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SURVEY ON THE MOST COMMON DISEASES CIRCULATING AMONG PIGEONS IN THE EASTERN PROVINCE, SAUDI ARABIA

Mahmoud H.A. Mohamed[1,2] and Adel M. Abdelaziz[3,4]

[1]Department Of Clinical Sciences, Collage Of Veterinary Medicine, King Faisal University, P.O. Box:400, Al-Hasaa, 31982, Saudi Arabia.
[2] Avian Diseases Research Center, Collage Of Veterinary Medicine, King Faisal University, P.O. Box:400, Al-Hasaa, 31982, Saudi Arabia.
[3]Faculty of Veterinary Medicine, Zagazig University, Veterinary Educational Hospital, 44511 Zagazig, Egypt
[4]Department of Avian Diseases, El-Ahsaa Veterinary Laboratory, Ministry of Environment, Water and Agriculture, El-Ahsaa,31982 K.S.A
Corresponding author's E-mail: mhmohammad@kfu.edu.sa

ABSTRACT

Molecular and microbiological approaches were used to investigate the common circulating pathogens in pigeon flocks**.** Sixty-nine flocks were investigated for viral, bacterial and parasitic infection evidence, including the histories, clinical signs and lesions. We reported that the seroprevalences of Newcastle disease viruses (NDVs), *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) were 50.57%, 7.25% and 8.69%. respectively; no avian influenza virus (AIV)–specific antibodies were detected. NDVs and pigeon paramyxoviruses (PPVs) were detected in 40.57% and 15.9%, respectively, of the pigeons**.** Multiple diseases were the predominant finding, with 60.87% of diseased flocks harboring multifactorial infections of viral, bacterial and parasitic pathogens. Concurrent viral and bacterial infections were reported in 15.94%, and viral and parasitic coinfections were found in 20.29%. Mixed infections of NDV with bacterial and parasitic diseases were detected in 21.74%. Bacterial and mycotic pathogens were detected in 22/69 (31.88%) and 5/69 (7.25%), respectively. *Salmonella* spp. were only detected in 3/69 (4.35%) of diseased flocks. Parasitic diseases were the most prevalent infections, either as a single infection or concurrently with others. Parasites including *Cestoda* spp., *Ascaris* spp., *Eimeria* spp., *Trichomonas gallinae* and external parasites(*Menopon gallinae, Pseudolynchia canariensis*) were reported in 12/69 (17.39%), 10/69 (10.49%), 10/69 (10.49%), 18/69 (26.1%) and 3/69 (4.35%), respectively. We concluded that pigeon flocks harbor many pathogens that may threaten the health of animals and humans; they are serious amplifiers and reservoir hosts of disease and, ultimately, may be serious biological hazards to the intensive poultry production sector and community. Pigeon vaccines should be developed. Strict biosecurity measures should be applied to protect pigeons and commercial poultry flocks.

Key words: Pigeon, diseases, PCR, isolation and identification, Saudi Arabia

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INTRODUCTION

Small-scale pigeon flocks have become common in Saudi Arabia as pigeon farming has gained in popularity as a way of producing food, serving as a hobby and providing birds for racing. Data about diseases and the prevalence of pathogens in pigeon flocks are scarce. However, it has been found that pigeons represent a serious threat to the larger intensive production sector and to public health. The role of pigeons in the dissemination of diseases to domestic animals and humans has been documented (Alexander, 2011; Bucher and Ranvaud, 2006; Some particularly important animal pathogens can be transmitted by pigeons, such as *Salmonella pullorum*, *Salmonella pallinarum*, *Mycoplasma synoviae* (MS), *Mycoplasma gallisepticum* (MG), Newcastle disease viruses (NDVs) and the highly pathogenic avian influenza virus (AIV) (Alexander, 2011). Pigeons are infected with several types of bacterial, viral and parasitic diseases due to poor management, lack of hygienic housing, lack of vaccinations, seasonal variations, heat stress and humidity. Pigeons are a major reservoir for many viral, bacterial and parasitic pathogens, especially NDVs, *Salmonella*, MG, MS and AIV, and can threaten the health of poultry, domestic animals and, mainly, humans, as well as the general environment (Alexander, 2011; Hemida *et al.*., 2019; Islam *et al.*., 2020;). Pigeons may have subclinical infections that can lead to the spreading of certain diseases for a certain period of time and over

large distances without any visible clinical signs (Catroxo *et al.*., 2011).

Birds of the family Columbidae (pigeons and doves) have been accused of being reservoir hosts for velogenic strains of NDVs all over the globe (Brown and Bevins, 2017; Dimitrov *et al.*., 2016;). Previous findings proved that pigeons are natural reservoirs and carriers of all APMV-1 pathogen types except viscerotropic velogenic viruses, the natural reservoirs of which have not been identified (Dimitrov *et al.*., 2016). Pigeon *paramyxovirus serotype 1* (PPMV-1), a class II genotype VIb avian *Avulavirus,* is host-speccific to *Columbiform* birds including pigeons (Brown and Bevins, 2017; Dimitrov *et al.*., 2016;). Pigeons can acquire infection with chicken NDV through direct contact and act as a reservoir host and disseminate infection to other poultry species in the opposite direction (Ellakany *et al.*., 2019).

Ten species in the genus Avipoxvirus (APV) have been described according to their host species: pigeonpox, sparrowpox, canarypox, fowlpox, psittacinepox, juncopox, mynahpox, quailpox, starlingpox and turkeypox (Moyer *et al.*., 2000). Avipoxvirus is the only genus in the subfamily Chordopoxvirinae that can infect nonmammalian avian hosts, including more than 374 wild avian species worldwide (Manarolla *et al.*., 2010; Williams *et al.*., 2021). Avian poxviruses are highly species specific, but some strains can cross species or family barriers. Fowlpox has two clinical forms: the mild cutaneous (dray) pox, characterized by nodular skin lesions on featherless skin, and the more severe diphtheritic (wet) pox, seen on the upper respiratory tract and digestive tube (Manarolla *et al.*., 2010;).

MG and MS are important bacterial pathogens for commercial poultry flocks and are repeatedly recovered from respiratory infections in pigeons, so that close contact between pigeons and poultry flocks must be avoided (Tsai and Lee, 2006). Previous studies recorded the presence of MG and MS in a wide variety of feral and domestic birds (Dhondt *et al.*., 2014;; Stipkovits and Szathmary, 2012;). Salmonellosis in pigeons is usually caused by serogroup B strains of *Salmonella typhimurium* var. Copenhagen of phage types DT2, PT4, PT46 and PT99 (Pasmans *et al.*., 2008; Teske *et al.*., 2013) and may lead to severe disease. The most typical signs of salmonellosis are greenish diarrhea, loss of appetite, thirst, debilitation, weight loss and, finally, death. Arthritis, panophthalmitis, torticollis and some neurological symptoms; a high mortality rate for nestling squabs in the first week of life; and embryo deaths can also be noticed. Hepatosplenomegaly, enteritis, liver hemorrhages and small abscesses in internal organs are the main postmortem lesions. The long-term survival of *Salmonella* in host macrophages is responsible for chronic carriers and the likelihood of a reservoir status. Carrier pigeons with subclinical chronic infection occasionally disseminate *Salmonella* spp. in droppings to the surrounding environment and contaminate poultry feed and water, which are the main sources of infection in pigeons and other poultry flocks and humans (Pasmans *et al.*., 2008).

