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Molecular Detection of Fancy Birds Parasites for Clinical Diagnosis and Epidemiology - A Review

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Abstract

Fancy birds in Pakistan and all around world play a pivotal role in the economy and social associations with community. These birds includes pigeons, peacock, ducks, love birds, chicken, doves and parrots. This study is about fancy birds and their parasites that cause different diseases to other birds as well as infection to human beings. Bird's parasites can be isolated and detected by using different techniques i.e. direct and indirect faecel examination and microscopy, flotation techniques. Molecular techniques with phylogenetic analysis and Polymerase Chain Reaction (PCR). The most prevalent *ectoparasites* are *Ceratophyllus columbae, Pseudolynchia canariensis, Menopon gallinae, Lipeurus caponis, Knemidokoptes pilae, Dermanyssus gallinae, Argas persicus, Menacanthus stramineus* and *Goniocotes gallinae*. However, the *endoparasites* include *Trichomonas gallinae, Eimeria spp., Ascaridia columbae, Cryptosporidium meleagridis, Raillietina echinobothrida, Heterakis gallinarum, Syngamus trachea, Davainea proglottina* and *Capilaria. spp.* The diagnosis of parasites is utmost needed for the strategic control and treatment of infections to prevent huge economic loses and mortality. It is concluded that fancy birds harbor various ecto- and *endoparasites* that contribute zoonotic diseases to the people who are in contact with them. Prevalence of these parasites is very high and fewer studies are available on the subject to address the impact and importance of their role in zoonosis. The purpose of this manuscript is to review the fundamental significances of studies on zoonotic potential between birds and their impact on veterinary professionals, birds and public health.

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Introduction

Birds are significant for environment just as for human beings, they play an imperative part in each living thing present on earth [1]. There are approximately 18,000 species of the birds across the world and almost 787 bird species have been found in Pakistan (https://en.wikipedia.org/wiki/List_of_birds_of_Pakistan)[2]. According to Charles Robert Darwin, all fancy strains were originated from Rock Dove by artificial selection. The favorite hobby in the people of Pakistan is to rear the beautiful and lovely birds as a decorative purpose and to fascinate public and also enhance the attractiveness of their houses. These birds are commonly called fancy birds like Pigeons, parrots, peacock, doves, duck, love birds and chicken. Breeding of fancy birds is also starting through domesticating them in birdcages [3].

Primary selective breeding of pigeons was seen in Egypt. The breeding of pigeons is also common in Pakistan, which is domesticated by many people and started about 10000 years ago [4]. The expression of "Domestic or Pet bird" assigns birds kept and reproduce for a solely fancy usage. This classification incorporates and will allude to primarily Passeriformes (for example sparrows, finches and canaries), corresponding called songbirds [5]. We can easily keep them in captivity according to their specific requirements of specie for a prolonged time by giving special attention. The climate conditions of Pakistan are very favorable for a variety of birds as they can easily adjust [3]. During the last several years, Karachi has become the largest bird breeding and exporting region followed by Lahore [6].

According to business point of view, these birds are very beneficial as total cost of their rearing is very small due to huge number of resources in Pakistan. Production cost is very small but it pays nearly 75-85% profit from them [3]. On the other hand, due to the lack of attention and improper handling, these fancy birds can be infected by any disease-causing agent [3].

The purpose of this review is to provide detailed information about zoonotic potential between birds and their impact on veterinary professionals, birds and public health. It also discuss studies for clinical diagnosis at molecular level.

Zoonotic potential

In Pakistan, the most common diseases are candida, cocci, paratyphoid, adenovirus, ornithosis, worms, canker, and paramyxovirus, avian trichomonosis [3]. Similarly, these living beings are expected importers as well as transmitters of Zoonotic infections. Some of them could critically infect human being, as chlamydophilosis, Newcastle Disease (ND), Bursal disease, salmonellosis or even exceptionally pathogenic avian flu AHSNI [7]. Even though non-comprehensive, targets edifying by the depiction of a few instances of bird human relation, the danger experienced by bird vendors as well as youngsters. Public health outcomes are deliberated, and accentuation are made on few vector borne infections, identified as rising or which are disparaged, similar to those sent by the red mite *dermanyssus gallinae*. At last, Cleanliness and biosecurity, just as counteraction rules are created a perception suggested by Boseret et al. [8].

