CHAPTER-1: INTRODUCTION

'Pet bird' designates those birds which are housed and bred for an exclusively ornamental purpose. This category includes mainly passeriformes (e.g. canaries, finches, sparrows etc.) and psittaciformes (parrots, parakeets, budgerigars, love birds etc.) (Mitchell and Mark, 2008). Pet birds rearing are considering an emerging sector in Bangladesh. This trendy pass-time hobby has already become a large commercial endeavor among the youth and also contributes to the national economy. Although there are no data available about the number of exotic and pet bird species, the number could be more than 30 according to the 'Chattogram Pet bird Breeders Association'. Many of the species were imported from abroad and bred afterwards to fulfill the demand of pet lover and aviculturist. One of the major impediments of this rising sector in Bangladesh is the occurrence of various types of diseases. These birds suffer from different types of diseases like endoparasitic infections (e.g. cestodes, trematodes, nematodes, protozoa) (Urquhart, 1996) including blood-borne protozoan diseases (e.g. Avian malaria, Haemoproteusis, Leukocytozoonosis) (Hellgren et al., 2004) and bacterial diseases (e.g. Colibacillosis, Salmonellosis) (Scheutzs et al., 2005) are more common.

The host and parasite relationship is an ancient one with an affirmation of parasites of intestine as cretaceous dinosaurs (Poinar, 2006). Nutrients are taken from the hosts by parasites and cause for pathological damage which is responsible for lacking host fitness by reducing reproduction, survival and growth (Holmstad et al., 2005). At the same time individual birds may be infected with different parasites. In birds, gastro intestinal parasites belong to various groups of helminths, such as cestodes, trematodes, nematodes and protozoa like *Eimeria, Giardia, Isospora* etc. Helminth parasites can cause for significant effects on bird populations. Like, in case of (*Lagopus lagopus*) willow ptarmigans that reduced annual growth, reduced host body condition and increase chick mortality with three various species of helminth parasitic infection (Holmstad et al., 2005). In most of the cases infected birds remained asymptomatic, other than in severe condition, clinical signs varied from anorexia, dull, depression, decreased feed conversion ratio, reduce body weight, diarrhea, melaena, obstruction of the intestines with a mass of worms reduced egg production, catarrh, anemia, weakness, paralysis, poor

feathering followed by death (Harrison et al., 2006). Endoparasites also cause for influencing physiological processes (Holmstad et al., 2005) which influence on normal body function and reproduction. Lack of hygiene, captive condition, and direct contact with human and environmental conditions such as humidity, rainfall, and temperature influences to maintain parasites populations in the host. Some parasites of bird have a zoonotic potential and cause for human diseases through direct or indirect contact with infected specimens (Ruff M, 1993). The probability of transmission of zoonotic diseases are influenced by many factors e.g. latent period, time of infection, population density, stability of the agent when exposed to the environment, animal handling, route of infection and virulence (Ruff M, 1993). Bad ventilation, wet litter, high density of birds, improper coccidiostat or other anthelmintic consumption and contaminated feeders that influences the infection of intestinal parasites (Ruff M, 1993). To the best of vigorous literature, there is no information or geographical distribution of parasitic diseases among bird species searches in Bangladesh.

For the identification of several endoparasites in birds faecal and sputum samples examination performed. As some stages of certain parasites (e.g. Coccidia) are shed subsequently, sampling faeces over several days is advisable (Globokar et al., 2017; Chitty and Lierz, 2008). The output of examination of sample must always be interpreted together with the sample quality and case history. The goal of this study is to examine a huge individual of faecal samples of pet birds under laboratory conditions by means of direct smear, flotation and sedimentation.

Other than gastrointestinal parasite, blood parasite like *Haemoproteus* spp., *Plasmodium* spp., *Leucocytozoon* spp. are another major hindrance to growth, production and reproduction and cause for increase mortality of birds (Beadell et al., 2006). *Haemoproteus* spp. are intraerythrocytic parasites which are vector transmitted and closely related to the vertebrate's malarial parasites. But unlike *Plasmodium* spp., rather than circulating erythrocytes, they undergo asexual reproduction (merogony) within tissues. Haemoproteus are the most common and widespread hemoprotozoa of birds, but their potential significance as disease agents is mostly vague. Some species of *Haemoproteus* cause severe myositis in avian hosts which is highly pathogenic.

Avian malaria caused by protozoan parasites in the genus *Plasmodium* is a common mosquito-transmitted disease of wild birds. Infections occurred by more than 40 species that differ widely in geographic distribution, pathogenicity, host range, and vectors. *Plasmodium,* of the avian species has developmental and morphological characteristics approximately similar with the genera *Leucocytozoon* and *Haemoproteus*, but the presence of (merogony) asexual reproduction in circulating erythrocytes which differentiate it from both. There are numerous reports of acute, pathogenic infections with *Plasmodium* in individual birds. Epizootics reports are rare and maximum are associated with captive birds and abnormal host–parasite relationship as well as introductions of parasites or mosquito vectors to remote islands of the world. Among all, one of the most widely distributed avian malaria species is *Plasmodium relictum* (Beadell et al., 2006), which has an important role as a limiting factor in the current availability of native Hawaiian forest birds (Woodworth et al., 2005; Foster et al., 2007).

Leucocytozoonosis are caused by various species of Apicomplexa under the genus *Leucocytozoon,* which is a vector-borne protozoan disease of birds. Only a few are known to be pathogenic to their hosts among many species of *Leucocytozoon*. Avian groups at risk include galliforms, raptors, waterfowl, ostriches and pigeons (Bennett et al., 1993; Valkiūnas et al., 2005). Many species cause huge mortality in domestic birds and poultry, and *Leucocytozoon simondi* causes localized epizootics in geese wild ducks (Herman et al., 1975). They are transmitted by black flies (Simuliidae), other than *L. caulleryi*, which is transmitted by biting midges under the family Ceratopogonidae, closely related to species of the genera *Plasmodium* and *Haemoproteus* and similar life cycles (Valkiūnas et al., 2005).

Escherichia coli and *Salmonella* spp. are Gram-negative, facultative anaerobic bacterium, which belongs to the Enterobacteriaceae family. Gastrointestinal tract is the main habitat and part of the normal intestinal microbiota in humans, birds and other mammals. They have the survival capability in the environment for long periods of time and can propagate in water as well as soil (Ishii et al., 2006). *Salmonella* spp. and some strains of *E. coli* have pathological significance and cause for remarkable chick mortality (Lillehaug et al., 2005).

Antimicrobial resistance (AMR) is a global concern for animal and human health (Scheutzs et al., 2005). Now a days, it is noted that increasing interest in bacterial resistant to antibiotic and resistance genes detection from different bird species. The resistance level is propagated in commensal bacteria with the pathogenic one now a days (Magiorakos et al., 2012). Pet birds are expected to be exposed directly to antimicrobials, due to use of different antibiotic among them. Wide ranges of antibiotic are used such as colistin sulphate, amoxicillin, ceftrioxon, gentamicin, streptomycin, azithromycin, ciprofloxacin, enrofloxacin and different combined antibiotic by pet owner by themselves as malpractice in pet birds in Chattogram metropolitan area to prevent disease. This type of extensive use of antibiotic contributes to emergence resistance gene among commensal as well as pathogenic bacteria (Jakobsen et al., 2010). As a result of wide use of antibiotic with poor healthcare system *E. coli* isolates resistant to ciprofloxacin, amoxicillin, streptomycin, azithromycin, colistin sulphate, tetracycline in farm animal and chicken (Agabou et al., 2016). As a results of chromosomal mutation, acquired resistance to polymyxins in naturally susceptible bacteria. bla_{TEM} for ampicillin resistance gene, *tetA* for tetracycline resistance gene, *sul2* for sulfur drug resistance gene, *gyrB* for ciprofloxacin resistance gene had been found *E. coli* infection in chicken (Sarker et al., 2019). The release of resistant antibiotic along with the resistant gene from bird facilitates the public health crisis (Sarker et al., 2019).

Specific objectives:

a) To determine the occurrence of endoparasites (e.g. cestodes, trematodes, nematodes, protozoa) in different pet birds species in Chattogram Metropolitan area, Bangladesh.
b) To investigate the occurrence of blood protozoan diseases (e.g. Avian malaria, Leukocytozoonosis and Haemoproteusis) in pet birds.
c) To assess the antibiotic resistance against *E. coli* and *Salmonella* spp. isolated from pet birds.
d) To determine the antibiotic resistance genes (bla_{TEM}, *tetA, tetB, tetC, sul1, sul2, gyrA, gyrB, parC and CTX-M-2*) in *E. coli* and *Salmonella* spp.

CHAPTER-2: REVIEW OF LITERATURE

2.1.1. Epidemiology of gastrointestinal parasites in birds

Coccidian parasite

Although there may be a high prevalence of oocyst shedding in the population, most wild birds infected with *Isospora* spp. have subclinical infections and as a result there is no identified symptoms. For instance, the coccidian parasite prevalence can be up to 90% in free living adult blackbirds in Germany (Misof, 2004). In a New Zealand study a 40% prevalence of coccidia among clutches of song thrushes (*Turdus philomelos*), blackbirds (*Turdus merula*) and starlings was found with no identified dissident impact on young birds, with nestling weight, clutch size, fledging success measured, tarsus length and plasma carotenoid concentration (Cassey and Ewen, 2008). The effect of subclinical infections on wild host health and reproduction has not been fully established, although decreased reproductive performance, an eleven general decline of condition and increased susceptibility to other infections might occur. Only when encountered with stress, e.g. capture stress, birds those are free-ranging observed to be seriously affected by the disease (Gill and Paperna, 2008). For instance, 97% died of black siskins (*Carduelis atrata*) from proliferative visceral *Isospora* infection after 2 months of transportation from Bolivia to Italy where the stress was a factor (Giacomo et al., 1997). It has been observed that greenfinches inoculated with multiple "strains" of *Isospora* developed higher infection intensities than single parasite species infection (Horak et al., 2006).

Trematode

Environmental conditions that affect the distribution of their intermediate hosts influence the geographical distribution of trematodes. These features include abiotic variables of the lentic environment such as size, salinity, characteristics of the sediments and average depth and biotic variables such as vegetation cover. For instance, a trematode as an intermediate host uses a particular species of mollusk may only occur where that species is available. Uvulifer ambloplitis's hosts (kingfishers) include fish, snails, and birds as intermediate host (Soulsby and L., 1982). This trematode distribution depends on the

availability of all three hosts. Monostomes infection occurs in a large variety of hosts such as *Catatropis verrucosa* and *Notocotylus attenuatus*. *Hypoderaeum conoideum* and *Echinostoma revolutum* are almost entirely inclined to infection in ducks and their close relatives (Hoeve and Scott, 1988). The American coot (*Fulica americana*) can serve as the definitive host and exotic European faucet snail (*Bithynia tentaculata*) can serve as the intermediate host, for *L. polyoon*, *C. bushiensis*, and *S. globulus* parasites. In both Europe and the US, *C. bushiensis* and *S. globulus* share similar waterfowl hosts. (Hoeve and Scott, 1988) reported about coinfections with *S. globules* and *C. bushiensis*. Within 3–8 days after infection by all three parasites can produce mortality in the avian host. Profligate of avian trematodes all over the world can possible by cross migration and movement of birds following the breeding season and through annual migrations.

Cestode

On the basis of avian and intermediate hosts and successful transmission of the parasites, the geographic distribution of cestodes dependent. At local scale, cestode species may be present in some areas and absent in others due to availability of migratory birds where cestode species may be acquired on the breeding grounds. Cestode may be transported from wintering areas to Asia by migratory birds. If parasite life spans are short and local transmission is not possible, some cestode species may persist while others may disappear (Wallace and Pence, 1986).

On the range of species every parasite has limits it can infect. An infection is possible only if physiological (compatibility) criteria and a series of environmental (contact) are met. Some species of cestodes infect less diverse species likewise species of Schistotaenia are specific to grebes (Stock and Holmes, 1987).

Nematode

Gapeworms (*Syngamus* spp.) have been reported globally in temperate to cold temperate and tropical climates. Species of *Ascaridia* and *Heterakis* are widely distributed all over the world except Antarctica. Ascarid nematodes have been found at least 139 host species and *Heterakis* in at least 107 captive and wild bird species. Some species are cosmopolitan (e.g., *Ascaridia columbae, A. galli, Heterakis dispar, H. gallinarum*),

which are seen worldwide distribution. In specific geographic regions, some specific species found (e.g., *Ascaridia geei* in China, *Heterakis pavonis* in Japan) (Atkinson et al., 2008).

Porrocaecum spp. is cosmopolitan in distribution for instance *Porrocaecum ensicaudatum* is a cosmopolitan parasite of passerine birds. *Porrocaecum depressum* also distributed all over the world and approximately 47 species of birds infected by it (Morgan and Schiller, 1950). In two or more avian species, maximum *Capillaria* species have been reported and a few are known to cosmopolitan distribution (e.g., *E. contortus* infects in nine orders of birds) because of the wide geographic range of the numerous hosts they infect. Some capillarids are only found in areas where suitable hosts are present so have a narrower host range. For instance, *Eucoleus frugilegi* infects a range of species in the family corvidae while *Pterothominx moraveci* infects only port lincoln parrots (*Barnardius zonarius*) (Morgan and Schiller, 1950).

2.1.2. Some prevalence data of gastrointestinal parasites in birds

Psittaciformes

In general, parasitism of captive parrots in Europe is low. Outdoor aviary birds may be more predisposed to parasitism due to increased access to the ground and potentially to faeces of wild birds (Harcourt-Brown and Chitty, 2005). The most frequent parasites found in this group were *Capillaria* and *Ascaridia* eggs (3.2% and 2.6% respectively), though at significant lower rates compared to columbiformes and galliformes (Globokar et al., 2017). *Capillaria* eggs had been reported in one st. vincent parrot (*Amazona guildingii*) (Deem et al., 2008) and in 5/33 parrots (African grey parrots, white cockatoos, macaws, and parakeets) in previous studies (Chitty and Lierz, 2008). *Ascaridia* eggs had been reported in 4/33 psittacines (lori, love bird, white cockatoo, amazon parrot) (Patel et al., 2000). Five *Ascaridia* spp. are regarded specific to parrots: *Ascaridia hermaphrodita*, *Ascaridia sergiomeiari*, *Ascaridia ornate* (Neotropical area), *Ascaridia nicobarensis* (Oriental area) and *Ascaridia platyceri* (Australia). In Globokar et al., (2017), *Ascaridia* eggs were detected in cockatoo, cockatiel and budgerigar (1.4–2.7%) with a higher occurrence than previously reported in single parakeets, cockatiels, amazon parrots and

African grey parrots before (Tsai et al., 1992); and the occurrence of *A. platyceri* (20%) in budgerigars, African grey parrots and eastern rosella in Poland (Balicka-Ramisz et al., 2007).

Galliformes

In Germany, the most prevalent parasite genera were *Eimeria* oocysts 53.9% (CI = 51.2–56.6%), *Capillaria* eggs 38.3% (CI = 35.7–41%), *Ascaridia* eggs 31.2% (CI = 28.7–33.8%), (Globokar et al., 2017) which is in accordance to other studies (Tomza-Marciniak et al., 2014). *Eimeria* spp. was the most prevalent parasite recorded in broilers (77.78%; 95% CI = 65.28–87.36), layers (49.02%; 95% CI = 35.55–62.60), cockerels (45.16%; 95% CI = 28.47– 62.72), indigenous chickens (23.88%; 95% CI = 19.54–28.67), and turkeys (52.17%; 95% CI = 32.13–71.70) (Ola-Fadunsin et al., 2019a). *Raillietina* spp. was detected as the most prevalent helminth species (86–92 %) followed by *A. galli* (70–86 %), and *H. gallinarum* (70–76 %) in in Narsingdi district, Bangladesh (Ferdushy et al., 2016).

Passeriformes

In Germany, 18.1% (CI = 14.4– 22.2%) *Isospora* oocysts, 3.5% (CI = 1.9–5.8%) *Eimeria* oocysts, 5.3% (CI = 3.3–8.0%) *Capillaria* eggs, 0.5% (CI = 0.1–1.8%) *Ascaridia* eggs, 0.5% (CI = 0.1– 1.8%) *Cyathostoma* eggs, 2.5% (CI = 1.2–4.6%) *Syngamus* eggs, 1.3% (CI = 0.4–2.9%) *Raillietina* eggs, 5.5% (CI = 3.5–8.2%) *Porrocaecum* eggs and 1.0% tape worm eggs were detected (Globokar et al., 2017). In 58 species and 21 families of the order passeriformes, two species of *Eimeria* and 81 species of *Isospora* have been reported (Berto et al., 2011). Globokar et al. (2017) found the occurrence of *Isospora* oocysts was 11.5% in canaries and therefore below the occurrence in canaries examined in Brazil (50.5%) (De Freitas et al., 2003), and in Elazığ province in Turkey (28.1%) (Eşaki et al., 2012). Likewise, the prevalence of *Isospora bocamontensis* has been reported to be high (44.5%) in captive yellow cardinals (*Gubernatrix cristata*) and may be even higher in free-ranging passerines (Urquhart, 1996). *Isospora canaria* and *Isospora serini* have been described in canary birds (Box, 1970) with *I. serini* representing an exception to the coccidian life-cycle. In corvids of the present study, a

high rate of parasitism was found (37.5% for *Porrocaecum* eggs and 26.8% for *Capillaria* eggs). Little is known about the pathogenic effects of *Capillaria* spp. in these avian hosts besides the formation of diphtheritic membranes in the gastrointestinal tract and emaciation caused by *Capillaria contorta* (Helmboldt et al., 1971).

Columbiformes

In Germany, the occurrences of 62.5% (CI = 60.5–64.5%) for *Eimeria* oocysts, 24.8% (CI = 23.0–26.6%) for *Capillaria* eggs, 16.6% (CI = 15.0–18.2%) for *Ascaridia* eggs and 0.4% (CI = 0.2–0.8%; n = 10) for *Trichostrongylus* eggs were identified. One pigeon shed *Syngamus* spp. eggs (Globokar et al., 2017). Previous studies reported lower prevalences (2.5–40.2%) of *Eimeria* oocysts in domestic pigeons than in this survey (Radfar et al., 2012). In contrast, Dovč et al. (2004) noted a high prevalence (71.9%) of *Eimeria* oocysts in free-living pigeons. Regarding nematode infections, higher prevalences (55.6–67.2%) of *Capillaria* eggs have been reported in pigeons previously, compared to the current study (Tanveer et al., 2011). The occurrence of *Ascaridia columbae* eggs (16.6%) in the present study was in agreement with studies conducted in Iran and Brazil, but lower than the prevalences (30–42%) observed in other studies (Senlik et al., 2005; Mushi et al., 2000).

2.1.3. Pathology and pathogenesis of gastrointestinal parasites in birds

Coccidian parasite

At post mortem examination, clinically affected birds commonly show reduce body mass, intestinal wall get thickened and excessive mucus with liquid gut content as well as there may be haemorrhage and necrosis in the mucosa of intestine. In cases of extraintestinal infection in their life cycle, extraintestinal findings may include discoloration of the spleen and splenomegaly and due to bile retention green colouration of liver and pinpoint necrosis as well as hepatomegaly, in canaries and black siskins. Martinez and Muñoz, (1998) observed a liver that was pale yellow in colour and found blood in the abdomen of a hybrid passerine. It is observed that hihi that died of coccidiosis suffered from a different body condition, ranging from in term of condition thin to good (Twentyman, 2001). The heavily infected hihi birds had a swollen abdomen; livers that were either

smaller than normal in size or swollen and sometimes small spots were visible on the livers and congested soiled vent.

Generally, it is observed at histopathologic examination that in the lamina propria of the intestine infiltration of mononuclear inflammatory cell and in the liver and spleen species that have extraintestinal stages. The inflammatory cells are mainly monocytes, a few plasma cells and lymphocytes (Martinez and Muñoz, 1998). Presence of coccidian sexual stages in the intestinal epithelium and in the lamina propria was observed in a case of *Atoxoplasma* in a hybrid passerine (Martinez and Muñoz, 1998). Infiltration of mononuclear cells were also identified at the glands of Lieberkühn in crypts, and a huge number of oocysts in fresh smears of, duodenum and jejunum contents in infected black siskins (Giacomo et al., 1997). Some fewer researchers working with variety of passerines observed that within the spleen, with an overall cellular increase there is signs of activation of the lymphoid follicles, and infiltration occured into the liver through centrilobular ways and around portal triads (Giacomo et al., 1997). Some authors have proved that in infected captive canaries, pronounced bile duct hyperplasia with perivascular accumulations of mononuclear cells, particularly in the periportal areas and microgranulomas in infected livers (Sánchez-Cordón et al., 2007). In infected black siskins, the lungs were also generally congested, swollen and infiltrated with exudate and intense hyperaemia with numerous mononuclear cells adhering to the walls of the blood vessels (Giacomo et al., 1997).

In greenfinch, within parasitophorous vacuoles in the cytoplasm, indenting the nucleus, of leucocytes in the blood vessels in the small intestine, there are merozoites of an *Atoxoplasma* species (Hudson and Lee, 1988). Merozoites were found predominantly in the spleen, in case of Israeli sparrows but rarely in the peripheral blood, multifocal necrosis the visceral organs (Gill and Paperna, 2008). The lamina propria contained giant cells, moderate numbers of lymphocytes, and proliferating fibroblasts. Sometimes coccidian sexual stages also present in the lamina propria, and there was a loss of epithelium and the intestinal villi were flattened. In infected hihi's liver there had scattered, sometimes perivascular, excessive lymphoid foci (Twentyman, 2001).

Trematodes

Host tissue damaged by the parasites due to the ingestion of tissue. Inflammation and immune-mediated pathology had been seen due to host immune responses in different organ on the basis of infection.

Liver, Bile Ducts, and Gall Bladder: Inflammation of the bile duct epithelium, blockage of the duct, enlargement of the bile duct lumen, and backflow of bile into the liver, leading to dystrophic changes in the liver of the infected avian host. Lesions have been reported from infections of metorchis bilis in white-bellied sea-Eagles (Krone et al., 2006). Most infected birds had numerous white, dark green or black foci on enlarged livers with irregular capsular surfaces. Microscopic lesions consisted of granulomas composed of multinucleated giant cells, other inflammatory cells surrounding necrotic debris, eggs, pigment, occasionally bacterial colonies with fibrous connective tissue. Trematode can migrate via the bile duct, pancreatic duct, and ureter to reach pancreas and kidney (Kuiken et al., 1999).

Kidneys: Distention and thickening of their walls of the collecting tubules of infected kidney and in the parenchyma there are extensive cellular infiltration of infected birds. Blue-winged macaws (*Primolius maracana*), white-eared parakeets (*Pyrrhura leucotis*), blue and yellow macaws (*Ara ararauna*), and ring necked pheasants (*Phasianus colchicus*) infected with *P. bragai* had irregular cortical surfaces on enlarged kidneys with brown-yellow discoloration. Microscopic lesions consisted of granulomatous nephritis, lympho plasmacytic infiltrate with some epithelioid macrophages with few heterophils and included an interstitial, multi focal to coalescent (Luppi et al., 2007).

Air Sacs: The air sacs of snail kites (*Rostrhamus sociabilis*) were infected by the *Bothrigaster variolaris* (Cyclocoelidae) (Cole et al., 1995). The air sacs were opaque and in the folds and angles of the tissues tan granular deposits had accumulated. Mild granulomatous airsaculitis, peribronchitis, pyogranulomatous bronchitis with mild squamous metaplasia of the epithelium of lungs were observed.

Gastrointestinal Tract: The character of the lesions depends on the species of trematode, specific site of infection and the number of parasites. *Echinostoma* spp. can cause mild to

severe enteritis in birds. Lesions are generally mild to severe ulcerative hemorrhagic enteritis, characterized by thickening of the intestinal wall and a fibrinous to caseous core of necrotic debris that blocks the lumen of the intestine due to intestinal trematode infection (Friend et al., 1999). Epizootics in waterfowl in the US caused by three gastrointestinal parasites namely *Sphaeridiotrema globulus*, *C. bushiensis,* and *L. polyoon*. Balloon structure of the jejunum and ileum with cyanotic appearance caused by infection of *S. globulus*. Foci of hemorrhage on the serosal surface observed. Both ceca can be affected and may distend, elongated and externally appear to be dark. Whitish caseous plaques and numerous hemorrhagic areas, ulceration, generalized mucosal necrosis, and firm, irregular cores may be present appeared internally (Gibson et al., 1972). Infection with *Ribeiroia* caused enlargement and reddening around the orifices, grayish exudates on the surface and superficial ulceration of the proventriculus. Histologically, outer portion is necrotic with a polymorphonuclear leukocytic infiltration (Kocan and Locke, 1974).

Eyes: Intense inflammatory response and erosion and ulceration of the conjunctival membrane were evident in histological sections of infected areas of the eye. Diffuse conjunctivitis was present in a swan goose when infected with of *P. gralli (Anser cygnoides*) (Ebbs et al., 2018).

Oviduct: In white-throated sparrows (*Zonotrichia albicollis*) infection of the oviduct with *P. marcrochis* results in distention and exudates accumulation, inflammation, catarrhal to a fibrinous exudates or a caseous mass may be present in the oviduct lumen. Oviduct ruptures leads to peritonitis with possible organ adhesions in the abdomen (Ebbs et al., 2018).

Cestode

They cause intestinal blockage, localized damage to the intestinal wall, inflammation or irritation of the intestinal lining or damage to the gizzard lining (Gastrotaenia). *Gastrotaenia* infection causes lesions on the lining of gizzard roughened, friable areas with ecchymoses and necrosis. In willow ptarmigan infected with *Raillietina spp.* causes intestinal hypertrophy (Thomas, 1986). In duck, infection by *C. infundibulum* caused

shortening of villi (Kishore and Sinha, 1989). Infection with *Otiditaenia macqueeni* in red-crested bustards (*Eupodotis ruficrista*) responsible for accummolation of small inflammatory nodules which is consist of, lymphoid cells, plasma cells, macrophages and clumps of hemosiderin. *C. megalops* cause uterus inflammation, heterophils infiltration in the walls of the ureters and in the kidneys of duck (Wobeser, 1997). During larval migration from the gut to internal organs, or musculature, transitory damage occurs and chronic lesions had been seen in the tissues. Pleurocercoid larvae cause fatalities followed by beath in captive pink-backed pelicans (*Pelecanus rufescens*) (Toplu et al., 2006).

Nematode

Trichostrongylus: Adult worms cause flattening of the epithelial cells, atrophy, and trauma of the caeca (Hudson and Lee, 1988). In chronic cases, the cecal contents may become yellowish white cheesy consistency and heavy infections may cause hemorrhagic typhlitis. *T. tenuis* leads to a loss of body condition, increased mortality (Dobson and Hudson, 1992).

S. trachea: The lungs and trachea in chickens and birds is the main pathological site. An experimental infection shows that any gross lesions do not appear in the lungs of pheasants until 7–9 days after infection but microscopic lesions are seen as early as 3 days PI (Fernando et al., 1991). Approximately 0.2 mm in diameter visible white foci can be seen in lungs due to larval migration through the lungs. In microscopically increase in the number of lymphocytes within the parabronchi, cuffing of larger vessels, connective tissue. Inflammation and secretory products can be seen in infection sites. Pale tracheal nodules form at the point of attachment had been seen in ring-necked pheasants (Fernando et al., 1991).

Ascaridia and *Heterakis*: The ceca infected with *H. gallinarum* may exhibit petechiation, congestion, thickening and of the mucosa, cecal abscesses, nodular typhlitis and intussusceptions in ring-necked pheasants (Menezes et al., 2003). Nodule can be surrounded by macrophages, lymphocytes, fibroblasts, plasma cells, giant cells and epithelioid cells (Balaguer et al., 1992).

Ascarid nematodes causes intestinal obstruction, destruction of villi, irritate the mucosal lining, dilation of the crypts of Lieberk¨uhn, edema, hyperemia, hemorrhage and and lymphoid cells and eosinophils had been observed. Acute catarrhal enteritis, intestinal dilation caused by infections with *A. perspicillum* in birds (Rao et al., 1991).

Contracaecum and *Porrocaecum*: In the alimentary tract of the infected birds can produce a severe inflammatory response. They can migrate within the walls of the proventriculus, esophagus, or intestine, peritonitis, ulceration, anemia followed by death (Huizinga, 1991).

Capillaria Nematodes: Causes inflammation, epithelial necrosis, sloughing with occasional ulceration, dilatation of the crop or esophagus, thickening of mucosa, fibrino necrotic plaques, and exudation in the upper gastrointestinal tract by *Capillaria* species and dehydration, extending of diphtheritic membrane from the oral cavity to the proventriculus (Helmboldt et al., 1991). In the oral cavity, pharynx, and esophagus thick, granular, yellow exudates had been seen (Clausen, 1991).