Candidiasis, crop mycosis or thrush is one of the most common mycotic diseases in pigeons, especially nonimmunocompetent squabs. Candidiasis, is caused by the wide spread and opportunistic yeast *Candida albicans*. Candidiasis is often accompanied with other concurrent infections or trauma. *Candida* transmitted directly through ingestion of crop milk and contaminated feed and water or from the environment. Clinical signs of candidiasis include off food, diarrhea, depression, regurgitation, crop distention with elevated morbidity and mortality percent. Postmortem lesions of candidiasis include ulcerative and proliferative white plaques, commonly known as a Turkish towel appearance. Pseudomembranes that can be easily peeled off may be present. Lesions are mainly present in the oral cavity, crop and esophagus (Crespo *et al.*., 2018).

Trichomoniasis is a common disease in pigeons caused by the flagellated protozoan *Trichomonas gallinae* (Levine, 1995) in young squabs and adults. Squabs may acquire infection vertically in the first day of life through crop milk and horizontally through the sharing of contaminated food and water (Bunbury *et al.*., 2007). *T. gallinae* infection may have high mortality rates of up to 66% in young squabs and a high incidence of latent infection (up to 90%) in adults (Bunbury *et al.*., 2007).

Coccidiosis is the most common intestinal protozoal disease in pigeons; it is caused by nine species of the genus *Eimeria* and one species of the genus *Isospora*. *Eimeria columbae*, *Eimeria columbarum* and *Eimeria labbeana* are the most common pathogenic species associated with varying degrees of virulence in domestic pigeons (*Columba livia domestica*) and rock pigeons (*Columba livialivia*) (Krautwald-Junghanns *et al.*., 2009). Mixed infections are commonly reported and increase the degree of pathogenicity (Balicka-Ramisz and Pilarczyk, 2014; Krautwald-Junghanns *et al.*., 2009). The aim of our study was to determine the prevalence of different pathogens among pigeon flocks using various molecular, microbiological and parasitological techniques.

MATERIALS AND METHODS

Ethical approval: All applicable institutional guidelines for the care and use of animals were followed. All animal experiments were conducted according to the Institutional animal care and Use committee, Zagazig University (Institutional animal care and Use committee) Approval number: ZU-IACUC/2/F/115/2023.

Sampling: Over a 2-year period (January 2020 to March2022), pigeons presented to the avian clinic, VTH, KFU, Alhasa, Eastern region, Saudi Arabia, were screened for different pathogens. For a total of 207 pigeons from 69 pigeon flocks the histories and clinical signs were recorded on a structured questionnaire (Supplementary Table 1). Serum samples were taken; tracheal, cloacal, conjunctival and oropharyngeal swabs were made; and skin lesions and tissues, including lung, heart, liver, spleen and brain tissues, were sampled, in addition to intestinal and crop contents (Table 1). The samples were collected, transported and prepared according to the procedures outlined by OIE (2021

Clinical and postmortem examination: Pigeons were examined for clinical signs and then euthanized by cervical dislocation following standard procedures (Anon, 2013). A diagnostic postmortem inspection was conducted, and the main lesions were systematically described. Tissues, organs and swab samples were processed and prepared for molecular and microbiological testing.

Serological examination: Serum samples were tested for the presence of specific antibodies against NDV, avian influenza type A, MG and MS by the IDEXX NDV, AIV type A, MG and MS Ab ELISA kits (IDEXX, USA). For MG and MS, the rapid serum plate agglutination test was used as a screening test with a commercial colored antigen (SPAFAS MG and SPAFAS MS plate antigen) as recommended by the manufacturer. Serum samples were tested by the hemagglutination inhibition (HI) test for an indirect NDV diagnosis according to standard methodologies (OIE, 2021).

Isolation and identification: The collected samples were examined bacteriologically for the presence of *Escherichia coli* and *Salmonella* spp. Samples from the heart, liver and spleen were cultured on 5% sheep blood agar, MacConkey agar and brain–heart infusion agar (Oxoid, Thermo Fisher Scientific, USA) and incubated aerobically at 37^{o}C for 24 hours. Isolates were identified based on colony morphology, gram staining, catalases and the oxidase test (Quinn *et al.*., 2002). They were identified biochemically using the Vitek 2 Compact System (BioMérieux, France). The analysis was applied and interpreted based on the manufacturer's recommendations.

RNA extraction: A tissue specimen was homogenized by a TissueLyser machine (Qiagen, Hilden, Germany) using silica beads. Tracheal and cloacal swab samples were thoroughly vortexed for 2 minutes. Viral RNAs were extracted using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche Diagnostic Gmbh, Mannheim, Germany) according to the manufacturer's instructions. Extracted viral RNAs were stored at –20^{O}C until used in a subsequent molecular procedure.

Detection and identification of ND and AI viruses: One-step RT-PCR was carried out using the Qiagen RT-PCR kit. The primers employed (Table 2) amplified a 535-bp fragment of the fusion protein (F) gene of NDV (Radwan *et al.*., 2013) or a 244-bp fragment of the M2 gene of AIV (Mady *et al.*., 2010).

Detection of pigeon poxvirus: A P4b gene-specific PCR was carried out in a thermal cycler. The primers described in Table 2 were used to amplify 578 bp of the P4b gene of PPV (Lee and Lee, 1997).

For histopathological examination, tissue samples (skin wart nodules from the head, eyelids and oral cavity) of birds (0.5 to 1 cm^3) were fixed in 10% buffered formalin, impregnated in paraffin wax overnight and sectioned into 4-µm–thick sections. After deparaffinization, the sections were stained with HandE as described by Kiernan (2008).

DNA extraction for MG and MS identification: The total genomic DNA was extracted using the MagNA Pure Compact Nucleic Acid Isolation Kit1 (Roche Diagnostic Gmbh, Mannheim, Germany) according to the manufacturer's instructions. Tracheal swabs were suspended in 1 ml of phosphate buffered solution (PBS; three tracheal swabs per sample) and spun at 13,000 rpm for 30 minutes. The supernatant was carefully removed, and the resultant cell pellet suspended in 25 ml of PCR-grade water. The cell suspension was heated in a dry block at 110°C for 10 minutes and placed on ice for at least 10 minutes. After boiling, the lysate was centrifuged at 13,000 rpm for 2 minutes to remove debris. The supernatant containing DNA was collected and stored at –20°C until used (Rasoulinezhad, 2017).

Investigation of gastrointestinal helminths: The gastrointestinal tract was extracted and dissected in a Petri dish containing PBS solution to facilitate the detachment of worms from the intestinal wall and contents. All worms were harvested under a binocular microscope and preserved in 70% ethyl alcohol. Collected worms were identified microscopically based on their morphological features (Gomes *et al.*., 2004).

Floatation technique (FT): Intestinal contents were exposed to the floatation technique to identify gastrointestinal tract helminths and oocystic intestinal protozoa (Presswell and Lagrue, 2016).

Direct smear and wet mount: Direct smears from the crop and oral cavity were examined under a light microscope. *T. gallinae* were examined under a light microscope at 400× by wet mount as described by Qiu *et al.*. (2017).

Table 1. Summarize the techniques used in diseases' diagnosis.

