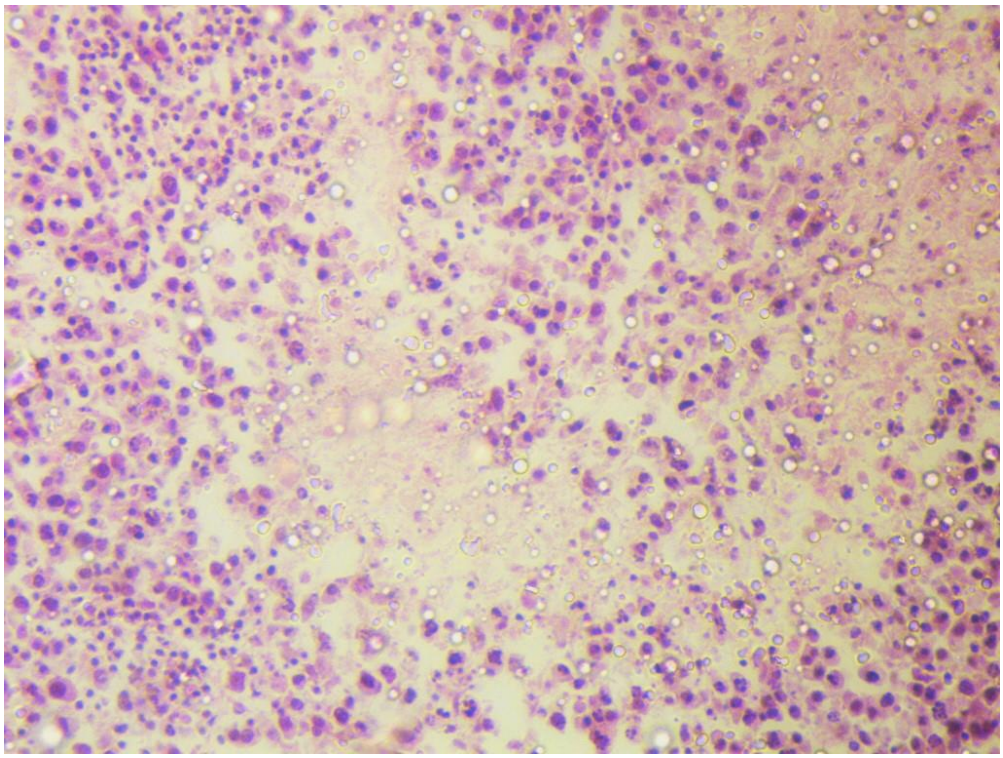


Figure 42: Presence of necrosis and tissue debris

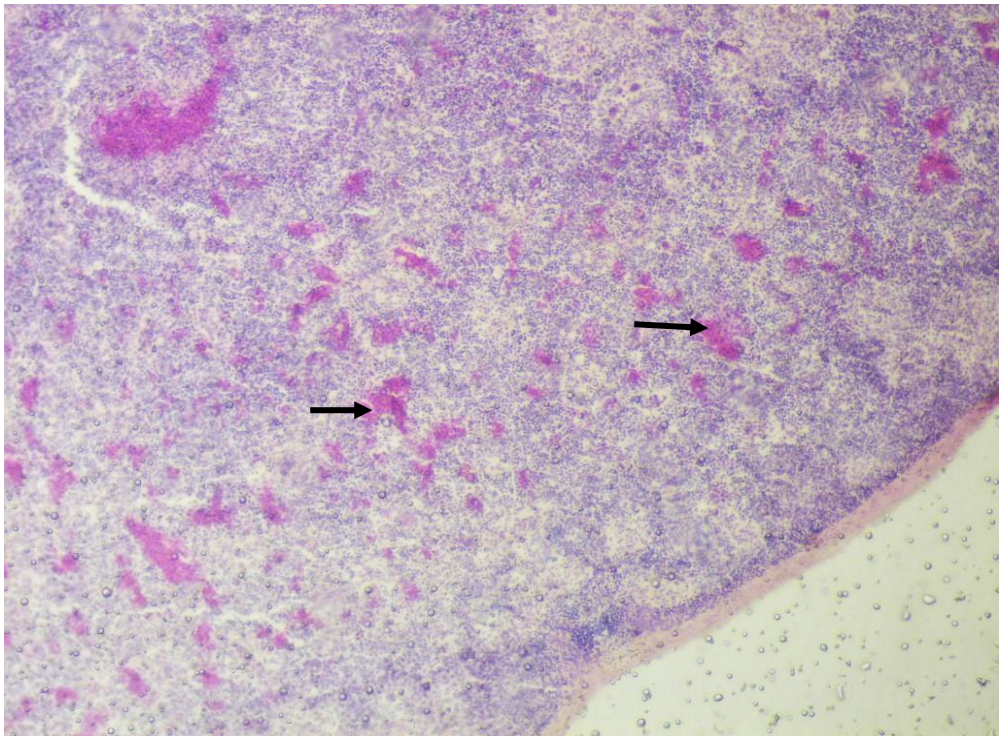


Figure 43: Overall congestion present in the spleen

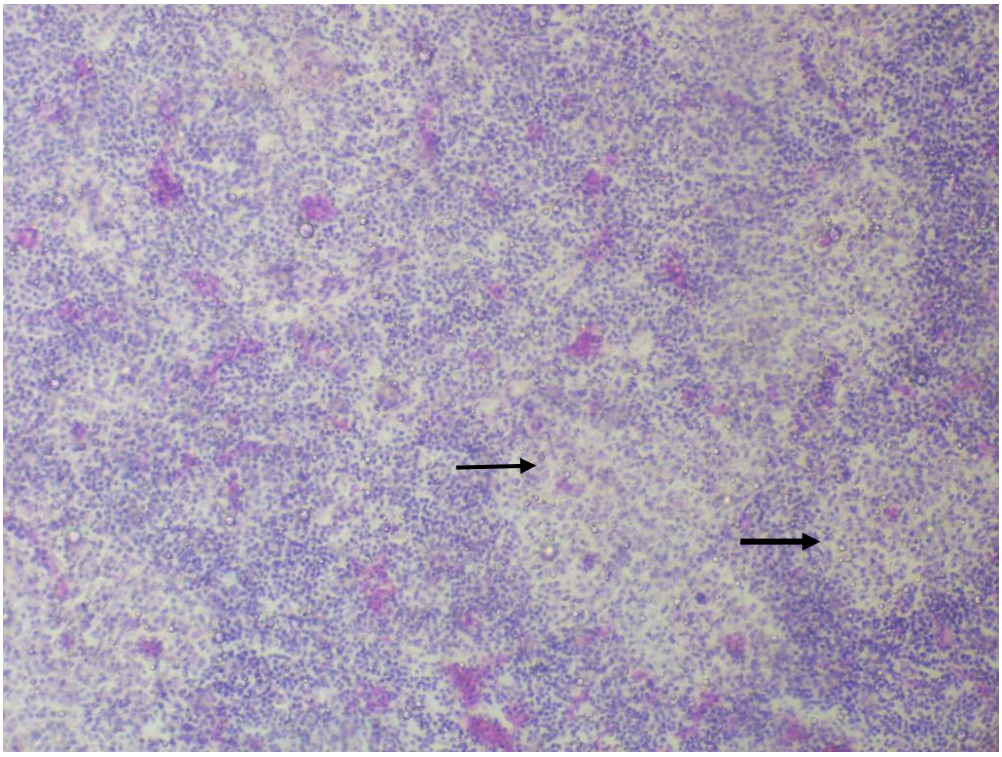


Figure 44: Depletion of lymphocyte in white pulp area

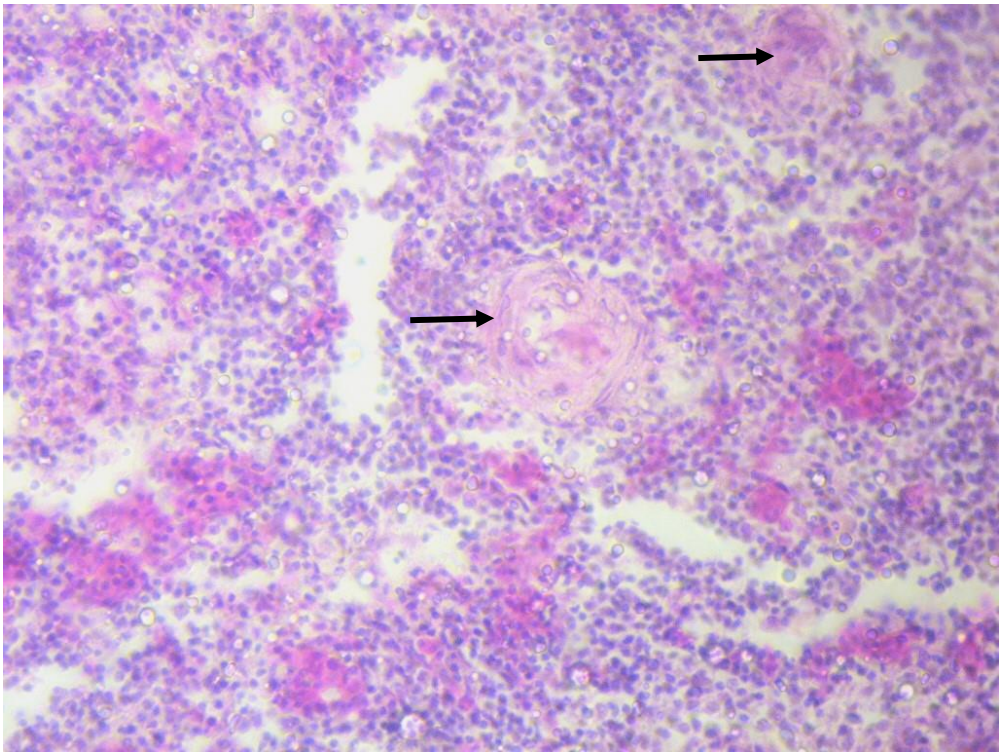


Figure 45: Destruction of lymphocyte in white pulp

These histopathological alterations provide insights into the pathological processes taking place within the organs, underscoring the multi-organ involvement and severity of the disease condition.

Table 4.3 Microscopic lesions among samples

Tissue sample (No. of samples)	Lesions	Number of observations
Liver (30)	Congestion Hemorrhage Inflammatory cellular infiltration Necrosis Fatty change Kupffer cell hyperplasia	25 (83.33%) 27 (90%) 17 (56.67%) 23 (76.67%) 10 (33.33%) 5 (16.67%)