2.1.4. Identification and diagnosis of gastrointestinal parasites in birds

Coccidian parasite

Subspherical shape is usually observed in case of passerine *Isospora* oocysts. Occasionally, smooth ellipsoidal, pale yellowish to colourless, without a micropyle single layered wall, or at the sporulated status, with one or more polar granules generally. Ovoid to lemon shaped sporocysts usually seen, and possess a stieda body within the thick walled (Martinez and Muñoz, 1998). An important way to distinguish between avian and mammalian *Isospora* spp. is The Stieda body, which is the plug on one end of the sporocyst (Soulsby and L., 1982).

Oocyst morphology

Faecal samples examination is the most common method for diagnosing coccidial infections. Direct smear and flotation techniques are used to detect oocysts of these parasites isolated from the faecal material, and either a sugar or a salt solution with a certain specific gravity helps to float of the oocysts and sink of faecal debris which is

heavier. Sugar (sucrose) or salt solutions (ZnSO4, ZnCl2, or NaCl) are used as flotation media and hygrometer is used to check the specific density of the solution. There is variability in shape and size of both sporocysts and the oocysts they contain among in the same species (Hudson and Lee, 1988). Morphology used to identify infections with different species. For instance, Twentyman, (2001) identified various sizes and shapes oocysts of coccidia in hihi. *Isospora*- type oocysts contain two sporocysts when they are sporulated, and each sporocyst contains four sporozoites. The caryospora sporulated oocyst contains 8 sporozoites within a single sporocyst. Stieda bodies have seen at the sporocysts as typical for oocysts of the family Eimeriidae.

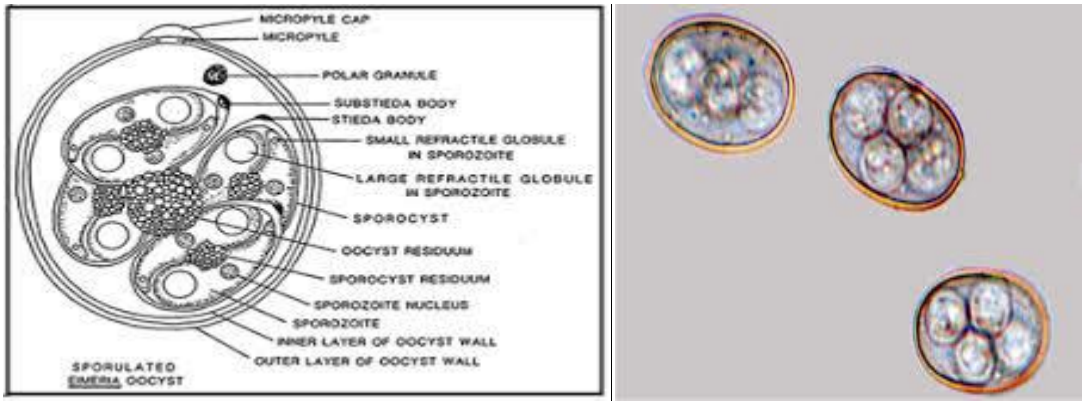


Figure 1: *Eimeria* sporulated oocyst (Soulsby and L., 1982)

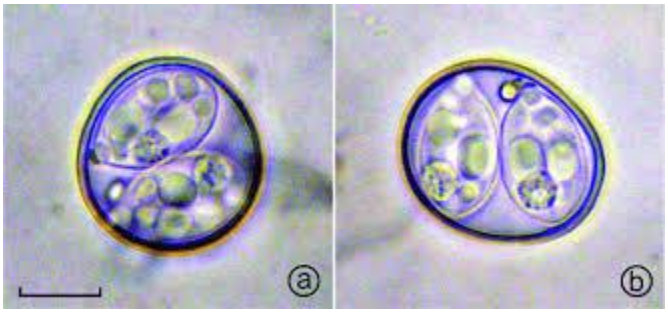


Figure 2: *Isospora* sporulated oocyst (Soulsby and L., 1982)

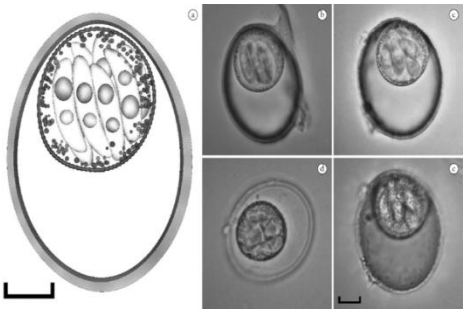


Figure 3: *Caryospora* sporulated oocyst (Soulsby and L., 1982)

Diagnosis of extraintestinal stages of coccidia:

In some species, within a complete life cycle there are an extraintestinal stages found in various organs. In some cases, asexual stages of *Isospora* can be observed at parasitophorous vacuole within mononuclear phagocytes, namely extraintestinal merozoites that located close to the host cell nucleus. Usually, in each parasitized cell, one merozoite can be seen. The merozoites contain a remarkable nucleus, a conoid, few rhoptries (mainly secretory organelles), a single mitochondrion and numerous elongated micronemes (specialized organelles important for gliding motility and host cell invasion) which observed through ultrastructural examination (Sánchez-Cordón et al., 2007). Sometimes, in Kupffer cells hepatocytes and merozoites can be found and through liver biopsy, it helps to diagnose as early as five days post infection period (Box, 1970).

Although asexual stages in monocytes and lymphocytes from heart blood of as early as 5 days after infection by post-mortem is positive, but peripheral blood smears examined as generally negative. In the heart blood, the presence of organisms associated with the existence of several parasites in other extra-intestinal tissues (Box, 1970). PCR method has become more useful for mononuclear cell stages detection. Identification of *Isospora* spp. from the blood of right jugular vein was done by (Adkesson et al., 2005) and 38.3% of their birds examined as positive through PCR technique. PCR is also used to determination of *Isospora* infections from extraintestinal tissue of an infected bird. Spleen, liver and mononuclear cells are probably the most valuable tissues for PCR testing after postmortem due to the parasite's predilection site (Atkinson et al., 2008).

Trematode

For identification anatomical location of adult trematodes is an important clue. Birds should be closely examined for cloacal prolapsed or soiling around the vent that may indicate infection with intracloacal or intestinal flukes and the conjunctival discharges may indicate eye flukes infection for instance *Philophthalmus* spp. Microscopic identification of eggs in the stool helps to diagnosis of a trematode. Comparatively small, with an operculum containing either an embryo or, in mature eggs, a ciliated miracidium indicate the trematode eggs (Soulsby and L., 1982). In contrast, eggs usually have thin

shells containing either a morula in unembryonated eggs or a recognizable larval worm indicate nematode infection. They do not have operculum, but egg of some species contains well-defined structural modifications (Soulsby and L., 1982). Eggs that have thickened walls and contain a larva (anonchosphere), which possesses six hooklets identified as cestode. A partially developed embryo or acanthor indicate acanthocephalan eggs. The schistosome's eggs do not have an operculum, but have terminal or lateral spines. No preservatives are needed if fecal samples are examined within less than 72 hours. Unless air is excluded from the container some eggs may embryonate or hatch during this time. The faecal sample should be fixed in 10–15 volumes of 10% formalin to maintain faecal samples longer than 3 days. After fixing and staining adult worms by using traditional morphological methods the genus and species of the trematode can be determined (Pritchard et al., 1982). Trematodes that passed in the feces or recovered at necropsy require relaxation before fixation. Relaxation is done by chilling the worms in refrigerator overnight or places them into 5–10% ethyl alcohol at room temperature, with the least handling. The relaxed worms can be fixed in preferably alcohol–formalin–acetic acid fixative or 10% formalin (Pritchard et al., 1982).

Cestode

In the faeces of the host the presence of eggs, gravid proglottids, or cestode fragments is diagnostic for cestode infection. On the basis of egg morphology and proglottid structure tetrabothriidean and cyclophyllidean cestodes can be distinguished from diphyllobothriid cestodes. Hard operculate shells of the eggs of diphyllobothriid but may be difficult to distinguish from trematodes. The presence or absence of the rostellar hooks on the scolex and their number, shape, and size required to identification in species level. The position, size, and shape of components of the male such as the number of testes and their spatial relationships within the mature proglottid and female reproductive systems are also important (Khalil, 1994). *Coanotaenia* spp. generally filled with haxacanth embryo and filamentous appendage. *Raillietina* spp. eggs are polygonal in shape with darker interior and transperant exterior zone with very thick surface (Soulsby and L., 1982).

Nematode

Eggs or larvae in feces are the main clue of infection. By a postmortem examination, diagnosis based on infective stages should be confirmed to verify the presence of adult nematode, in their specific infection site. Bipolar plugs is distinct of the eggs of *Trichuris* and *Capillaria* and clear to a deep golden color; the surface ranges from smooth to variably textured (Soulsby and L., 1982).

Eggs of *Syngamus trachea* have bipolar with clearly visible opercula at both ends and approximately 80–100 μm ×50–60 μm. When freshly passed each egg contains a morula. Eggs of *Capillaria* spp. superficially resemble to *S. trachea*. But the *Capillaria* spp. eggs have a thicker (and frequently brownish) eggshell, possess pronounced polar plugs and comparatively smaller (<60 μm in length) and when passed contain a single cell (Soulsby and L., 1982).

Eggs of *H. gallinarum* and *A. galli* can be distinguished from one another on the basis of size and shape (Urquhart et al., 1996). The measurement of *A. galli* eggs are 73-92 by 45-57 microns, looks like oval with thick smooth shell (Soulsby and L., 1982). *Porrocaecum* spp. eggs are oval, thick shelled with puncture surface and measurement is (88-123) by (59-88) microns (Soulsby and L., 1982).

2.2.1. Blood parasites of birds

Russian zoologist V. Ya. Danilewsky first noticed *Haemoproteus* spp. which infect birds on unstained blood smears along with other intra-erythrocytic hemosporidian parasites as character as variable in shape and size, colourless, clear, transparent vacuoles, in which are present several refractile glossyblack granules" (Beadell et al., 2006). After discover of Giemsa staining to differentiate host cells from parasites (Sehgal et al., 2005), the broad host range and variety of these parasites became evident, but their life cycles, host specificity, and vectors were unknown. After a long time, it was identified that *Haemoproteus columbae* of doves and pigeons which can be transmitted by the bite of hippoboscid flies and the discovery that under the genus of Culicoides, ceratopogonid flies can transmit other species of *Haemoproteus* (Khan and Fallis, 1970). Coatney at 1936 first discover the genus *Haemoproteus* and *hostindex* which included about 45

species of *Haemoproteus*, and maximum described from birds. Few reports with *Haemoproteus saoharovi* were concerned solely with its prevalence in nature. The initial description of the parasite was by MacNeal at 1904 who obtained specimens from the blood of the mourning dove, *Zenaidura macroura*. Identification of *H. saoharovi* from the mourning dove *Zenaidura macroura* was done from blood parasites in Nebraska birds found *H. saoharovi* in two mourning doves.

Plasmodium in the avian species played a cardinal role as models for human malaria while they were first noticed as common intraerythrocytic parasites in wild birds. The early years of this field have been reviewed in detail by Valkiūnas et al., (2009), and it is obvious that most of the field of human malariology were connected in one way with avian parasites. The number of documents of large-scale epizootics by avian malaria over the past 100 years were surprisingly slight, in captive penguins (Fix et al., 1988), in wild ciconiiformes in Venezuela (Vincent and Wilson, 1980) and native Hawaiian forest avian species (Warner, 1988).

Sakharoff did first work on *Leucocytozoon* and established morphological guideline of leucocytozoids of magpies, crows, and rooks in Georgia. Then Ziemann stated a description of leucocytozoids from a little owl (*Athene noctua*). He elucidated the *Leukocytozoon danilewskyi* and which was the first in stain blood films; his demonstration contained color depict of the gametocytes of his found leucocytozoid. Berestneff describe the genus name *Leucocytozoon* in several species from rooks, owls, and crows, but first definition of genus *Leucocytozoon* done by the Sambon. Leucocytozoid *(Akiba caulleryi*) which is transmitted by biting midges other than black flies (Greiner et al., 1975). The gametocytes of this parasite developed in erythrocytes as well (Desser, 1967). Consequencely and independently studied that transmission of *L. simondi* to ducks by simuliid black flies and *L. smithi* to turkeys. Bennett and Laird were active in commencing large collection of the literature on blood parasites of avian species and also a huge collection of more than 64,000 preparations (stained blood films) from different countries. Type material for many *Leucocytozoon* species is contained in the accumulation of the center (Bennett and Lopes, 1980).

In recent time, it seems wise to use both traditional morphologic (microscopic) techniques as well as molecular technique to diagnose *Haemoproteus, Plasmodium, Leucocytozooon* spp. and explore their phylogenetic relationships such as has been done by different scientists (Beadell et al., 2006) and (Sehgal et al., 2006).

2.2.2. Some prevalence data of blood parasites in birds

Hellgren et al., (2004) reported *Haemoproteus* from avian blood were detected as 1.2% of a single lineage by PCR method. From Costa Rica 4.8% was detected as the prevalence of *Haemoproteus* in birds (Valkiunas et al., 2004). 3.5% in birds from São Paulo State, Brazil (Bennett and Lopes, 1980); in Japanese wild birds 5.1% (Murata, 2002); 7.7% was detected in west African rainforest birds (Sehgal et al., 2005). In maximum case, the previous study conducted in wild birds or parasite detected as microscopical examination. Primary vectors known for *Haemoproteus* spp. is Hippoboscid flies and *Culicoides* spp. (Diptera: Ceratopogonidae).

In case of *Plasmodium* spp. (Bennett and Lopes, 1980) reported 1.8% was the infection rate in birds from São Paulo State, Brazil, 1.7% in Japanese wild birds (Murata, 2002), 0.6% of birds from Costa Rica (Valkiunas et al., 2004), 1.9% in birds from Madagascar (Savage et al., 2009), 3% in passerine birds from central New Jersey (Kirkpatrick and Suthers, 1988), 3.6% birds from a neotropical savanna in Brazil (Fecchio et al., 2011).

Leococytozoon infection is also common indifferent birds spp. (Bennett and Lopes, 1980) found out the prevalence rate was 0.06% from birds of São Paulo State, Brazil; 0.3% from birds from Costa Rica (Valkiunas et al., 2004) and slightly lower than nearctic-neotropical passerine birds which is 1.3% (Garvin et al., 2006), west African rainforest birds 4.6% (Sehgal et al., 2005) and 4% from naturally infected birds (Valkiunas et al., 2009).

2.2.3. Transmission of blood parasites in birds

The possibilities of a natural vector being responsible for transmission were discussed and an extensive survey of the ectoparasites of these birds was undertaken. For *Haemoproteus* species, transmission occurred by Culicoides among haemoproteids,

prepatent periods range, 14 days for *H. danilewskii* of blue Jays (Garvin et al., 2006), 11 to 14 days for *Haemoproteus velans* of woodpeckers and from 11-12 days for *Haemoproteus belopolskyi* of Blackcaps (*Sylvia atricapilla*) (Khan and Fallis, 1970). The prepatent period ranges from 14 days for *Haemoproteus palumbis* of Common wood-pigeons (*Columba palumbus*) and about 17 -37 days for *H. columbae* of Rock Pigeons (Baker, 1966). Transmission occurred by Culicoides of *Haemoproteus* species in which merozoites in circulating erythrocytes develop to mature macrogametocytes and microgametocytes, within approximately 5–10 days that encircle the erythrocyte nucleus. After first appearing in the circulation, gametocyte numbers jump up high in the peripheral circulation approximately within 10–20 days and then the number goes declined. Transmission occurred by ceratopogonid flies among *Haemoproteus* spp. is seasonal and limited to their range like in the spring and summer months (Bennett et al., 1993), but in subtropical habitats can occur throughout the year where suitable vectors are present year round in Florida and most likely other parts of the world (Atkinson et al., 1991). In contrast, at temperate North America, seasonal transmission occurred of *H. columbae* by hippoboscid flies and closely correlated with variation in vector availability, generally up going in the winter and summer months and due to vector density decreases, the number goes decline (Klei et al., 1975). In spite of being similar of man and birds malarial parasites, were not identical at all (Farmer, 1964). The way of transmission of malaria parasite was observed, in transferring *Plasmodium* to sparrows through using gulex mosquitoes. Need a suitable invertebrate host for bird-to-bird transfer. Observation related to host-parasite relationships turn labyrinthine when the vector is not unidentified. By the bite of the hippoboscid *Haemoproteus columbae* Kruse is normally transmitted from pigeon to pigeon. A species of *Omithornyia latreillewas* indicated to be a vector of *H. columbae* in wood pigeons, *Columba palumbus*, *Lophortyx californica*, California quail, may suffered a severe malaria-like malaise caused by *Haemoproteus lophortyx* in England.

In temperate climates most transmission of *Plasmodium* in birds takes place during the summer and spring season, but the dynamics of infection in tropical parts is not clearly known. *P. relictum* transmission at lower elevations can take place over the year in Hawaii (Woodworth et al., 2005), but at higher elevations it is more seasonal where

rainfall and temperature have significant effects on vector availability (Ahumada et al., 2004). On the other hand, at subtropical Florida *P. hermani* transmission in wild turkeys is limited primarily early fall and late summer when the number of the (*Culex nigripalpus*) primary vector, goes to high. As is the case with *Haemoproteus*, both the spatial and seasonal patterns of transmission depend on availability of suitable mosquito vectors and susceptible avian hosts. Among migratory species, recent evidence indicates that transmission of some species of *Plasmodium* and other haemosporidian parasites can occur on both the breeding and the wintering grounds, leading to increases in parasite dispersal (Hellgren et al., 2007). Based on surveys by microscopy, prevalence of *Plasmodium* is four to five times lower than either *Haemoproteus* or *Leucocytozoon*. From more than 2000 birds sample, the overall prevalence of less than 4% in North America (Greiner et al., 1975). Prevalence of *Plasmodium* differed in specific physiographic regions of the continent, ranging as high as almost 10% in the southeastern US to less than 1% in the arctic barrens (Greiner et al., 1975).

Sporozoites of *Leukocytozoons* can be transmitted into the blood stream by biting flies. Black flies are responsible for transmission of *Leucocytozoon*. The transmission of parasites in different geographic range of the parasite is restricted to the behavioral factors, range of the susceptible vectors and ecological factors. For instance, due to a number of abiotic and biotic factors, including physiological and behavioral chacteristics of both vectors and hosts. In Florida *Cnephia ornithophilia* might be either does not feed on these birds or from non migratory ducks and geese spatially separated. *Leucocytozoon* transmission relies on the availability of gametocytes in the peripheral blood of the birds and presence of appropriate vectors and to infect that hosts. In temperate climates area, that is gained through “spring relapse” (Khan and Fallis, 1970). Some colonial-nesting birds have higher prevalence of infection and higher diversity of leucocytozoids (Tella, 2002). Where host density is high, efficiency of transmission also high. Migratory waterfowl are exposed to a more diverse community of parasites, so there has a higher risk of infection (Figuerola and Green, 2000). Leucocytozoids transmission also rely on number of abiotic factors including environmental conditions, particularly rainfall, the flow of running water (running water is necessary for black fly vectors to reproduce), humidity and temperature (Adler et al., 2004).

2.2.4. Diagnosis of blood parasites from birds

For diagnosis of *Haemoproteus* and *Plasmodium* spp. the gold standard is thin blood smear with giemsa-stained in which the presence of erythrocytic gametocytes with clear golden-brown or black pigment granules are demonstrated and which *Plasmodium* species indicated by lacking of erythrocytic meronts. Each and every species are described by host specificity and intra-erythrocytic gametocytes morphology. Molecular techniques are commencing to be applied to define the individual parasite ancestry and to differentiate of genera. High sensitivity of the molecular techniques make them valuable for recognizing birds with very low intensity infections, but these techniques have not been clarified to the point where they can be used to differentiate each and every species. Current studies recommend that this may in time be viable (Hellgren et al., 2007).

***Haemoproteus* species**

Haemoproteus spp. might be hard to differentiate from bird *Plasmodium* spp., particularly in inveterate infections where number of circulating gametocytes is low and where it may be difficult to identify whether the intracellular meronts distinguishing of *Plasmodium* are absent or present. Various modern sets of primers discovered to amplify the mitochondrial genome portions can differentiate *Leucocytozoon* from *Plasmodium* and *Haemoproteus* (Hellgren et al., 2004) or from each other, all three genera following restriction digests of PCR products (Beadell et al., 2006). Furthermore, PCR products sequencing is requisite for identifying each and every parasite lineages and determining phylogenetic relationships among the parasites. Only tissue stages morphology is difficult for exact diagnosis of parasite. Meronts which is thin-walled branching or oval features of some species of columbiform, haemoproteids those are alike in morphology to tissue stages of both *Plasmodium* and *Leucocytozoon*. Megalomeronts of *Leucocytozoon* may be hard to differentiate from those of *Haemoproteus*. To solve of these problem, the recent use of molecular techniques may help. It is appearance that, *Haemoproteus* is antigenically identical from *Plasmodium* and in rock pigeons crude antigen extracts have been used to develop an ELISA test for *H. columbae* (Graczyk et al., 1994). With other avian haemoproteids the sensitivity and specificity of this serological test are not known,

but in birds with low intensity of infections**,** they may prove useful for making genus level diagnosis.

Haemoproteus nasimii

The blood of *C. livia* revealed gamogonic stages of *Haemoproteus*. The microgametocyte means the male is separable from the macrogametocyte (female) by its diffuse and larger nucleus. The concentration of the parasite was sparse (in general) (1–6 pars/100 RBC) but sometimes a higher rate of erythrocytes parasitization was detectable (10–20 pars/100 RBC) (Zajac et al., 2012). The parasite mainly infected two adjoining cells, at a time there was close adjacent of cells parasitized with macro and micro gametocyte. Immature gametocyte: The young and immature forms (8.4 × 3.7 μm) develop lateral to the host cell nucleus and have no contact with the host cell membrane or the host cell nucleus. Mature form: Mature forms could be differentiated into macrogametocytes (randomly scattered granules, nucleus with clear margins) and microgametocytes (granules polar, nucleus diffused with cytoplasm (Zajac et al., 2012).

Macrogametocyte: Macrogametocytes are elongated bean shaped, usually laterally situated to the erythrocyte nucleus. The parasite spread the poles of the infected erythrocyte but never surrounds its nucleus. The edges of the gametocyte were closely smooth and rarely amoeboid. Dissimilarities in the shape of the macro gametocyte were picturesque. Sometimes, a large space could be seen in the central zone between the gametocyte and the host cell membrane. In these cases, the gametocyte was broad at the ends and thin in the central zone. The nucleus of the parasite was median and stained as pink with Giemsa's stain, averaging 1.8 μm in length and 1.6 μm in width in its size. The mature form size varied from 13.0 to 16.0 μm in length and 4.0–6.9 μm in width in which average length 13.9 μm and width 4.7 μm. The parasite occupied almost 75% of the host cell and occusionally completely filled the host cell cytoplasm (Zajac et al., 2012).

Microgametocyte: They were slightly smaller than the macrogametocyte and placed laterals to the host cell nucleus similar to the macrogametocyte. The terminals of the parasites are somewhat rounded and the margin entire. The gametocytes adjoined to the host cell membrane at the polar zone but occasionally, in the central zone. A growing

microgametocyte occupied the poles of the affected erythrocyte and might replace its nucleus towards the pole in exterior. A nucleated erythrocyte could also contain micro gametocyte (Hellgren et al., 2007). Mature forms cytoplasm was fairly granular and stained lightly with Giemsa's stain or sometimes it was lack of color. The parasitic granules were placed at the poles of the parasite and are yellow–brown or black in appearance. The nucleus of the parasite was adhere and not easily differentiable from the cytoplasm of the parasite. Microgametocytes size range from 13.0 to 15.0 µm in length and 4.0 to 6.0 µm in width (average 14.0 µm in length and 4.3 µm in width). Mature form of microgamatocyte settled the major part of the infected RBC (Zajac et al., 2012).

Haemoproteus pastoris

The gametocytes look like amoeboid structure, accrue to the nucleus of the RBC and filling the erythrocyte up to their both poles. They displaced the erythrocyte nucleus slightly. The measurement of the macrogametocytes is 14.575 ± 0.4 µm × 4.12 ± 0.3 µm. Macrogametocyte nucleus is compact measuring 3.15 ± 0.02 µm × 2.575 ± 0.1 µm. In the cytoplasm 9 to 17 small reddish pigment granules were seen scattered. *Plasmodium* genus belongs to the family Plasmodiidae under the order Haemosporidia (Corradetti et al., 1963).

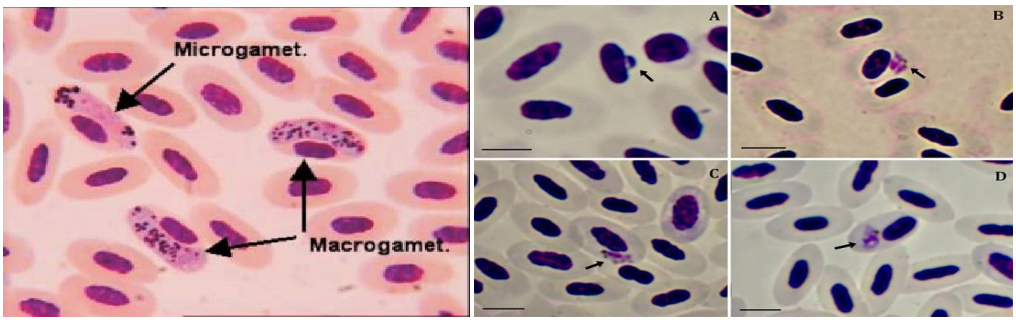


Figure 4: *Haemoproteus* spp.

***Plasmodium* species**

For *Plasmodium*, individual species are identified by shape and size of intraerythrocytic meronts and gametocytes, changes in morphology of the host erythrocyte, number of merozoites produced by mature meronts, and other biological characteristics such as

morphology, and location of exoerythrocytic meronts, host range, susceptibility to species of mosquitoes etc. (Valkiūnas et al., 2005). Since from blood smears, most identification are made, life history and features may be not known, and it becomes necessary to find huge amount of mature meronts and gametocytes on a smear for accurate identification of morphology of parasite. While it was known to early malariologists that by blood inoculation *Plasmodium* can be passed to a new host, (Herman et al., 1975) first apply this method in wild birds to diagnose *Plasmodium.* Blood from an infected host to an uninfected host of the same species is passed by intravenous, intramuscular or intra-peritoneal inoculation, and from the inoculated host for several weeks after injection blood smears are prepared. An acute phase infection will often result and meronts and gametocytes can be found at morphological analysis, if the host is susceptible to the parasites. Blood can be collected when parasitemia is high, treated with glycerin or dimethyl sulfoxide, aliquoted, and frozen in liquid nitrogen for repetative examination (Garnham, 1966). When *P. relictum* passed by sporozoites from silver gulls (*Larus novaehollandiae*) to canaries and sparrows, the morphology of gametocyte and merozoite changed remarkably. Mature meronts had 10 merozoites and gametocytes were elongate and in gulls. Morphology was more identical of *P. relictum* in sparrows and mature meronts had on average 14 merozoites and gametocytes were or oval or round. Some other reports explained that when parasites are inoculated into atypical hosts, morphological changes occur (Laird and Van Riper, 1981). To solve these problems, to diagnose avian malaria molecular methods with PCR primers to ribosomal and mitochondrial genes may help. Those have extremely low parasitemia PCR methods may still miss infections, although they have higher sensitivity (Valkiūnas et al., 2005), but real-time methods to may solve these problems. Nevertheless, PCR products sequencing is required for recognizing individual parasite lineages and to phylogenetic relationships determine.

Plasmodium species produces an insoluble golden brown or black deposit of haemozoin pigments in the parasite cells were confirmed by (Friend and Franson, 1999) from *C. livia* clearly place it in the subgenus Haemameoba, the morphological characteristics of *Plasmodium* species discovered with round or oval gametocytes, schizonts in mature

erythrocytes which is being larger than the host cell nucleus and with erythrocytic gametocytes lacking noticeable cytoplasm (Zajac et al., 2012).

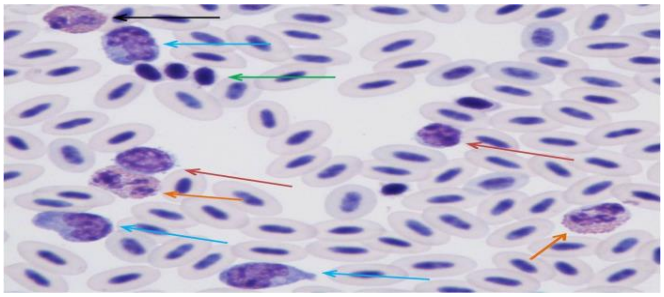


Figure 5: *Plasmodium* spp.

The entire host cell cytoplasm can be filled by mature gametocytes. In macrogametocyte, pigmented granules scatteredly distributed and small in number while in microgametocytes, they are large in number and cluster at one end of the parasite. The size of Macrogametocytes average is 7.8 × 7.7 µm and microgametocytes 7.8 × 7.6 µm. All gametocytes seen were pigmented (Zajac et al., 2012).