Diagnostic test	Sample type	Total no. of flocks / bird / samples	+Ve Results	Percent of +Ve	Pathogen(s)
Real time RT- PCR	Trachea, Lung, Liver, Spleen, ,and Brain	69 (207) 207 *	84 / 123	40.57 %	ND , AI, MG, and MS
	Tracheal , Cloacal swab	69 (207) 138 ¥	66 / 72	47.83 %	
	Skin warts Nodules	11 (33) 33	33/0	100%	PPV
Bacterial culture	Trachea and Cloacal swab	69 (207) 414	162/252	39.13%	*Salmonella spp., E.coli, Candida spp. ,Staphylococcus aureus* and *Aspergillus spp.*
	Eye swab	15 (207) 45	32 / 13)(	71.1%	
	Liver, heart, spleen	69 (207) 621	243/378	39.13 %	
Detection of antibodies	Serum	69 (207) 207	138/69	66.67 %	NDV, AI, MG, and MS
Full GIT Examination	GIT	69 (207) 207	66/141	31.88%	GIT Helminths (Cestodes spp and Ascaries spp.)
Floatation technique (FT)	GIT content	69 (207) 207	96/111	46.38 %	Gastrointestinal Helminths and Coccidia
Wet mount	Croup content and swab	69 (207) 207	54/153	26.1%	*Trichomonas gallinae*

Table 2. Primers

Pathogen	Primer- name		Sequence	T*a*	Size	References
NDV	APMV1-F	F	5`-ATGGGCYCCAGACYCTTCTAC-3`	60^{O} c	535 bp	Radwan *et al.*., (2013)
		R	5`-CCTGAGGAGAGGCATTTGCTA-3`			
AIV	AIV M2	F	5'- CTTCTAACCGAGGTCGAAACG -3'	52^{O} c	244bp	(Mady *et al.*., 2010)
		R	5'- AGGGCATTTTGGACAAAGCGTCTA -3'			
PPOX	P4b gene	p2fPF	5'- CAGCAGGTGCTAAAC AACAA-3'	60°C	578 bp	Lee and Lee (1997)
		p2fPR	5'- CGGTAGCTTAACGCC GAATA-3'			
MG	mgC2	F	5`-CGC AAT TTG GTC CTA ATC CCC AAC A -3`	72^{O} c	237 bp	Hnatow *et al.*., (1998)
		R	5`-TAA ACC CAC CTC CAG CTT TAT TTC C-3`			
MS	16S rRNA	F	5`-GAG AAG CAA AAT AGT GAT ATC A-3`	72^{O} c	214bp.	Pérez, *et al.*., (2011)
		R	5`- CAG TGG TCT CCG AAG TTA ACA A-3`			

RESULTS

Clinical signs and postmortem examination: Pigeons in investigated flocks were found to be suffering from one or more of the following signs: weakness, loss of weight, greenish or watery diarrhea, inability to fly, offensive odor from cheesy caseated material in the oral cavity, wart nodules on the head and eyelids, unilateral eye swelling, occulonasal discharge, respiratory signs and nervous signs, such as recumbence, wing and leg paralysis, circling, signs of opisthosomas, neck paralysis and star gazing. Off food, thirsty, enlarged crop filled with fluid and gases, and some were suffering from sour crop. External parasites were observed in 6/69, or 8.69%, of the flocks. The mortality rate among the examined flocks ranged from 1.5% to 34%. Lesions varied according to the type of infections, in NDV-infected flocks the predominant lesions were brain congestion and ulcers and hemorrhages on the proventriculus, intestine and cecal tonsils in NDV-infected flocks. An impacted sour crop with small white pustules on a thickened crop wall was found in pigeons with candidiasis. Wart nodules and scars on the head and eyelids suggested PPV, and white or yellowish cheesy material with a foul odor in the oral cavity was characteristic of trichomoniasis caused by *T. gallinae*. Conjunctivitis, eye swelling and occulonasal discharge were common findings in infections due to NDV, PPV, *Staphylococcus aureus*, *E. coli* and MG. Enteritis, mucoid or hemorrhagic intestinal contents and a thick intestinal wall were pathognomonic for coccidiosis, as was the presence of a large number of adult gastrointestinal tract helminths such as *Ascaris* spp. and *Cestoda* spp. Enteritis, watery greenish diarrhea and hepatosplenomegaly with multiple white necrotic foci on the liver and other organs were suggestive of *Salmonella* infection. Grayish nodules on the lungs and air sacs were commonly seen in cases of aspergillosis. Pigeon flocks infected with cestodes showed greenish diarrhea, recumbence, thirst, debilitation, head shaking and severe nervous signs, especially in mixed infections with NDV.

The detailed clinical findings, lesions and mortality rates were reported in Supplementary Table 1.

Serological survey: Out of 69 pigeon flocks evaluated with rapid serology testing, MG- and MS-specific antibodies were detected in 7.25% and 8.7%, respectively. NDV-specific antibodies were detected in 35/69, or 50.72%, of examined nonvaccinated pigeon flocks using the HI test. No influenza A virus–specific antibodies were detected (Table 3).

Molecular detection: On molecular testing, NDVs were detected in 28/69, or 40.57%; PPV in 11/69, or 15.9%; MG in 2/69, or 2.89%; and MS in 3/69, or 4.35 %. Viral infections were found in a total of 56.5% of the birds. Tables (1, 3 and 4).

Bacterial investigation: Bacterial and mycotic infections were found in 31.88% and 7.25%, respectively, of examined flocks. The most common bacterial pathogen was *E. coli* (13/69, 18.84%), followed by *S. aureus* (6/69, 8.69%) and *Salmonella* spp. (3/69, 4.35%). *Candida* spp. were detected in 4/69, or 5.79%, of flocks, while *Aspergillus* spp. were detected in one flock (1.45%) with a history of the repeated administration of antibiotics for 3 months. Eye infections were recorded in 15 flocks, and 71.1% of eye infections had bacterial causes, including *S. aureus*, *E. coli* or MG. Enteritis caused by *Salmonella* spp. was reported in 4.35% of pigeon flocks **(**Tables 3 and 4).

Parasitic infestations: *Ascaris* spp. and *Cestodes* were detected in 14.49% and 17.39% respectively during necropsy. A 52.17 % of investigated pigeon flocks were infested with either single pathogen (7/69, or 10.14%) or with concurrent viral and bacterial infections (42.03%). Based on FT, *Ascaris* spp., *Cestoda* spp. and *Eimeria* spp. oocysts and external parasites were detected microscopically in 14.49%, 17.39% and 14.49%, respectively, of examined pigeon flocks. *T. gallinae* was detected in 18 (26.1%) of examined pigeon flocks using the wet mount technique**.** External parasites(*Menopon gallinae, Pseudolynchia canariensis)*were investigated microscopically in 6/69, or 8.69% **(**Tables 1, 3 and 4).

