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First analysis of beak and feather disease of psittacine in Nineveh province, Iraq

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Abstract

Parrots are susceptible to the viral infection known as Beak and Feather Disease of the Psittacine (PBFD). The causative virus belongs to the *Circoviridae* family under taxonomic genus *Circovirus*. In the province of Nineveh, no previous research has found Circovirus in parrots. Therefore, the purpose of this investigation was to ascertain whether the virus is present in parrots. using cytopathology, histopathology, PCR techniques and genetic sequence from feather follicles. The results showed that 47% of the parrot birds were infected with the disease, which showed brittleness of feathers, loss of its weight, feather blade shattering from its follicles, and the cytological and histological diagnosis showed the presence mixed cell inflammation and cytoplasmic inclusion bodies with atrophy of the feather follicles with necrosis and degeneration in the liver cells. Circovirus was detected by PCR with 717 bp. Targeting VP1 capsid genes from 20 positive samples were sequenced and documented in the GenBank-NCBI under accession numbers of OQ925390 and OQ925391. The phylogenetic tree diagram reveals that the sequences of the VP1 capsid gene has identical to 100% genetic match with the strains utilized Poland and Brazil fixed in the Gen Bank.

DOI: 10.33899/ijvs.2023.141118.3104, @Authors, 2024, College of Veterinary Medicine, University of Mosul. This is an open access article under the CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0/).

Introduction

Psittacine beak and feather disease (PBFD), a viral disease, affects all parrots from the Old World and the New World (1). Etiological agent, is a taxonomic member of the Circoviridae family that affects chicken, pigeons, and other birds in addition to poultry (2). which causes PBFD has a genomic size of between 1992 and 2018 nucleotides. It has a diameter of 14-16 nm and is a circular or icosahedral singlestranded DNA virus (3,4). The BFDV is currently assumed relating to the Circoviridae family. Identical to circoviruses The BFDV virus is protected by an icosahedral, spherical, non-enveloped virion with a tiny, circular single-stranded DNA (ssDNA) genome that is approximately 2.0 kb in size (5-7). In order to access the host cell's transcriptional machinery, BFDV targets the nucleus (8,9). There are numerous tissues known to be BFDV replication sites, including the epidermis, liver, gastrointestinal tract, and bursa of Fabricius (10,11). Despite the fact that the BFDV capsid antigen is present in the spleen, thymus, thyroid, parathyroid, and bone marrow (12). Since Circo virus has existed in Australasia for at least 10 million years, it is the major viral infection of Psittaciformes there (13), and Australia has a reputation for being the virus's most likely source (14). Currently, the BFDV has been identified in over 78 psittacine bird species throughout the world, including around 25 non-psittacine bird species, as well as no less than 38 of the 50 native parrot species of Australia, within captivity and in the wild (15,16). BFDV is unlikely to be transmitted vertically, despite the fact that the importance of the vertical spread of the avian circovirus has been debatably discussed in the literature, as viral DNA may be found in chicken embryos from infected birds (17). The illness manifests as an immunological syndrome with symmetrical, permanent both beak and claw loss and feather loss malformations, ultimately resulting in death (18). Per acutely, varying from rapid mortality, especially in newborns (19), refers to a short-lived stage in nestlings and fledglings marked by feather dystrophy, diarrhea, weakness, and despair that ultimately results in death within 1-2 weeks (20). The lack of immunity brought on by a PBFD viral infection typically results in secondary viral, fungal, bacterial, or parasitic diseases (21,22). The majority of other clinical signs and symptoms, such as PBFD viral infections are not necessarily the cause of increased white blood cell counts, which are caused by secondary infections (23). Histopathology can be used to examine basophilic intranuclear and intracytoplasmic inclusion bodies to diagnose PBFDV infection, however viral DNA detection is required for a definitive diagnosis (24).

This study aimed to determine the disease prevalence, symptoms, cytological, histopathological changes and to identify the genetic makeup and pattern of the virus that causes the circus infection in parrot birds in the Iraqi city of Mosul.

Materials and methods

Ethical approve

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of College of Veterinary Medicine at University of Mosul in ethical approval code UM.VET.2021.068 in 3/10/2021.

Study birds of parrot

A forty-two psittacine transferred to a laboratory for poultry diseases in the Veterinary Teaching Hospital belong to College of Veterinary Medicine, University of Mosul. This study was conducted between November 2021 and November 2022 with a variety of clinical manifestation based on parrot health condition.