Host nucleus: The gametocyte displaced the host cell nucleus. Nucleus migrated to the one pole of the cell occasionally. NDR was 0.3 with a range of 0.1–0.5 in the host cell. Exo-erythrocytic stages: The prevalence of exoerythrocytic forms in the blood is highly variable, sometimes being frequent or usually quite sparse. They are usually round in shape and may be seen escaping from the RBC or lying free in the plasma (Zajac et al., 2012)

***Leucocytozoon* species**

For diagnosis of *Leucocytozoon* spp. we should examining stained thin blood films made from peripheral circulation of blood and matching the gametocytes characteristic (Valkiūnas et al., 2005) and he explain the methods of making and staining procedure of thin blood smears on the slide. The species can be determined by the host involved, identifying the gametocytes morphologic and metric features and by through literature review. Including measurements, a contemporary monograph explained by (Valkiūnas et al., 2005), accommodate huge amount of information which was collected from the world literature on blood protozoans. For diagnosis, consideration should be taken including

observation of clinical signs (mainly anemia), typical gross and histologic lesions, and gametocytes *of Leucocytozoon* spp. in the blood should be identified (Wobeser, 1997). In chicken, to detect antibodies against *L. caulleryi* some serological tests had explained at the early 1970s. Some serological tests only for diagnosis of *L. caulleryi* are counter-immunoelectrophoresis, agar gel precipitation (Mori and T, 1972), immunofluorescence (Isobe. T and Akiba, 1982), enzyme-linked immunosorbent assay (ELISA), immunoblot analysis.

Two subgenera: Akiba and *Leucocytozoon* under the genus *Leucocytozoon* considering the vector availability. *Leucocytozoon caulleryi* is the only known member of the subgenus Akiba and as its vectors that uses members of the genus Culicoides. Genus *Simulium*, act as vector for the remaining host species. After an extensive examination of species in the order Falconiformes declared that the only valid species infecting this order was *L. toddi* said by Greiner and Kocan in 1977 (Zajac et al., 2012).

Morphology: Mainly macrogametocytes and microgametocytes and present as few in number, measured as 10.8 ± 1.34 μM × 8.7 ± 1.43 μM. The cytoplasm is dark blue with many small vacuoles presence as coarsely granulated over the cytoplasm. The pigment granules are seen in small round organelles (Zajac et al., 2012).

Leucocytozoon macleani

In the mononuclear leucocytes and erythroblasts, merozoites enter and form elongated and ovoid (1p x 15ban) gametocytes (24 x 4 Am). With elongated gametocytes, host cells (67 x 6 ', cm) become spindle-shaped in which beside the parasite the nuclei appearing as thin bands (Zajac et al., 2012).

Leucocytozoon simondi

Elongated shaped gametocytes measure 14 - 22 gm, while found in monocytes and lymphocytes and round when found in the RBC. With a long, thin nucleus along one side with elongation of host cell (45 - 55ym long) in length. The microand macro-gametocytes found inside round host cells (Zajac et al., 2012).

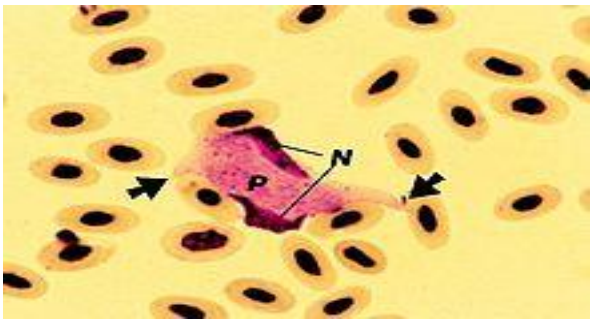


Figure 6: *Leucocytozoon* spp.

Leucocytozoon smith

Only in leucocytes, the gametocytes are found appear rounded before becoming elongated, 20- 22 um in length. The host cells become elongated, up to 50 um in length only after infection (Zajac et al, 2012).

2.2.5. Public health significance

Haemosporida and Plasmodiidae, under genus *Plasmodium* inhabit all major groups in terrestrial vertebrates of the world. Particularly due to the capability of variety of species to propagate and perform life cycles in different bird species belonging to various families and orders, among them bird malaria parasites is a unusual group (Clark et al., 2014). The same is true for invertebrate hosts (vectors) of these parasites (Santiago-Alarcon et al., 2012). Many species of avian *Plasmodium* use Culicidae mosquitoes belonging to different genera (Culex, Coquillettidia, Aedes, Mansonia, Culisetta, Anopheles, Psorophora) for completing sporogony and transmission (Njabo et al., 2009). This is not the case in mammalian malaria parasites whose are transmitted mostly by *Anopheles* species (Ejiri et al., 2011). Furthermore, sporogony of many avian *Plasmodium* parasites is completed relatively fast in susceptible vectors at relatively low temperatures (Valkiūnas et al., 2015). These characters influence to the world distribution of some avian malaria infections, which can transmitted in warm and cold climates including polar areas of the world (Howe et al., 2012). Four families of haemosporidians were identified on the basis of current taxonomy. These are Plasmodiidae, Haemoproteidae, Leucocytozoidae and Garniidae. Blood stages of species of

Plasmodium are particularly similar to those of relatively rare haemosporidian parasites of the genera Fallisia and Garnia of the family Garniidae (Bennett et al., 1993).

Evidence are increasing with pathogenicity in tissue stages of birds by *Plasmodium* has been remarkably miscalculated while huge evidence still bolster this scene for the rodent and primate malarial parasites. Even more, avian malaria is often a more severe disease than human malaria (Valkiunas et al., 2004). The intensity of malaise due to given ancestry of *Plasmodium* often varies significantly in various species of birds, from any clinical symptoms absence to high mortality because of broad vertebrate host specificity, and the same *Plasmodium* spp. can infect distantly related birds (Beadell et al., 2006).

2.3. *E. coli and Salmonella* species from faecal culture of bird and their antibiotic resistance

2.3.1. Enterobacteriaceae family

Different Gram-negative bacteria such as *Salmonella*, *Escherichia coli*, *Shigella*, *Klebsiella* and *Yersinia pestis* form a large family called *Enterobacteriaceae*. Some other pathogenic bacteria in this family include *Serratia*, *Proteus*, *Citrobacter* and *Enterobacter*. Enterobacteriaceae family is the only representative in the order Enterobacteriales which is under the phylum Proteobacteria and the class Gammaproteobacteria (Bergey and Brenner, 1984).

2.3.2. Morphology of *E. coli and Salmonella* species

Escherichia coli (*E. coli*) is Gram-negative, straight cylindrical rods measuring 1.1-1.5 x 2.0-6.0 μm, capsulated, lactose fermenter, non-acid fast, oxidase negative, aerobic and facultative anaerobic, non spore former, rendered motile by peritrichous flagella, or non-motile an enteric bacilli (Scheutzs and F., 2005) and remains as commensal in the lower intestine of human, animals and birds (Abdullah et al., 2010).The taxonomy of *E. coli* is summarized below:

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: Escherichia

Species: *Escherichia coli* (Scheutzs and F., 2005).

Salmonellae are Gram-negative, facultative anaerobes, straight rods not exceeding 1.5 micrometers in width; usually motile by peritrichous flagella and maximum contract common fimbriae which is type-1 ssociated with mannose-sensitive adhesive properties (Max, 1997). A high proportion of hydrophobic amino-acids containing fimbrillin subunits helps to form these fimbriae. According to the Kauffmann-White scheme, *Salmonella* are routinely classified by serotype on the basis of expression of three surface antigens. Those antigens are the somatic O antigen, the capsular Vi antigen and the flagella HI and H2 antigens (Scott et al., 2002). The absence of flagella may consequently affect complete identification of the serotype. For instance, *Salmonella enterica* serovar Typhimurium exhibits morphological differences depending on the peptone ingredient of the culture medium. However, as the primary nutrient in media containing soy-based peptone, *Salmonella* possesses a normal flagellated morphology (Gray et al., 2006).

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: Salmonella

Species: *Salmonella* spp. (Knodler and Elfenbein, 2019)

2.3.2.1. Strains of *E.coli*

Escherichia coli, as commensal bacteria which found in intestinal microflora of a variety of animals including man. All the strains are not harmless and some can cause detrimental effect on body as well as fatal diseases in animals and birds (Bélanger et al., 2011). They are the predominant facultative organism in the gastrointestinal tract of birds and animals. Due to the presence of virulence factors, pathogenicity associated genes and specific colonization factors pathogenic forms of *E. coli* can cause a variety of diarrhoeal diseases in hosts. Pathogenic *E. coli* is classified as extra-intestinal pathogenic *E. coli* (ExPEC) (caused for sepsis and airsaculitis in birds) (Cunha et al., 2014), and intestinal pathogenic (or diarrheagenic *E. coli* - DEC) (Russo and Johnson, 2000). Based on the mechanisms diarrheagenic *E. coli* - DEC *strains* have been divided into six pathotypes (Bélanger et al., 2011).

a) Verocytotoxigenic *E. coli* (VTEC)
b) Enterotoxigenic *E. coli* (ETEC)
c) Enteroinvasive *E. coli* (EIEC)
d) Enteropathogenic *E. coli* (EPEC)
e) Enteroaggregative *E. coli* (EAggEC)
f) Diffusely adherent *E. coli* (DAEC)

Among all strains of *E. coli,* ETEC is most commonly caused for diarrhoea in young (Franck et al., 1998). These strains produce heat stable and heat-labile enterotoxins which are plasmid-mediated enterotoxins. Heat-stable enterotoxin has two subtypes: STa and STb. Bovine and ovine ETEC isolates usually produce STa toxin (Blanco, 1991). Similarly there are two major subtypes of heat-labile enterotoxin, designated as LT-I and LT-II, with no cross-reactivity. Strains that express LT-I are pathogenic to both animals and birds, whereas LT-II is found primarily in animal isolates not in human (Qadri et al., 2005). Similarly, The DAEC and EAEC are increasingly identified as emerging pathotypes responsible for acute diarrhoea (Dovč et al., 2004). A fragment of a 60–65 MDa virulence plasmid, referred to as DNA probe pCVD432, has been used to recognize EAEC in polymerase chain reaction process (Schmidt et al., 1995). Similarly, based on their afimbrial adhesive sheaths (Afas), which are encoded

by gene clusters comprising *afaA*, *afaB*, *afaC*, *afaD* and *afaE*, DAEC strains can be identified through PCR (Le Bouguénec et al., 2001).

a) Verocytotoxigenic *E. coli* (VTEC)

VTEC/ Shiga toxin producing *E. coli*, STEC are responsible for cytotoxins production within host cells that disrupts protein synthesis. These toxins are synonymously either called Shiga toxins (Stx) because of their similarity with the toxin produced by *Shigella* dysenteriae or verocytotoxins (VT), because of their activity on Vero cells. The terms VTEC and VT will be used throughout this document.

Enterohaemorrhagic *E. coli* (EHEC) are a subset of VTEC which is known as human pathogens. Fig-15, represent EHEC as a subset of EPEC and VTEC, they can produce VT and have the ability to form attaching and effacing lesions on epithelial cells in the intestinal tract. EHEC also possess an approximately 60-MDa "EHEC plasmid". The most important EHEC (and VTEC) serotype in public health terms is *E. coli* O157. VTEC infection results in symptoms ranging from mild diarrhoea to severe bloody diarrhea which occurs via the faecal-oral route. The disease in humans causing of VTEC is associated with express VT (Mcdaniel et al., 1995).

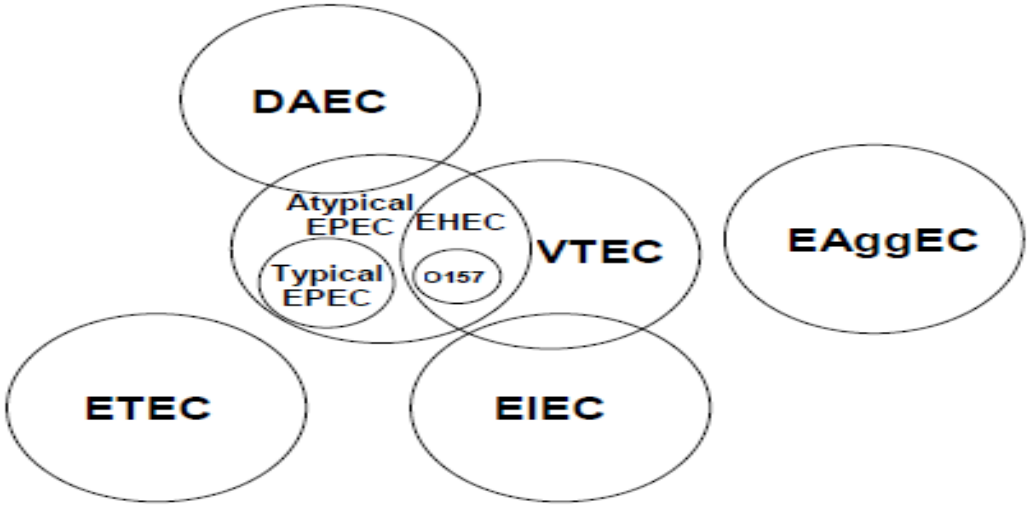


Figure 7: Venn diagram illustrating the relationships between *E. coli* pathotypes causing diarrhoeal disease (Mcdaniel et al., 1995).

b) Enteropathogenic *E. coli* (EPEC)

After the ingestion of EPEC, the organisms causing either watery or bloody diarrhea, low grade fever and vomiting by append to the intestinal epithelial cells. Any classic toxins cannot be produced by EPEC. Their virulence technique associated with interference with host cell signal transduction and the formation of A/E lesions. Virulence factors located on the EPEC adherence factor (EAF) (bfp which codes for bundle-forming pili, BFP) and on the LEE (such as eae which codes for intimin and esp which codes for secreted proteins) are responsible for this process. Contamination occurred by EPEC in food and Water have been linked to EPEC infection and transmission (Mcdaniel et al., 1995).

c) Enterotoxigenic *E. coli* (ETEC)

ETEC are responsible for diarrhea in travelers throughout the world. Infection with ETEC leads to watery diarrhea with abdominal cramps, sometimes with headache (fever is usually absent) and nausea. On infection, ETEC first attach to the small intestinal epithelium through one or more colonization factor antigens (CFA) and the expression of one or more heat labile (LT) or heat- stable (ST) enterotoxins. These enterotoxins lead to watery diarrhoea through hinderance of absorption of sodium and stimulation to chloride secretion. Distinct groups of the enterotoxins present for the for the heat labile, LTI and LTII – encoded for on the chromosome and heat stable, STa (STI) and STb (STII) – encoded for on plasmids. Infection occurs when a person ingests food or water contaminated with ETEC bacteria (Mcdaniel et al., 1995).

d) Enteroinvasive *E. coli* (EIEC)

EIEC need minimal contact for transmission through the faecal-oral route. After ingestion of EIEC, the bacteria infiltrate the epithelial cells of the intestine caused for mild form of dysentery characterized by the appearance of mucus and blood in the faeces of the host.

The respective genes need to infiltrate into host cells are clustered on a 220 kb virulence-associated invasion plasmid in EIEC strains and several plasmid-encoded

proteins expression required for the complete virulence phenotype of Enteroinvasive *E. coli*. In the ipa operon, these invasion plasmid antigens (Ipa) proteins are encoded. Through mechanical vectors such as flies or contaminated food or water, EIEC infection can occur (Mcdaniel et al., 1995).

e) Enteroaggregative *E. coli* (EAggEC)

EAggEC are associated with acute or persistent watery, mucoid, diarrhoeal illness with little to no fever and an absence of vomiting especially in developing countries. By which mechanisms EAggEC cause diarrhoea and pathogenicity factors are rarely known. Aggregative adherence to intestinal mucosa of EAggEC is mediated by either aggregative adherence fimbrae I (AAF/I) or AAF/II, which are encoded for by aggR genes. EAggEC can also produce an enteroaggregative heat-stable toxin (EAST1) similar to ST and which is responsible for the clinical signs. astA genes encode the EAST1 on a plasmid (Mcdaniel et al., 1995).

f) Diffusely Adherent *E. coli* (DAEC)

DAEC are responsible for urinary tract infections but its role as a causative agent of diarrhoea is controversial. DAEC with variable virulent organisms are making heterogeneous groups. Their adherence to Hep-2 cells in a diffuse pattern helps to recognize them. DAEC are divided into two classes, those that express an adhesin involved in diffuse adherence and, those which harbor afimbrial adhesins (Afa)/Drori antigen adhesins which is a potential cause of infantile diarrhea. DAEC infection is characterized by wrapping around the adherent bacteria by the growth of long finger-like cellular projections (Mcdaniel et al., 1995).

Table 1: Pathotypes of *E. coli* with their virulence factors (Mcdaniel et al., 1995).

Pathotype	General features	Principal virulence factors
EPEC	EPEC was the first pathotype of *E. coli* to be described.	Type III secretion system, intimin, Tir, EspA, EspB, EspD, EspF Pathogenicity island LEE, (McDaniel et al., 1995)

ETEC	The organism is an important cause of childhood and travelers' diarrhea in developing countries.	□ Heat-stable toxin (STa, STb) □ Colonization factor antigens (CFA)
EHEC	EHEC causes bloody diarrhea (hemorrhagic colitis) and hemolytic uremic syndrome (HUS).	□ The key virulence factor for EHEC is Stx, which is also known as verocytotoxin. The Stx family contains two subgroups Stx1 and Stx2 (VT) □ Type III secretion system, intimin, Tir
EIEC	Diarrhea caused by EIEC	□ Invasion plasmid (pINV)
EAEC/ EAggC	It is a cause of persistent diarrhea in children and adults in both developing and developed countries.	□ Toxins (Pic, ShET1, EAST) □ Aggregative adherence fimbrieae (AAFs) EAEC flagellin
DAEC	DAEC have been implicated as a cause of diarrhea in several studies, particularly in children >12 months of age (Nataro and Kaper, 1998)	□ Fimbrial adhesin F1845

2.3.2.2. *Salmonella* genus, species, subspecies, serotypes

Within the family of Enterobacteriaceae Salmonella organisms are Gram-negative facultative anaerobic rods shaped organism (Steve Yan et al., 2003). Generally, due to the presence of peritrichous flagella all the members of this genus are motile except *Salmonella* Gallinarum and *Salmonella* Pullorum, which are lacks of flagella. But under

special medium conditions the motility can be induced in *Salmonella* Pullorum (Holt and Chaubal, 1997). At 35°C to 37°C *Salmonella* grow optimally, use citrate as the sole carbon source; catabolize a variety of carbohydrates into acid and H2S gas. In these process they decarboxylate lysine and ornithine to cadaverine and putrescine sequentially (Hailu and Kebede, 2016). Generally *Salmonella* can catabolize glucose and lysine, but failed to metabolize sucrose, urea and lactose. Atypical Salmonella biotypes cannot decarboxylate lysine or that readily use lactose, sucrose (Reid et al., 1993) and urea because of widespread exchange of genetic elements between compatible bacterial strains in the environment (Morita et al., 2006). They are chemo-organotrophic organisms, having both a fermentative and a respiratory type of metabolism (Reid et al., 1993). In the group many serotypes are closely related to each other by flagellar and somatic antigens. Among the flagellar antigens group most strains show diphasic variation.

The genus *Salmonella* comprises two species: (1) *Salmonella enterica* and (2) *Salmonella bongori* (formerly called *Salmonella* enterica subspecies bongori V)

Salmonella enterica divided into six subspecies: (a) *Salmonella enterica* subspecies Enterica (I), (b) *Salmonella enterica* subspecies Salamae (c) *Salmonella enterica* subspecies Arizonae (Ilia), (d), *Salmonella enterica* subspecies Diarizonae (III b), (e) *Salmonella enterica* subspecies Houtenae (IV) and (f) *Salmonella enterica* subspecies Indica (VI).

On the basis of antigenic variation and on differential character, species and subspecies can be distinguished into 2501 serovars (Solari et al., 2003). According to their somatic (O) and flagellar (H) antigens, the members of the *Salmonella* species devided into more than 2541 serotypes (Brenner et al., 2000). World Health Organization (WHO), collaborating with centre for reference and research defined and maintained the antigenic formulae of *Salmonella* serotypes on *Salmonella* at the Pasteur Institute and new serotypes are listed in the annual updates of the Kauffmann-White scheme (Brenner et al., 2000).

Table 2: *Salmonella* species, subspecies, serotypes and their usual habitats, Kauffmann White scheme (Brenner et al., 2000).

***Salmonella* species and subspecies**	**No. of serovars within subspecies**	**Usual habitat**
Salmonella enterica subsp. Enterica (I)	1454	Warm blooded animals
Salmonella enterica subsp. Salamae (II)	489	Cold blooded animals and the environment
Salmonella enterica subsp. Arizonae (III a)	94	Cold blooded animals and the environment
Salmonella enterica subsp. Diarizonae (III b)	324	Cold blooded animals and the environment
Salmonella enterica subsp. Hautenae (IV)	70	Cold blooded animals and the environment
Salmonella enterica subsp. Indica (VI)	12	Cold blooded animals and the environment
Salmonella bongori (V)	20	Cold blooded animals and the environment

2.3.3. Pathogenesis and transmission of *E. coli* and *Salmonnella* species

E. coli

The most prevalent opportunistic enterobacteria considered *Escherichia coli* in captive animals and related with systemic disease in birds (Mattes et al., 2005). Avian pathogenic *Escherichia coli* (APEC) caused for sepsis and airsaculitis, which are considered as Extraintestinal pathogenic *E. coli* (ExPEC) pathotype (Pontes et al., 2018). In birds, the pathogenesis of enteritis by *E. coli* is still vague, but there may have a public health risk by the presence of diarrheagenic strains. Enteropathogenic *E. coli* (EPEC) and Shiga toxin-producing *E. coli* (STEC) represent at least six pathotypes of human diarrheagenic *E. coli* that affect birds and so they have zoonotic significance (Farooq et al., 2009).

Former study reported that the intestinal flora of healthy psittacines is primarily filled wih Gram positive bacteria (Bangert et al., 1988). In case of bacterial enteritis resulting in intestinal disorders or septicemia and are considered pathogenic or opportunistic Gram-negative bacterial infection (Becker Saidenberg et al., 2012). Gram-negative bacteria may also be transient in the gastrointestinal tract and has little significance as long as the normal microbiota remains intact. While disturbing of resident microbiota, cause for proliferation of these Gram-negative bacteria results in disease. For a long period, it is considered that *Escherichia coli* are a commensal inhabitant in the intestine without high pathogenic potential. However, numerous intestinal and extraintestinal diseases harboring *E. coli* associated virulence factors have been identified and molecular biology techniques have classified the virulence factors into pathotypes (Max, 1997).

The most prevalent diarrheagenic *E. coli* (DECs) in psittacine birds are enteropathogenic *E. coli* (Becker Saidenberg et al., 2012). Farm, pet and wild animals are recognized as possible reservoirs and sources of aEPEC infection for humans (Almeida et al., 2012). Some other DECs of human clinical importance have also been recognized, such as EAEC and EHEC, which were identified in captive psittacine birds (Marietto-Gonçalves, 2011). In recent years, the demand for psittacine breeding has increased and this growth continues to at upstairs. In these consequences, as occurs in domestic animals, the contact of man and psittacine is increasingly closer. So there create opportunity of microorganisms mutual transmission. Considering the health risk from Enterobacteriaceae family, researches have raised concern on the pathogenic organism profligation in environment as well as psittacine birds (Lopes et al., 2016).

***Salmonella* species**

In case of salmonellosis in caged birds the zoonotic potential has been identified (Orós et al., 1998). In psittacine birds clinical signs of salmonellosis vary from mild enteritis to a severe illness characterized by anorexia, lethargy, diarrhea, crop stasis and dehydration. Resulting from systemic disease, without presenting clinical signs, acute death may also occur (Orosz et al., 1992). By ingestion of contaminated water or food by infected rodents, wild birds, or domesticated species, salmonellosis in caged birds may occur. In housing condition, an infected bird is another common source to other healthy birds.

Flies, cockroaches, people, parasites beetles, and fleas may act as vectors. Salmonella can live for 28 months in avian feces and survive for extended periods on dirt and wood (Hechinger and Lafferty, 2005). In most outbreaks the incubation period is 1 wk (Amy et al., 1989). Salmonellosis in psittacine birds usually involved the organism *Salmonella typhimurium* var. Copenhagen followed by *Salmonella enteritidis* (Orosz et al., 1992). The prevalence of *Salmonella typhimurium* var. Copenhagen is very low, probably 1%–2% in most populations in captive psittacine birds (Grimes and Arizmendi, 1992). Salmonellosis outbreaks associated with high mortality occurred and has been reported in lorikeets (Panigrahy et al., 1979).

In case of acute (24 hr) or peracute case the most common clinical signs were fluffed appearance, Lethargy and dyspnea or dead without any presenting any signs. On necropsy common gross findings included reddening and congestion of the lungs (with hemorrhage in some cases), hepatomegaly and infection of the intestinal serosal surface. Sometimes, cardiac petechiae, splenomegaly, fibrinous adhesions to the hepatic surface, atrial dilation, and petechial hemorrhages on the serosal surface of the proventriculus were observed. Histologic changes indicative of acute, overwhelming bacterial septicemia, bacterial emboli (liver, spleen, lung, kidney, proventriculus), including fibrinonecrotic hepatitis and splenitis, enteritis, pulmonary congestion and hemorrhage were observed (Panigrahy et al., 1979).

2.3.4. Antimicrobial susceptibility testing

Against antibiotics bacteria exhibit two types of resistance, namely acquired and intrinsic resistance. Any species that was resistance to an antibiotic before it was introduced to it intrinsic resistance. If the species was originally susceptible to any antibiotic followed by become resistant called acquire resistance. Through exchange of genetic materials among closely related species or mutation bacteria can acquire antibiotic resistant. Resistance to antibiotics at sudden acquisition poses difficulties in treating infection. It is even more significant problem that resistance to several different antibiotics at the same time. Different methods of antibiotic susceptibility testing are:

a. Quantitative Methods

b. Qualitative Methods

c. Automated Susceptibility Tests

d. Newer Non-Automated Susceptibility Tests

e. Molecular Techniques

In disc diffusion method, on an agar plate standardized bacterial isolate is spread followed by paper disc containing specific concentration of antibiotics are placed. Then the petridish incubated overnight at 37°C. Isolate does not grow around the disk if the bacteria are susceptible to the antibiotic thus forming a zone of inhibition. Bacteria those are resistant to an antibiotic grow up to the margin of disk. The diameter of zone of inhibition must be measured and result read from the Kirby Bauer chart as sensitive, intermediate or resistant.

Detection of gene coding for resistance to one or several drugs by techniques such as PCR and DNA hybridization can be used as Molecular Techniques (Sarker et al., 2019).

2.3.5. Development and transmission of antimicrobial resistances

Obtaining antibiotic resistance, mechanism can be divided into natural and acquired resistance (McDermott et al., 2003). Bacteria become intrinsically resistant means natural resistance. Acquired resistance is often caused by the acquisition of mobile genetic elements, which carry the antimicrobial resistance genes or mutations in chromosomal genes. The biochemical and genetic aspects of antibiotic resistance mechanisms described by (Suskovic et al, 2008) in bacteria. The biochemical aspects can be divided into four categories: (i) antimicrobial inactivation, direct inactivation of the active antimicrobial molecule; (ii) target modification, alteration of the sensitivity to the antimicrobial by modification of the target; (iii) efflux pumps and outer membrane permeability changes, reduction of the concentration of drug without modification of the compound itself; or (iv) target bypass, some bacteria become refractory to specific

antimicrobials by bypassing the inactivation of a given enzyme. The genetic aspects can be divided into vertical evolution and horizontal evolution (Suskovic et al, 2008). Vertical evolution is acquired by chromosomal mutation and selection. Bacterial strains carrying resistance conferring mutations are selected for because susceptible strains die, while newly resistant strains survive and grow. In horizontal gene transfer, bacteria develop resistance through the acquisition of new genetic material from other resistant organisms. Horizontal gene transfer may occur between strains of the same species or between different bacterial species or genera. Mechanisms of genetic exchange include conjugation, transduction, and transformation. During each of these processes, transposons facilitate the transfer and incorporation of the acquired resistance genes into the host's genome or plasmids (Suskovic et al, 2008).

CHAPTER-3: MATERIALS AND METHOD

3.1. Description of study areas

The study was conducted in topographically metropolitan areas of Chattogram city. The selection of the study areas are based on high frequency of inclination of pet bird's ownership in Chattogram metropolitan area.

Figure 8: Area for sample collection (Metropolitan areas of Chittagong)

3.2. Study period

The study was undertaken for a period of 10 months starting from June, 2019 to March, 2020 where first 4 months were spent on sample collection followed by lab work subsequent 6 months period.

3.3. Selection of birds and sampling

For faecal sample we observed 3766 birds and collected 549 total pool samples representing 5 orders and 23 species from pet birds were kept in household and aviary shop. A prototype questionnaire was used to record information like owner's name and address, species of bird, deworming history, presence or absence of lice or mite etc.