Many infections that are transferred in humans by birds are named zoonotic disease. Parrot fever is also called Psittacosis and a zoonotic infectious disease in humans, which is orthosis, spread by a bacterium known as *Chlamydia psittaci* and constricted from infected parrots (cockatiels, macaws and budgerigars), sparrows, hens, ducks, pigeons and also from other birds species [9].

Recommendations

According to previous studies, to minimize the risk of diseases or infections in Antarctica, some recommendations are made including ‘‘establishment of serum banks’’ and arrange ‘‘central clearinghouse for evidence on suspected disease prevalence’’. But unfortunately, these recommendations have not been applied yet. To avoid outbreaks or make decisions, these two commendations must be conformed to enhance our understanding regarding the health of mammals and bird populations in Antarctica [10].

We can prevent zoonotic infections by adopting a simple hygiene environment, which humans and birds share. We should be careful and always sanitized or wash hands after dealing with birds. As every bird is not harboring such infections but it is necessary to be safe and healthy [9].

Parasites of fancy birds

The term ‘‘parasite’’ is originated from the Greek ‘‘parasitos’’ means individuals that eat at the side of other and generally with harmful impacts on the host. The life cycle of parasites can be divided as direct and indirect transmission i.e. from host to host or from one to another respectively [11].

Types of parasites

Protozoa, arthropods and helminths i.e. cestodes, trematodes and nematodes are the parasites of parrots termed *ectoparasites* and *endoparasites* [12].

Infections by parasites in birds

Some parasites occur as primary or opportunistic pathogens that may be effective means causing little injury or uneffective means causing infection or death. These parasites may affect the organ systems like muscles, kidneys, skin, gastrointestinal tract, respiratory tract and blood [12]. Opportunistic parasites are present in rabbits as well as also common in humans or animals i.e. Microsporidia [13].

In the 19th century, *Trichomonas gallinae* and *Tetratrichomonas gallinarum*, are the members of Trichomonadidae family which are important parasites found in the birds [14]. *Trichomonas* is widely spread disease caused by single-celled protozoan parasites i.e. *Trichomonas gallinae*. Parasitic infections can harmfully effect human health and indigenous animal suitability [15]. In the protection of bird species, parasites are one of the significant concerns due to the cause of wildlife health problems and death. Parasites are found on equally wild and caged bird species, which causes several infections e.g. avian malaria, ornithosis or psittacosis and bird flu [16].

Cryptosporidiosis is the primary protozoan infections in birds. It shows as either a respiratory or gastrointestinal disease and it influences countless avian species across a few regions. The purpose of this survey is to investigate the fundamental consequences of studies on cryptosporidiosis between birds and the significance of these outcomes to veterinary medication and public health [17].

Studies also conducted on the complaints of black flies i.e. *Simulium spp.*, which attacks several species of poultry and increases the rate of mortality and morbidity in affected groups. While black flies can cause infection directly like breakdown of cardiopulmonary and anaphylactoid responses. In poultry birds, some species of protozoans i.e. *Leucocytozoon spp.* are identified which are transferred by black flies and may cause injury and death [18].

In domestic birds, a common parasitic infection is Heterakidosis which is caused by the species of *Heterakis* including *Heterakis gallinarum*, *H. isolonche*, and *H. dispar*. Mostly noted that in *galli*naceous birds the superlative defined species is *H. gallinarum* while *H. dispar* stays the major species in waterfowl [19]. But as compared to wild birds, caged birds are more susceptible to parasites because wild birds can leave hostile atmosphere and naturally handle health contests. The evaluation of this study covers all the aspects i.e. epidemiology, aetiology, pathogenesis, clinical symptoms, injuries, analysis, cure and inhibition of the parasites [20].