Detection of multiple infections: Multiple infections were commonly recorded. The overall prevalence of mixed co-infections was 60.87%, including viral, bacterial and parasitic infections. Concurrent viral and bacterial infections were recorded in 15.94% of pigeons and concurrent viral and parasitic infections in 20.29%. NDVs were detected with other bacterial and parasitic infections in 21.74%, and PPVs were detected with other infections such as *S. aureus, E. coli,* MG, *T. gallinae* and *Cestoda* spp. in 10.14% of investigated flocks. The most prevalent mixed infection was with *T. gallinae*, at 21.74%, followed by NDV and *Cestoda* spp. (8.7%). Co-infections with NDV and MS were observed in three pigeon flocks representing 4.35%. The most prevalent bacterial infection was *E. coli* (18.84%) in combination with other viral, bacterial and parasitic infections. *Salmonella* spp. were recorded only in three pigeon flocks (4.35%) with *E. coli* and *Coccidia* spp. oocytes (Fig1, 2).

Histopathological findings: In thick paraffin sections of skin wart nodules from the head and eyelids stained with HandE, large eosinophilic intracytoplasmic inclusion bodies were seen in many cells of the stratum spinosum layer of the epidermis**(Fig 3).**

1

Table 3. Prevalence of detected pathogens among the examined flocks

Pathogen	Method of detection	Nature of Sample	Number of examined flocks/Samples	Number of positive flock / samples	Prevalence (%)
NDV	RT-rtPCR	Tracheal and cloacal swabs	69 / 138*	28/ 56	40.57 %
		Trachea, lung, spleen, brain	69/ 207	28/84	40.57%
	HI test	Serum samples	69/207	35/105	50.72 %
AIA	RT-rtPCR	Tracheal and cloacal swabs	69	0	0 %
		Tissue samples	69	0	0%
	HI and ELISA test	Serum samples	69	0	0%
PPV	-PCR	Warts and nodules	69	11	15.9%
	- Histopathology	Warts and nodules	11	11	
MG	RtPCR	Tracheal swabs	69	2	2.89 %
	RSA	Serum	69/207	5/15	7.25 %
MS	RtPCR	Tracheal swabs	69	3	4.35%
	RSA	Serum	69/207	6/18	8.7%
Salmonella spp.	Bacterial culture	Cloacal swab	69 / 207	3/9	4.35%
		Liver, heart, spleen	69 /621	3/27	4.35%
E.coli	Bacterial culture	Cloacal swab	69 / 207	13/39	18.84%
		Liver, heart, spleen	69 /621	13/117	18.84%
Staph. aureus	Bacterial culture	tracheal swab	69/207	6/18	8.69 %
		Eye swab	15/45	6/18	
Candida spp.	Bacterial culture	Oral and croup swab	69(207)	4/12	5.79 %
Aspergillus spp	Bacterial culture	Air sac swab,	69	1	1.45%
T.Gallinae	wet mount	Croup swab and content	69/207	18/54	26.1 %
Cestodes spp.	GIT inspection and FT*	Intestinal content	69/207	12/36	17.39%
Ascaries spp.	GIT inspection and FT*	Intestinal content	69/207	10/30	14.49%
Eimeria spp.	Floatation test	Intestinal content	69/207	8/24	11.59%
External parasites*	Microscopic examination of parasites		69	6	8.69 %

* Menopon gallinae, Pseudolynchia canariensis

Table 4. Molecular Detection, Bacterial Isolation and Microscopic detection of infected pigeon flocks

	Viral infection																**Bacterial infection**										**Parasitic infection**						**total**
	Single			Mixed													Single		Mixed								single		mixed parasitic				
				viral + bacterial						viral +parasitic			viral + bacteria + parasitic						Bacterial		bacteria+ parasite											
	ND	PPV	AI	PPV+ staph.	ND + E. coli	PPV+ E. coli	PPV + MG	NDV + MS.	NDV + Aspergillosis	PPV+ Cestodes spp+ T. Gallinae	NDV + Cestodes spp.	NDV + Ext .parasites	NDV + T. Gallinae + MS	NDV + staph.spp. + Ascaries SPP.	PPV+ staph. Spp.+ T. Gallinae.	Salmonella spp.	E. co li	Salmonella +E.coli	E. .coli + Staph.	E. .coli + Candida spp.	MG + E. coli	salmoella + Eimeria spp.	E.coli + Eimeria spp. + Ascaries SPP	E.coli + T.gallinae + Cestodes spp.	T. Gallinae + candida spp.	Cestodes spp.	Eimeria spp.	T. Gallinae	T. Gallinae+ Ascaries+ Ext .parasites	T.gallinae + Eimeria spp.+ Cestodes ssp.	T.gallinae + Eimeria spp. + Ascaries SPP	
Flock	13	4	0	2	1	1	1	2	1	2	6	3	1	1	1	1	2	1	2	1	1	1	3	1	3	2	2	3	3	1	3	69
Total		17					8				11			3			3			5			8				7			7		69
Total %																																