Table 3: Sample collection from birds

Order	Bird species	Scientific name	Faecal sample for GI parasite detection		Faecal sample for bacterial isolation and AMR	Blood for hemo parasite detection
			Bird observed	(n)	(n)	(n)
Psittaciformes	Budgerigar	*Melopsittacu sundulatus*	3189	290	90	138
	Cockatiel	*Nymphicus hollandicus*	132	38	0	36
	Parrot	*Psittaciformes (Unspecified)*	29	22	14	25
	Lovebird	*Agapornis*	33	20	5	42
	Galah	*Eolophus roseicapilla*	29	19	10	0
	Blue-fronted Parrot	*Amazona aestiva*	25	17	0	0
	African Gray Parrot	*Psittacus erithacus*	19	14	0	0
	White Cockatoo	*Cacatua alba*	29	11	3	0
	Yellow Crested Cockatoo	*Cacatua sulphurea*	18	9	0	0
	Rose-ringed Parakeet	*Psittacula krameri*	12	8	14	0
	Horned Parakeet	*Eu nymphicus cornutus*	32	8	5	0
	Macaw	*Ara macao*	16	8	0	50
	Eastern	*Platycercus*	17	7	3	0

	Rosella	*eximius*				
	Lories	*Loriini*	11	5	0	20
	Red Winged Parrot	*Aprosmictus erythropterus*	6	4	0	0
	Blue-and-yellow macaw	*Ara ararauna*	4	3	0	0
Passeriformes	Canary	*Serinus canaria domestica*	34	17	0	0
	Finch	*Haemorhous mexicanus*	42	13	3	0
	European Goldfinch	*Carduelis carduelis*	32	10	0	0
	Gouldian Finch	*Erythrura gouldiae*	22	7	0	0
Columbiformes	Pigeon	*Columba livia domestica*	28	14	0	0
Galliformes	kadaknat	*Gallus gallus domesticus*	6	4	2	0
Aqila	Eagle	*Aquila*	1	1	1	0
Total sample (N)			3766	549	150	311

3.4. Sample collection and preservation

Two different types of biological samples (blood and faeces) were collected during this study and an individual bird was considered as a sampling unit for blood sample but pool sample (sample from group of birds) consider sample unit for faecal sample.

Faeces (approximately 2 gm) were collected directly pressed from cloaca or very raw defecation not more than one hour and stored in 2 types of plastic containers. Then, one type of container was filled with formalin (10%) and refrigerated at 4^0C temperature

which was further used for coproscopical parasitic examination. Fresh faeces were kept another container for bacteriological examination like *E. coli and Salmonella* spp. culture and detection of antibiotic resistance pattern among them.

Smear was prepared from blood obtained from leg nail cutting with sterile nail cutter after making aseptic by 70% alcohol. First, a thin smear was prepared by taking a drop of blood on the glass slide and then spread by another slide. The slides were air dried and fixed by 100% methyl alcohol for 3-5 minutes. Another drop of blood absorbed by FTA card to do PCR from the suspected blood parasite positive sample detected from the slide of blood smear.

Figure 9: Sample collection from different household and pet shop

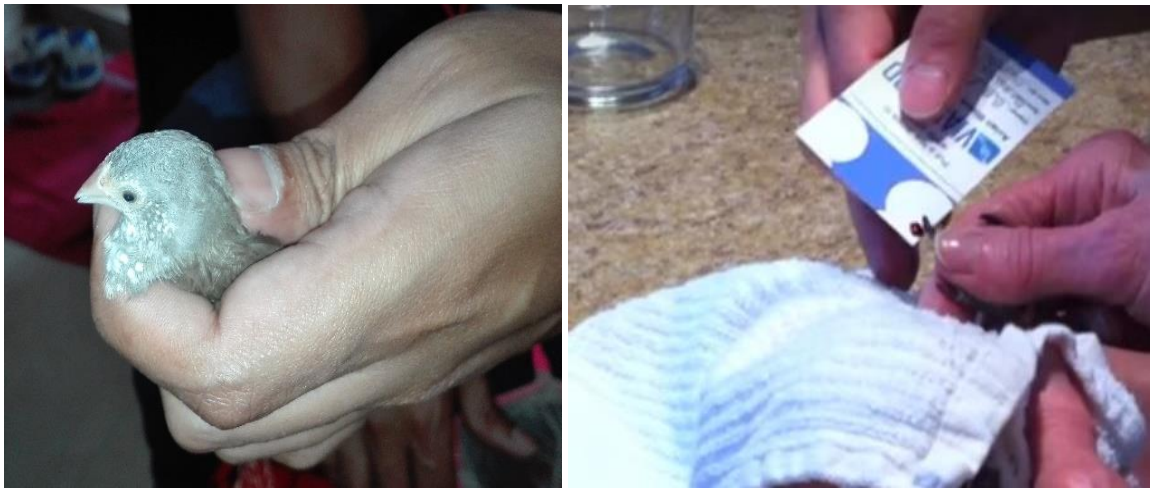
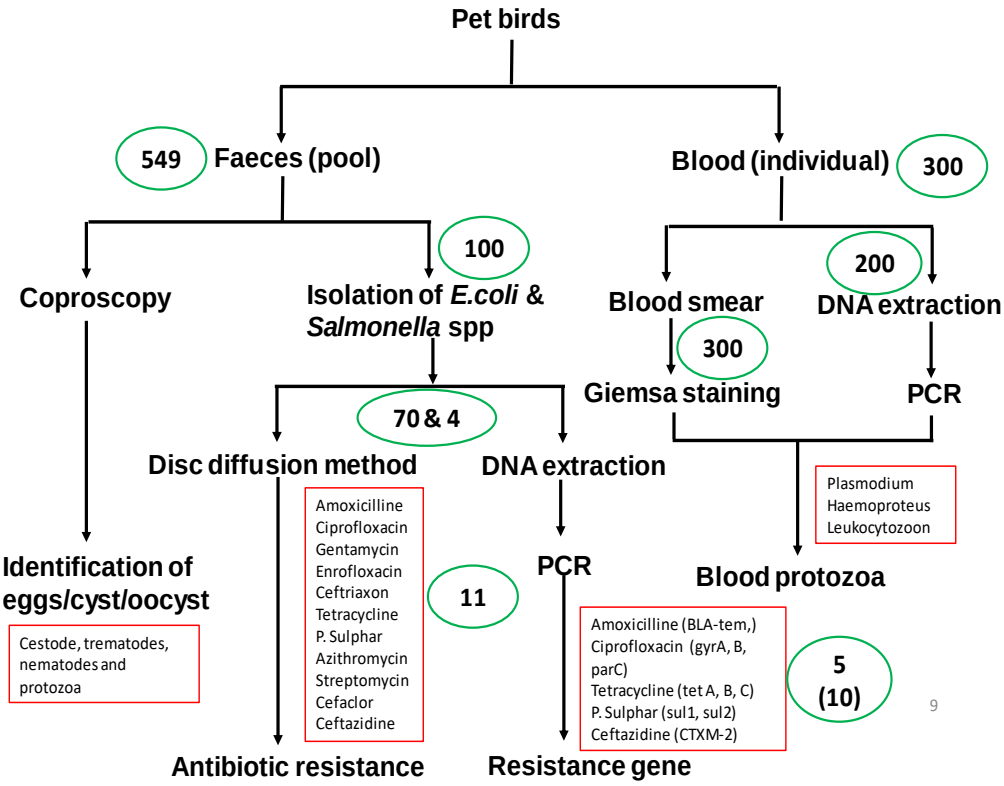


Figure 10: Restraining and blood collection from finch bird

3.5. Experimental study design



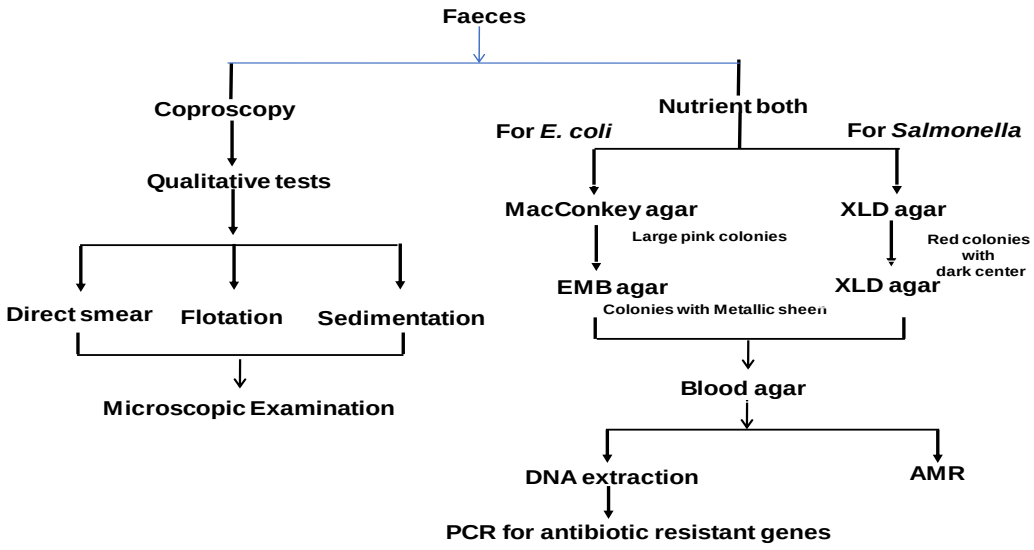


Figure 11: Experimental design of the study

3.6. Examination and tests of samples

3.6.1. Faecal samples examination for endoparasite detection

In addition to gross examination of faecal samples (colour, consistency, odour, blood or mucus, etc.) three different types of qualitative tests, namely direct smear, floatation and sedimentation techniques were used to examine the faecal samples. For flotation, sugar solution were used and specific gravity was 1.28 and Centrifugal sedimentation used for trematode egg detection. At least, two smears were prepared from each sample for each test to identify the morphological characteristics of eggs, cyst, oocysts etc. (Urquhart, 1996). Micrometry was also performed for the conformation of the egg size (Zajec et al., 2012).

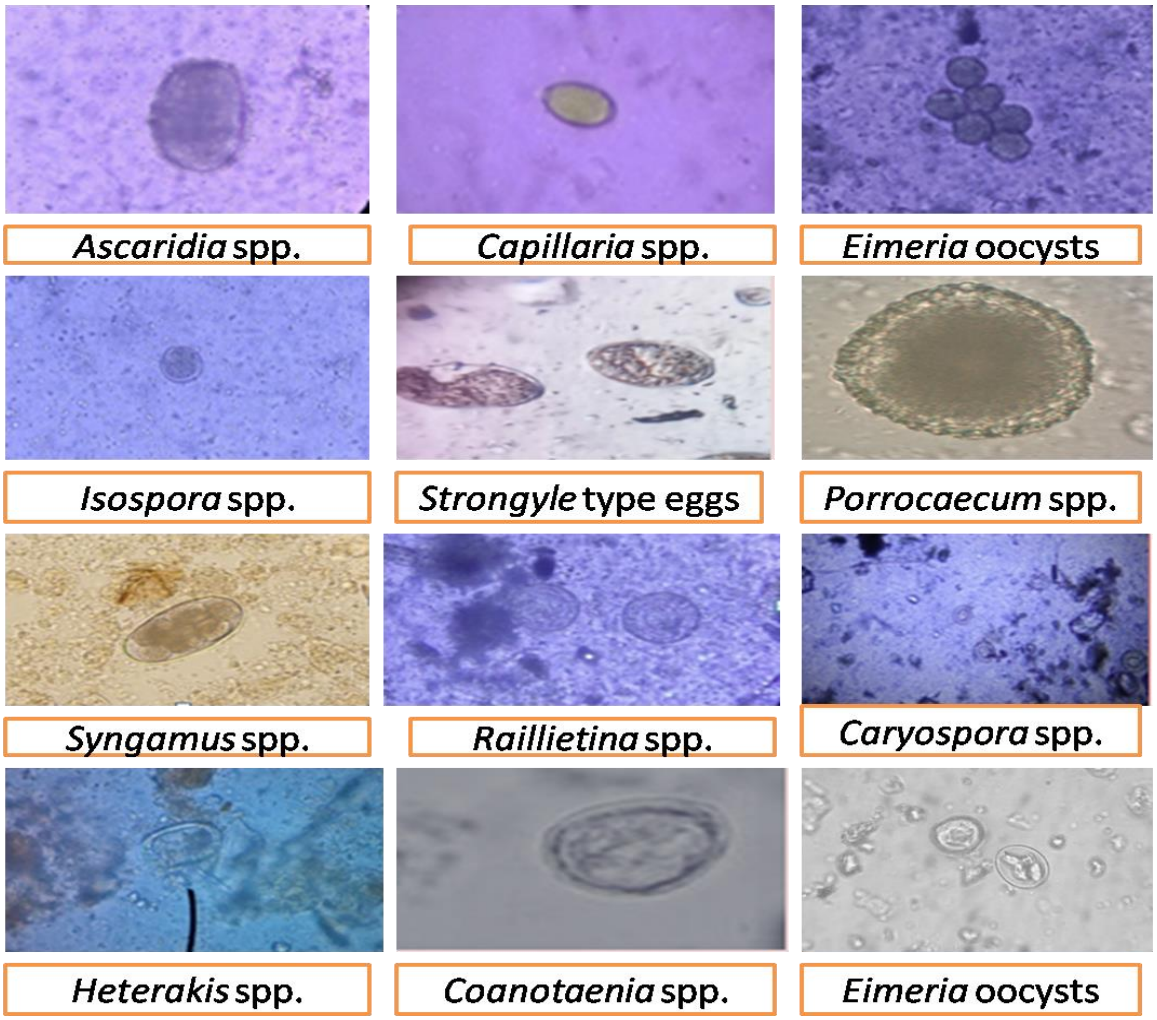


Figure 12: Different types of egg of gastrointestinal parasite.

3.6.2.1. Blood Smears Examination

All tests were accomplished at the Parasitology laboratory, CVASU. The prepared thin blood smears (Dwight et al, 2002) were stained with the Giemsa stain for 25-30 minutes (Urquhart, 1996). After rinsing with water, the stained blood smears were air dried and examined under binocular microscope (X100) with immersion oil for the identification of blood parasites (Urquhart, 1996; Soulsby and L., 1982).

3.6.2.2. DNA extraction from blood sample

DNA was extracted from blood sample by using Favorprep™ blood genomic DNA extraction mini kit (FAVORGEN from Taiwan) (Cat. No: FABGK001) following manufacturer's protocol given as bellow:

The filter paper with dried blood spot was chopped and took into fresh sterile microcentrifuge tube. An amount of 200µl phosphate buffered saline was added into the sample & mixed thoroughly by vortexing. Incubation of the mixture was performed at 70°C till the blood was dissolved. Then, 20µL protinase-k was added and shortly vortexed for 10 seconds. After that 200µL FABG (Favorgen blood genomic) buffer was added into tube and mixed by vortexing. Incubation was done at 70°C for 15 min and vortexed the tube in every 5 min. Then, 200µL absolute ethanol was added into sample and centrifuged at 4000 rpm for 1 minute then placing a new FABG mini column in a collection tube. Mixed sample was transferred in FABG mini column and Centrifuged at 6000 RCF for 1 min. Washed FABG column with 400µL wash buffer 1 by centrifuging at 18000 RCF for 30 sec then discarded flow through. Again, washed FABG column with 750µL wash buffer by centrifuging at 18000 RCF for 30 sec and discarded flow through. Centrifuge process was performed for an additional 3 min at 18000 RCF for drying the filter membrane of the column. Shifting of the FABG mini column were done to a new elution tube. 40µL elution buffer was added on the membrane directly and let it 2 minutes for incubation. Centrifuge was done at 18000 RCF for 1 min. The DNA sample were collected in new tube and stored DNA at -20°C

3.6.2.3. Primer and PCR assay for blood parasite detection

Table 4: Cycling conditions used during PCR for detection of blood parasite detection

Sl. No.	Steps		Temperature and time
1	Initial denaturation		95°C for 15 min
2	Cyclic denaturation		94°C for 30 sec
3	Cyclic annealing	(35 cycles)	59°C for 90 sec
4	Cyclic extension		72°C for 30 sec
5	Final extension		72°C for 10 min
6	Final holding		4°C

3.6.2.4. Multiplex PCR assay primer details for blood parasite

Table 5: Primer sequence for blood parasites detection

	Parasite genus	Primer name	Primer sequence (5′ to 3′)	Product size (bp)	Reference
Multiplex PCR primers	*Plasmodium*	PMF	CCTCACGAGTCGATCAGG	377–379	Ciloglu et al., 2019
		PMR	GGAAACCGGCGCTAC		
	Haemoproteus	HMF	ATTGGATGTCAATTACCACAATC	525–533	
		HMR	GGGAAGTTTATCCAGGAAGTT		
	Leucocytozoon	LMF	TGGAACAATAATTGSATTATTTACAYT	218	
		LMR	AACATATCATATTCCATCCATTTAGATTA		

3.6.3.1. Isolation of *E. coli* and *Salmonella* species

For initial detection of *E. coli* from a faecal sample, it was enriched into 5 ml of BPW that was prepared according to the manufacturer's instructions (Oxoid, England) for enrichment. Then it was incubated at 37°C for 24 hours**.** After the enrichment, 1 loopful enriched broth was inoculated into MacConkey agar (Oxoid, England) and incubated at 37˚C for 24-48 hours. After incubation, plates were checked for the presence of characteristic colonies (Dark pink coloured raised colony) suspected to be *E. coli*. Suspected positive colony was streaked on the EMB agar (selective media for *E. coli*) where characteristic metallic sheen indicated positive one. Characteristic positive colonies yielded on the EMB, further grown in blood agar for 24 hours followed by preservation at -20°.

For *Salmonella* isolation, after enrichment from BPW and nutrient broth, inoculation was done on XLD agar followed by again XLD agar characteristic black colonies with dark centre. Then it was grown in blood agar and preserved at -20°.

3.6.3.2. Antimicrobial susceptibility testing

Antibiotic resistance was determined by a disk diffusion method on Mueller-Hinton agar (Oxoid) according to the Clinical Laboratory Standards Institute (CLSI, 2018). Bauer-Kirby disk-diffusion procedure (Bauer, 1966) was used to determine the microbial resistance. Mueller-Hinton (MH) agar was prepared according to the manufacturer`s instructions (Oxoid, UK). A bacterial turbidity equivalent to 0.5 McFarland standards was used as inoculums for each isolate. By adding 0.5ml of 1% (11.75g/L) BaCl2.2H2O to 99.5ml of 1% (0.36N) H2SO4 the McFarland standard was prepared (Carter, 1990). The antibiotic resistance pattern for the isolates tested against the antibiotic was determined considering the zones of inhibition as 'resistant (R)', 'intermediately resistant (I)', and 'sensitive (S)' as recommended by the (CLSI, 2018). For an isolate to test, a sterile swab was dipped into the inoculums and rotated against the side of the tube with firm pressure. Then after removing the excess fluid from the swab, the dried surface of a MH agar was inoculated by streaking the swab three times over the entire agar surface rotating the plates approximately at 60 degrees for each time to ensure an even distribution of the inoculums.

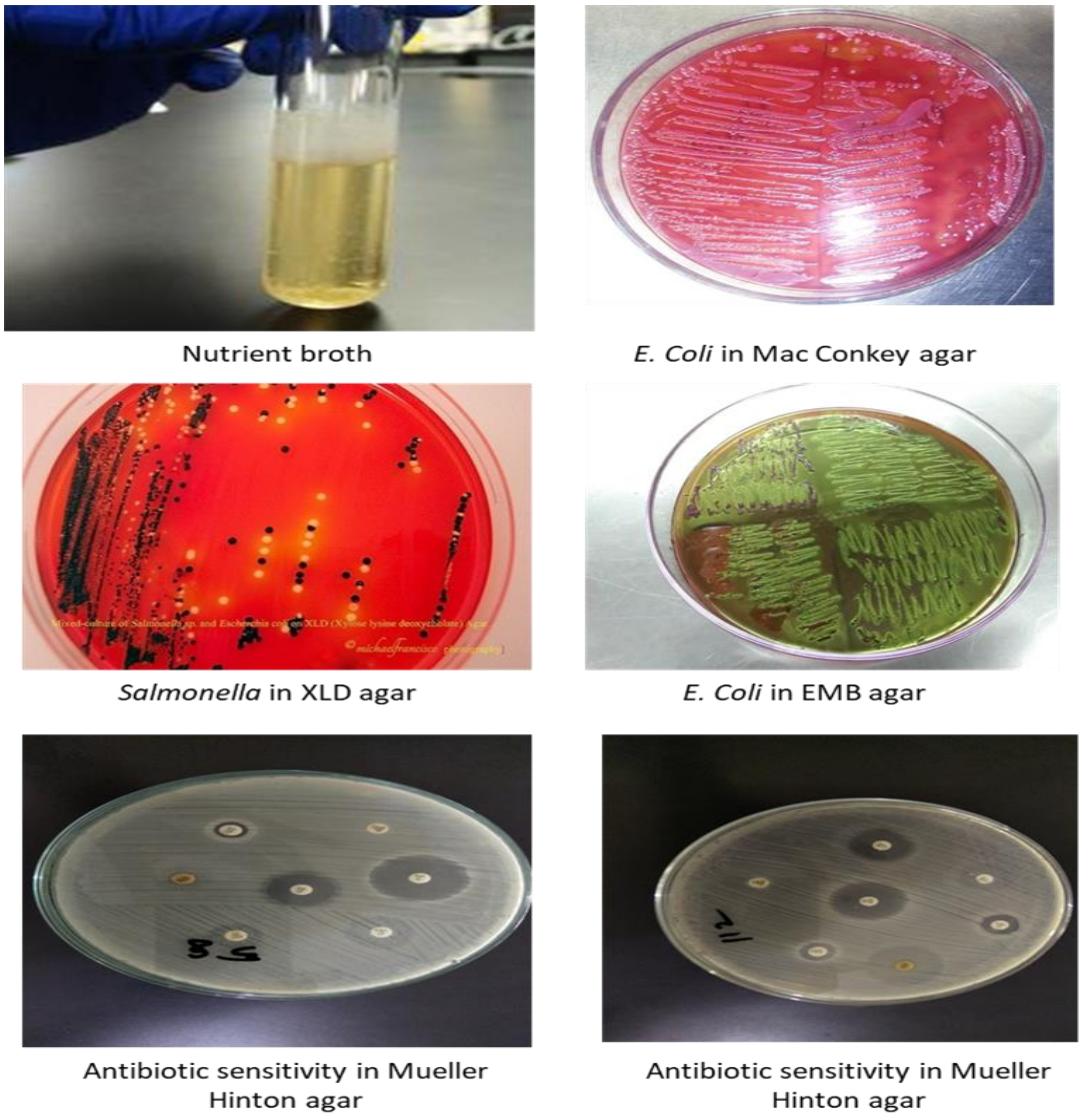


Figure 13: Bacteria culture and sensitivity to antibiotic

The antimicrobial disk was then placed on the surface of the inoculated agar. Disk was placed carefully on the surface of the agar with a gentle pressure to make a complete contact. Then, the agar plate was incubated at 35^{o}C for 16 to 18 hours. At the end of incubation, the size of zone of inhibition around a micro-disk was measured with a digital slide calipers and the result was recorded.

Table 6: Zone of inhibition and their corresponding interpretation

Group of antimicrobial agent	Antimicrobial agent	Disc code and content (µg)	Zone diameter, nearest whole mm			Manufacturer
			S	I	R	
Penicillin	Amoxicillin	AMP 10	≤13	14-16	≥17	Oxoid, UK
Cephalosporin	Ceftriaxone	CRO 30	≤17	20-22	≥23	
Amino glycosides	Gentamicin	CN 10	≤12	13-14	≥15	
	Streptomycin	STP 10	≤11	12-14	≥15	
Tetracycline	Tetracycline	TE 30	≤11	12-14	≥15	
Fluoroquinolones	Ciprofloxacin	CIP 5	≤15	16-20	≥21	
	Enrofloxacin	ENR 5	≤14	15-17	≥18	
Folate pathway inhibitor	Trimethoprim-sulfomathoxazole	SXT 25	≤10	11-15	≥16	
Polymyxins	Colistin sulfate	CT 25	≤10		≤11	
Macrolide	Erythomycin	E 15	≤13	14-22	≥23	
	Azithromycin	AZM 15	≤13	14-17	≥23	

((CLSI, 2018), Manufacturer guideline was followed in case of Colistin sulfate)

R'=resistance, 'I'=intermediate, 'S'=sensitive

3.6.3.3. DNA extraction from *E coli* and *Salmonella* species

Genomic DNA of *E. coli* isolates was extracted by boiling. The isolates stored at -80°C were thawed in room temperature, inoculated onto 5% citrated bovine blood agar and incubated at 37°C for 24 hours. Afterwards, for each isolate, for 100 µl deionized water was taken in Eppendorf tube and using a sterile inoculating loop 5 to 6 fresh colonies from the agar plate were picked up and transferred to the eppendorf tube. Then the tubes were vortexed to make a homogenous cell suspension, subjected to boiling at 99.9°C for 15 min and immediately thereafter cooled by placing them on ice for 7 min. Then the Eppendorf tubes with the cell suspension were centrifuged at 10000 rpm for 5 minutes

and 50 µl of supernatant containing DNA was collected in another sterile Eppendorf tube and stored at -20°C until testing. An aliquot of 0.5 µl of template DNA was used for PCR.

3.6.3.4. Primers and PCR assay

Oligonucleotide primers used for PCR to detect genes, reagents for corresponding PCR, amounts of different reagents and thermal conditions used were shown in Table 4, 5, 7 and 8 respectively. Oligonucleotid primers used for detection of antibiotic resistance genes in *E. coli* strains isolated from bird faeces.

Table 7: Primer details and annealing temperature for antibiotic resistance gene determination

Gene	Primer name	Primer sequence (5´-3´)	Annealing (ºC)	Fragment size (bp)	References
tetA	*tetA L*	GGCGGTCTTCTTCATCATGC	64	502	Lanz et al., 2003
	tetA R	CGGCAGGCAGAGCAAGTAGA			
tetB	*tetB L*	CATTAATAGGCGCATCGCTG	64	930	Lanz et al., 2003
	tetB R	TGAAGGTCATCGATAGCAGG			
tetC	*tetC L*	GCTGTAGGCATAGGCTTGGT	65	888	Lanz et al., 2003
	tetA R	GCCGGAAGCGAGAAGAATCA			
bla$_{TEM}$	*bla$_{TEM\ F}$*	TAC GATACGGGAGGG CTTAC	50	716	Belaaouaj et al., 1994
	bla$_{TEM\ R}$	TTCCTGTTTTTGCTCACCCA			
sul1	*sul1 F*	CGGCGTGGGCTACCTGAACG	68	779	Lanz et al., 2003
	sul1 R	GCCGATCGCGTGAAGTTCCG			

sul2	*sul2 F*	CGGCATCGTCAACATAACCT	59	721	Lanz et al., 2003
	sul2 R	TGTGCGGATGAAGTCAGCTC			
gyrA	*gyrA L*	TACCGTCATAGTTATCCACGA	60	312	Wiuff et al., 2000
	gyrA R	GTACTTTACCGCCATGAACGT			
gyrB	*gyrB L*	GTCCGAACTGTACCTGGTGG	60	281	Wiuff et al., 2000
	gyrB R	AACAGCAGCGTACGAATGTG			
parC	*parC L*	TGGGATCCAAACCTGTTCAGC GCCGCATT	60	261	Wiuff et al., 2000
	parC R	CGGAATTCGTGGTGCCGTTAAGC AAA			
CTX-M-2	*CTX-M-2-F*	ATGATGACTCAGAGCATTCG	52	876	Bertrand et al., 2006
	CTX-M-2-R	TCAGAAACCGTGGGTTAC			

Table 8: Contents of each reaction mixture of PCR used to detect different gene

Content Name	**Amount**
Thermo Scientific Water (Nuclease free)	10.5µl
Thermo Scientific dream Taq PCR Master Mix (2x) Ready to use	12.5 µl
Forward Primer	0.5 µl
Reverse Primer	0.5 µl
DNA template	1µl
Total volume	= 25 µl

3.7. Visualization of PCR product

1.5 % agarose gel was prepared by Agarose I (Molecular Biology Grade) powder (Thermo Scientific^{TM}) according to the following steps:

0.75 gm of Agarose (Molecular Biology Grade) powder (Thermo Scientific^{TM}) was mixed thoroughly with 50 mL of 1X TAE buffer in a conical flask and boiled in a microwave oven until agarose dissolved. Then the agarose mixture was cooled at 50°C in a water bath and 4µL of ethidium bromide was added to the mixture. Gel casting tray was assembled by sealing the ends of gel chamber with provided rubber and placed appropriate number of combs in the gel tray. The agarose-TAE buffer mixture was poured into the gel tray and kept for 20 minutes at room temperature for solidification. Then the combs were removed and the gel was shifted into an electrophoresis tank filled with 1X TAE buffer and kept until the gel is drowned completely. An amount of 5 µL of PCR product for each sample was loaded into each gel well. An amount of 3 µL of 1 kb DNA marker (O' Gene Rular 1 kb plus) was used to compare the amplicon size of the gene product and the electrophoresis was run at 110 volts and 80 mA for 30 minutes. Finally, the gel was examined by using a UV transilluminator for image acquisition and analysis.