Factors in the transmission of diseases

Pathogenic load increased in atmosphere where cohort and birdcage parrots alive together due to close bounds. The risk of infection and disease becomes larger due to the highest exposure of birds to parasites and microbes, which may leads to increased pathogenic load. The discussion about the parasitic and microbial infection in companion or aviary parrots leads to cover their origins, pathogens, diagnosis, treatment, and some of the supplementary risk factors [12]. Subclinical infections may be caused by parasitic diseases, which found in birds even they occur in small amount [21].

Age and sex of game birds are also identified and ensured substantial ($p > 0.05$) effect in gastrointestinal parasitic diseases. According to these results, it was determined that birds, regardless of age, season, sex, cure, gastrointestinal organisms are the sever hazard to birds in Dhaka (Municipality), Bangladesh [22].

Different factors involved in the transmission of parasites between the hosts are significant for observation of animal parasites before they effectively arise in people and increasing the effectiveness of projects for the control and management of zoonotic infections. For multi-host infectious diseases, a developed study conduct about wildlife ecology, parasite sharing and prevalence that could be expanded and merged into active surveillance structures. These techniques reflect developing interdisciplinary methods by significant capacity for the detection of upcoming zoonotic parasites and unidentified reservoirs of present zoonosis, policies for the decrease of parasite frequency and transmission between hosts, and reducing the load of contagious infections [23].

The properties of pathogens and parasites are not afar by Antarctic birds. Though, widespread infection for bird's populations but the latent environment significances in Antarctica have established slight attention [24]. Available data about disease was evaluated by Andre´s and Marı´a [10]. Parasites and its infection were also analyzed on Antarctic birds. Host species, pests and pathogens or topographical areas data is still imperfect and information about the environmental effects on inhabitants which consists of how birds react towards parasites and pathogens are almost not existing. To control the prevalence of outbursts, this data is essential and also support in managing process [10].

So, it was suggested that further study is required to found overall patterns of chronological and longitudinal differences and also determine how these patterns can affect pathogens or microbes and parasites [25]. In the perspective of pathogenicity of trichomonads, virulence factors were studied in restricted data, which varies significantly, and showing specific strain heterogeneity of the parasites. In future, resistant parasites become more challenging. Presenting further standardized genetic analysis and surveys focused on the host-pathogen contact should be supportive to illuminate virulence factors that might lead to new perceptions of handling [26].

Epidemiology of fancy bird parasites

Identification and prevalence of parasites (*ectoparasites* and *endoparasites*) in local pigeons (*Columba livia*) in Tripoli; Libya. Every pigeon was analyzed for *ectoparasites*. The analysis showed that 55% (55/100) were diseased with *T. gallinae* whereas 76% (76/100) with *Haemoproteus spp.* The frequency of ecto-parasitic invasion was 89% as in *Columbicloa columbae* (82%), *Goniodes gallinae* (18%), *Menopon gallinae* (3%) and *Pseudolynchia canariensis* (1%). While in examined pigeons the intestinal helminths prevalence ewas 56% (56/100). Three species of Nematoda i.e. 18% *Heterakis gallinarum*., 22% *Ascaridia galli* and 4% *Capillaria spp.* and also three species of Cestoda i.e. 2% *Raillietina* tetragona, 32% R. *echinobothrida* and 4% *R. cesticillus* were detected. From this study, it was concluded that pigeons infected with different types of parasites and highlight that hosts of helminths are pigeons of veterinary significance and also cause diseases in other avian hosts [27]. The prevalence of endo and ecto parasites in some fancy birds is given in the following (Table 1).