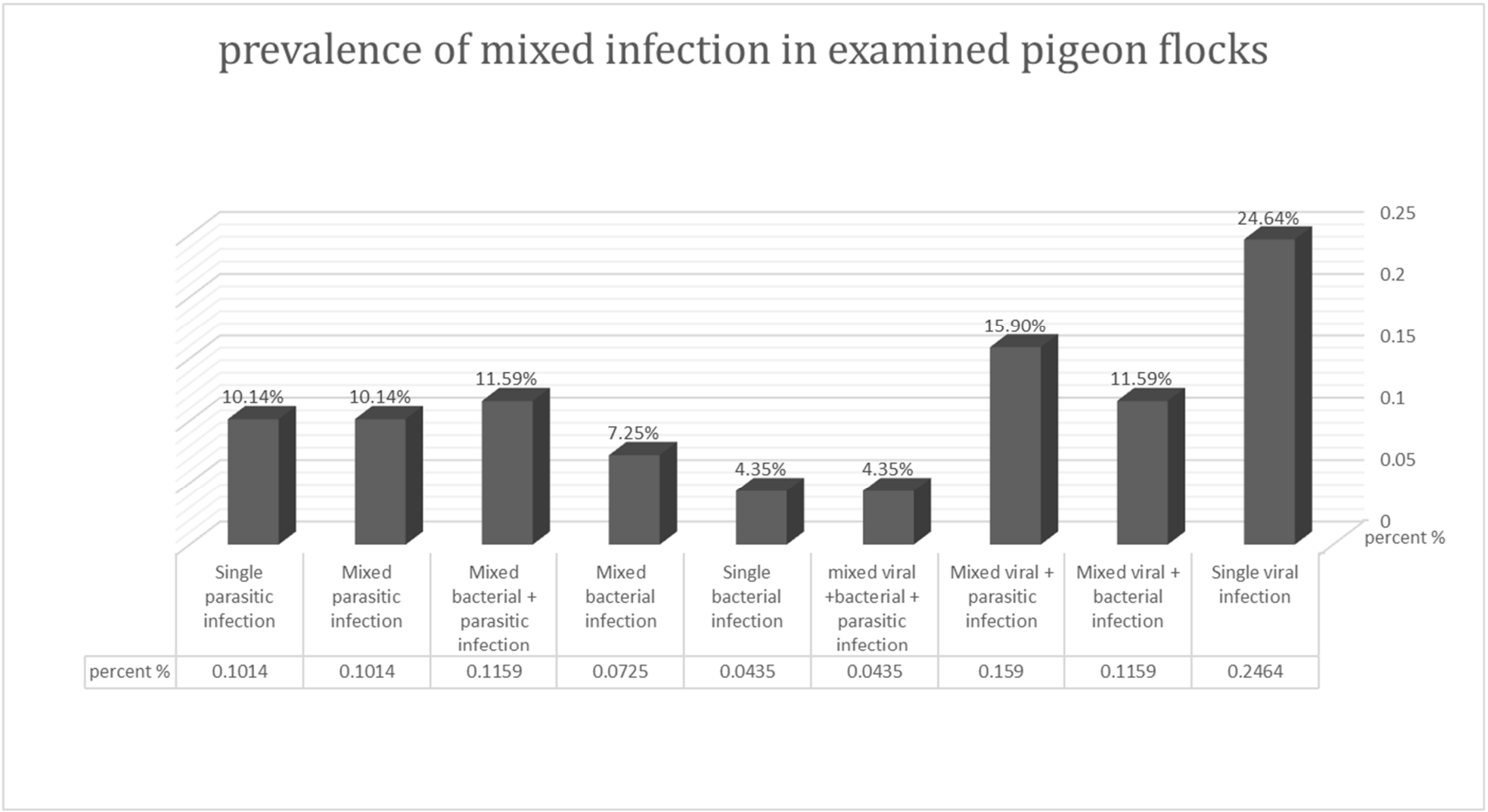

Fig 1. Prevalence of mixed infection in examined pigeon flocks

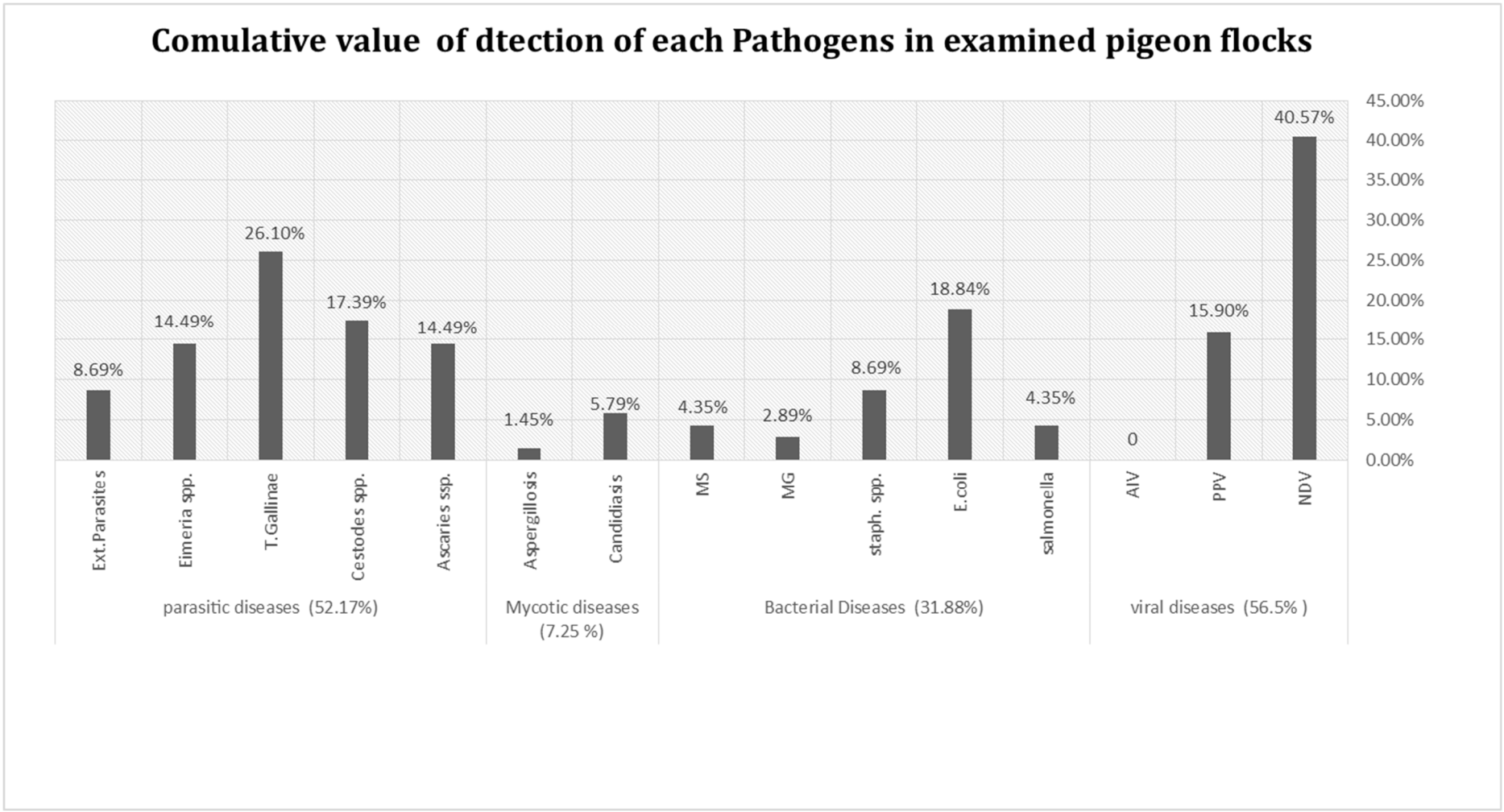

Fig 2. Cumulative value of detection of each Pathogens in examined pigeon flocks

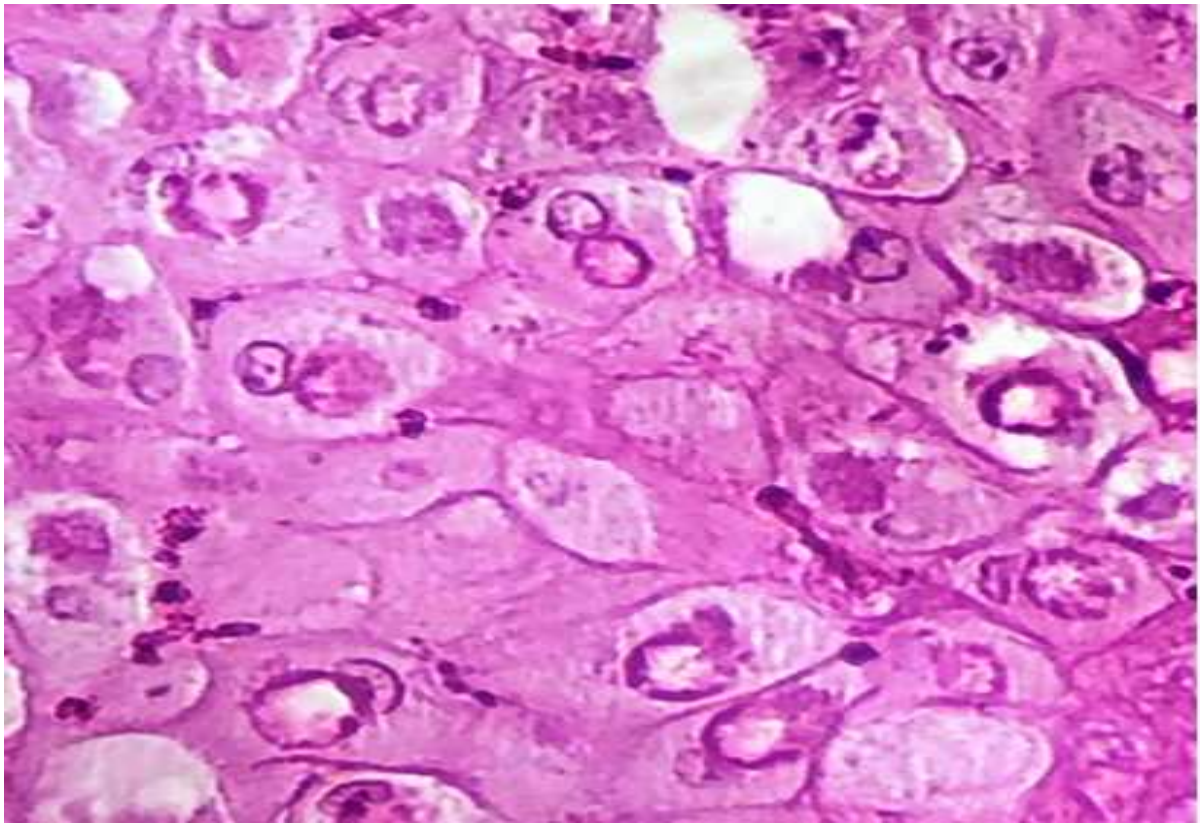

Fig3: Skin of pigeon showed large eosinophilic intracytoplasmic inclusion bodies (Bollinger bodies) (arrows) in many cells of the stratum spinosum layer of the epidermis (H&E staining x400).

DISCUSSION

Pigeon farming has recently gained in popularity worldwide. Pigeons are traditionally reared as a source of food, to serve as a hobby, and for racing. Using molecular and microbiological approaches, we investigated the pathogens circulating among pigeon flocks. Serologically, the prevalence of NDV, AIV, MG and MS were 50.72%, 0%, 7.25% and 8.7%, respectively, among the investigated flocks. In Bangladesh, a higher percentage of viral diseases was reported, ranging from 47.3% to 70.43% according to the season of the year (Islam *et al.*., 2020). In Taiwan, a seroprevalence of NDV (43.3%), MS (1.8%) and MG (1.3%) was recorded without any clinical signs (Tsai and Lee, 2006). This may be due to the close contact between pigeons and other poultry flocks (Abdelaziz *et al.*., 2019; Tsai and Lee, 2006). Previous screening by rapid serological testing (RST) showed that of 240 pigeons, 3.3% and 2.5%, respectively, were positive for MS and MG in Sào Paulo State, Brazil (Ferreira *et al.*., 2016). Pigeons play a role in the transmission of infection to other poultry by direct contact or contamination of poultry feed with pigeon droppings (Tsai and Lee, 2006). A higher seroprevalence of MG, 39%, was reported in pigeons in Sokoto, Nigeria, and other African countries (Mera *et al.*., 2020). MG and MS are important pathogens that imposed a great hazard for the poultry industry and are associated with respiratory infections in pigeons, so direct contact and entrance of pigeons to poultry farms must be prevented (Tsai and Lee, 2006).

NDVs (40.57%) and PPVs (15.9%) were ranked as the most prevalent viral infections in this study. Single infections with NDV or PPV were recorded in 18.84% and 5.8%, respectively, in pigeons, while disease complexes with other bacteria and parasites were recorded in 31.88% of pigeon flocks. *Paul et al..* (2015) recorded a high prevalence of viral infections in pigeon flocks (25.5%), especially PPV (18.92%) and NDV (6.57%). Islam *et al.*. (2020) recorded that the prevalence of viral diseases in young and adult pigeons was 66.06% and 53.31%, respectively. NDV class II genotypes II, VI and VII were widely isolated from pigeons (Liu *et al.*., 2006; Qin *et al.*., 2008; Snoeck *et al.*., 2013; Wang *et al.*., 2015). The high prevalence and detection rates of virulent NDVs from pigeons highlighted the importance of pigeons in the maintenance and circulation of NDV infections (Hirschinger *et al.*., 2019, and Ellakany *et al.*. (2019). In this study, NDV infections were mainly associated with severe nervous system signs. Marlier and Vindevogel (2006) reported that the clinical signs recorded in pigeons and neurotropic NDV in chickens were similar (i.e., tremors of the neck and wings, torticollis, paralysis and ataxia). Greenish diarrhea and mild respiratory signs were frequently recorded, as well. Guo *et al.*. (2014) and Ellakany *et al.*. (2019) noticed only mild respiratory symptoms in pigeons infected naturally with velogenic