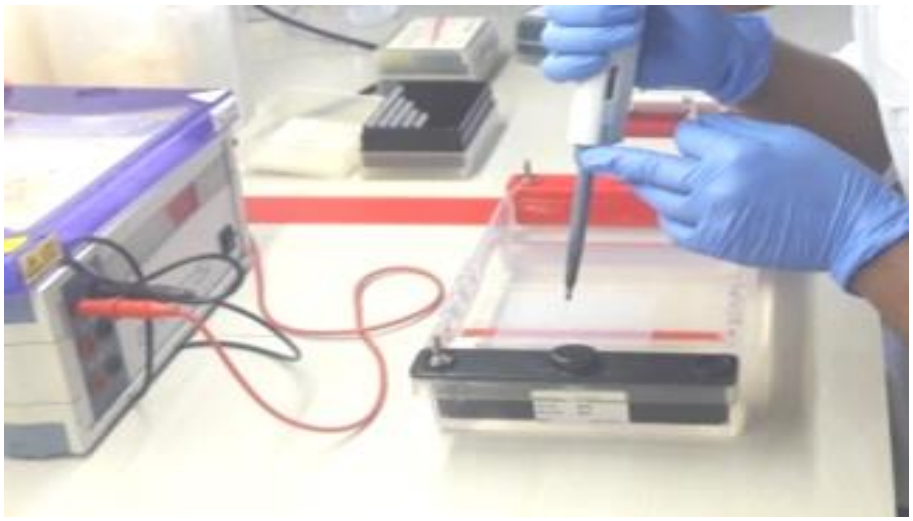


Figure 14: Loading PCR product in 1.5% agarose gel**c**

3.8. Statistical analysis

All data were entered into a spreadsheet of Microsoft Excel 2007 for making graphs and transferred to (https://epitools.ausvet.com.au/ciproportion) software for data summary and descriptive statistics.

CHAPTER-4: RESULTS

4.1. Occurrence of gastrointestinal parasites in pet birds

Out of total 549 faecal samples, 104 were found positive for different parasites by direct smear, faecal sedimentation and faecal flotation which represent the occurrence is 18.9%; CI = 15.7– 22.5% and which carried protozoal oocyst, nematode, cestode but not any trematode eggs. From the total samples, protozoal oocyst was 10.9%, (CI = 8.40–13.8%). Within them, *Eimeria* spp. 7.7% (CI=5.6-10.2%), *Isospora* spp. 2.9% (CI=1.7-4.7%), *Caryospora* spp. 0.4% (CI=0-1.3%). Among nematode, *Ascaridia* infection rate to total sample was 3.5% (CI=2.1-5.4%), *Capillaria* spp. 1.7% (CI=1-3%), *Heterakis* spp. 0.91% (CI=0-2), *Porrocaecum* and *strongyl* type both were 0.72% *(*CI=0-2%), *Coanotaenia* and *Syngamus* these two were 0.36 % (CI=0-1%), and *Raillietina* spp. 0.18% (CI=0-1%).

At psittaciformes group, the prevalence of *Eimeria* spp. among budgerigar was the most, which was 8% (CI=5-12%) followed by *Isospora* spp. infection 4% (CI=2-7%). *Ascaridia* infection was 1.03% (CI=0-3%) and *Porrocaecum* spp. found 1.03% (CI=0.21-3%). Prevalence of *Eimeria* species infection was most which is 10.5% (CI=4-24%) in cockatiel bird followed by 7.9% (CI=3-21%) in each by *Capillaria* spp. and *Isospora* spp. In case of galah, there was no other infection other than *Ascaridia* which is 5.2% (CI=1-25). Parrots carried infection by *Ascaridia* spp. at 9.09% (CI=3-28%) rate. Identification of *Eimeria* spp. at blue fronted parrot were 5.88% (CI=1-27%) and in love birds 10% (CI=3-30%) followed by *Ascaridia* spp. 5% (CI=1-24%) and in Macow at 12.5% (CI=2-47%) rate.

The precise results of the passeriformes are listed in Table 9. Within passeriformes order, canary infected by *Eimeria* and *Porrocaecum* spp. at the same rate 5.88% (CI=1-27%). *Eimeria* and *Ascaridia* occurred in European goldfinch at the same infection rate which is 10% (CI=2-40%). *Heterasis*, strongyle type eggs and *Eimeia* oocysts were detected at 7.69% (CI=1-33%) and *Ascaridia* at 15.38% (CI=4-42%) in finch. In Accipitriformes order we collected only one sample which was eagle and detected with *Eimeria* oocyst. Pigeon under the columbiformes order, *Eimeria* detected 41.42% (CI=8-48%), *Ascaridia*

28.57% (CI=12-55%), and *Capillaria*, *Heterakis*, Strongyle type eggs identified at same rate 7.14% (CI=1-31%).

Order	Scientific Name	Common Name	No of Samples examined	No of Birds Examined	Percent occurrence (%), Confidence interval (95%) and Number of positive samples										
					Eimeria	*Isospora*	*Caryospora*	*Ascaridia*	*Capillaria*	*Heterakis*	*Porrocaecum*	*Strongyle type*	*Syngamus*	*Coanotaenia*	*Raillietina*
					Protozoal Cyst			Nematode						Cestode	
Psittaciformes	*Melopsittacus sundulatus*	Budgerigar	290	3189	(8) (5-12) 23	(4) (2-7) 12	(.34) (0-2) 1	(1.03) (0-3) 3	(.34) (0-2) 1	-	(1.03) (.21-3) 3	(.34) (0-2) 1	(.34) (0-2) 1	(.68) (0-2) 2	-
	Nymphicus hollandicus	Cockatiel	38	132	(10.5) (4-24) 4	(7.9) (3-21) 3	-	-	(7.9) (3-21) 3	-	-	-	-	-	-
	Cacatua alba	*White cockatoo*	11	29	-	-	-	(9.09) (2-42) 1	-	(9.09) (2-42) 1	-	(9.09) (2-42) 1	-	-	-
	Cacatua sulphurea	Yellow-crested cockatoo	9	18	(11.1) (2-43) 1	(11.1) (2-43) 1	-	-	-	-	-	-	-	-	(11.11) (2-43) 1
	Eolophus roseicapilla	Galah	19	29	-	-	-	(5.2) (1-25) 1	-	-	-	-	-	-	-
	Psittaciforme							(9.09)							

	s (Unspecified)	Parrots	22	29	-	-	-	(3-28) 2	-	-	-	-	-	-	-
	Aprosmictus erythropterus	Red winged Parrot	4	6	-	-	-	-	(25) (5-70) 1	-	-	-	-	-	-
	Psittacus erithacus	African Gray Parrot	14	19	(7.14) (1-31) 1	-	-	(7.14) (1-31) 1	-	(7.14) (1-31) 1	-	-	-	-	-
	Amazona aestiva	Blue – fronted Parrot	17	25	(5.88) (1-27) 1	-	-	-	-	-	-	-	-	-	-
	Agapornis	Lovebird	20	33	(10) (3-30) 2	-	-	(5) (1-24) 1	-	-	-	-	-	-	-
	Loriini	Lories	5	11	-	-	-	-	-	(20) (4-62) 1	-	-	-	-	-
	Eu nymphicus cornutus	Horned Parakeet	8	32	(12.5) (2-47) 1	-	-	-	-	-	-	-	-	-	-
	Psittacula krameri	Rose-ringed parakeet	8	12	-	-	(12.5) (2-47) 1	-	-	-	-	-	-	-	-
	Platycercus	Eastern			(14.2)										

	eximius	Rosella	7	17	(3-51) 1	-	-	-	-	-	-	-	-	-	-
	Ara macao	Macaw	8	16	-	-	-	(12.5) (2-47) 1	-	-	-	-	-	-	-
	Ara ararauna	Blue-and-yellow macaw	3	4	(33.3) (6-79) 1	-	-	-	-	-	-	-	-	-	-
Passeriformes	*Serinus canaria domestica*	Canary	17	34	(5.88) (1-27) 1	-	-	-	-	-	(5.88) (1-27) 1	-	-	-	-
	Carduelis carduelis	European Goldfinch	10	32	(10) (2-40) 1	-	-	(10) (2-40) 1	-	-	-	-	-	-	-
	Haemorhous mexicanus	Finch	13	42	(7.69) (1-33) 1	-	-	(15.38) (4-42) 2	-	(7.69) (1-33) 1	-	(7.69) (1-33) 1	-	-	-
	Erythrura gouldiae	Gouldian Finch	7	22	-	-	-	(14.28) (3-51) 1	(7.14) (3-51) 1	-	-	-	-	-	-
Accipitriformes	*Aquila*	Eagle	1	1	(100) (21-100) 1	-	-	-	-	-	-	-	-	-	-

Columbiformes	*Columba livia domestica*	Pigeon	14	28	(21.42) (8-48) 3	-	-	(28.57) (12-55) 4	(7.14) (1-31) 1	(7.14) (1-31) 1	-	(7.14) (1-31) 1	-	-	-
Galliformes	*Gallus gallus domesticus*	Kadaknath	4	6	(25) (5-70) 1	-	-	(25) (5-70) 1	-	-	-	-	(25) (5-70) 1	-	-
Total			549	3766	(7.65) (6-10) 42	(2.91) (2-5) 16	(.36) (0-1) 2	(3.46) (2-5) 19	(1.27) (1-3) 7	(.91) (0-2) 5	(.72) (0-2) 4	(.72) (0-2) 4	(.36) (0-1) 2	(.36) (0-1) 2	(.18) (0-1) 1

Table 9: Coproscopy and endoparasite detection result chart.

4.2. Occurrence of blood parasites in pet bird's species

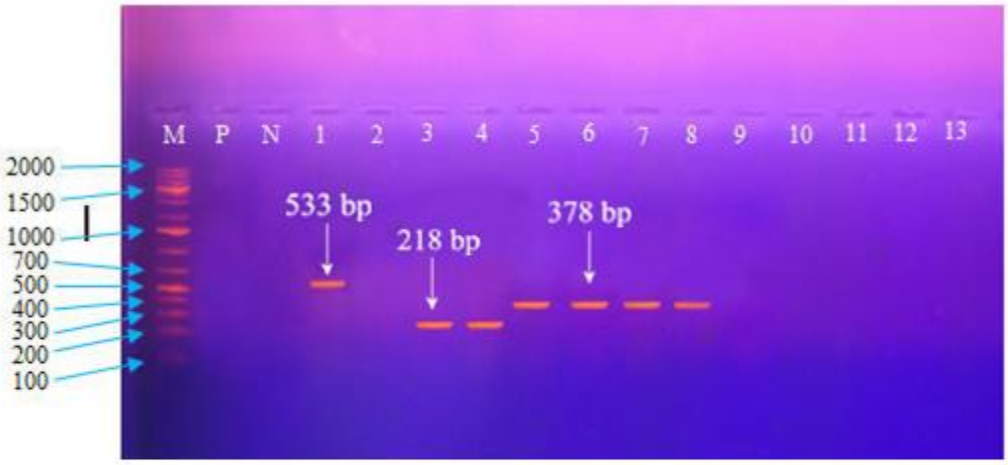


Figure 15: PCR results of *Haemoproteus, Leucocytozoon* and *Plasmodium* at genus level. Lane M: 2 kb DNA ladder; Lane P: positive control (absent, we had not any positive control from pet bird sample); Lane N: negative control; Lane 1: *Haemoproteus,* sized (533bp) amplicon; Lane 2-4: *Leucocytozoon*, sized (218bp) amplicon; Lane 5-8: *Plasmodium*, sized (378bp) amplicon.

Table 10: Occurrence of blood parasite detection chart

Order	Scientific Name	Common name	Sample No.	Percent occurrence (%), Confidence interval (95%) and Number of positive samples		
				Leucocytozoon	*Plasmodium*	*Haemoproteus*
Psittaciformes	*Melopsittacus undulatus*	Budgerigar	138	0	(0.7) (0.4-3.9) 1	(0.7) (0.4-3.9) 1
	Nymphicus hollandicus	Cockatiel	36	(2.3) (0.1-14.5) 1	0	0
	Agapornis	Lovebird	42	0	(2.4) (0.1-12.6) 1	0
	Loriini	Lories	20	0	0	0
	Psittaciformes (Unspecified)	Parrot	25	(4) (0.1-20.4) 1	0	0
	Ara macao	Macaw	50	0	(4) (0.5-13.7) 2	0
Toal			311	(0.64) (0.4-2.3) 2	(1.3) (0.35-3.26) 4	(0.32) (0.4-1.8) 1

Total 311 blood samples were collected from different types of birds. Among them budgerigar (138), cockatiel (36), lovebird (42), lories (20), parrot (25) and macaw (50) were selected. For the PCR cycle, the amplifications yielded products of different sizes for each genus (218 bp for *Leucocytozoon*, 378 bp for *Plasmodium*, and 533 bp for *Haemoproteus*) detection. There were no interference between each primer pairs and no overlapping bands among the genera. One *Haemoproteus* spp., four *Plasmodium* spp. and two *Leucocytozoon* spp. were confirmed by PCR. Total 7 sample were found positive which represented the occurrence of blood parasitic disease was 2.25% (CI=0.91-4.58%).

Among the total blood sample, 0.32% (CI=0.4-1.8%) was the occurrence of *Haemoproteus* spp. and only one positive found in budgerigar, so prevalence was 0.7% (CI=0.4-3.9%) in budgerigar. Prevalence of the *Plasmodium* spp. in all birds was 1.3% (CI=0.35-3.26%) in where 0.7% (CI=0.4-3.9%) in budgerigar, 2.4% (CI=0.1-12.6%) in lovebird and 4% (CI=0.5-13.7%) in macow. 0.64% (CI=0.4-2.3) sample from all bird species were detected as leococytozoon infection. Among them cockatiel was infected 2.3% (CI=0.1-14.5%), parrot 4% (CI=0.1-20.4) and there was no other species identified as leococytozoonosis.

4.3.1. Isolation of *E. coli*

The isolation of *E. coli* from faecal samples from pet birds is shown in table 11. A total number of 150 samples were collected, 90 from budgerigar, 5 each from lovebirds and horned parakeets, 14 from rose ring parakeet, 14 from parrots, 3 each from eastern rosella, finch and cockatoo, 10 from galah and 1 from eagle and 2 from kadaknath type chicken. 73 samples (48.7%) (CI=40.4-57%) of them were positive for *E. coli*. The highest isolation rate of *E. coli* from faecal samples of rose ring parakeet 71.4% (CI=41.9-91.6%) followed by galah 60% (CI=26.2-87.8). The occurrence rate of *E. coli* in GI tract of parrot was 50% (CI=25.1-80.8%). From budgerigar, the isolation rate of *E. coli* was significant which was 45.6% (CI=35.-56.4%), from horned parakeet and love birds the rate was 40%.

Table 11: Occurrence of *E. coli* infection in pet birds

Bird	Total sample	Positive samples	Occurance (%)	95% CI
Budgerigar	90	41	45.6	35.-56.4
Horned Parakeet	5	2	40	5.5-85.3
Rose ring parakeet	14	10	71.4	41.9-91.6
Parrot	14	7	50	25.1-80.8
Lovebird	5	2	40	5.5-85.3
Eastern Rosella	3	1	33.3	8-90.6
Finch	3	1	33.3	8-90.6
Cockatoo	3	1	33.3	8-90.6
Eagle	1	1	100	4.5-100
Kadaknath	2	1	50	1.3-98.7
Galah	10	6	60	26.2-87.8
Total	150	73	48.7	40.4-57

4.3.2. Antimicrobial resistance of *E. coli*

From the total *E. coli* positive sample (N=73), the occurrence of antimicrobial resistance (shown in table 12) have been found. 71.23% isolates demonstrated resistance to amoxicillin and 86.30% in tetracycline, ceftriaxone (91.78%), trimethoprim-sulfomathoxazole 75.34% and ciprofloxacin 25%, and gentamicin 1.37%. There was no resistance found against colistin sulfate. Most sensitive is colistin sulfate followed by gentamicin and the rate was 98.63% and 97.26%, respectively. About 57.53% *E. coli* isolates displayed sensitive to ciprofloxacin.

Table 12: Antibiotic susceptibility testing of *E. coli* isolates

Antibiotic class	Antibiotics	S(%)	I(%)	R(%)
Beta lactam	Amoxicillin	6.8	21.92	71.23
Cephems	Ceftriaxone	2.23	5.48	91.78
Amino glycosides	Gentamicin	97.26	1.37	1.37
Tetracycline	Tetracycline	8.22	5.48	86.30
Fluoroquinolones	Ciprofloxacin	57.53	26.03	16.44
Folate pathway inhibitor	Trimethoprim-sulfomathoxazole	9.59	15.07	75.34
Polymyxins	Colistin sulfate	98.63	1.37	0

R'=resistance, 'I'=intermediate, 'S'=sensitive

4.3.3. Antibiotic resistance pattern for *E. coli*

This study represents that 90.41% of the *E. coli* isolates were multidrug resistance (MDR). This term defined, antimicrobial resistance shown by a species of microorganism to at least one antimicrobial drug in three or more antimicrobial categories. Even 56.16% of isolates showed resistance to 4 to 6 class of antimicrobial drugs. AML-CN-CIP-CRO-OT-SXT combindly showed resistance in one sample which is 1.37% (CI=1.4-7.4%) and had highest MAR index which was 0.86 (MAR index is calculated as the ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics the organism is exposed to). AML-CIP-CRO-OT-SXT

combindly showed resistance in a rate of 13.70% (CI=6.8-23.8%) and had 0.71 MAR index. AML-CRO-OT-SXT pattern identified as 38.35% resistance (CI=27.2-50.5%) with 0.57 MAR index and AML-CRO-SXT pattern recognized as 9.59% resistance (CI=3.9-18.8%) with 0.43 MAR index. CRO-OT-SXT pattern displayed resistance as 13.70% (CI=6.8-23.8%) with 0.43 MAR index.

Table 13: Antibiotic Resistance Pattern for *E. coli*

Pattern	Ratio = Percentage	95% CI	MAR Index
AML-CN-CIP-CRO-OT-SXT	1/73=1.37	1.4-7.4	0.86
AML-CIP-CRO-OT-SXT	10/73=13.70	6.8-23.8	0.71
AML-CRO-OT-SXT	28/73=38.35	27.2-50.5	0.57
AML-CRO-SXT	7/73=9.59	3.9-18.8	0.43
CIP-CRO-OT	1/73=1.37	1.4-7.4	0.43
CIP-CRO-OT-SXT	1/73=1.37	1.4-7.4	0.57
CRO-OT-SXT	10/73=13.70	6.8-23.8	0.43
CRO-OT	1/73=1.37	1.4-7.4	0.29
AML-CRO-OT	7/73=9.59	3.9-18.8	0.43
AML-CIP-CRO-SXT	1/73=1.37	1.4-7.4	0.57
AML-OT	2/73=2.74	0.3-9.5	0.29
AML	2/73=2.74	0.3-9.5	0.14
OT	2/73=2.74	0.3-9.5	0.14
Total	100%		

(AML=Amoxicillin, CN=Gentamicin, CIP= Ciprofloxacin, AZM= Azithromycin, CRO= Ceftriaxone, OT= Tetracycline, SXT= Trimethoprim-sulfomathoxazole)

4.3.4. Antimicrobial resistance genes from *E. coli*

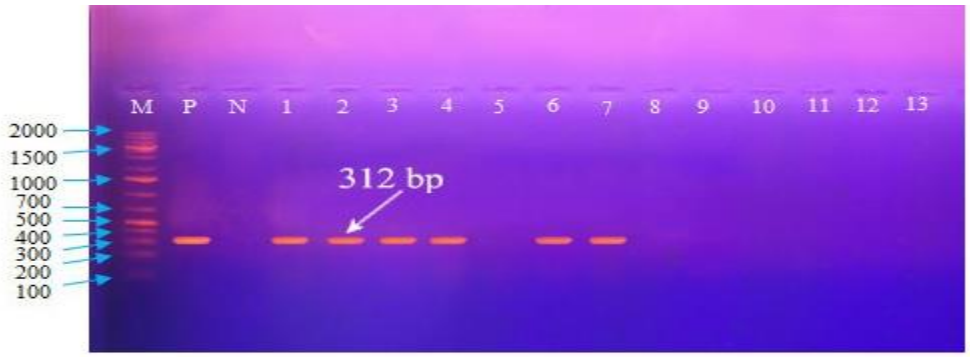


Figure 16: PCR results of gyr A gene from *E. coli*. Lane M: 2 kb DNA ladder; Lane P: positive control for gyr A; Lane N: negative control; Lane 1-7: gyr A gene-sized (312bp) amplicon.

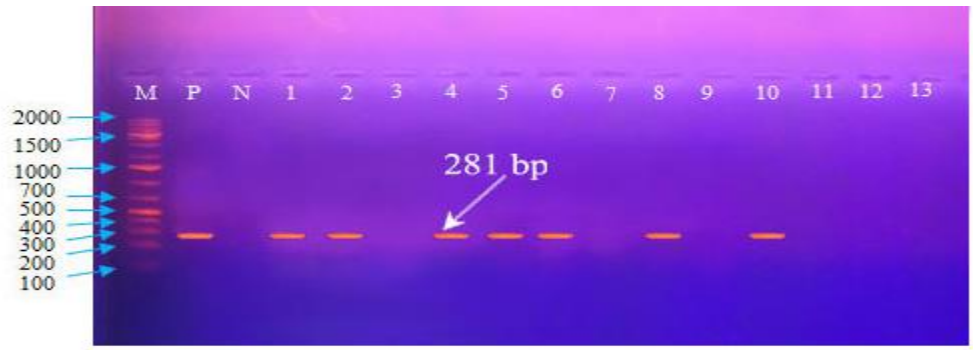


Figure 17: PCR results of gyr B gene from *E. coli*. Lane M: 2 kb DNA ladder; Lane P: positive control for gyr B; Lane N: negative control; Lane 1-10: gyr B gene-sized (281bp) amplicon.

Figure 18: PCR results of par C gene from *E. coli*. Lane M: 2 kb DNA ladder; Lane P: positive control for par C; Lane N: negative control; Lane 1-5: par C gene-sized (261bp) amplicon.

Figure 19: PCR results of tetA gene of *E. coli*. Lane M: 2 kb DNA ladder; Lane P: positive control for tetA; Lane N: negative control; Lane 1-5: tetA gene-sized (502bp) amplicon.

Figure 20: PCR results of tet B gene of *E. coli*. Lane M: 2 kb DNA ladder; Lane P: positive control for tet B; Lane N: negative control; Lane 1-2: tet B gene-sized (930bp) amplicon.

Figure 21: PCR results of CTX-M-2 gene from *E. coli*. Lane M: 2 kb DNA ladder; Lane P: positive control for CTX-M-2; Lane N: negative control; Lane 1-6: CTX-M-2 gene-sized (876bp) amplicon.

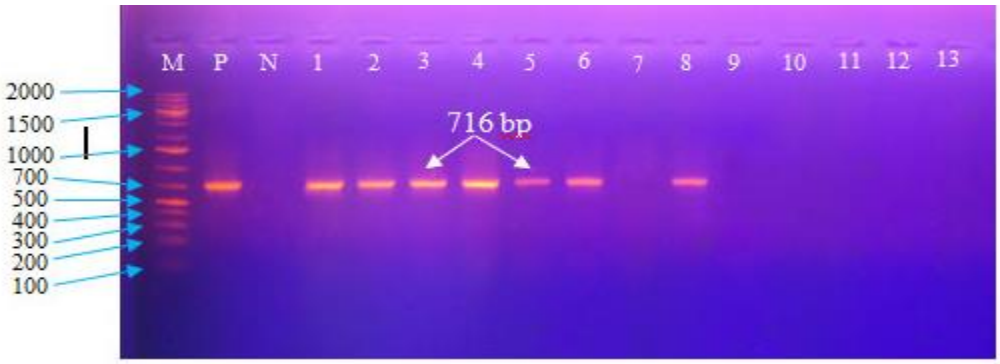


Figure 22: PCR results of bla_{TEM} gene from *E. coli*. Lane M: 2 kb DNA ladder; Lane P: positive control for bla_{TEM}; Lane N: negative control; Lane 1-8: bla_{TEM} gene-sized (716bp) amplicon.

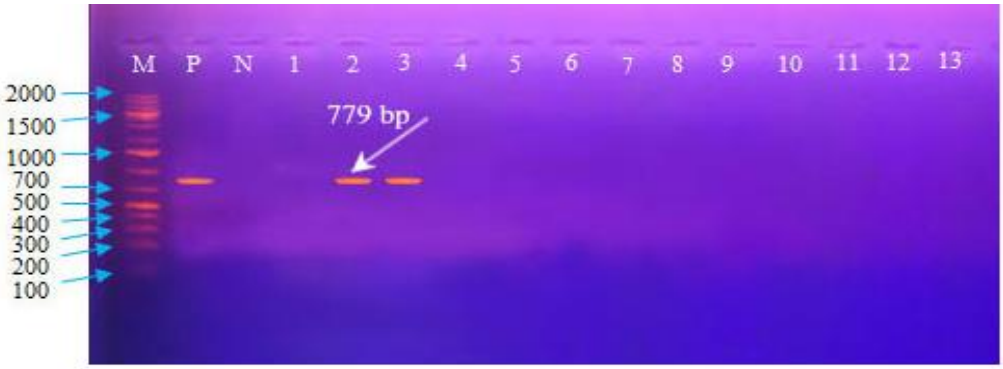


Figure 23: PCR results of sul 1 gene from *E. coli*. Lane M: 2 kb DNA ladder; Lane P: positive control for sul 1; Lane N: negative control; Lane 1-3: sul 1gene-sized (779bp) amplicon.

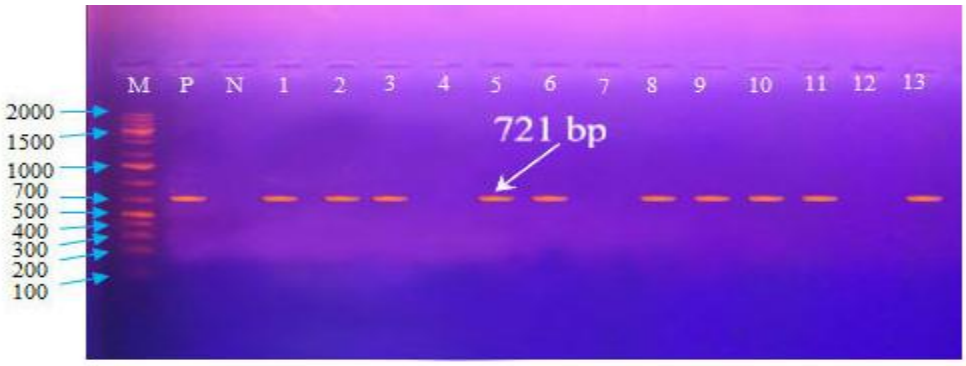


Figure 24: PCR results of sul 2 gene from *E. coli*. Lane M: 2 kb DNA ladder; Lane P: positive control for sul 2; Lane N: negative control; Lane 1-13: sul 2 gene-sized (721bp) amplicon.

The PCR product sizes of *bla$_{TEM}$, tetA, tetB, tetC, gyrA, gyrB, parC, CTX-M-2, sul1 and sul2* genes were 716, 502, 930, 888, 312, 281, 261, 876, 716 and 721 bp, respectively. Results displaying all genes were positive in different isolates. Out of 12 ciprofloxacin resistant isolates, 10 gave positive amplicons for the *gyrB* gene which is highest occurrence of antibiotic resistance gene 83.3% (CI=51.6-97.9%), *gyrA* was 66.7% (CI=34.9-90.1%), *parC* 25% (CI=5.5-57.2%). Occurrence of *bla$_{TEM}$* for amoxicillin was 15.4%. The *tetA* was (7.9%) floolwed by *tetB* (1.6%) for tetracycline. CTX-M-2 for ceftriaxone was 10.5% (CI=4-21.5%) and *sul1*(3.6%) (CI=0.4-12.5) and *sul2* (41.5%) (CI=28.7-55.9%) represent the occurrence of antibiotic resistance gene from the resistance antibiotics.

Table 14: Occurrence of antibiotic resistance genes in *E. coli*

Resistant antibiotic	Antibiotic resistant gene	No. of Resistant antibiotics	No. of resistant gene	Occurance (%)	95% CI
Amoxicillin	bla$_{TEM}$	52	8	15.4	6.9-28.1
Tetracycline	tet A		5	7.9	2.6-17.6
	tet B	63	1	1.6	0-8.5
	tet C		0	0	0-5.7
Ciprofloxacin	gyr A		8	66.7	34.9-90.1
	gyr B	12	10	83.3	51.6-97.9
	par C		3	25	5.5-57.2
Ceftriaxone	CTX-M-2	67	6	10.5	4-21.5
Trimethoprim-	Sul 1	55	2	3.6	0.4-12.5
sulfomathoxazole	Sul 2		23	41.8	28.7-55.9

4.4.1. Isolation of *Salmonella* species

The isolation of *Salmonella* spp. from faecal samples from pet birds is shown in table 15. A total number of 150 samples were collected, 90 from budgerigar, 5 each from lovebirds and horned parakeets, 14 from rose ring parakeet, 14 from parrots, 3 each from eastern rosella, finch and cockatoo, 10 from galah and 1 from eagle and 2 from kadaknath types chicken. The occurrence rate of *Salmonella* spp. was (2.7%). The highest isolation rate of

Salmonella spp. from faecal samples of lovebird 20% (95% CI=5.0-71.6) followed by budgerigar 3.3% (95% CI=0.7-9.4).