Lung (20)	Congestion Inflammatory cellular infiltration Edema	15 (75%) 15 (75%) 8 (20%)
Liver (12)	Congestion Thickening of blood vessel RE cell hyperplasia Multiple focal area of necrosis	8 (66.67%) 5 (41.67%) 6 (50%) 4 (33.33%)

Chapter 5: Discussion

This study was conducted on 60 commercial broiler chickens to observe the pathological changes resulting from *E. coli* infection. Initially, 60 birds were presumptively diagnosed with colibacillosis, and their organs were preserved for further examination. Out of the 60 deceased birds, 45 tested positive for *E. coli* bacteria in bacterial culture. Notably, the colony characteristics observed in all isolated *E. coli* strains, such as the production of a metallic sheen on EMB agar and the presence of rose-pink colonies on MacConkey agar, were consistent with previous studies (Sharada et al., 2001). Among the 60 locally isolated *E. coli* strains, 45 were chosen for testing using housekeeping primers adk-f and adk-r, targeting the *E. coli* housekeeping adenyl kinase gene. The resulting 536-bp amplicons were confirmed through 1% agarose gel electrophoresis. Similar findings were reported by other researchers (Wirth et al., 2006), emphasizing the specificity of this 536-bp sequence to *E. coli*. According to PCR results, 37 cases were confirmed as *E. coli*, accounting for 61.67%.

Variations in gross pathological lesions were evident across different organs in various age groups. The majority of infections were observed within the age range of 0 to 2 weeks. Complex infections were predominant, with airsacculitis noted in 61.67% of cases, omphalitis in 35%, perihepatitis in 33.33%, pericarditis in 58.33%, septicemia in 21.67%, and enteritis in 31.67% of cases.