Prevalence in some samples of pet birds was analyzed by faecel flotation method. Generally, 35.6% of the birds parasites i.e. 42.2% of zoo birds and 27% of domestic birds, including *Ascaridia* (6.8%), *Strongyles* (5.5%), *Strongyles*-Capillarids (8.9%), *G. duodenalis* Assemblage A (5.3%), Coccidia (4.1%), *Cryptosporidium* (4%), *Porrocaecum*-Capillarids (2%), *Porrocaecum* (2.7%), and *Syngamus*-Capillarids (0.7%). As compared to domestic birds, zoo birds were most probable to harbor different diseases whereas indicative birds to be parasitized [28]. Therefore, Clinicians should be conscious of the public health suggestions posed by *Cryptosporidium spp.* and zoonotic *G. duodenalis* Collections in caged birds [29].

Some findings in the occurrence of intestinal parasites of poultry discovered that 62% were diseased with diverse species of parasites including *Ascaridia galli*; coccidian; *Heterakis gallinae* (C ecal worm); *Syngamus trachea* (Gapeworms); *Capillaria annulata* (Thread worm) and Tape worm. *Ascaridia*, *galli* was the most dominant species (17.2%) among the helminthes. It was suggested that a sustainable control methodology after the determination of high prevalence of diverse diseases and parasitism could be a major imperative to creation in the study area [30].

Generally, occurrence of protozoa and GI helminths were documented as 11.32% and 26.05% correspondingly. The occurrence was most noteworthy in the laying birds continued all together by agonizing and developing birds. Economically elevated birds, regular lodging framework, manual taking care of and watering structures, crumbed-feed and ruined surface were found having positive measurable relationship with the GI parasitism in layer birds of the analysis zone. This information won't just be useful for the little holder poultry growers to regulate their cultivating rehearses yet additionally for the arrangement and chiefs to execute techniques that can limit the danger of GI parasitism in business just as lawn poultry raising frameworks [31].

A study conducted about the prevalence of 60 game birds with intestinal parasites, these birds including parrot (*Psittaci formes*), dove (*Streptopelia chinensis*), budgerigar (*Melopsittac usundulatus*), cockatoo (*Cacatuidae*) and teeter (*Franocdinus pondicerianus*) [32].

According to the study of Albeshr & Alrefai, [33], it was discovered that genetypic diversity and occurrence of *Trichomonas gallinae* in Riyadh, Saudi Arabia. Among domestic and wild pigeons, the ratio of diseases was compared that caused by *T. gallinae* then infections were identified that significantly complex in domestic pigeons. For the first time, the variety of *T. gallinae* strains were discovered in the birds of Saudi Arabia and concluded that among Riyadh bird's the ribotypes A or C are dominant [33].

Coprologic examination discovered that the general frequency of intestinal parasitic disease (45%), in this (21.67%) *Ascaridia galli*, (10%) for *Balantidium coli* and (13.33%) for *Eimeria spp.* The epidemiology of *Ascaridia galli* recorded as (28.7%) teeter, (22.22%) budgerigar and (16.6%) parrot individually. In budgerigar, *Eimeria spp.* was 16.67% and in cockatoo 16.67% but in parrot whereas *Ascaridia galli* existed maximum as 25%. On the other hand, the epidemiology of *Balantidium coli* 44.44% was maximum in the dove as compared to *Ascaridia galli* 22.22%. In the teetar, the occurrence of *Ascaridia galli* remained maximum as 28.57% as compared to *Eimeria spp* 14.23% [22].

The study also conducted on poultry birds including domestic chicken (*Gallus gallus domesticus*) because of infection of birds by illnesses, affecting living creatures as well as parasites [34]. This research based on the occurrence of intestinal parasites in indigenous and unusual varieties of chickens in Pankrono-Kumasi (Ashanti Region of Ghana). The percentage prevalence of local strains recorded as 76.0%, which making them more liable strain to intestinal parasites. Therefore, it was suggested that farmers should educate on farm supervisory practices that will decrease the threat of disease and aid to enhance production which fulfill the request of customers [35].

Sample of domestic birds including 4 pigeons (*Columbia livie*), 6 ducks (*Anas sparsa*), and 13 chickens (*Gallus gallus* domestica) were screened for ecto- and endo-parasites. Results showed that all chickens as 100% and some pigeons like 50% were detected to be diseased by endo-parasites and also calculated their prevalence rate. From this study, it was reported that ecto-and endo-parasites are main significances of the bird's wandering and feeding ways [36].