Table 15: Occurrence of *Salmonella* spp. infection in pet birds

Bird	Total sample	Positive samples	Occurance (%)	95% CI
Budgerigar	90	3	3.3	0.7-9.4
Lovebird	5	1	20	5.0-71.6
Horned parakeets	5	0	0	0-52.2
Rose ring parakeet	14	0	0	0-23.2
Parrot	14	0	0	0-24.7
Eastern Rosella	3	0	0	0-70.8
Finch	3	0	0	0-70.8
Cockatoo	3	0	0	0-70.8
Eagle	1	0	0	0-82.2
Kadaknath	2	0	0	0-82.2
Galah	10	0	0	0-30.8
Total	150	4	2.7	0.7-6.7

4.4.2. Antimicrobial resistance of *Salmonella* species

The occurrence of antibiotic resistance for the *Salmonella* spp. (N=4) isolates originated from pet birds is shown in table 16. 50% isolates demonstrated resistance to amoxicillin and tetracyclin in each, ceftriaxone (100%), gentamicin 25%, and azithromycin 75%. There was no resistance found against colistin sulfate, ciprofloxacin, enrofloxacin and trimethoprim-sulfomathoxazole.

Table 16: Antibiotic susceptibility testing of *Salmonella* isolates

Antibiotic class	Antibiotics	S(%)	I(%)	R(%)
Beta lactam	Amoxicillin	25	25	50
Cephems	Ceftriaxone	0	0	100
Amino glycosides	Gentamicin	75	0	25
Tetracycline	Tetracycline	50	0	50
Fluoroquinolones	Ciprofloxacin	100	0	0
	Enrofloxacin	100	0	0
Folate pathway inhibitor	Trimethoprim-sulfomathoxazole	75	25	0
Polymyxins	Colistin sulfate	100	0	0
Macrolide	Azithromycin	25	0	75

R'=resistance, 'I'=intermediate, 'S'=sensitive

4.4.3. Antibiotic resistance pattern for *Salmonella* species

The occurrence rate of multidrug resistance in *Salmonella* species was 25%. Among the total 9 tested antibiotics, the organism showed resistance to 5. AML-CN-AZM-CRO-OT pattern showed resistance as 25% (CI=0.6-80.6%) and had 0.55 MAR index (MAR index is calculated as the ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics the organism is exposed to). Bacteria having **MAR index ≥ 0.2** originate from a high risk source of contamination where several antibiotics are used. AZM-CRO identified as 50% (CI=6.8-93.2%) resistance.

Table 17: Antibiotic resistance pattern for *Salmonella* spp.

Pattern	Percentage	95% CI	MAR Index
AML-CN-AZM-CRO-OT	1/4=25%	0.6-80.6	0.55
AZM-CRO	2/4=50%	6.8-93.2	0.22
CRO	1/4=25%	0.6-80.6	0.11
Total	100%		

(AML=Amoxicillin, CN=Gentamicin, AZM= Azithromycin, CRO= Ceftriaxone, OT= Tetracycline)

4.4.4. Antimicrobial resistance genes for *Salmonella* species

The PCR product sizes of *bla*$_{TEM}$, *tetA*, *tetB*, *tetC* and *CTX-M-2* genes were 716, 502, 930, 888 and 876 bp respectively. Results displaying none sample are positive in those genes.

Table 18: Occurrence of antibiotic resistance genes in *Salmonella* spp.

Resistant antibiotic	Antibiotic resistant gene	No. of resistant antibiotics	No. of resistat gene	Occurrence (%)	95% CI
Amoxicillin	*bla*$_{TEM}$	1	0	0	0-97.5
Tetracycline	*tetA*		0	0	0-84.2
	tetB	2	0	0	0-84.2
	tetC		0	0	0-84.2
Ceftriaxone	*CTX-M-2*	4	0	0	0-60.2

CHAPTER-5: DISCUSSION

5.1. Gastrointestinal parasites of birds

Through faecal microscopical examination like direct smear, sedimentation and flotation from more than 500 samples including more than 20 species of pet birds divulge a high occurrence of parasitic infections in Chattogram region of Bangladesh. From different article some prevalence data of endoparasitic infection in pet bird species similar to this study findings (Papini et al., 2012; Badparva et al., 2015; Globokar et al., 2017; Lima et al., 2017; Ola-Fadunsin et al., 2019b). In different order of birds, the parasitic species infection differed significanly between them. The recognition of parasites in each group will be discussed separately. It is noted that there is no trematodal infection diagnosed in pet birds in this area. For the completion of life cycle of treamtode, required one or more intermediate host which could be snail or others group (Hechinger and Lafferty, 2005; Ebbs et al., 2018). No trematodal infection might be due to captive raring of pet birds and has no opportunity to completion of life cycle for trematode.

In Psittaciformes group, the infection of different parasites is low because of regular deworming process by the owners of pet birds. Birds from outdoor might be more infected and predisposed to various parasites due to more excess to soil as well as no deworming process (Harcourt-Brown and Chitty, 2005). *Caryospora, Porrocaecum, Syngamus* and *Coanotaenia* species shows conspicuously low infection in Psittaciformes in contrast to other avian orders in this study. *Eimeria* and *Isospora* are most frequently found this group 7.25% and 3.31% respectively, which is approximately similar with (Papini et al., 2012) 6.3% (*Eimeria*) and (Globokar et al., 2017) 0.49% *Isospora* spp. infection. The infection rate of *Isospora* in psittaciformes group is lower from passeriformes and columbiformes group of this study, but higher than Northeastern Brazil which was 1.53% (Lima et al., 2017). Globokar et al., (2017) found most frequent infection in this group is *Capillaria* and *Ascaridia* eggs (3.2% and 2.6%). These variations might be due to sample number fluctuation in two research group or variation in feed ingreadient or due to environmental variation like, different temperature and humidity in two countries. Niilo, (1970) said that, at low temperature coccidian parasite cannot survive in environment and so, in New Zealand, coccidian parasitic infection

found lower rate in comparison to Bangladesh has higher temperature and humidity. Another cause is that, pet bird owners frequently use anthelmintics rather than anti-coccidial drugs in Bangladesh. We found 70% owners used anthelmintics within 6 months of sample collection but only 5% served anti-coccidial drugs. Even feeding practice might cause for infection type variation among different groups of birds like, in budgerigar, cockatiel, parrot etc. feeds are commonly provided in elevated feeding dishes, while feed intake from potentially contaminated ground in pigeons and chickens.

In psittaciformes group, the *Ascaridia* spp. infection found (in budgerigar, cockatoo, galah, parrots, lovebird and macaw) at 2.07% rate, which is approximately similar with Globokar et al., (2017) 3.2%; in northeastern Brazil 1.53% (Lima et al., 2017) and slightly higher occurrence than previously reported in single parakeets, cockatiels, parrots (Tsai et al., 1992). However, the infection rate was much lower than the infection rate of *A. platyceri* (20%) in budgerigars, eastern rosella and african grey parrots in Poland (Balicka-Ramisz et al., 2007), 15.9% in psittaciformes group in Italy (Papini et al., 2012) and *A. galli* in zoo parrots in Pakistan (26.1%). Patel et al., (2000) reported that, *Ascaridia* eggs had been identified in 4/33 psittacines (white cockatoo, lore, amazon parrot, love bird).

Capillaria spp. infection occurred in cockatiel, budgerigar and parrot at 1.03% rate which is mostly similar with 2.6% in New Zealand (Globokar et al., 2017) and 1.6% in Italy (Papini et al., 2012) and 0.6% in Northeastern Brazil (Lima et al., 2017). *Capillaria* eggs also found in one St. vincent parrot (*Amazona guildingii*) (Deem et al., 2008) and in (Chitty and Lierz, 2008) identified in 5/33 parrots (African grey parrots, white cockatoos, macaws and parakeets). There is very few information known about the pathogenicity of *Capillaria* species. In the gastrointestinal tract, diphtheritic membranes form and caused for less absorption of feed consequently emaciation occurred (Helmboldt et al., 1971).

Heterakis infection in this group is 0.62% which is mostly similar with Lima et al., (2017) was 1.02% in Northeastern Brazil. Strongyl type and *Syngamus* species eggs found 0.41% and 0.21% respectively and his findings slightly less than from (Papini et al., 2012) 1.6% both. *Porrocaecum* infection found 0.62% and these results similar with Falconiformes and Accipitriformes groups is 1.96% (Globokar et al., 2017)

In Passeriformes group, *Ascaridia* is the most frequent infection found and the occurrence is 8.51% for this group. In pet and zoo birds from Italy, 15.9% *Ascaridia* infection found by (Papini et al., 2012); which is slightly more than this study. (Globokar et al., 2017) found 0.50% ascaridial infection in New Zealand and is less than our findings, which might be due to management as well as environmental fluctuation. Coccidial infection, mainly the *Eimeria* found 6.38% in Passeriformes group which is same to (Papini et al., 2012) findings and slightly more than (Globokar et al., 2017) findings is 3.51%. As the oocysts shedding rate fluctuate during the day, the late afternoon is supposed to increase oocyst shedding, and is the proper time for sample collection (López et al., 2007). In the present study, for Passeriformes group; *Capillaria, Heterakis, Porrocaecum* and Strongyle types eggs found at similar rate 2.13%. *Capillaria* infection in passeriformes group found 5.27% in New Zealand and *Porrocaecum* is 5.52% (Globokar et al., 2017). Occurrence of strongly type infection 1.6% in Italy (Papini et al., 2012) and 1.78% in Northeastern Brazil (Lima et al., 2017).

In Columbiformes group, parasitic infection in faecal samples is very common. Different retrospective review reported higher as well as lower occurrence of different parasites. Occurrence of *Eimeria* oocysts in domestic pigeon is 21.42% in this survey. (Radfar et al., 2012) found various rate of infection of coccidian parasites depending on different season (2.5–40.2%). Occurrence of coccidial infection was 5.40% in Northeastern Brazil (Lima et al., 2017). In Bangladesh, at Chattogram Metropolitan Area, prevalence of *Eimeria* species infections in pigeon was 11% (Krishna Ghosh, 2014). High prevalence (71.9%) of *Eimeria* oocysts observed in free-living pigeons in the city of Ljubljana (Dovč et al., 2004) and at Ilorin, North Central Nigeria was 64.71% (Ola-Fadunsin et al., 2019b). Some variation in findings might be due to living standard of pigeons likewise allowed or not to go out of cage as well as anthelmintic therapy provided or not. Even high oocyst counts (3000–20 000 oocysts/g faeces) were detected in pigeon with poor body condition in different flock (Wallis, 1991). Virulence of infection depends on the species in case of *Eimeria* likewise, especially in young *Eimeria columbae, Eimeria labbeana, Eimeria columbarum* etc. (Balicka-Ramisz et al., 2007). In the present study, *Ascaridia* species eggs (28.57%) was in concordant with studies conducted in Rajshahi division of Bangladesh was 31.74% (Rahman et al., 2019),

22.81% (Khanum et al., 2018), 28.33% (Begum and Sehrin, 2013), 35% in Chittagong metropolitan area (Krishna Ghosh, 2014). In different other studies occurrences is observed 30–42% (Al-Barwari and Saeed, 2012; Tanveer et al., 2011). Occurrence of each type of parasitic infection *Capillaria*, *Heterakis*, Strongyle type eggs is 7.14% in this study. *Capillaria* infection found in 13.51% and strongly type is 4.04% in Northeastern Brazil (Lima et al., 2017). 2.7% *Capillaria* infection found in Iran (Badparva et al., 2015). In Bangladesh, *Capillaria* 22% and *Heterakis* 13% infection at Chattogram metropolitan area from pigeon (Krishna Ghosh, 2014); *Capillaria* 18% in Rajshahi division (Khanum et al., 2018). So, our findings are more or less similar of others findings.

In Galliformes of our experiment, the occurrence of parasite genera were *Eimeria*, *Ascaridia*, and *Syngamus* within only 4 faecal samples from ornamental galliformes chickens and which is very similar with Globokar et al., (2017) and Tomza-Marciniak et al., (2014). Different other study represented that, these parasitic genus are very common in galliformes groups (Hoque et al., 2014; Ferdushy et al., 2016). Seven different *Eimeria* species is habitat in specific region of intestines of chicken (*Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria tenella, Eimeria mitis, Eimeria brunette, Eimeria praecox*) (Williams et al., 2009) and *E. tenella* and *E. necatrix* are most pathogenic species and cause for high mortality and infection. So, according to (Alam et al., 2014), regular deworming at three months interval with appropriate anthelmintics is recommended against both nematodes and cestodes, anti coccidial drugs recommend for coccidiosis as well.

5.2. Blood parasites of birds

Through nested PCR examination from 312 samples including 6 species of psittaciformes group of pet birds expressed fewer occurrences of blood parasitic infections. In our study, the occurrence of *Haemoproteus* is 0.32%, *Plasmodium* 1.3% and *Leococytozoon* 0.64%. (Hellgren et al., 2004) reported *Haemoproteus* from avian blood were detected as 1.2% of a single lineage by PCR method. His finding is approximately similar of our finding. From Costa Rica 4.8% was detected as the prevalence of *Haemoproteus* in birds (Valkiunas et al., 2004). 3.5% in birds from São Paulo State, Brazil (Bennett and Lopes, 1980); in Japanese wild birds 5.1% (Murata, 2002); 7.7% was detected in West African rainforest birds (Sehgal et al., 2005). In maximum case, the previous study conducted in wild birds or parasite detected as microscopical examination. Primary vectors known for *Haemoproteus* spp. is Hippoboscid flies and *Culicoides* spp. (Diptera: Ceratopogonidae). They are not common in our country might be consequencely the occurrence of *Haemoproteus* is also low.

In case of *Plasmodium* spp. the infection rate was 1.3% which is similar to (Bennett and Lopes, 1980) 1.8% in birds from São Paulo State, Brazil, 1.7% in Japanese wild birds (Murata, 2002), 0.6% of birds from Costa Rica (Valkiunas et al., 2004), 1.9% in birds from Madagascar (Savage et al., 2009), 3% in passerine birds from central New Jersey (Kirkpatrick and Suthers, 1988), 3.6% birds from a neotropical savanna in Brazil (Fecchio et al., 2011).

0.64% is the occurrence rate of *Leococytozoon* in our study. Our findings is slightly higher than (Bennett and Lopes, 1980) findings 0.06% from birds of São Paulo State, Brazil; 0.3% from birds from Costa Rica (Valkiunas et al., 2004) and slightly lower than nearctic-neotropical passerine birds which is 1.3% (Garvin et al., 2006), west African rainforest birds 4.6% (Sehgal et al., 2005) and 4% from naturally infected birds (Valkiunas et al., 2009). Due to less availability of the species of ornithophilic black flies that serve as vectors for *Leucocytozoon* species might be one cause of less infection of *Leococytozoon* to pet birds.

The results or outcomes of a study depend on some factors such as, method of detection of parasites, geographical distribution, vector availability, sample focus on pet or wild birds, management and caring system to birds etc. In Bangladesh, previously there was no report published on the blood parasitic infection on pet birds. Compare to others prevalence findings from different area of the world, our occurrence rate findings is low, because of pet birds are reared in intensive care with regular anthelmintic treatment and not much vector available in the apartment due to use of mosquitoes and fly repellent, results in very little transmission of the blood parasites.

5.3. *E. coli* and *Salmonella* species and their antibiotic resistance

In this study, faecal samples collected from different household pet birds were investigated to determine the occurrence and antimicrobial resistance patterns of *E. coli* and *Salmonella* spp. Associated with this resistance, possible corresponding genes were also examined. *E. coli* and *Salmonella* isolates found in household pet birds have acquired resistance against multiple classes of antimicrobials including amoxicillin, ceftriaxone, and tetracycline in case of both *E. coli* and *Salmonella* spp. and gentamicin in *Salmonella* spp. as well as ciprofloxacin, trimethoprim-sulfomathoxazole in case of *E. coli*; indicating the widespread circulation of multi-drug resistant. Third generation cephalosporins and fluoroquinolones are critically important antimicrobials considered by World Health Organization (WHO, 2016) and therefore resistance to these antimicrobials is worrisome.

The complexity of antibiotic resistance from pet birds to human is not fully identified. Unfortunately, there is paucity in data related to AMR in pet birds although huge amount of study is performed regarding AMR. In our study, we found the overall prevalence of *E. coli* from pet birds of household that was 48.7%. This finding was almost similar to Lopes et al., (2018) who found 55% prevalence of *E. coli* from psittaciformes, 50% in piciforms and same % in finch birds. Gopee et al., (2000) and Diren Sigirci et al., (2020) showed 68% and 37.7% isolates were positive in *E. coli* in pet birds. It is reported that 48% was the *E. coli* isolation rate from cloacal sample of psittacine birds (Corrêa et al., 2013). In case of pigeon, the occurrence is 24% in Bangladesh (Hasan et al., 2014). These

variations may be due to the difference in sampling size, use of antibiotics, detection method and geographical distribution.

The prevalence of *Salmonella* species from faecal sample in our finding is 2.7%. This finding is similar to Lopes et al., (2014) 1.65% from Brazil, 0.9% of pet birds from Trinidad (Seepersadsingh and Adesiyun, 2003) and 1.63% from Turkey (Sareyyüpoğlu et al., 2008). In case of Allgayer et al., (2008) the occurrence % increase up to 13.2% in PCR based technique but none of them was positive by standard microbiological techniques. It may be due to the greater sensitivity of PCR than standard bacteriological techniques (Allgayer et al., 2008). From Bangladesh, no other research article has been published yet relating pet bird's species. But from pigeon faeces the infection rate is 27.50% (Hosain et al., 2013), 37.5% from cloacal swabs (Saifullah et al., 2016). So, the occurrence rate is higher than our findings. This higher rate of infection might be lack of hygienic management (as they are not like as adorable to owner as pet ornamental birds) and allowed to go out for flying and feeding purpose.

To determine the antimicrobial susceptibility the *E. coli* isolates were subjected to seven antimicrobial agents. Results revealed 91.78% resistance to ceftriaxone, 86.30% to tetracyclin, 75.34% to trimethoprim-sulfomathoxazole, 71.23% to amoxicillin, and 16.44% to ciprofloxacin. Tetracycline resistance in *E. coli* in our findings was 86.30% which is similar to 84% in pet birds from turkey (Diren et al., 2020), 60% from Trinidad (Gopee et al., 2000), 65.2% from Turky (Diren et al., 2019), 69% in pigeon from Bangladesh (Hasan et al., 2014), 41% from the cloacal swab of cockatiels (Pontes et al., 2018). Tetracyclin is commonly using in our country both in veterinary and human practice.

Trimethoprim-sulfomathoxazole has also oral efficacy as broad spectrum drug in birds. (Diren et al., 2019) reported 38% resistance to SXT, 46% (Diren et al., 2020), 100% (Varriale et al., 2020) and 38% in pigeon in Bangladesh (Hasan et al., 2014). 30% *E. coli* resistance to ciprofloxacin group from healthy pet cockatiels (Pontes et al., 2018), 13% from pet birds (Diren et al., 2020). 27% *E. coli* got resistance to ciprofloxacin in pigeon from Bangladesh (Hasan et al., 2014). In our study, we did not find any gentamicin

resistance against *E. coli*, although about 2% was in intermediate stage. Other study also finds little resistance like 7% pet birds of turkey (Diren et al., 2020), 4% reported by Pontes et al., (2018) in captive birds.

Amoxicillin got resistance 82% against *E. coli* in captive cockatiels (Pontes et al., 2018) which is very similar our findings 71.23%. (Yılmaz and Dolar, 2017) found 100% resistance from cage birds. 87.5% amoxicillin resistance found in psittacidae group and 47.5% in columbidae group (Varriale et al., 2020) and 72.7% in captive pigeon (Borges et al., 2017). 91.78% *E. coli* got resistance to ceftriaxone in our findings. 63.6% in captive pigeon (Borges et al., 2017). Cephalothin got resistance 100% found against *E. coli* in captive birds (Gopee et al., 2000; Yılmaz and Dolar, 2017).

In case of *Salmonella* species, we have found only 4 positive samples and resistance against ceftriaxone (100%), azithromycin (75%), tetracycline and amoxicillin both are 50% and gentamycin (25%). *Salmonella* from cloacal swabs of pigeons identified as 100% sensitive to ciprofloxacin and 75% to gentamicin; in contrast, more than 80% resistance to ceftriaxone and amoxicillin (Saifullah et al., 2016). Cephalothin (30-67%) found as resistance from wild birds of Bangladesh (Al Faruq et al., 2016). From pigeon sample of Bangladesh 80% of the *Salmonella* isolates were sensitive to ciprofloxacin followed by sulphamethoxazole (70%), gentamicin (60%) and in contrast, resistant to amoxicillin (90%) and tetracycline (60%) (Hosain et al., 2013). These findings are very similar to our findings. Some fluctuation may be due to managemental system as well as antibiotic exposure to the birds.

This study revealed high levels of resistance to antimicrobial agents. Pet birds owner treat their birds at any clinical sign with antibiotics like ciprofloxacin, amoxicillin mostly. The antibiotic resistance load of birds vastly influence by the human activity like antibiotic malpractice in any clinical symptoms without veterinary supervision. Strains of *E. coli* and *Salmonella* species may have generally high susceptibility to colistin sulfate, gentamicin, and enrofloxacin but high resistance to amoxicilline, ceftriaxone, and tetracycline while moderately susceptible to sulphonamides, ciprofloxacin. This study represents that 90.41% of the *E. coli* isolates were multidrug resistance (MDR). This term

defined, minimum 3 different groups of antibiotic combined show resistance. Even 56.16% of isolates show resistance to 4 to 6 class of antimicrobial drugs. From companion birds 67% MDR was detected from *E. coli* isolates (Diren et al., 2020); 59% reported (Pontes et al., 2018) in captive birds. There are some articles reported 100% of their tested antibiotics were MDR in poultry (Rahman et al., 2011; Sarker et al., 2019) and from captive pigeons found in 72.7% isolates (Borges et al., 2017). Due to excess use of antibiotics, MDR strain of *E. coli* apparently occurring with high frequently (Van Den Bogaard and Stobberingh, 2000).

In case of *Salmonella* spp., we found 25% of isolates are MDR and they are 5 classes of microbial drugs. Krawiec et al., (2017) found more than 50% isolates MDR against *Salmonella* in free living birds. In pigeon from Bangladesh, MDR found against salmonellosis (Saifullah et al., 2016). Al Faruq et al., (2016) found MDR in case of wild birds of Bangladesh.

Where several antibiotics are used, bacteria originate these area have more than 0.2 MAR index and it is the ratio of resistance antibiotics to total number of antibiotics used. It is very alarming that our study revealed that our all MDR have more than 0.2 MAR index. MDR results will be helpful for veterinarian to treat pet birds in Bangladesh.

Huge number of different antibiotic resistance gene found in our *E. coli* isolates. 15.4% of amoxicillin resistance isolates positive for bla_{TEM} gene; *tetA* (7.9%) and *tetB* (1.6%) for tetracyclin; *gyrA* (66.7%), *gyrB* (83.3%) and *parC* (25%) for ciprofloxacin; CTX-M-2 (10.5%) for ceftriaxone; *sul1* (3.6%) and *sul2* (41.4%) for trimethoprim-sulfamethoxazole. Beta-lactam resistance gene (bla_{CTX-M}) (52%), *tetB* (80%) were detected from *E. coli* isolates of cage birds (Yılmaz and Dolar, 2017). 33% of sulfonamide resistance gene *sul1, sul2, sul3* were identified from captive Cockatiels (Pontes et al., 2018). *tetA, sul2* and bla_{TEM} gene was identified in poultry in Chattogram region of Bangladesh (Sarker et al., 2019). *gyrA, gyrB, parC, CTX-M-2* resistance gene found against *E. coli* from healthy chicken (Vounba et al., 2019; Temmerman et al., 2020). In case of *Salmonella* spp, we tested PCR for bla_{TEM} gene, *tetA, tetB, tetC, CTX-*

M-2 in relevant antibiotic resistant samples but could not find any positive gene from them. This might be due to very small size for antibiotic resistant samples.

It is possible to hypothesize that pet bird, especially those positive for the zoonotic *E. coli* or *Salmonella* spp. could be zoonotic sources of infection to humans and other domestic animals. Antibiotic resistance gene transfer by R factors is also known to occur widely in Gram-negative bacteria, especially the coliforms (Anderson, 1968). So, antibiotic malpractice should be avoided in term of public health concern.

CHAPTER-6: CONCLUSION

This study, as our knowledge for the first time in Bangladesh provides the novel information about gastro intestinal and blood parasitic infection as well as antimicrobial resistance pattern of *E. coli* and *Salmonella* spp. strains isolated from pet birds.

From 549 faecal pool sample it is revealed that, the occurrence of gastrointestinal parasitic infection was 18.9%. Among them, *Eimeria* spp. occurrence was the most significant rate 7.7%. Within the nematode infection *Ascaridia* spp. was the most prominent one which was 3.5% followed by *Capillaria* spp. 1.7% and *Heterakis* spp. 0.91%. *Porrocaecum* spp. *and* strongyl type both are 0.72%. Cestode infection is very rare in which *Coanotaenia* spp. 0.36% and *Raillietina* spp. 0.18%. The most important thing is that we did not get any trematode infection and this may be due to mollusk is needed to complete life cycle as intermediate host and direct contact to them is not possible.

Randomly 311 blood samples were collected for blood parasite detection by PCR method. The study objective was to identify *Haemoproteus, Plasmodium* and *Leucocytozoon* at genus level. The occurrence of blood parasitic infection was 2.25%. Among them 0.32% is the occurrence of *Haemoproteus* spp., the *Plasmodium* spp. was 1.3% and *Leucocytozoon* spp. was 0.64%. Due to rearing pet birds as ornamental purpose, owner take care about them in term of management like regular anthelmintics therapy, uses of mosquito repellent, less vector availability resulting low infection in both gastro intestinal as well as blood parasites occurrence.

A total of 73 *E. coli* isolates were isolated from 150 samples tested. More than 91% isolates were resistant to ceftriaxone, 86.3% to tetracycline and 75.34% to sulfomethoxazole-trimethoprim. Most sensitive antibiotic to *E. coli* is colistin sulfate 98.63% followed by gentamicin 97.26%. Majority of *E. coli* isolates were multidrug resistant and carried the resistant gene bla_{TEM}, *tetA, tetB, sul1, sul2, gyrA, gyrB, parC* and *CTX-M-2*. The multi-drug resistant *E. coli* can disseminate the resistance genes horizontally to other environmental bacteria and could expose a risk to public and animal health.

2.7% (n=4/150) is the occurrences of *Salmonella* spp. All four isolates were resistant to ceftriaxone, three to azithromycin, two to amoxiciline and tetracycline. All isolates have showed sensitive to ciprofloxacin, enrofloxacin and colistin sulfate. For the management and rearing system of pet birds in captivity, the present findings are valuable for the guidance to pet bird's owner focusing on restriction on antibiotic malpractice and would be act as mentor to veterinarian to treat pet birds in Bangladesh.

CHAPTER-7: RECOMMENDATION AND FUTURE PERSPECTIVE

The study time and the funding were limited. We covered our research on the pet birds of Chattogram metropolitan area which may not reflect the total scenario of Bangladesh. For greater understanding of GI, blood parasitic infection in pet birds, it is recommended to sampling from all over the country. For better finding of AMR, huge amount of sample should be tested. MIC should be done for better understanding for colistin resistance identification. Due to affection of people to pet birds rearing for ornamental purpose, research preference should be more in this field to identify potential disease in birds as well as to control the zoonotic diseases.

REFERENCES

Abdullah, A. S., Khan, M. S., Alam, M., Haq, F. and Hassan, J. 2010. Isolation and molecular characterization of Escherichia coli from goat of apparently healthy and clinical cases. Bangladesh Journal of Microbiology. 27(1): 14–17.

Adkesson, M. J., Zdziarski, J. M. and Little, S. E. 2005. Atoxoplasmosis in tanagers.. Journal of Zoo and Wildlife Medicine. 36(2): 265–272. https://doi.org/10.1638/03-091.1

Adler, P. H. ; Currie, D. C. and Wood, D. M. 2004. The black flies (Simuliidae) of north America. Cornell University Press.