Severe gross pathological alterations were evident in the liver, manifesting as congestion, necrotic foci, fibrinous exudate on the liver surface, adhesions, and rounded edges. The gall bladder showed swelling, and abdominal fluid accumulation was observed. These observations are consistent with (Kumar et al., 2013), who reported similar liver surface changes. Avian colibacillosis, as documented by (Renu et al., 2012), exhibited a thick fibrinous layer on visceral organs. The heart displayed congestion, variable fibrinous layer deposition on the pericardium, and adhesions to the chest cavity, in line with Nakamura et al., (1985), Gangane et al., (2006), Renu et al., (2012), and Kumar et al., (2013). Air sacs were characterized by cloudiness with a thin to thick fibrin layer, more pronounced in thoracic than abdominal air-sacs. These findings align with Sylvester et al., (2005), who reported airsacculitis in field outbreaks, and Gangane et al., (2006), observing congestion and cloudiness in air-sacs post APEC inoculation. Kumar et al., (2013) reported fibrinous deposition on air sacs in older birds

during natural colibacillosis outbreaks, indicating its association with outbreak severity. Renu et al., (2012) identified cloudiness of air-sacs as a significant pathological lesion in chicken colibacillosis.

Gross lung lesions ranged from mild congestion to edema and consolidation. Macroscopically, the spleen exhibited a slight enlargement, along with varying degrees of congestion and isolated necrotic foci in more severe cases. These findings are in agreement with the research of Nakamura et al., (1985) and Kumar et al., (2013). In the intestine, our study identified enteritis and hemorrhage in the duodenal area. Shah et al., (2019) documented distinct macroscopic lesions in cases of omphalitis, characterized by unabsorbed yolk material (sac) and congested intestinal mucosa, aligning with our findings. Conversely, colisepticaemia cases in our study exhibited specific macroscopic lesions such as fibrinous pericarditis, perihepatitis, and airsacculitis.

The histopathological changes identified in the liver comprised congestion, cellular swelling, individualization of hepatocytes, and distortion of hepatic cords. These findings align with research by Hooda et al., (2011), Kumar et al., (2013), Goyal et al., (2004), Gangane et al., (2006), and Hooda et al., (2011). In Kumar et al.,'s (2013) investigation, there was a notable presence of fibrinous exudate on the liver, including heterophils, lymphocytes, inflammatory cells, fibrin, and degenerative alterations in hepatocytes, which corresponds to the observations in our study. Hooda et al., (2011) documented hepatic sinusoid dilatation, RBCs in sinusoids, vacuolation, hepatocyte degeneration, hyperplasia of Kupffer cells, congestion, hemorrhages, and fatty changes. Similarly, our study noted slight hyperplasia of Kupffer cells in the microscopic analysis.

Microscopic examination of the lungs shows different levels of hemorrhage and congestions. Mild cases showed mild heterophil infiltration in air spaces, while severe outbreaks exhibited focal areas of inflammatory cell proliferation aligned the findings of Kumar et al., (2013). The spleen exhibited diverse alterations, encompassing different levels of hemorrhage, congestion, localized infiltration of inflammatory cells, reduction of lymphoid components, and the presence of whitish focal areas with necrosis and reticulo-endothelial cell proliferation. These findings align with the

research of Hegazy et al., (2010), Kumar et al., (2013), both of whom documented a reduction in lymphocytes within lymphoid organs in instances of *E. coli* infection.

In the course of this study, our sampling strategy was formulated on the foundation of a presumptive diagnosis, meticulously derived through the observation of clinical signs and comprehensive evaluation of gross lesions during post-mortem examinations. This preliminary diagnostic approach aimed to identify potential cases of the targeted disease. Following the initial presumptive diagnosis, a thorough confirmatory diagnostic phase ensued, employing both bacterial culture and Polymerase Chain Reaction (PCR) techniques. These methods were chosen for their precision and reliability in confirming the presence of the disease-causing agent.

Upon completion of the confirmatory diagnostic procedures, our analysis revealed an impressive diagnostic accuracy rate of 61.67%. This substantiates the robustness of our presumptive diagnostic approach, affirming its capability to effectively and reliably identify instances of the investigated disease.

Chapter 6: Conclusion

Post-mortem examinations and histopathological examinations revealed notable findings in chicken's indicative of different forms of colibacillosis. The livers displayed coagulation-type focal necrosis, with infiltration of heterophils, lymphocytes, and macrophages mainly in the portal area. There was particular concern about fatty changes in the liver. Microscopic examination of the lungs revealed congestion, hemorrhage, and edema. The spleen showed scattered pyknosis of lymphocytes, destruction of white pulp, and proliferation of reticuloendothelial (RE) cells. These findings encompassed cloudiness and thickening of air sacs, congestion and thickening of the liver capsule, and congestion with consolidation in some cases of the lungs and spleen. These pathological manifestations collectively underscore the systemic nature of colibacillosis.