NDV. Our studies confirmed the fact that pigeons were definitely potential reservoirs and amplifiers of velogenic NDV infections for commercial poultry flocks. Therefore, new vaccines should be developed, and strong biosecurity instructions must be applied to limit the risk of free living pigeon in NDV outbreaks.

Poxvirus infection is not cmmonly considered a main cause of death for all diseased birds, but its proliferation in the upper digestive tract and respiratory airway, in addition to eye lesions, may increase the risk of accidents, predation and acquired secondary infection that could aggravate the lesions (van Riper and Forrester, 2007). The diphtheritic form of pox on theoral cavity, pharynx, trachea tube and lungs leads to debilitation, emaciation and ending with death (Harlin and Wade, 2009).

In our investigation, the common bacterial diseases were recorded in 31.88% of infected flocks and single bacterial infections in 4.35%. Bacterial diseases in association with other viral (15.9%), bacterial (7.25%) and parasitic (15.9%) pathogens were reported in investigated birds. A high prevalence of bacterial infections among pigeon flocks were reported worldwide. Islam *et al..*, 2020 and Paul *et al..*, 2015 recorded prevalence of bacterial diseases in pigeon flocks of 22.48% and 24.30%, respectively.

Salmonella spp. infections associated with severe clinical signs and lesions had a low prevalence (4.35%); these results were consistent with the observations of Kaczorek-Łukowska *et al.*. (2021) and Stenzel *et al.*. (2013) of prevalences of 5.47% and 7%, respectively, of *Salmonella* spp. in domestic pigeons. A similar prevalence (0.9% to 3.7%) was recorded in Germany by Teske *et al.*. (2013). In India, of 150 samples from pigeons screened for *Salmonella* organisms, 12 *Salmonella* isolates were recovered with an 8% prevalence (Dutta *et al..*, 2013). In Bangladesh, Islam *et al.*. (2020) recorded *Salmonella* spp. in young and adult pigeons at a prevalence of 5.05% and 4.52%, respectively. However, Paul *et al.*. (2015) isolated *Salmonella* spp. from 20.32% of pigeons in Bangladesh. *Salmonella* species in Egypt were isolated from squabs and adult pigeons at 5% and 3.5%, respectively (Ammar *et al..*, 2014). There is a great convergence in the results of and observations on the prevalence of fungal infections (7.25%) with the results and observations of Paul *et al.*. (2015), who recorded fungal infections in 5.18% of pigeon flocks.

The overall prevalence of parasitic diseases was 52.17%. The present investigation revealed a high prevalence of infestation with gastrointestinal tract helminths (31.88%), especially *Cestoda* spp. (17.39%) and *Ascaris* spp. (14.49%). In another study in Medina, Saudi Arabia, the recorded prevalence of Ectoparasites was 88.9% to 100%, *Cestoda* spp. was 10.71% and *Ascaris* spp. was 3.57% (Ali *et al..*, 2020). Mehmood *et al.*. (2019) reported a total of 36.67% of pigeon flocks were positive for gastrointestinal tract helminths, including *Raillietina* spp. (25%) and *Ascaridia* spp. (5%). Many studies worldwide demonstrated a high prevalence of parasitic infestations among pigeon flocks (Al Quraishy *et al..*, 2019; El-Dakhly *et al..*, 2019; Islam *et al..*, 2020; Paul *et al..*, 2015; Sivajothi and Sudhakara, 2015; Yousafzai *et al..*, 2021). A high percentage of parasitic infestations (72.7%) and likely mixed infestations (31.8%) were recorded in pigeon flocks and were thought to be due to large amounts of infected droppings and/or intermediate hosts (Sivajothi and Sudhakara, 2015).