Table 1: Prevalence of parasites in some fancy birds.

Birds	Species of parasites	Prevalence (%)	Sex	Country/Region	Reference
Pigeon (*Columba livia*)	*C. columbae*	35	-	Benin, Nigera	[36]
		82	-	Tripoli, Libya	[27]
		63.8	-	Zaria, Nageria	[53]
		66.9	M	Zaria, Nageria	[53]
		60.2	F	Zaria, Nageria	[53]
		86.66	-	KPK, Pakistan	[54]
		56.36	-	Iran	[55]
	G. gallinae	18	-	Tripoli, Libya	[27]
	P. canariensis	1	-	Tripoli, Libya	[27]
		37.1	-	Zaria, Nageria	[53]
		38.6	M	Zaria, Nageria	[53]
		35.4	F	Zaria, Nageria	[53]
		36.36	-	Iran	[55]
	Menopon gallinae	15	-	Benin, Nigera	[36]
		6.3	-	Zaria, Nageria	[53]
		3	-	Tripoli, Libya	[27]
		3.1	M	Zaria, Nageria	[53]
		9.7	F	Zaria, Nageria	[53]
		21.81	-	Iran	[55]
	Goniodes dissimilis	20	-	Benin, Nigera	[36]
		10.8	-	Zaria, Nageria	[53]
		10.2	M	Zaria, Nageria	[53]
		11.5	F	Zaria, Nageria	[53]
	Dermanyssus gallinae	1.6	M	Zaria, Nageria	[53]
		3.5	F	Zaria, Nageria	[53]
	Lipeurus caponis	25	-	Benin, Nigera	[36]
		16.36	-	Iran	[55]
	Chelopistes meleagridis	5	-	Benin, Nigera	[36]
	Trichomonas gallinae	56	-	Saudi Arabia.	[33]
		67.27	-	Iran	[55]
		75.78	-	Bursa, Turkey	[27]
	Eimeria labbeana	23.63	-	Iran	[55]
	Cryptosporidium meleagridis	2.7	-	Iran	[37]
		3.63	-	Iran	[55]