In essence, the consistency noted between macroscopic lesions, histopathological examination, and molecular diagnosis provides robust validation for relying on postmortem findings as a dependable approach to diagnose Colibacillosis. This thorough methodology enhances diagnostic accuracy and underscores the significance of postmortem investigations for recognizing and confirming diseases.

Chapter 7: Limitations

Our study is subject to several limitations. Firstly, due to constraints in both time and resources, we conducted the research on a relatively small scale. Consequently, we were unable to explore all forms of colibacillosis for sampling or comprehensive study, and our investigations are confined to what is readily available and accessible to us. Our emphasis lies in studying the discernible forms of colibacillosis that we can locate and currently have at our disposal. Secondly, the research conducted in Bangladesh was constrained to a specific geographical area, and the isolation of bacteria and PCR analysis focused solely on colibacillosis, without considering other bacterial infections. A more comprehensive understanding of farm conditions could be achieved by encompassing farms from various regions across the country. Thirdly, our study concentrated exclusively on broiler chickens, overlooking the substantial presence of layer and backyard chickens in the broader poultry production system of Bangladesh.

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Appendix

Reagents and solutions used in staining of tissue sections

Harris Hematoxylin	**1% stock alcoholic eosin**
Hematoxylin crystals…….. 5 gm. Alcohol 100% .....,..... 50 ml. Ammonium or potassium alum….. 100 gm. Distilled water……..……. 1000 ml. Mercuric oxide……..... 2.5 gm. After preparation of the stain 2-4 ml glacial acetic acid per 100 ml of solution was added. Stain was filtered before use.	Eosin Y ……...…. 1 gm. Distilled water ……...… 20 ml. Dissolve and add; Alcohol 95% ……..…. 80 ml. For working solution 1 part of Eosin stock solution was mixed with 3 parts of 80% alcohol. Just before use 0.5 ml of glacial acetic acid per 100ml of stain solution was added.
Acid alcohol	**Ammonia water**
Alcohol 70%……..1000 ml. Hydrochloric acid, concentrated ……10 ml.	Distilled water……1000 ml. Ammonium hydroxide, 28%.....2-3 ml.

❖ Staining procedure

i. Deparaffinization:
   a. Xylene …….2 changes, 5-10 minutes each.
ii. Rehydration through graded alcohol:
   a. Alcohol 100%.....2 changes, 5 minutes each.
   b. Alcohol 95%.....2 minutes.
   c. Tap water ……5 minutes.
iii. Harris hematoxylin……..10-15 minutes.
iv. Rinse in tap water……. 10 minutes.

v. Differentiate in acid alcohol……3-10 quick dips.
vi. Wash in tap water…… 5 minutes.
vii. Ammonia water (for bluing)……. 3-5 dips.
viii. Wash in tap water……. 10 minutes.
ix. Eosin…… 15 seconds to 2 minutes.
x. Alcohol 95% …… 2 changes, 2 minutes each.
xi. Alcohol 100% …… 2 changes, 3 minutes each.
xii. Xylene …… 2 changes, 2 minutes each.
xiii. Cover slip was placed on stained tissue after putting DPX.

The slides were then dried at room temperature and examined under microscope.

Biography

DR. Sadia Jahan is a veterinarian from Nagarpur Upazilla in Tangail, Bangladesh. She is the daughter of Md. Shahjahan Shiraz and Mrs. Anjuman Ara. She successfully completed her Secondary School Certificate (SSC) Examination in 2012 and her Higher Secondary Certificate (HSC) Examination in 2014. In 2020, she graduated with a Doctor of Veterinary Medicine (DVM) degree from Chattogram Veterinary and Animal Sciences University in Chattogram, Bangladesh. She is currently pursuing a Master of Science in Veterinary Pathology at the Department of Pathology and Parasitology, Faculty of Veterinary Medicine at CVASU. She is a candidate for the degree of Master of Science in Veterinary Pathology.