Agabou, A., Lezzar, N., Ouchenane, Z., Khemissi, S., Satta, D., Sotto, A., Lavigne, J. P. and Pantel, A. 2016. Clonal relationship between human and avian ciprofloxacin-resistant Escherichia coli isolates in north-eastern Algeria. European Journal of Clinical Microbiology and Infectious Diseases. 35(2): 227–234. https://doi.org/10. 1007/s10096-015-2534-3

Ahumada, J. A., Laoointe, D. and Samuel, M. D. 2004. Modeling the population dynamics of Culex quinquefasciatus (Diptera: Culicidae), along an elevational gradient in Hawaii. Journal of Medical Entomology. 41(6): 1157–1170. https://doi. org/10. 16 03/ 0022-2585-41.6.1157

Al-Barwari, S. and Saeed, I. 2012. The parasitic communities of the rock pigeon Columba livia from Iraq: component and importance. Türkiye Parazitolojii Dergisi/ Turkish Society for Parasitology. 36(4): 232–239. https://doi.org/10. 5152/tpd. 2012 .56

Al Faruq, A., Hassan, M. M., Mejbah Uddin, M., Rahman, M. L., Rakib, T. M., Alam, M. and Islam, A. 2016. Prevalence and multidrug resistance pattern of Salmonella isolated from resident wild birds of Bangladesh. International Journal of One Health. 2(3): 2455–8931. https://doi.org/10. 14202/ IJOH.2016.35-41

Alam, M., Mostofa, M., Khan, M., Alim, M., Rahman, A. and Trisha, A. 2014. Prevalence of gastrointestinal helminth infections in indigenous chickens of selected areas of Barisal District, Bangladesh. Bangladesh Journal of Veterinary Medicine. 12(2): 135–139. https://doi.org/10.3329/bjvm.v12i2.21275

Allgayer, M. C., Lima-Rosa, C. A. V., Weimer, T. A., Rodenbusch, C. R., A. F., Oliveira, S. D. and Canal, C. W. 2008. Molecular diagnosis of Salmonella species in captive psittacine birds. Veterinary Record. 162(25): 816–819. https://doi.org/10.1136/ vr.162.25.816

Almeida, P. M. P. de, Arais, L. R., Andrade, J. R. C., Prado, E., Irino, K. and Cerqueira, A. 2012. Characterization of atypical enteropathogenic Escherichia coli (aEPEC) isolated from dogs. Veterinary Microbiology. 158(3–4): 420–424. https:// doi.org /10.1016/j.vetmic.2012.02.021

Amy, L., Shima and Kent G. Osborn. 1989. An epornitic of Salmonella typhimurium in a collection of lories and lorikeets. Journal of Zoo and Wildlife Medicine. 20(2): 373–376.

Anderson, E. S. 1968. The ecology of transferable drug resistance in the entero bacteria. Annual Review of Microbiology. 22(1): 131–180. https://doi.org/10. 1146/ annurev. mi.22.100168.001023

Aragao, H. D. B. 1916. Researches on Haemoproteus columbae. Brasil-Medico. 30(45): 353–354.

Atkinson, C. T., Thomas, N. J., & Hunter, D. B. Eds. .2008. Parasitic diseases of wild birds (J. W. & Sons. (ed.); 1st ed.).

Atkinson, C. T., Van Riper III, I., C. 1991. Pathogenicity and epizootiology of avian haematozoa: Plasmodium, Leucocytozoon, and Haemoproteus. Oxford: Oxford University Press.p.212

Badparva, E., Ezatpour, B., Azami, M. and Badparva, M. 2015. First report of birds infection by intestinal parasites in khorramabad, west Iran. Journal of Parasitic Diseases. 39(4): 720–724. https://doi.org/10.1007/s12639-014-0427-5

Baker, J. R. 1966. Haemoproteus palumbis sp. nov. (Sporozoa, Haemosporina) of the english wood-pigeon columba p. palumbus. The Journal of Protozoology. 13(3): 515–519. https://doi.org/10.1111/j.1550-7408.1966.tb01954.x

Balaguer, L., J. Romano, J. M. Nieto. and Jhosef, P. F. 1992. Nodular typhlitis of pheasants caused by heterakis isolonche: further evidence of a neoplastic nature. Journal of Zoo and Wildlife Medicine. 23(2): 249–253.

Balicka-Ramisz, A., Tomza-Marciniak, A., Pilarczyk, B., Wieczorek-Dabrowska, M. and Bakowska, M. 2007. Intestinal parasites of parrots. Wiadomości Parazytologiczne. 53(2): 129–132. https://europepmc.org/article/med/17912808

Bangert, R. L., Widders, P. R., Stauber, E. H. and Ward, A. C. 1988. A survey of aerobic bacteria and fungi in the feces of healthy psittacine birds. Avian Diseases. 32(1): 46–52. https://doi.org/10.2307/1590947

Bauer, & AW. 1966. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol. 45: 149–158. https://ci.nii.ac.jp/naid/10024190092

Beadell, J. S., Ishtiaq, F., Covas, R., Melo, M., Warren, B. H., Atkinson, C. T., Bensch, S., Graves, G. R., Jhala, Y. V, Peirce, M. A., Rahmani, A. R., Fonseca, D. M. and Fleischer, R. C. 2006. Global phylogeographic limits of Hawaii's avian malaria. Proceedings of the Royal Society B: Biological Sciences. 273(1604): 2935–2944. https://doi.org/10.1098/rspb.2006.3671

Becker Saidenberg, A., Robaldo Guedes, N. M., Fernandes Seixas, G. H., da Costa Allgayer, M., Pacífico de Assis, E., Fabio Silveira, L., Anne Melville, P. and Benites, N. R. 2012. A survey for escherichia coli virulence factors in asymptomatic free-ranging parrots. ISRN Veterinary Science. 201(2): 1–6. https://doi.org/10.5402/ 2012 /984813

Begum, A. and Sehrin, S. 2013. Gastrointestinal helminths in pigeon Columba livia (Gmelin, 1789). Journal of the Asiatic Society of Bangladesh, Science. 38(1): 93–98. https://doi.org/10.3329/jasbs.v38i1.15324

Belaaouaj, A., Lapoumeroulie, C., CaniÃ§a, M. M., Vedel, P., Krishna moorthy, R. and Paul, G. 1994. Nucleotide sequences of the genes coding for the TEM-like Î²-lactamases IRT-1 and IRT-2 (formerly called TRI-1 and TRI-2). FEMS Microbiology Letters. 120(1–2): 75–80. https://doi.org/10.1111/j.1574-6968. 1994. tb07010.x

Bélanger, L., Garenaux, A., Harel, J., Boulianne, M., Nadeau, E. and Dozois, C. M. 2011. Escherichia coli from animal reservoirs as a potential source of human extra intestinal pathogenic E. coli. FEMS Immunology & Medical Microbiology. 62(1): 1–10. https://doi.org/10.1111/j.1574-695X.2011.00797.x

Bennett, Gordon F. and Lopes, O. de S. 1980. Blood parasites of some birds from São Paulo state, Brazil. Memórias Do Instituto Oswaldo Cruz. 75(1–2): 117–134. https://doi.org/10.1590/s0074-02761980000100012

Bennett, G. F., Peirce, M. A. and Ashford, R. W. 1993. Avian haematozoa: Mortality and pathogenicity. Journal of Natural History.27(5): 993–1001. https://doi.org/10. 1080/ 00222939300770621

Bergey, D. H. and Brenner, D. J. 1984. Bergey's Manual® of systematic bacteriology.

Berto, B. P., Flausino, W., McIntosh, D., Teixeira-Filho, W. L. and Lopes, C. W. G. 2011. Coccidia of new world passerine birds (Aves: Passeriformes): A review of Eimeria schneider, 1875 and isospora schneider, 1881 (Apicomplexa: Eimeriidae). Systematic Parasitology. 80(3):159–204. Springer. https://doi.org/ 10.1007/s11230-011-9317-8

Bertrand, S., Weill, F. X., Cloeckaert, A., Vrints, M., Mairiaux, E., Fraud, K., Dierick, K., Wildemauve, C., Godard, C., Butaye, P., Imberechts, H., Grimont, P. A. D. and Collard, J. M. 2006. Clonal emergence of extended-spectrum β-lactamase (CTX-M-2)-producing Salmonella enterica serovar virchow isolates with reduced

susceptibilities to ciprofloxacin among poultry and humans in Belgium and France (2000 to 2003). Journal of Clinical Microbiology. 44(8): 2897–2903. https://doi.org /10.1128/JCM.02549-05

Blanco, J. 1991. Enterotoxins, colonization factors and serotypes of enterotoxigenic Escherichia coli from humans and animals. https://www.researchgate. net/publi cation/21470476

Borges, C. A., Maluta, R. P., Beraldo, L. G., Cardozo, M. V., Guastalli, E. A. L., Kariyawasam, S., DebRoy, C. and Ávila, F. A. 2017. Captive and free-living urban pigeons (Columba livia) from Brazil as carriers of multidrug-resistant pathogenic Escherichia coli. Veterinary Journal. 219: 65–67. https://doi.org/10.1016 /j.tvjl. 2016 .12.015

Box, E. D. 1970. Atoxoplasma associated with an isosporan oocyst in canaries. The Journal of Protozoology. 17(3): 391–396. https://doi.org/10.1111/j.1550-7408.1970. tb04700.x

Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R. and Swaminathan, B. 2000. Salmonella nomenclature. Journal of Clinical Microbiology. 38(7): 2465–2467. https://doi.org/10.1128/jcm. 38.7. 2465-2467.2000

Carter GR, C. J. 1990. Diagnostic procedures in veterinary bacteriology and mycology (5th ed.). Veterinary bacteriology Technique. San Diego, Calif: Academic Press, c1990.

Cassey, P. and Ewen, J. G. 2008. Relationships between nestling condition and variability in coccidian prevalence among three species of wild-nesting birds in New Zealand. Australian Journal of Zoology. 56(2): 75. https://doi.org/10.1071/ZO08001

Chitty, J. and Lierz, M. 2008. BSAVA manual of raptors, pigeons and passerine birds. BSAVA Manual of Raptors, Pigeons and Passerine Birds.

Ciloglu, A., Ellis, V. A., Bernotienė, R., Valkiūnas, G. and Bensch, S. 2019. A new one-step multiplex PCR assay for simultaneous detection and identification of avian haemosporidian parasites. Parasitology Research. 118(1): 191–201. https://doi.org /10.1007/s00436-018-6153-7

Clark, N. J., Clegg, S. M. and Lima, M. R. 2014. A review of global diversity in avian haemosporidians (Plasmodium and Haemoproteus: Haemosporida): new insights from molecular data. International Journal for Parasitology. 44(5): 329–338. https:// doi.org/10.1016/j.ijpara.2014.01.004

Clausen, B. 1991. Causes of mortality among free ranging gyrfalcons in Iceland. Journal of Wildlife Diseases. 17(1). https://doi.org/10.7589/0090-3558-17.1.105

CLSI. 2018. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical Infectious Diseases. 49(11)

Cole, R. A., Thomas, N. J. and Roderick, C. L. 1995. Bothrigaster variolaris (Trematoda: Cyclocoelidae) infection in two Florida snail kites (Rostrhamus sociabilis plumbeus). Journal of Wildlife Diseases. 31(4): 576–578. https://doi.org/10.7589 /0090-3558-31.4.576

Corradetti, A., Garnham, P. C. C. and Laird, M. 1963. New classification of the avian malaria parasites. Parassitologia. 5(1): 1–4.

Corrêa, I. M. d. O., Flores, F., Schneiders, G. H., Pereira, L. Q., Brito, B. G. and Lovato, M. 2013. Detecção de fatores de virulôncia de escherichia coli e análise de salmonella spp. em psitacídeos. Pesquisa Veterinaria Brasileira. 33(2): 241–246. https://doi.org/10.1590/S0100-736X2013000200017

Cunha, M. P. V., De Oliveira, M. G. X., De Oliveira, M. C. V., Da Silva, K. C., Gomes, C. R., Moreno, A. M. and Knöbl, T. 2014. Virulence profiles, phylogenetic background, and antibiotic resistance of Escherichia coli isolated from turkeys with airsacculitis. Scientific World Journal. 12(23): 25-34 https://doi.org/10.1155/ 2014 /289024

Deem, S. L., Ladwig, E., Cray, C., Karesh, W. B. and Amato, G. 2008. Health assessment of the ex situ population of st vincent parrots (Amazona Guildingii) in St vincent and the grenadines. Journal of Avian Medicine and Surgery. 22(2): 114–122. https://doi.org/10.1647/2007-022R.1

De Freitas, M. F. L., De Oliveira, J. B., De Brito Cavalcanti, M. D. and De Freitas, D. A. 2003. Occurrence of coccidiosis in canaries (Serinus canarius) being kept in private captivity in the state of Pernambuco, Brazil. Parasitologia Latinoamericana. 58(1–2): 86–88. https://doi.org/10.4067/S0717-77122003000100017

Desser, S. S. 1967. Schizogony and gametogony of Leucocytozoon simondi and associated reactions in the avian host. The Journal of Protozoology. 14(2): 244–254. https://doi.org/10.1111/j.1550-7408.1967.tb01992.x

Diren Sigirci, B., Celik, B., Başaran Kahraman, B., Bagcigil, A. F. and Ak, S. 2019. Tetracycline resistance of enterobacteriaceae isolated from feces of synanthropic birds. Journal of Exotic Pet Medicine. 28(4): 13–18. https://doi.org/10.1053 /j.jepm. 2017.12.003

Diren Sigirci, B., Celik, B., Halac, B., Adiguzel, M. C., Kekec, I., Metiner, K., Ikiz, S., Bagcigil, A. F., Ozgur, N. Y., Ak, S. and Basaran Kahraman, B. 2020. Antimicrobial resistance profiles of Escherichia coli isolated from companion birds. Journal of King Saud University Science. 32(1): 1069–1073. https://doi.org/10. 1016/j.jksus. 2019.09.014

Dobson, A. P. and Hudson, P. J. 1992. Regulation and stability of a free-living host-parasite system: Trichostrongylus tenuis in red grouse. II. population models. The Journal of Animal Ecology. 61(2): 487. https://doi.org/10.2307/5339

Dovč, A., Zorman-Rojs, O., Vergles Rataj, A., Bole-Hribovšek, V., Krapež, U. and Dobeic, M. 2004. Health status of free-living pigeons (Columba livia domestica) in the city of Ljubljana. Acta Veterinaria Hungarica. 52(2): 219–226. https://doi.org/10 .1556/AVet.52.2004.2.10

Dwight, D. B., Charles, M., Hendrix, D. and Lindsay, S. C. B. 2002. Feline clinical parasitology (1st ed.).

Ebbs, E. T., Loker, E. S. and Brant, S. V. 2018. Phylogeography and genetics of the globally invasive snail physa acuta draparnaud 1805, and its potential to serve as an intermediate host to larval digenetic trematodes. BMC Evolutionary Biology. 18(1): 1–17. https://doi.org/10.1186/s12862-018-1208-z

Ejiri, H., Sato, Y., Kim, K. S., Tsuda, Y., Murata, K., Saito, K., Watanabe, Y., Shimura, Y. and Yukawa, M. 2011. Blood meal identification and prevalence of avian malaria parasite in mosquitoes collected at kushiro Wetland, a subarctic zone of Japan. Journal of Medical Entomology. 48(4): 904–908. https://doi.org/10. 1603/ ME11053

Eşaki, C., Özer, E., Ecmel, S. and Özer, E. 2012. Isospora species (I. canaria, Isospora sp.) in canaries (Serinus canarius, Linnaeus). Animal Science. 36(2): 197–200. https://doi.org/10.3906/vet-1003-312

Farmer, J. 1964. Gizzard lesions associated with Haemoproteus sacharovi infections of pigeons. Lowa Academy of Science. 71(1): 321. https://scholarworks. uni.edu/pias /vol71 /iss1/80

Farooq, S., Hussain, I., Mir, M. A., Bhat, M. A. and Wani, S. A. 2009. Isolation of atypical enteropathogenic Escherichia coli and shiga toxin 1 and 2f-producing Escherichia coli from avian species in India. Letters in Applied Microbiology. 48(6): 692–697. https://doi.org/10.1111/j.1472-765X.2009.02594.x

Fecchio, A., Lima, M. R., Silveira, P., Braga, É. M. and Marini, M. Â. 2011. High prevalence of blood parasites in social birds from a neotropical savanna in Brazil. Emergience medical universe. 111(2): 132–138. https://doi.org/10.1071/MU10063

Ferdushy, T., Hasan, M. T. and Golam Kadir, A. K. M. 2016. Cross sectional epidemio logical investigation on the prevalence of gastrointestinal helminths in free range chickens in Narsingdi district, Bangladesh. Journal of Parasitic Diseases. 40(3): 818–822. https://doi.org/10.1007/s12639-014-0585-5

Fernando, M. A., Stockdale, P. H. and Remmler, O. 1991. The route of migration, development, and pathogenesis of Syngamus trachea (Montagu, 1811) Chapin, 1925, in pheasants. The Journal of Parasitology. 57(1): 107–116. https://doi.org/10. 2307/3277762

Figuerola, J. and Green, A. J. 2000. Haematozoan parasites and migratory behaviour in waterfowl. Evolutionary Ecology. 14(2): 143–153. https://doi.org/10.1023/ A:1011 009419264

Fix,', A. S., Waterhouse, C., Greiner, E. C. and Stoskopv, M. K. 1988. Plasmodium relictum as a cause of avian malaria in wild caught magellanic penguines. Journal of Wildlife Diseases. 24(4): 610–619. https://doi.org/10.7589/0090-3558-24.4.610

Foster, J. T., Woodworth, B. L., Eggert, L. E., Hart, P. J., Palmer, D., Duffy, D. C. and Fleischer, R. C. 2007. Genetic structure and evolved malaria resistance in Hawaiian honeycreepers. Molecular Ecology. 16(22): 4738–4746. https://doi.org/10 .1111/j. 1365-294X.2007.03550.x

Franck, S. M., Bosworth, B. T. and Moon, H. W. 1998. Multiplex PCR for enterotoxi genic, attaching and effacing, and shiga toxin-producing Escherichia coli strains from calves. Journal of Clinical Microbiology. 36(6): 1795–1797. https://doi.org/ 10.1128/jcm.36.6.1795-1797.1998

Friend, M., Franson, J. 1999. Field manual of wildlife diseases (field procedures and diseases of birds) (1999th–001 ed.).

Garnham, P. C. C. 1966. Malaria parasites and other haemosporidia. Malaria parasites and other haemosporidia.

Garvin, M. C., Szell, C. C. and Moore, F. R. 2006. Blood parasites of nearcticneotropical migrant passerine birds during spring trans-Gulf migration: Impact on host body condition. Journal of Parasitology. 92(5): 990–996. https://doi.org/10.1645/GE-758 R.1

Giacomo, R., Perrucci, S., Ennio, T., Giorgina, V. C., Giovanni, B. and Giacomo, R. 1997. Mortality in black siskins (Carduelis atrata) with systemic coccidiosis. Journal of Wildlife Diseases. 33(1): 152–157. https://doi.org/10.7589/0090-3558-33.1.152

Gibson, G. G., Broughton, E. and Choquette, L. P. 1972. Waterfowl mortality caused by Cyathocotyle bushiensis Khan, 1962 (Trematoda: Cyathocotylidae), St. Lawrence River, Quebec. Canadian Journal of Zoology. 50(11): 1351–1356. https://doi.org/10.1139/z72-184

Gill, H. and Paperna, I. 2008. Proliferative visceral Isospora (atoxoplasmosis) with morbid impact on the israeli sparrow passer domesticus biblicus Hartert, 1904. Parasitology Research. 103(3): 493–499. https://doi.org/10.1007/s00436-008-0986-4

Globokar, M., Fischer, D. and Pantchev, N. 2017. Occurrence of endoparasites in captive birds between 2005 to 2011 as determined by faecal flotation and review of literature. Berliner Und Münchener Tierärztliche Wochenschrift. 5(130): 11–12.

Gopee, N. V., Adesiyun, A. A. and Caesar, K. 2000. A longitudinal study of Escherichia coli strains isolated from captive mammals, birds, and reptiles in Trinidad. Journal of Zoo and Wildlife Medicine. 31(3): 353–360. https://doi.org/10.1638/1042-7260(2000)031[0353:alsoec]2.0.CO;2

Graczyk, T. K., Cranfield, M. R. and Shiff, C. J. 1994. Extraction of Haemoproteus columbae (Haemosporina: Haemoproteidae) antigen from rock dove pigeons (Columba livia) and its use in an antibody ELISA. Journal of Parasitology. 80(5): 713–718. https://doi.org/10.2307/3283250

Gray, V. L., O'Reilly, M., Müller, C. T., Watkins, I. D. and Lioyd, D. 2006. Low tyrosine content of growth media yields aflagellate Salmonella enterica serovar Typhimurium. Microbiology. 152(1): 23–28. https://doi.org/10.1099/mic.0.28442-0

Greiner, E. C., Bennett, G. F., White, E. M. and Coombs, R. F. 1975. Distribution of the avian hematozoa of North America. Canadian Journal of Zoology. 53(12): 1762–1787. https://doi.org/10.1139/z75-211

Grimes, J. E. and Arizmendi, F. 1992. Survey of clinical psittacine bird sera for Salmo nella typhimurium agglutinins. Avian Diseases. 36(3): 813–815. https://doi. org/10. 2307/1591791

Grimes, James E. and Arizmendi, F. 1995. Salmonella typhimurium agglutinins in exotic bird sera in the USA. International Journal of Veterinary Diagnosis Investigation. 7(12): 34-39

Hailu, T. and Kebede, B. 2016. Isolation of non-typhoidal Salmonella from sheep faeces in eastern hararghe, Ethiopia. Microbiology. 12(31): 23–28 https://doi.org/10.4172/ 2471-9315.1000120

Harcourt-Brown, N., & Chitty, J. 2005. BSAVA manual of psittacine birds. BSAVA Manual of Psittacine Birds., Ed.2.

Harrison, G. J., Lightfoot, T. L. 2006. Clinical avian medicine. (1):234. http://avian medi cine. net/publication_cat/clinical-avian-medicine/

Hasan, B., Islam, K., Ahsan, M., Hossain, Z., Rashid, M., Talukder, B., Ahmed, K. U., Olsen, B. and Abul Kashem, M. 2014. Fecal carriage of multi-drug resistant and extended spectrum β-lactamases producing E. coli in household pigeons, Bangladesh. Veterinary Microbiology. 168(1): 221–224. https://doi.org/10.1016/ j.vetmic.2013.09.033

Hechinger, R. F. and Lafferty, K. D. 2005. Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. Proceedings of the Royal Society B: Biological Sciences. 272(1567): 1059–1066. https://doi.org/10.1098 /rs pb.2005.3070

Hellgren, O., Waldenström, J. and Bensch, S. 2004. A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood. Journal of Parasitology. 90(4): 797–802. https://doi.org/10.1645/GE-184R1

Hellgren, O., Križanauskiene, A., Valkiunas, G. and Bensch, S. 2007. Diversity and phylogeny of mitochondrial cytochrome B lineages from six morphospecies of avian Haemoproteus (Haemosporida: Haemoproteidae). Journal of Parasitology. 93(4): 889–896. https://doi.org/10.1645/GE-1051R1.1

Helmboldt,', C. F., Eckerlin, R. P., Penner, 1 R. and Wyand', D. S. 1991. The pathology of capillariasis in the blue jay. Journal of Wildlife Diseases. 8(7): 213-217. https://doi. org/10.7589/0090-3558-7.3.157

Herman, C. M., Barrow, J. H. and Tarshis, I. B. 1975. Leucocytozoonosis in Canada geese at the seney national wildlife refuge. Journal of Wildlife Diseases. 11(3): 404–411. https://doi.org/10.7589/0090-3558-11.3.404

Hoeve, J. and Scott, M. E. 1988. Ecological studies on Cyathocotyle bushiensis (Digenea) and Sphaeridiotrema globulus (Digenea), possible pathogens of dabbling ducks in southern Québec. Journal of Wildlife Diseases. 24(3): 407–421. https://doi.org/10. 7589/0090-3558-24.3.407

Holmstad, P. R., Hudson, P. J. and Skorping, A. 2005. The influence of a parasite community on the dynamics of a host population: a longitudinal study on willow ptarmigan and their parasites. Oikos. 111(2): 377–391. https://doi.org/10.1111 /j.00 30-1299.2005.13640.x

Holt, P. S. and Chaubal, L. H. 1997. Detection of motility and putative synthesis of flagellar proteins in Salmonella pullorum cultures. Journal of Clinical Microbiology. 35(4): 134-138.

Hoque, M. A., Hassan, M. M., Haque, E., Shaikat, A. H., Khan, S. A., Alim, A., Skerratt, L. F., Islam, A., Tun, H. M., Dissanayake, R., Day, T. K., Debnath, N. C. and Yamage, M. 2014. A survey of gastro-intestinal parasitic infection in domestic and wild birds in Chittagong and Greater Sylhet, Bangladesh. Preventive Veterinary Medicine. 117(1): 305–312. https://doi.org/10.1016/j.prevetmed.2014.07.012

Horak, P., Saks, L., Karu, U. and Ots, I. 2006. Host resistance and parasite virulence in greenfinch coccidiosis. Journal of Evolutionary Biology. 19(1): 277–288. https://doi.org/10.1111/j.1420-9101.2005.00988.x

Hosain, M. S., Islam, M. A., Khatun, M. M. and Dey, R. K. 2013. Prevalence and antibiogram profiles of Salmonella isolated from pigeons in Mymensingh, Bangladesh. Microbes and Health. 1(2): 54–57. https://doi.org/10. 3329 /mh.v1i2. 14090

Howe, L., Castro, I. C., Schoener, E. R., Hunter, S., Barraclough, R. K. and Alley, M. R. 2012. Malaria parasites (Plasmodium spp.) infecting introduced, native and endemic New Zealand birds. Parasitology Research. 110(2): 913–923. https://doi.org/10. 1007/s00436-011-2577-z

Hudson, P. J. and Lee, D. L. 1988. Primary and secondary infections of the domestic chicken with Trichostrongylus tenuis (Nematoda), a parasite of red grouse, with observations on the effect on the caecal mucosa. Journal of Parasitology. 97(1): 89–99. https:// doi.org/10.1017/S0031182000066774

Huizinga, H. W. 1991. Contracaeciasis in pelicaniform birds. Journal of Wildlife Diseases. 7(3): 198–204. https://doi.org/10.7589/0090-3558-7.3.198

Ishii, S., Ksoll, W. B., Hicks, R. E. and Sadowsky, M. J. 2006. Presence and growth of naturalized Escherichia coli in temperate soils from lake superior watersheds. Applied and Environmental Microbiology. 72(1): 612–621. https://doi.org/10. 1128/ AEM.72.1.612-621.2006

Isobe, T. and Akiba, K. 1982. Indirect immunofluorescent antibody test in chicken leucocytozoonosis. National Institute of Animal Health Quarterly. 22(4): 163–169. https://europepmc.org/article/med/6225953

Isobe, Takashi, Shimizu, S. Y., Yoshihara, S. and Suzuki, K. 1998. Immunoblot analysis of humoral immune responses to Leucocytozoon caulleryi in chickens. Journal of Parasitology. 84(1): 62–66. https://doi.org/10.2307/3284531

Jakobsen, L., Spangholm, D. J., Pedersen, K., Jensen, L. B., Emborg, H. D., Agersø, Y., Aarestrup, F. M., Hammerum, A. M. and Frimodt-Møller, N. 2010. Broiler chickens, broiler chicken meat, pigs and pork as sources of ExPEC related virulence genes and resistance in Escherichia coli isolates from community-dwelling humans and UTI patients. International Journal of Food Microbiology. 142(1–2): 264–272. https:// doi.org/10.1016/j.ijfoodmicro.2010.06.025

Khalil, L. F. 1994. Keys to the cestode parasites of vertebrates. Preventive Veterinary Medicine. 117(1): 305–312

Khan, R. A. and Fallis, A. M. 1970. Relapses in birds infected with species of Leucocytozoon. Canadian Journal of Zoology. 48(3): 451–455. https://doi.org/10. 11 39/z70-077

Khanum, T., Rahman, S., Rahman, M. M., Khaton, R., Ahmed, S., Kabir, A., Sarder, U. and Baree, M. A. 2018. Prevalence of gastrointestinal parasitic diseases and associated risk factors in domestic pigeons (Columba livia) in Rajshahi division of Bangladesh. Bangladesh Livestock Journal. 1(5): 37–39. https://doi.org/10.1007 /s12 639-012-0112-5

Kirkpatrick, C. E. and Suthers, H. B. 1988. Epizootiology of blood parasite infections in passerine birds from central New Jersey. Canadian Journal of Zoology. 66(11): 2374 -2382. https://doi.org/10.1139/z88-352

Klei, T. R. and D. L. D. 1975. Seasonal occurrence of Haemoproteus columbae Kruse and its vector Pseudolynchia canariensis Bequaert. Journal of Wildlife Diseases. 11(3): 130–135.