	Ascaridia columbae	11.3	-	Zaria, Nigeria	[53]
		10.2	M	Zaria, Nigeria	[53]
		12.4	F	Zaria, Nigeria	[53]
		27.8	M	Lahore, Pakistan	[56]
		40	F	Lahore, Pakistan	[56]
		21.81	-	Iran	[55]
	Ascaridia galli	22	-	Tripoli, Libya	[27]
		3.3	-	Zaria, Nigeria	[53]
		3.1	M	Zaria, Nigeria	[53]
		3.5	F	Zaria, Nigeria	[53]
		7.27	-	Iran	[55]
	R. echinobothrida	85	-	Benin, Nigera	[36]
		32	-	Tripoli, Libya	[27]
		10.8	-	Zaria, Nigeria	[53]
		11	M	Zaria, Nigeria	[53]
		10.6	F	Zaria, Nigeria	[53]
		18.18	-	Iran	[55]
	A. cuneate	5	-	Benin, Nigeria	[36]
		0.8	M	Zaria, Nigeria	[53]
		0.9	F	Zaria, Nigeria	[53]
	C. contorta	10	-	Benin, Nigera	[36]
	H. gallinarum	18	-	Tripoli, Libya	[27]
		3.3	-	Zaria, Nigeria	[53]
		3.1	M	Zaria, Nigeria	[53]
		3.5	F	Zaria, Nigeria	[53]
Chicken (*Gallus gallus domesticus*)	*Argas persicus*	62.72	-	Sulaimani, Iraq	[57]
		7.46	-	Benin, Nigera	[36]
	Menacanthus stramineus	72.92	-	Sulaimani, Iraq	[57]
	Goniocotes gallinae	54.17	-	Sulaimani, Iraq	[57]
	Goniodes gigas	17.91	-	Benin, Nigeria	[36]
		39.58	-	Sulaimani, Iraq	[57]
	Menopon gallinae	22.39	-	Benin, Nigeria	[36]
		37.5	-	Sulaimani, Iraq	[57]
	Cuclotogaster heterographus	4.48	-	Benin, Nigeria	[36]
		10.42	-	Sulaimani, Iraq	[57]
	Liperus caponis	17.91	-	Benin, Nigeria	[36]
	Leucocytozoon sp.	0	-	Diyala's localities ,Iraq	[58]
		13	-	Layyah, Punjab, Pakistan	[58]
	Haemproteus sp.	13.2	-	Diyala's localities ,Iraq	[58]
		24.4	-	Layyah, Punjab, Pakistan	[58]
	Plasmodium sp.	2.6	-	Diyala's localities ,Iraq	[58]
		31.5	-	Layyah, Punjab, Pakistan	[58]
	Ascaridia galli	1.15	-	Benin, Nigeria	[36]
		32.5	-	Kumasi Ghana	[35]
		21.29	-	Faisalabad, Pakistan	[59]
		31	-	Sulaimani, Iraq	[57]
	Heterakis gallinarum	19	-	Kumasi Ghana	[35]
		2.81	-	Faisalabad, Pakistan	[59]
		21.66	-	Tabriz, Iran	[58]
		81	-	Sulaimani, Iraq	[57]
	Prosthogonimus species	1.5	-	Kumasi, Ghana	[35]
	Davainea proglottina	5.38	-	Benin, Nigeria	[36]
		2	-	Kumasi Ghana	[35]
		3.45	-	Sulaimani, Iraq	[57]
	Raillietina spp	9.5	-	Kumasi Ghana	[35]
		7.5	-	Iran	[37]
		55.17	-	Sulaimani, Iraq	[57]
	Amaebotaenia sphenoides	19.7	-	Benin, Nigeria	[36]
		10.34	-	Sulaimani, Iraq	[57]

	Eimeria species	1	-	Iran	[37]
		50	-	northern Jordan	[58]
	Capilaria. Spp	14.5	-	Kumasi Ghana	[35]
		1.72	-	Sulaimani, Iraq	[57]
Duck	*Anaticola cassicornis*	100	M/F	Dhaka, Bangladesh	[60]
	Lipeurus caponis	100	M/F		
	Goniocotes hologaster	75	M		
		100	F		
	Menopon gallinae	93.75	M		
		100	F		
	Menacanthus stramineus	62.5	M		
		71.43	F		
	Holomenopon leucoxanthum	56.25	M		
		64.28	F		
	Goniocotes gigas	37.5	M		
		35.71	F		
	Colpocephalum turbinatum	25	M		
		50	F		
	Echinoparyphium recurvatum	25	M		
		35.71	F		
	E. elegans	18.75	M		
		28.57	F		
	E. trivolvus	12.5	M		
		14.28	F		
	Echinostoma revolutum	18.75	M		
		21.43	F		
	P. longicirratus	18.75	M		
		35.71	F		
	H. lanceolata	43.75	M		
		78.57	F		
	H. columbae	62.5	M		
		85.71	F		
	R. bonini	62.5	M		
		64.28	F		
	R. cesticillus	56.25	M		
		57.14	F		
	R. echinobothrida	50	M		
		50	F		
	C. digonopora	31.25	M		
		50	F		
	Sobolevicanthus sp	25	M		
		35.71	F		
	A. galli	43.75	M		
		85.71	F		
Peacock	*Menacanthus stramineus*	10.89	-	Bahawalpur Zoo	[61]
	Columbicola columbae	9.9	-		
	Echidnophaga gallinacean	6.93	-		
	Argus persicus	5.94	-		
	Menopon sp.	7.14	-	Ben Aknoun, Algeria	[62]
	Menacuntus sp	28.57	-		
	Colpocephalum tausi	50	-		
	Amyrsidea minuta	7.14	-		
	Lipeureus caponis	7.14	-		
	Amidostomum sp.	20.8	-		
	Capillaria sp	25	-		
	Chilomastix sp.	16.7	-		
	Cooperia sp	4.2	-		
	Cyathostoma branchalis	8.3	-		
	Eimeria sp.	58.3	-		