Clinical examination

The parrot birds were clinically examined and the morbidity rate has been determined according to the following formula

Morbidity rate = infected parrot/total*100.

Sample collection

After euthanasia of infected Parrot, the swab was been taken from root of feather, skin, bursa of Fabricia and liver, in order to prepare for histopathology examined (25), then swabs were taken from the roots of the feathers and prints

from the liver, and they were prepared for staining with May-Grunwald Giemsa stain (26). Finally partial pulp of feather kept samples were immediately frozen at -20°C for further molecular assessment.

Viral DNA extraction

Viral DNA was extracted using Add Bio's (Korea) AddPrep Viral Nucleic Acid Extraction Kit in accordance with the manufacturer's instructions.to take samples of feathers and extract the DNA of the beak and feather disease virus. Affected feather samples weighing 20 mg were broken up into little pieces and put in a 1.5 ml microcentrifuge tube. Next, 250 µl of nuclease-free water was added, ground using a special pestle for 15 seconds, and then the tube was centrifuged at 13,000 rpm for 30 seconds. After that, 350 µl of Lysis Solution and 200 µl of the supernatant were combined, and 3.5 µl of -mercaptoethanol was added. The mixture was then thoroughly mixed by pulse vortexing for 15 seconds. The lysate was then mixed well by pulse vortexing for 15 seconds with 150 µl of isopropanol added. The flow-through was once more discarded, and 500 µl of Washing 2 Solution was added to the spin column before centrifuging it for 1 minute at 13,000 rpm. The spin column was thereafter transferred to the fresh 1.5 ml microcentrifuge tube, 100 µl of Elution Solution was then added, and the mixture was allowed to sit for at least one minute. Centrifugation at 13,000 rpm for one minute was employed to elute the viral nucleic acid, which was then kept at -20°C until use.

Polymerase chain reaction

Polymerase chain reaction was performed using specific primers as in table 1 (27). The primers were obtained from (Macrogen Co, Korea). The VP1 capsid gene was amplified using PCR. Amplification was performed under the following circumstances utilizing a Bio-Rad thermocycler (Bio-Rad, USA): one cycle lasting 10 minutes at 95°C, followed by 35 cycles at 95°C for 45 seconds, 60°C for 45 seconds, and 72°C for 45 seconds. A 20- µ1 PCR reaction containing 2 µl of DNA and 10 l of master mix (2x conc., AddBio Inc., South Korea) were used in the reaction. The last extension was then programmed for a cycle at 72°C for 7 minutes. Before The processes were cooled to 4°C before gel electrophoresis could start. The amplified products were verified in a 1.5% agarose gel prepared with 1x Tris-borate-EDTA buffer and colored with a secure red DNA coloring solution (GeNetBio, South Korea).

Table 1: Primer sequences utilized for amplification of beak and feather disease virus

Primer Name	Primer Sequence 5' - 3'	Size (bp)	Reference
BFDV-F	AACCCTACAGACGGCGAG	717	27
BFDV-R	GTCACAGTCCTCCTTGTACC	/1/	21

The PCR reaction mixtures were prepared using HS Prime Taq Premix (2X) (AddBio, Korea). The PCR was carried out using a thermal cycler (T100 BioRad, USA). The PCR cocktail mixes were made in 20 l comprising a final concentration of 1X Taq Master Mix, 1 M of each primer, and 2 l of DNA template (2 ng/l). The cycling conditions including temperature and time required 35 cycles of polymerase activation for 10 minutes at 95°C, followed by 45 seconds of denature at 60°C, one minute of extension at 72°C, and a final five minutes of extension at 72°C.

Electrophoresis of agarose gels and documentation

Electrophoresis in divide the amplified products, 1.5% agarose gel electrophoresis was used and assess the quality of the recovered DNA. Each PCR result was placed into the agarose gel's well in a volume of 5 l. The electrophoresis was performed using an electrophoresis tank from BioRad in the United States that contained 1X TBE buffer and a power source from MP 300V for 1 hour at 80 V. The standard molecular weight marker was a 5 μl (AddBio, Korea) 100 bp DNA marker.