Knodler, L. A. and Elfenbein, J. R. 2019. Salmonella enterica. Trends in Microbiology. 27(11): 321-325. https://doi.org/10.1016/j.tim.2019.05.002

Kocan, A. A. and Locke, L. N. 1974. Some helminth parasites of the American bald Eagle. Journal of Wildlife Diseases. 10(5): 345-350. https://doi.org/10.7589/0090-3558-10.1.8

Krawiec, M., Rusiecki, S., Kuczkowski, M. and Wieliczko, A. 2017. Antimicrobial susceptibility of Salmonella spp. strains isolated from free-living birds. Polish Journal of Veterinary Sciences. 20(4): 635–642. https://doi.org/10.1515/pjvs-2017-0080

Krishna Ghosh, K. 2014. Prevalence of ecto and gastrointestinal parasitic infections of pigeon at Chittagong metropolitan area, Bangladesh. The Journal of Advances in Parasitology. 5(4): 9–11. https://doi.org/10.14737/journal.jap/2014/1.1. 9.11

Krone, O., Stjernberg, T., Kenntner, N., Tataruch, F., Koivusaari, J. and Nuuja, I. 2006. Mortality factors, helminth burden, and contaminant residues in white-tailed sea eagles (Haliaeetus albicilla) from Finland. Ambio. 35(3): 98–104. https://doi.org/10.1579/0044-7447(2006)35[98:MFHBAC]2.0.CO;2

Kuiken, T., Leighton, F. A., Wobeser, G. and Wagner, B. 1999. Causes of morbidity and mortality and their effect on reproductive success in double-crested cormorants from Saskatchewan. Journal of Wildlife Diseases. 35(2): 331–346. https://doi.org/10.7589/0090-3558-35.2.331

Laird, M. and Van Riper, C. 1981. Questionable reports of Plasmodium from birds in Hawaii, with the recognition of P. relictum ssp. capistranoae as the avian malaria parasite. Journal of Parasitology. 45(23): 159–165.

Lanz, R., Kuhnert, P. and Boerlin, P. 2003. Antimicrobial resistance and resistance gene determinants in clinical Escherichia coli from different animal species in Switzerland. Veterinary Microbiology. 91(1): 73–84. https://doi.org/10.1016/S0378-1135(02)00263-8

Le Bouguénec, C., Lalioui, L., Du Merle, L., Jouve, M., Courcoux, P., Bouzari, S., Selvarangan, R., Nowicki, B. J., Germani, Y., Andremont, A., Gounon, P. and Garcia, M. I. 2001. Characterization of AfaE adhesins produced by extraintestinal and intestinal human escherichia coli isolates: PCR assays for detection of afa adhesins that do or do not recognize Dr blood group antigens. Journal of Clinical Microbiology. 39(5): 1738–1745. https://doi.org/10.1128/JCM.39.5.1738-1745.2001

Lillehaug, A., Bergsjø, B., Schau, J., Bruheim, T., Vikøren, T. and Handeland, K. 2005. Campylobacter spp., Salmonella spp., verocytotoxic Escherichia coli, and antibiotic resistance in indicator organisms in wild cervids. Acta Veterinaria Scandinavica. 46(1–2): 23–32. https://doi.org/10.1186/1751-0147-46-23

Lima, V. F. S., Bezerra, T. L., Deandrade, A. F., Ramos, R. A. N., Faustino, M. A. da G., Alves, L. C. and Meira-Santos, P. O. 2017. Parasitos gastrointestinais de aves exóticas que vivem em cativeiro no estado de Sergipe, Nordeste do Brasil. Revista Brasileira de Parasitologia Veterinaria. 26(1): 96–99. https://doi.org/10.1590/ S198 4-29612016080

Lopes, E. S., Cardoso, W. M., Albuquerque, Á. H., Teixeira, R. S. C., Salles, R. P. R., Bezerra, W. G. A., Rocha e Silva, R. C. and Vasconcelos, R. H. 2014. Isolation of Salmonella spp. in captive psittaciformes from zoos and a commercial establishment of Fortaleza, Brazil. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia. 66(3): 965–968. https://doi.org/10.1590/1678-41626643

Lopes, E. de S., Maciel, Albuquerque, Á. H. de, Vasconcelos, R. H., Machado, D. N., Bezerra, W. G. A. and Santos, I. C. L. 2016. Isolamento de Salmonella spp. e Escherichia coli de psittaciformes: relevância em saúde pública. Arquivos Do Instituto Biológico. 83(3): 35-43. https://doi.org/10.1590/1808-1657000602014

Lopes, Elisângela S., Maciel, W. C., Medeiros, P. H. Q. S., Bona, M. D., Bindá, A. H., Lima, S. V. G., Gaio, F. C. and Teixeira, R. S. C. 2018. Molecular diagnosis of diarrheagenic Escherichia coli isolated from psittaciformes of illegal wildlife trade. Pesquisa Veterinaria Brasileira. 38(4): 762–766. https://doi.org/10.1590/1678-5150-PVB-5083

López, G., Figuerola, J. and Soriguer, R. 2007. Time of day, age and feeding habits influence coccidian oocyst shedding in wild passerines. International Journal for Parasitology. 37(5): 559–564. https://doi.org/10.1016/j.ijpara.2006.12.014

Luppi, M. M., de Melo, A. L., Motta, R. O. C., Malta, M. C. C., Gardiner, C. H. and Santos, R. L. 2007. Granulomatous nephritis in psittacines associated with parasitism by the trematode Paratanaisia spp. Veterinary Parasitology. 146(3–4): 363–366. https://doi.org/10.1016/j.vetpar.2007.03.011

MacNeal, W. J. 1904. The life-history of Trypanosoma lewisi and Trypanosoma brucei. Universität of Michigan.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas and Monnet, D. L. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection. 18(3): 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Marietto-Gonçalves, G. A. 2011. Presence of a human diarrheagenic Escherichia coli clone in captivity kept psittacidaes. The Open Microbiology Journal. 5(1): 72–75. https://doi.org/10.2174/1874285801105010072

Martinez, F. and Muñoz, E. 1998. Atoxoplasma spp. in a hybrid passerine (Serinusï¿¿ canariusï¿¿× Carduelisï¿¿cannabina). Avian Pathology. 27(4): 420–422. https://doi.org/10.1080/03079459808419362

Mattes, B. R., De, S., Consiglio, A. S., De Almeida, B. Z., Guido, M. C., Orsi, R. B., Da Silva, R. M., Costa, A., Ferreira, A. J. P. and Knöbl, T. 2005. Influência da biossegurança na colonização intestinal por Escherichia coli em psitacídeos. Influencia da biosseguranca na colonizacao intestinal por escherichia coliempsita cideos. In Arq. Inst. Biol 2(1): 342.

Max Sussmann. (1997). Escherichia coli Mechanisms of Virulence. University Press, Cambridge, UK.

Mcdaniel, T. K., Jarvis, K. G., Donnenberg, M. S. and Kaper, J. B. 1995. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. National Academy of Sciences of the United States of America. 92(5): 1664–1668. https://doi.org/10.1073/pnas.92.5.1664

McDermott, P. F., Walker, R. D. and White, D. G. (2003). Antimicrobials: Modes of action and mechanisms of resistance. International Journal of Toxicology. 22(2): 135–143. https://doi.org/10.1080/10915810305089

Menezes, R. C., Tortelly, R., Gomes, D. C. and Pinto, R. M. (2003). Nodular typhlitis associated with the nematodes Heterakis gallinarum and Heterakis isolonche in pheasants: Frequency and Pathology with Evidence of Neoplasia. Memorias Do Instituto Oswaldo Cruz. 98(8): 1011–1016. https://doi.org/10.1590/S0074-0276 200 3000800005

Minchin, E. A. 1912. An introduction to the study of the protozoa: with special reference to the parasitic forms. E. Arnold.

Misof, K. 2004. Diurnal cycle of Isospora spp. oocyst shedding in Eurasian blackbirds (Turdus merula). Canadian Journal of Zoology. 82(5): 764–768. https://doi.org/10. 1139/Z04-054

Mitchell, S. and Mark, B. 2008. Manual of exotic pet Practice .Cambridge University press.p. 342

Morgan, B. B. and Schiller, E. 1950. A Note on porrocaecum depressum (Zeder, 1800) (nematoda: anisakinae). Transactions of the American Microscopical Society. 69(2): 210. https://doi.org/10.2307/3223411

Morii, T. and T, M. 1972. Presence of antigen and antibodies in the sera of chicken infected with akiba caulleryi. Veterinary Parasitology. 146(3–4): 363–366. https:// doi.org /10.1016/j.vetpar.2007.03.011

Morita, M., Mori, K., Tominaga, K., Terajima, J., Hirose, K., Watanabe, H. and Izumiya, H. 2006. Characterization of lysine decarboxylase-negative strains of Salmonella enterica serovar Enteritidis disseminated in Japan. FEMS Immunology & Medical Microbiology. 46(3): 381–385. https://doi.org/10.1111/j.1574-695X.2006.00043.x

Mozgovoi, A. A. 1968. Ascaridata of animals and man and the diseases caused by them. 1: 283.

Murata, K. 2002. Prevalence of blood parasites in Japanese wild birds. Journal of Veterinary Medical Science. 64(9): 785–790. https://doi.org/10.1292/jvms.64.785

Mushi, E. Z., Binta, M. G., Chabo, R. G., Ndebele, R. and Panzirah, R. 2000. Helminth parasites of domestic pigeons (Columba livia domestica) in Sebele, Gaborone, Botswana. Published by the Agricultural Research Council, Onderstepoort Veteri nary Institute. 3(6): 521. https://repository.up.ac.za/handle/2263/19084

Nataro, J. P. and Kaper, J. B. 1998. Diarrheagenic Escherichia coli. Clinical Microbio logy. 2(1): 142–201. https://doi.org/10.1128/cmr.11.1.142

Niilo, L. 1970. Bovine coccidiosis in Canada. Canadian Veterinary Journal. 11(5): 91–98. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1695004/

Njabo, K. Y., Cornel, A. J., Sehgal, R. N. M., Loiseau, C., Buermann, W., Harrigan, R. J., Pollinger, J., Valkiunas, G. and Smith, T. B. 2009. Coquillettidia (Culicidae, Diptera) mosquitoes are natural vectors of avian malaria in Africa. Malaria Journal. 8(1): 1–12. https://doi.org/10.1186/1475-2875-8-193

Ola-Fadunsin, S. D., Ganiyu, I. A., Rabiu, M., Hussain, K., Sanda, I. M., Musa, S. A., Uwabujo, P. I. and Furo, N. A. 2019a. Gastrointestinal parasites of different avian species in Ilorin, North Central Nigeria. Journal of Advanced Veterinary and Animal Research. 6(1): 108–116. https://doi.org/10.5455/javar.2019.f320

Ola-Fadunsin, S. D., Ganiyu, I. A., Rabiu, M., Hussain, K., Sanda, I. M., Musa, S. A., Uwabujo, P. I. and Furo, N. A. 2019b. Gastrointestinal parasites of different avian species in Ilorin, North Central Nigeria. Journal of Advanced Veterinary and Animal Research. 6(1): 108–116. https://doi.org/10.5455/javar.2019.f320

Orós, J., Rodríguez, J. L., Fernández, A., Herráez, P., Espinosa De Los Monteros, A. and Jacobson, E. R. 1998. Simultaneous occurrence of Salmonella arizonae in a sulfur crested cockatoo (Cacatua galerita galerita) and iguanas. Avian Diseases. 42(4): 818–823. https://doi.org/10.2307/1592723

Orosz, S. E., Chengappa, M. M., Oyster, R. A., Morris, P. J., Trock, S. and Altekruse, S. 1992. Salmonella enteritidis infection in two species of psittaciformes. Avian Diseases. 36(3): 766–769. https://doi.org/10.2307/1591782

Panigrahy, B., Grimes, J. E., Rideout, M. I., Simpson, R. B., & Grumbles, L. C. 1979. Zoonotic diseases in psittacine birds: Apparent increased occurrence of chlamy diosis (psittacosis), salmonellosis, and giardiasis. Journal of the American Veter inary Medical Association. 175(4): 359–361. https://europepmc.org /article/ med/50 0464

Papini, R., Girivetto, M., Marangi, M., Mancianti, F. and Giangaspero, A. 2012. Endo parasite infections in pet and zoo birds in Italy. The Scientific World Journal. 2(4): 175-178. https://doi.org/10.1100/2012/253127

Patel, P. V, Patel, A. I., Sahu, R. K. and Vyas, R. 2000. Prevalence of gastrointestinal parasites in captive birds of Gujarat zoos. In case report zoos print journal. 15(10): 413.

Poinar, G. 2006. Evidence of intestinal parasites of dinosaurs fossil insects of mid-cretaceous. Burmese Amber View project. https://doi.org/10.1017/ S0031182 0060 00138

Pontes, P. S. de, Coutinho, S. D. A., Iovine, R. de O., Cunha, M. P. V., Knöbl, T. and Carvalho, V. M. de. 2018. Survey on pathogenic Escherichia coli and Salmonella spp. in captive cockatiels (Nymphicus hollandicus). Brazilian Journal of Microbiology. 49(4): 76–82. https://doi.org/10.1016/j.bjm.2018.05.003

Pritchard, M. H., and G. O. W. K. 1982. The Collection and preservation of animal parasites.

Qadri, F., Svennerholm, A. M., Faruque, A. S. G. and Sack, R. B. 2005. Enterotoxigenic Escherichia coli in developing countries: Epidemiology, microbiology, clinical features, treatment, and prevention. Clinical Microbiology. 18(3): 465–483. https:// doi.org /10.1128/CMR.18.3.465-483.2005

Radfar, M. H., Asl, E. N., Seghinsara, H. R., Dehaghi, M. M. and Fathi, S. 2012. Biodiversity and prevalence of parasites of domestic pigeons (Columba livia domestica) in a selected semiarid zone of South Khorasan, Iran. Tropical Animal Health and Production. 44(2): 225–229. https://doi.org/10.1007/s11250-011-0002-3

Rahman, M. M., Bashar, T., Rahman, M., Rabbi, F. A. and Noor, R. 2011. Enterotoxin profiling and antibiogram of Escherichia coli isolated from poultry feces in Dhaka district of Bangladesh. Journal of Microbiology. 1(1): 51–57.

Rahman, S., Khatun, R., Nahar, L. and Khanum, T. 2019. Chemotherapy of gastro intestinal parasitic diseases in domestic pigeons (Columba livia) in Rajshahi division of Bangladesh. Research in Agriculture Livestock and Fisheries. 6(2): 323–328. https://doi.org/10.3329/ralf.v6i2.43061

Rao A.T.; Acharjyo L.N. and Patnaik M.M. 1991. Pathology of Ascariasis in a pea fowl (Pavo cristatus) caused by Ascaridia perspicillum rudolf 1803. Indian Veterinary Journal. 58(7): 585.

Rausch, R. L. 1983. The biology of avian parasites. Academic Press. New York.p.342

Reid, R. L., Porter, R. C. and Ball, H. J. 1993. The isolation of sucrose-fermenting Salmonella mbandaka. Veterinary Microbiology. 37(1–2): 181–185. https://doi.org/ 10.1016/ 0378-1135(93)90192-A

Ruff M. 1993. External and internal factors affecting the severity of avian coccidiosis. http://avianmedicine.net/publication_cat/clinical-avian-medicine/

Russo, T. A. and Johnson, J. R. 2000. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of Escherichia coli: ExPEC. The Journal of Infectious Diseases. 181(5): 1753–1754. https://doi.org/10.1086/315418

Saifullah, M. K., Mamun, M. M., Rubayet, R. M., Nazir, K., Zesmin, K. and Rahman, M. T. 2016. Molecular detection of Salmonella spp. isolated from apparently healthy pigeon in Mymensingh, Bangladesh and their antibiotic resistance pattern. Journal of Advanced Veterinary and Animal Research. 3(1): 51–55. https://doi.org/10. 5455/ javar.2016.c131

Sánchez-Cordón, P. J., Gómez-Villamandos, J. C., Gutiérrez, J., Sierra, M. A., Pedrera, M. and Bautista, M. J. 2007. Atoxoplasma spp. Infection in Captive Canaries (Serinus canaria). Journal of Veterinary Medicine. 54(1): 23–26. https:// doi.org/10 .1111/j.1439-0442.2007.00909.x

Santiago-Alarcon, D., Palinauskas, V. and Schaefer, H. M. 2012. Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. Biological Reviews. 87(4): 928–964. https://doi.org/10.1111/j.1469-185X.2012. 002 34.x

Sareyyüpoğlu, B., Ok, A. Ç., Cantekin, Z., Yardimci, H., Akan, M. and Akçay, A. 2008. Polymerase chain reaction detection of Salmonella spp. in fecal samples of pet birds. Avian Diseases. 52(1): 163–167. https://doi.org/10.1637/8052-070507-Reg

Sarker, M. S., Mannan, M. S., Ali, M. Y., Bayzid, M., Ahad, A. and Bupasha, Z. B. 2019. Antibiotic resistance of Escherichia coli isolated from broilers sold at live bird markets in Chattogram, Bangladesh. Journal of Advanced Veterinary and Animal Research. 6(3): 272–277. https://doi.org/10.5455/javar.2019.f344

Savage, A. F., Robert, V. and Goodman, S. M. 2009. J Wildl Dis.: Blood parasites in birds from Madagascar. Journal of Avian Medicine and Surgery. 23(4): 333–334.

Scheutzs, F & Fraklin, M. 2005. Genus I. Escherichia. In Bergey's Manual of Systematic Bacterio logy. 2(3):342. Springer-Verlag. http://ci.nii.ac.jp/naid/10020269723/en/

Schmidt, H., Knop, C., Franke, S., Aleksic, S., Heesemann, J. and Karch, H. 1995. Devel opment of PCR for screening of enteroaggregative Escherichia coli. Journal of Clinical Microbiology. 33(3):123-128.

Scott, F., Threlfall, J. and Arnold, C. 2002. Genetic structure of Salmonella revealed by fragment analysis. International Journal of Systematic and Evolutionary Micro biology. 52(5): 1701–1713. https://doi.org/10.1099/00207713-52-5-1701

Seepersadsingh, N., Adesiyun, A. A. and Aleksic, G. 2003. Prevalence and antimicrobial resistance of Salmonella spp. in pet mammals, reptiles, fish aquarium water, and birds in Trinidad. Journal of Veterinary Medicine. 50(10): 488–493. https://doi.org /10.1046 /j.0931-1793.2003.00710.x

Sehgal, R. N. M., Jones, H. I. and Smith, T. B. 2005. Blood parasites of some west African rainforest birds. Journal of Veterinary Medical Science. 67(3): 295–301. https:// doi. org/10.1292/jvms.67.295

Sehgal, R. N. M., Valkiunas, G., Iezhova, T. A. and Smith, T. B. 2006. Blood parasites of chickens in Uganda and Cameroon with molecular descriptions of Leucocytozoon schoutedeni and Trypanosoma gallinarum. Journal of Parasitology. 92(6): 1336–1343. https://doi.org/10.1645/ge-927r.1

Senlik, B., Gulegen, E. and Akyol, V. 2005. Effect of age, sex and season on the prevalence and intensity of helminth infections in domestic pigeons (Columba livia) from Bursa Province, Turkey. Acta Veterinaria Hungarica. 53(4): 449–456. https:// doi.org/10.1556/AVet.53.2005.4.5

Sol, D., Jovani, R. and Torres, J.(2000. Geographical variation in blood parasites in feral pigeons: the role of vectors. Ecography. 23(3): 307–314. https://doi.org/10.1111/ j.1600-0587.2000.tb00286.x

Solari, C. A., Mandarino, J. R., Panizzutti, M. H. M. and Farias, R. H. G. 2003. A new Ssrovar and a new serological variant belonging to Salmonella enterica subspecies diarizonae. Memorias Do Instituto Oswaldo Cruz. 98(4): 501–502. https://doi.org /10.1590/S0074-02762003000400013

Soulsby, & L., E. J. 1982. Helminths arthropods and protozoa of domesticated animals. pp. 291. https://ci.nii.ac.jp/naid/10028216900

Steve Yan, S., Pendrak, M. L., Abela-Ridder, B., Punderson, J. W., Fedorko, D. P. and Foley, S. L. 2003. An overview of Salmonella typing: Public health perspectives. In Clinical and Applied Immunology Reviews. 4(3): 189–204. Elsevier. https://doi.org /10.1016/j.cair.2003.11.002

Stock, T. M. and Holmes, J. C. 1987. Host specificity and exchange of intestinal helminths among four species of grebes (Podicipedidae). Canadian Journal of Zoology. 65(3): 669–676. https://doi.org/10.1139/z87-104

Suskovic J, Dzidic S, K. B. 2008. Antibiotic resistance mechanisms in bacteria: Bio chemical and genetic aspects. Food Technology and Biotechnology. 1(46): 11–21.

Tanveer, M. K., Kamran, A., Abbas, M., Umer, N. C., Azhar, M. A. and Munir, M. 2011. Prevalence and chemo-therapeutical investigations of gastrointestinal nematodes in domestic pigeons in Lahore, Pakistan. Tropical Biomedicine. 28(1): 348

Tella, J. L. 2002. The evolutionary transition to coloniality promotes higher blood parasitism in birds. Journal of Evolutionary Biology. 15(1): 32–41. https://doi.org /10.1046/j.1420-9101.2002.00375.x

Temmerman, R., Garmyn, A., Antonissen, G., Vanantwerpen, G., Vanrobaeys, M., Haese brouck, F. and Devreese, M. 2020. Evaluation of fluoroquinolone resistance in clinical avian pathogenic Escherichia coli isolates from flanders (Belgium). Antibiotics. 9(11): 800. https://doi.org/10.3390/antibiotics9110800

Thomas, V. G. 1986. Body condition of willow ptarmigan parasitized by cestodes during winter. Canadian Journal of Zoology. 64(1): 251–254. https://doi.org/10. 1139/z86-039

Tomza-Marciniak, A., Pilarczyk, B., Tobianska, B. and Tarasewicz, N. 2014. Gastrointes tinal parasites of free-range chickens. In Annals of Parasitology. 60(4):481-488

Toplu, N., Sarimehmetoglu, O., Metin, N. and Eren, H. 2006. Pleural and peritoneal tetra thyridiosis in a peafowl. Veterinary Record. 158(3): 102–103. https://doi.org/10. 1136/vr.158.3.102

Tsai, S.S., Hirai, K. and Itakura, C. 1992. Histopathologica survey of protozoa, hel minths, and ascarids of importhd and local psittacine and passerine birds in Japan. Japanese Journal of Veterinary Research. 40(4): 161–174. https://eprints .lib.hoku dai.ac.jp/ dspace/handle/2115/2407

Tsiodras, S., Kelesidis, T., Kelesidis, I., Bauchinger, U. and Falagas, M. E. 2008. Human infections associated with wild birds. Journal of Infection. 56(2): 83–98. https:// doi. org/10.1016/j.jinf.2007.11.001

Twentyman, C. M. 2001. A study of coccidial parasites in Bili (Notiomystis cincta). Massey University. https://mro.massey.ac.nz/handle/10179/7151

Urquhart, G. M. 1996. Veterinary parasitology. Blackwell Science. https://agris.fao.org/ agris-search/search.do?recordID=US9631504

Valkiunas, G., Iezhova, T. A., Brooks, D. R., Hanelt, B., Brant, S. V., Sutherlin, M. E. and Causey, D. 2004. Additional observations on blood parasites of birds in Costa Rica. Journal of Wildlife Diseases. 40(3): 555–561. https://doi.org/10.7589/0090-3558-40.3.555

Valkiūnas, G., Anwar, A. M., Atkinson, C. T., Greiner, E. C., Paperna, I. and Peirce, M. A. 2005. What distinguishes malaria parasites from other pigmented haemospori dians? Trends in Parasitology. 21(8): 357–358. https://doi.org/10.1016/j.pt. 2005.06. 005

Valkiunas, G., Iezhova, T. A., Loiseau, C. and Sehgal, R. N. M. 2009. Nested cyto chrome B polymerase chain reaction diagnostics detect sporozoites of hemospori dian parasites in peripheral blood of naturally infected birds. Journal of Parasitology. 95(6): 1512–1515. https://doi.org/10.1645/GE-2105.1

Valkiūnas, G., Žiegytė, R., Palinauskas, V., Bernotienė, R., Bukauskaitė, D., Ilgūnas, M., Dimitrov, D. and Iezhova, T. A. 2015. Complete sporogony of Plasmodium relictum (lineage pGRW4) in mosquitoes Culex pipiens pipiens, with implications on avian malaria epidemiology. Parasitology Research. 114(8): 3075–3085. https://doi.org /10. 1007/s00436-015-4510-3

Van Den Bogaard, A. E. and Stobberingh, E. E. 2000. Epidemiology of resistance to antibiotics: Links between animals and humans. International Journal of Antimicro bial Agents. 14(4): 327–335. https://doi.org/10.1016/S0924-8579(00)00 145-X

Varriale, L., Dipineto, L., Russo, T. P., Borrelli, L., Romano, V., D'Orazio, S., Pace, A., Menna, L. F., Fioretti, A. and Santaniello, A. 2020. Antimicrobial resistance of Escherichia coli and Pseudomonas aeruginosa from companion birds. Antibiotics. 9(11): 780. https://doi.org/10.3390/antibiotics9110780

Vincent, H. M. and Wilson, R. J. M. 1980. Malarial antigens on infected erythrocytes. Transactions of the Royal Society of Tropical Medicine and Hygiene. 74(4): 501–507. https://doi.org/10.1016/0035-9203(80)90067-X

Vounba, P., Arsenault, J., Bada-Alambédji, R. and Fairbrother, J. M. 2019. Prevalence of antimicrobial resistance and potential pathogenicity, and possible spread of third generation cephalosporin resistance, in Escherichia coli isolated from healthy chicken farms in the region of Dakar, Senegal. Plos One. 14(3): 304. https:// doi.org/ 10.1371/journal.pone.0214304

Wallace, B. M. and Pence, D. B. 1986. Population dynamics of the helminth community from migrating blue- winged teal: loss of helminths without replacement on the wintering grounds. Canadian Journal of Zoology. 64(8): 1765–1773. https://doi. org /10.1139/z86-266

Wallis, A. S. 1991. Common conditions of domestic pigeons. British Medical Journal. 13(3): 95–100. Publishing Group. https://doi.org/ 10. 1136/inpract.13.3.95

Warner, R. E. 1988. The role of introduced diseases in the extinction of the endemic hawaiian avifauna. The Condor. 70(2): 101–120. https://doi.org/10.2307/1365954

WHO. 2016. Critically important antimicrobials in human and animal. Safe Food. 4(2): 19-24.

Williams, R. B., Marshall, R. N., Pagès, M., Dardi, M., & del Cacho, E. 2009. Patho genesis of Eimeria praecox in chickens: virulence of field strains compared with laboratory strains of E. praecox and Eimeria acervulina. Avian Pathology. 38(5): 359–366. https://doi.org/10.1080/03079450903186028

Wiuff, C., Madsen, M., Baggesen, D. L. and Aarestrup, F. M. 2000. Quinolone resistance among Salmonella enterica from cattle, broilers, and swine in Denmark. Microbial Drug Resistance. 6(1): 11–17. https://doi.org/10.1089 /mdr.2000.6.11

Wobeser, G. A. 1997. Diseases of Wild Waterfowl. Plenum Press. pp. 420

Wood, M. J., Cosgrove, C. L., Wilkin, T. A., Knowles, S. C. L., Day, K. P. and Sheldon, B. C. 2007. Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, Cyanistes caeruleus. Molecular Ecology. 16(15): 3263–3273. https://doi.org/10.1111/j.1365-294X.2007.03362.x

Woodworth, B. L., Atkinson, C. T., LaPointe, D. A., Hart, P. J., Spiegel, C. S., Tweed, E. J., Henneman, C., LeBrun, J., Denette, T., DeMots, R., Kozar, K. L., Triglia, D., Lease, D., Gregor, A., Smith, T. and Duffy, D. 2005. Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. National Academy of Sciences of the United States of America. 102(5): 1531–1536. https://doi.org/10.1073/pnas.0409454102

Yılmaz, E. Ş. and Dolar, A. 2017. Detection of extended-spectrum β-lactamases in Escherichia coli from cage birds. Journal of Exotic Pet Medicine. 26(1): 13–18. https://doi.org/10.1053/j.jepm.2016.10.008

Zajac, A.M., Conboy, G. editors. 2012. Veterinary clinical parasitology (8th ed.) pp. 67-120.

Appendix A:

Questionnaires for sample collection from pet birds in Chittagong

1. Sample no:
2. Date of collection:
3. Owners name:
4. Address:
5. Mobile no:
6. Type of birds:
7. How long you rare pet birds:
8. Health status of birds: a) healthy b) sick
9. If sick, what types of clinical sign did you observed:
10. Feed type:
11. Quality of drinking water supplied for birds: a) deep b) tape c)pond
12. Did you ever give anthelmintics: a) yes b) no
13. Type of anthelmintics you have supplied:
14. How long before you give anthelmintics to your birds:
15. Did you ever give anti-coccidial drug: a) yes b) no
16. Type of anti-coccidial drug you have supplied:
17. How long before you give anti-coccidial drug to your birds:
18. Did you ever give antibiotic drug: a) yes b) no
19. Did you frequently use antibiotics to your birds: a) yes b) no
20. What types of antibiotic you have used:

BRIEF BIOGRAPHY

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