Eimeria species were found in pigeon flocks (14.49%) either as single infections (2.89%) or concurrent infections with other pathogens (11.59%). Many research works reported that the prevalence rate of *Eimeria* spp. and *T. gallinae* in pigeons was similar in Bangladesh (Islam *et al..*, 2020; Paul *et al..*, 2015). In India, the prevalence of *Eimeria* spp. was much higher at approximately 31% (Sivajothi and Sudhakara, 2015). Another study by Mehmood *et al.*. (2019) reported a high prevalence of *Coccidia* spp. (58.3%) in domestic pigeons in India.

T. gallinae, the causative agent of canker, was recorded in 18/69, or 26.1%, of flocks either as a single pathogen (4.35%) or mixed with other pathogens (21.74%). Similarly, high prevalences rate of *T. gallinae* (40% and 11.95%) were recorded in Bangladesh and India by Mehmood *et al.*. (2019) and Paul *et al.*. (2015), respectively. Abed (2013) showed that the total infection rate in doves was 13% and in pigeons was 29%. Hamad and Hassan (2017) reported a total prevalence rate of *T. gallinae* 49.26% in domestic pigeons, with the lowest rate (30.4%) in zubaeri pigeons in Iraq. A higher frequency of *T. gallinae* was recorded in Iraq (66.98%) in adults and (33.02%) young pigeons (Layla Tahir Fadhil and Azhar Ali, 2019), and Jaafar (2014) reported *T. gallinae* in 64% of adults and 57% of young pigeons. The high prevalence rate may be related to the aggregation of pigeons at feeders or watering spots, and overcrowding and stress may enhance transmission (Villanua *et al..*, 2006).

The overall prevalence of complex infections in pigeons was 60.87%, including different etiological agents, either viral, bacterial or parasitic. The most common co-infections were viral and bacterial infections (15.94%), and the next most common were viral and parasitic infections (20.29%). This higher rate of mixed infections in pigeons may be compatible with the higher rate reported in poultry flocks in the same geographical district (Abdelaziz *et al..*, 2019).

In our study, the most common bacterial infections were *E. coli* infections. *E. coli* was the most common pathogen in mixed bacterial infections with *S. aureus* (2.9%), *Salmonella* (1.45%) and MG (1.45%) in pigeon flocks. Our previous studies found mixed infections with *E. coli* and MG in 9.1% of broiler flocks. MG was investigated in 14.5% and MS in 3.6% of backyard flocks mixed with *E. coli* and some viruses (Abdelaziz *et al..*, 2019). It was clear that mixed infections enhanced the pathogenicity of different diseases, resulting in severe clinical signs and elevated

mortality rates in pigeons (up to 34%). Previous studies by Mosleh *et al.*. (2017) and Abdelaziz *et al.*. (2019) confirmed that bidirectional synergistic effects occurred between concurrent mixed infections and augmented the pathogenicity of both. Mixed infections usually have severe clinical signs and higher mortality rates. Bacterial invasions secondary to diphtheritic pox lesions can enhance proliferative lesions, obstructing the upper digestive tract and respiratory airway and resulting in difficulty in eating and breathing. Death may occur due to suffocation (Harlin and Wade, 2009).

Candidiasis is often accompanied with other concurrent infections or trauma (Crespo *et al.*., 2018). Mixed infections with *Candida* and *E. coli* and *T. gallinae* were recorded in 4/69 pigeon flocks.

The discovery of a large number of previously recorded pathogens in poultry flocks (Abdelaziz *et al.*., 2019; Al-Ali *et al.*., 2018; Hemida *et al.*., 2019) and wild bird flocks (Hirschinger *et al.*., 2019) that were circulating in neighboring pigeon flocks was a clear affirmation that pigeons may act as a maintenance host and bridge host between domestic poultry flocks and other bird species, including wild free-living and migratory birds crossing the Arabian Peninsula.

Conclusion: It is concluded that pigeon flocks are a maintenance community that may harbor many pathogens that threaten other birds, animals and humans, such as NDVs, PPVs, *Salmonella* spp., avian pathogenic *E. coli*, MG, MS, mycotic infections and parasitic infestations. Pigeons may act as maintenance hosts and bridge hosts between domestic and wild birds. Our studies confirmed the fact that pigeons are potentially serious amplifiers of infections and reservoir hosts and that they pose a serious biological hazard for the intensive poultry production sector and community. New pigeon disease vaccines should be developed, and strong biosecurity instructions must be applied to slow the spread of infections from pigeons to other birds, animals and humans.

strategies.

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Supplementary Table (1): Details of Examined Pigeon Flocks.