Results

Clinical examination

The clinical examination show only 20 from 42 psittacine infected at morbidity rate 47% exhibit clinical signs which are PBFD loss of appetite, dehydration, depression, cachexia, dystrophic of primary and secondary feather, infected psittacine bird revealed zonal red discoloration contour feather in African grey parrot (Figure 1), parrot of budgerigar bird showed diffuse abnormal feather development with abnormal development and delamination of beak as well as loss of feather in wing, breast and flank (Figure 2). The main pathological lesions of diseases were classified according to the age and descriptions in (Table 2).

Cytological assay

Cytological examination of feather pulp revealed presence of mixed cell inflammation, aseptic cell inflammation with inclusion bodies of botryoid Intracytoplasmic inclusion body (Figures 3 and 4) from liver impression the cytological examination shows presence of mixed inflammation, heterophilic inflammation with inclusion bodies (Figures 5 and 6).

The microscopic examination of cytological responses can classify as depending on posture in noted that most of the samples collected from parrot birds were found in a state of condition mixed cell inflammation ten cases, four cases of inflammation of lymphoplasmocytic inflammation, also aseptic inflammation (Table 3).

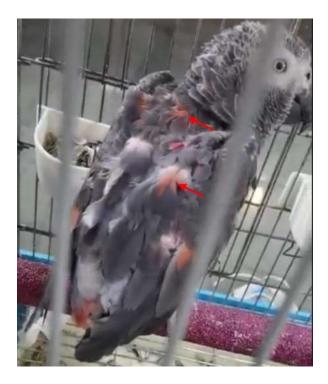


Figure 1: African grey parrot (*Psittacus erithacus*) zonal red discoloration contour feather that is typically of PBFD (red arrow).



Figure 2: Budgriger bird (*Melopsittacus undalatus*) showed symmetric feather dystrophy and loss in wing, breast and flank feather (black arrow).

Table 2: Categories of pathological lesions of feather and ages of parrot

Age	Number infected	Types of lesion description in feather	Morbidity
3 months	5	Annular constriction base of feather dysplasia with hemorrhage, pulpitis	25%
6 months	3	Severe dystrophy of wing, flank feather	15%
9 months	10	Pulpitis with feather destructive, hyperkeratotic sheaths	
1-1.5 year	2	Thick black pin feather on pectoral area, pulp hemorrhage	10%

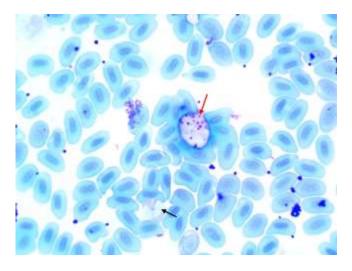


Figure 3: Feather pulp swap of budgriger bird (*Melopsittacus undalatus*) showed aseptic cell inflammation (red arrow) with inclusion bodies (black arrow) (MGG,1000X).

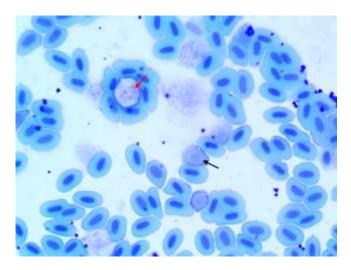


Figure 4: Feather pulp swap of budgriger bird (*Melopsittacus undalatus*) showed mixed cell inflammation (red arrow) with inclusion bodies (black arrow). (MGG,1000X).

Histopathology

The histopathological examination of PBFD samples in Budgerigar birds revealed small follicles with severe lymphocyte depletion and cluster of cell contain large botryoid inclusion bodies in bursa of Fabricia (Figure 7), the histopathological changes occurred from infection PBFD especially of skin revealed intracytoplasmic botryoid inclusion bodies, epidermal collar necrosis (Figures 8 and 9) the liver showed necrosis, acute hemolysis with presence of intracytoplasmic botryoid inclusion bodies (Figure 10).

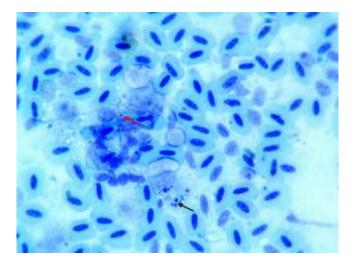


Figure 5: Liver impression of budgriger bird (*Melopsittacus undalatus*) showed mixed cell inflammation (red arrow) with inclusion bodies (black arrow). (MGG,1000X).