	Giardia sp	4.2	-		
	Strongyloides sp	16.7	-		
	Amidostomum sp	25	-		
	Capillaria sp	25	-		
	Eimeria sp	33.3	-		
Parrot (*Psittaci forms*)	*Ascaridia*	25	-	Dhaka	[22]
		26.14	-	Lahore, Pakistan	[63]

A cross-sectional study was conducted in 451 birds including sparrow, hen, pigeon and decorative birds. It was found that 157 (34.8 %), species were diseased by single or other variety of gastrointestinal parasites. In these species, there was no any trematode species but identified two cestoda, five protozoan parasites and two nematode species in the samples. The bird parasites identified as *Raillietina spp.* (4.2 %) and *Eimeria spp.* (7.1 %) were the most widely recognized helminthes and protozoa individually. Hence, overall study of the birds, it was investigated that there were 12 (2.7 %) and 6 (1.3 %) have two and three diverse infections correspondingly. So, we can say that in birds, intestinal parasitic diseases are common in west Iran. Therefore, future investigations are required to decide to which degree the contaminations impact mortality and execution of the birds [37].

Molecular detection of fancy bird parasites

To establish the relationships among isolates, molecular techniques were introduced for the presence and characterization of parasites after the method of choice i.e. light microscopy. For the isolation of trichomonads, a detailed study is required, which includes in vitro and in vivo analysis [38].

Occurrence and molecular classification of *Enterocytozoon bieneusi* and *Encephalitozoon spp.* in rabbits identified from inadequate epidemiology information. The total frequency of microsporidia syndrome existed as 24.8% through nested PCR targeting the Internal Transcribed Spacer (ITS) region of *E. bieneusi* and *Encephalitozoon spp.* individually. In the *Encephalitozoon* intestinalis (n = 16, 2.7%) and *Encephalitozoon* cuniculi (n = 34, 5.8%), it was found the most common species which was *E. bieneusi* (n = 90, 15.4%). In 0.9% rabbits, many infections were detected caused by *E. bieneusi* and *E. cuniculi*. Phylogenetic analysis was observed with the sequence of ITS region of *E. cuniculi*. These results provide initial information for observing microsporidia diseases in domestic rabbits and human beings [39].

After using a standard microscopy method, for the identification of *Trichomonas gallinae* infection in fancy pigeons, a pair of primers was designed i.e. TgF2/TgR2, which based on nuclear ribosomal DNA and used a molecular technique, which develop a PCR assay and their characteristics also identified by phylogenetic analysis. In this assay, it was detected a small amount of DNA which was only 15 pg. All the samples, which were positive *T. gallinae* in microscopic study, also identified positive in PCR assay then further confirmed by sequencing. Phylogenetic analysis and sequencing showed that positive samples of *T. gallinae* were identified as genotype B [40].

Madani & Peighambari, [41] described that in nested Polymerase Chain Reaction (PCR), 32 (12.6%) samples were positive for *Chlamydia psittaci* by gene (ompA) DNA using CTU/CTL primers and AluI restriction enzyme. In this study, total four restriction patterns were determined. Seven specimens totally resembled with the consequences of PCR-restricted fragment length polymorphism by the restricted sequencing of the ompA gene and also affirmed the existence of genotypes A and B and the two different impermanent genotypes I and J. *Chlamydia psittaci* and *Chlamydia abortus* were very closest with these new genotypes but from evolutionary point of view, specially genotype J was intermediate among *C. psittaci* and *C. abortus* [41].