	Flock ID	Flock size	sample size			MR %	clinical sings		Lesions	Diseases
			Serum	swab	Tissues		Rs	Ns		
1	1140	150	3	6	5	34%	-	+	Brain congestion,Hemorrhage on proveent. adult Cestodes spp. In intestine	NDV+CS
2	872	110	3	6	5	25.8	-	+	Brain congestion, hemorrhage on provent., green diarrhea	NDV
3	629	80	3	6	6	12.5	-	+	hemorrhage on provent., ceacal tonsils, brain congestion	NDV
4	1410	150	3	6	6	13.3	-	+	brain congestion and green diarrhea	NDV
5	345	160	3	6	10	15.6	+	+	bain congestion, , pneumonia. diarrhea.	NDV
6	58	40	3	6	12	23	+	+	bain congestion, pneumonia, diarrhea.	NDV
7	422	55	3	6	8	2.5	+	-	Enteritis. Liver and spleen congestion	E.coli
8	340	40	3	6	10	16.6	+	+	brain congestion, pneumonia, diarrhea, enteritis, hemorrhage on ceacl tonsils	NDV
9	983	70	3	6	10	3	+	-	enteritis, hemorrhage intestine mucosa	Eimeria spp.(ES)
10	1235	50	3	6	10	3.5	+	-	enteritis, hemorrhage intestine mucosa	Eimeria spp,(ES)
11	242	180	3	6	10	4	-	-	enteritis, hemorrhage intestine mucosa	cestodes spp.(CS)
12	57	200	3	6	12	7	+	-	hepatosplenomegaly and hemorrhage, white necrotic foci on liver	Salmonalla
13	86	85	3	6	10	10	+	-	hepatosplenomegaly and hemorrhage, white necrotic foci on liver,enteritis	E.coli +Salmonella
14	898	55	3	6	12	10	+	-	paralysis, brain congestion, liver congestion, air saculitis, adult Cestodes spp. In intestine	NDV+ Cestodes spp.
15	526	40	3	6	10	13	+	-	eye swelling and secretion, diarrhea, liver congestion,airsaculitis	*E.coli + MG*
16	527	35	3	6	10	3			bad odour white material in mouth and crop	T.gallinea (T.G)
17	539	700	3	6	8	18.57	+	+	paralysis, brain congestion, liver congestion, air saculitis, adult Cestodes spp. In intestine	NDV+CS.
18	673	450	3	6	7	6	-	-	white cheasy mat. In mouth	T.G
19	931	255	3	6	5	5			Enteritis with Ascaries worm, white crust in mouth + ext.parasites	T.G + AS+ ext. paras.
20	1035	135	3	6	8	17	+	+	Brain congestion, caseated material on air sacs with black spots	NDV+ Aspergillosis
21	1159	35	3	6	10	14.3	-	+	paralysis, brain congestion, liver congestion, air saculitis, adult Cestodes spp. In intestine	NDV+ CS.
22	1079	60	3	6	10	22	-	+	brain congestion, liver congestion, air saculitis,hemorrhage on provent, and ceacal tonsils	NDV+ E.coli
23	1148	80	3	6	10	9	+	-	hem. foci with adult cestodes in intestine, white crust in mouth	T.G+ES+ CS
24	1137	130	3	7	10	7	-	-	cheasy mat. In mouth, hem. Foci + Ascaries worm In intestine	T.G +ES+ AS
25	898	300	3	6	10	21	-	+	paralysis, brain congestion, liver congestion, air saculitis, adult Cestodes spp. In intestine	NDV+ CS.
26	837	132	3	6	10	23		+	Congestion of brain, heart, intestine, liver, caseation in oral cavity	NDV + T.G +MS
27	1010	180	3	6	10	20	-	+	Congestion of brain heart, lung, liver, hem.on ceacal tonsils	NDV
28	37	225	3	6	10	8	-	+	congestion liver, hem. Foci and Ascaries spp. worm In intestine	E.coli +ES+ AS
29	631	73	3	8	10	3		+	Congestion of lung, intestine, liver, crop filled with fluid and gases	E.coli + candida spp.
30	182	85	3	6	10	11		+	Congestion of brain, heart, hem. On ceacal tonsils and provent.	NDV
31	183	55	3	6	10	5			Wing paralysis, Enteritis, white necrotic foci on liver, diarrhea, thirst	Salmonella + ES
32	448	300	3	6	10	20	-	+	Congestion of brain, heart, hem. On ceacal tonsils and provent.	NDV
33	477	150	3	8	10	3	-	-	enteritis, congestion of liver and spleen, hem. On itestine and ascaries worm	E.coli +ES+ AS
34	478	50	3	8	10	3.2	-	-	enteritis, congestion of liver and spleen, hem. On itestine and ascaries worm	E.coli +ES+ AS

35	Fp1	120	3	6	10	2	+	-	Eye swelling and secretion, diarrhea, liver congestion	E.coli + staph. spp.
36	Fp2	110	3	6	11	7.5	+	-	warts on head, csetodes worm	PPV+ Cestodes spp. + T.G
37	Fp4	50	3	6	10	12	+	+	Recumbancy, brain congestion, ulcer on ceacal tonsils	NDV+ MS
38	Fp5	35	3	6	8	13.4			Warts on head , white material in mouthand, cestodes in intestine	PPV+ CS + T.G
39	Fp6	76	3	6	11	5.5			white cheasy material in mouth cavity	T.gallinae (T.G)
40	Fp7	66	3	6	12	6.2			white cheesy material in mouth ,crop filled with fluid and white crust	T.G + candida spp.
41	Fp8	155	3	6	12	9.5	-	+	brain congestion, liver congestion, air saculitis, adult Cestodes spp. In intestine	NDV+ CS.
42	Fp9	300	3	6	9	21.5	-	+	Brain congestion, hem. On prove., enteritis,	NDV
43	Fp10	40	3	6	11	4.5			Wart nodules and crust on head, eye secretion	PPV+ staph. spp.
44	Fp11	420	3	6	12	3.5			white cheasy material in mouth ,crop filled with fluid and white crust	T.G + candida spp.
45	Fp12	75	3	6	13	22			congestion on brain, lunge, eye seceation, and green diarrhea+ Ascaries worm	NDV+ Staph.spp +AS
46	Fp13	100	3	6	12	7.5	-	-	white cheasy material in mouth ,crop filled with fluid and white crust	T.G + candida spp.
47	Fp14	88	3	6	11	16.5			Brain congestion, hem. On proven. and green diarrhea + ext. parasites	NDV+ Ext. paras.
48	Fp15	62	3	6	11	12	+	+	Recumbancy, brain congestion, ulcer on ceacal tonsils	NDV
49	Fp16	140	3	6	10	18			Brain congestion, hem. On proven. and green diarrhea + ext. parasites	NDV+ Ext. paras.
50	Fp17	40	3	6	14	11.5	+	-	Wart on head, eye secretion, cheesy material on oral cavity	PPV+ Staph. Spp. + T.G
51	Fp18	50	3	6	10	10	+	+	Recumbancy, brain congestion, ulcer on ceacal tonsils	NDV
52	Fp19	35	3	6	15	8	+	-	Wart nodules and crust on head, eye and nasal secretion	PPV+ MG
53	Fp20	70	3	6	12	8			Brain congestion, hem. In intestine, green diarrhea	NDV
54	Fp21	133	3	6	10	5			white cheesy material in mouth and, Cestodes in intestine	T.G + E.coli + CS
55	9831	270	3	6	14	5	+	-	Small nodules and crust on head and eye lids	PPV
56	12352	210	3	6	10	6	+	-	Small nodules and crust on head and eye lids	PPV
57	2423	115	3	6	10	11	-	-	Small nodules and crust on head and eye lids	PPV
58	4450	305	3	6	8	19.5	-	+	Brain congestion, enteritis, arthritis, conjunctivitis	NDV+ MS
59	4451	152	3	6	8	7			Enteritis with Ascaries worm, white crust in mouth	T.G +ES +AS
60	4452	71	3	6	10	5			Small nodules and crust on head and eye lids	PPV
61	4453	49	3	6	10	2			Eye swelling and secretion, diarrhea, liver congestion	E.coli + staph.spp.
62	4454	311	3	6	10	5.6	-	+	Small nodules and crust on head, swellingg eye	PPV+ E.coli
63	4455	70	3	6	8	1.5			enteritis, liver, kidney, spleen congestion	E.coli
64	4456	50	3	6	8	6			Enteritis with Ascaries worm, white crust in mouth + ext.parasites	T.G + AS+ ext.para.
65	4457	44	3	6	8	8			Wart on head, eye and nasal secretion,	PPV + Staph.spp.
66	4458	65	3	6	8	5			white crust in mouth + Enteritis with Ascaries worm + ext.parasites	T.G + AS+ ext.para.
67	4459	90	3	6	12	9			white crust in mouth + Enteritis, hem., with Ascaries worm	T.G+ES+ AS
68	4460	300	3	6	10	13	-	+	Brain congestion, hem. In intestine, green diarrhea	NDV
69	4461	105	3	6	10	4.5			paralysis, brain congestion, enteritis , adult Cestodes spp. In intestine	Cestodes spp.

Ns = nervouse sings. Rs = respiratory sings MR= mortality rate .≠= age / day T.gallinae =(T.G) **AS= Ascaries spp. CS= Cestodes spp. ES= Eimeria spp. Ext. paras. - External parasites**