Due to the shortage of *H. dispar* arrangements, the phylogenetic relations among heterakids were not clear for a long time. The molecular data for *H. dispar* was examined with homological sequences by the restricted 18S rRNA gene and region ITS1-5.8SrRNA-ITS2. As, PCR (18S rRNA) product of *H. dispar* was about 800 bp, and PCR (ITS-5.8S-ITS2) product was approximately 920 bp, unusually smaller size paralleled to H. *gallinarum* product. The examination of BLAST of *H. dispar* 18S sequence indicated a 99% resemblance with the arrangements of *Heterakis gallinarum* and *Ascaridia galli*, A. nymphii (98%), while the sequence of *Heterakis* sp. was 94% (Bobrek et al, 2019)[42]. Phylogenetic analysis shows that the initial effort at the renewal of relations in this superfamily Heterakoidea which is based on 18S rDNA and ITS portion [43].

Rarely, in the non-psittacine birds, a small, non-enveloped, single stranded DNA viruse is present i.e. Circoviruses which was characterized at molecular level as in nested Polymerase Chain Reaction (nested-PCR) for the detection of rep gene of circoviruses. Then different varieties of circoviruses were isolated in pigeon samples [44].

From all birds, samples of brain, cardiac muscles and skeletal muscle were tested by the molecular technique i.e. PCR which targeting a small portion of the gene encoding a minor ribosomal unit (nPCR-18Sa). As a result of both skuas only two samples were positive by nPCR which shows closely related to homologous sequences. To identify the prevalence of disease and its influence on the health of aquatic wildlife, more studies required to isolate, identify and detect these parasites [45].

Analysis of *Leucocytozoon spp.* was done by the PCR and sequencing were more complex as a result of coinfection by two closely related haemosporidians i.e. *Haemoproteus spp.* and *Plasmodium spp.* In this study, it was investigated the outburst of black flies or association of haemosporodians and molecular identification done in both blood parasites and black flies [46]. Avian blood parasites, like *Plasmodium spp.* also, *Haemoproteus spp.*, were discovered globally and transferred by biting [47]. However just restricted data about the existence is accessible in the Republic of Korea (ROK) by PCR. Blood specimens were gathered from 118 wild birds of 27 species in the Chonbuk Province, ROK. However, 53 (45%) were affirmative by PCR focusing with the cytochrome b gene but using microscopic analysis of blood smears only 43 (36%) were positive for avian haemosporidia [48].

Through sequencing of PCR amplicons, 6 (11%) were distinguished i.e. *Plasmodium spp.* and 47 (89%) as *Haemoproteus spp.* Phylogenetic examination utilizing cytochrome b gene discovered that inhabitant and transient birds have very much like hereditary genealogies of the two parasites in ROK, offering the probability in traveler birds that may go about just like a middle person for the parasite between Asian nations [49]. Molecular screening of blood specimens from 109 entities by PCR discovered that indigenous source of disease is present by showing 6% of the examined birds were positive for malarial parasites [50].

In avian populations the family of protozoa i.e. *Haemoproteus* occurs enormously, normally found in the fringe blood of hosts from anyplace on the earth. By using molecular technique (PCR), the occurrence of *Haemoproteus columbae* was found in Iranian pigeons. The prevalence rate of *Haemoproteus columbae* was 23.18% (51/120) in this study [51].

Fancy pigeons which were infected with PiCV (circovirus) could be more sensitive for sever infections of respiratory and digestive tract. Young Pigeon Disease Syndrome (YPDS) has been closely linked with PiCV infection and categorized by high level of genetic recombination and positive selection, which play an important role in the evolution. Against PiCV infection, vaccines are not yet developed. For identifying anti-PiCV antibodies, some recombinant capsid proteins have been found which can be used in the manufacture of diagnostic experiments [52].

Conclusion

The present study confirmed our hypothesis: "occurrence of *ecto*- and *endoparasites*" in various fancy bird species exists in huge number, which causes sever health hazards and impact on human health across the world as well as in Pakistan. Strategies should be devised to regulate the rearing of fancy birds in controlled environment and bird keepers. Parasite control regimes should be strictly followed to avoid economic losses incurred both in birds and their fanciers.

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