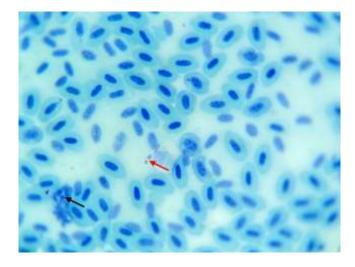


Figure 6: Liver impression of budgriger bird (*Melopsittacus undalatus*) showed aseptic cell inflammation (black arrow) with inclusion bodies (red arrow). (MGG,1000X).

Table 3: Categories of cytological responses

Sample no.	Inflammatory posture	Cytological diagnosis	
4	50 =heterophil with degenerative Phagocyte by leukocyte with most bacteria called phagosomes Squamous cell hyperplasia	Septic inflammation Variable amount of keratinous debris Lymphoid hematopoietic tissue	
10	50%> lymphocyte and heterophil Squamous cell hyperplasia and metaplasia	Mixed cell inflammation Intracytoplasmic inclusion bodies Keratinous debris Large number of uniform squamous epithelial cell Keratinized squamous epithelium	
2	>80% phagocytosis cellular with degenerative of heterophil Squamous cell hyperplasia	Heterophilic inflammation Intracytoplasmic inclusion bodies Keratinized squamous epithelium	
4	50%> multinucleated cell, macrophage, lymphocyte and heterophil Squamous cell hyperplasia and metaplasia	Lymphoplasmacytic inflammation Intracytoplasmic inclusion bodies Hyperplastic squamous epithelial cell Variable number of granulocytes	

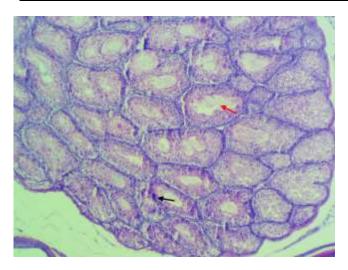


Figure 7: Bursa of Fabricia of budgerigar bird (*Melopsittacus undalatus*) with PBFD showed small follicle atrophied and lymphocyte depletion (red arrow), a cluster of cells contain large batryoid inclusion with macrophage (black arrow) (H& E.,10X).

Molecular analysis of PBFDv

PCR analysis reveal only 10 samples from 20 parrot forms are positive result for virus in 2 cases of African grey, one case of parakeet and seven of budgerigar bird for detection of viral nucleic acid in feather and blood. The diagnosed positive cases derived from psittacine forms to diagnosed PBFDv. The analysis of the gel revealed the presence of amplicons with a size of 717 bp (Figure 11).

DNA sequencing and sequencing of PBFDV

Phylogenic analysis of 2 positive samples based on VP1 capsid gene showed that the local under the accession

numbers of OQ925390 and OQ925391 reported a close-relationship to NCBI-BLAST by 100 % in strain VP1 capsid gene MW917239, ON502956, OL362209, MN175611 and MW174168 of Brazil strains, other close-relationship by 99% in strain MW917240 and by 97 % in strain ON502953 of Brazil strain. Which are in Poland also reported a close-relationship to NCBI-BLAST by 100 % in strain VP1 capsid gene JX221023, JX221022, JX221005, JX221017 and JX221020 strains (Figure 12).



Figure 8: Feather follicle budgerigar bird (*Melopsittacus undalatus*) with PBFD showed epidermal collar necrosis leading to constriction of feather shaft severe of intra cytoplasmic botryoid inclusion (red arrow) (H& E.,40X).

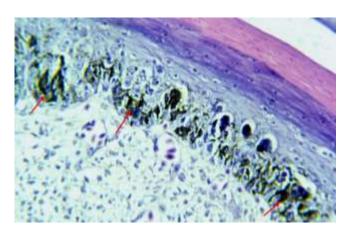


Figure 9: Feather follicle budgerigar bird (*Melopsittacus undalatus*) with PBFD showed epidermal collar necrosis of pulp cavity with numerous Basophilic globular severe of intra cytoplasmic botryoid inclusion bodies (red arrow) (H& E.,40X).

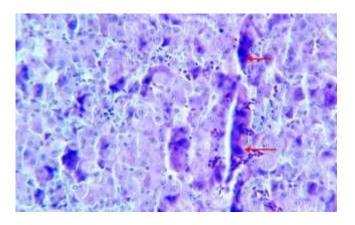


Figure 10: Liver from budgerigar bird (*Melopsittacus undalatus*) with PBFD showed small focal area of hepatocyte degeneration and necrosis, acute hemolysis, with presence of intracytoplasmic botryoid inclusion bodies (red arrow) (H& E.,40X).

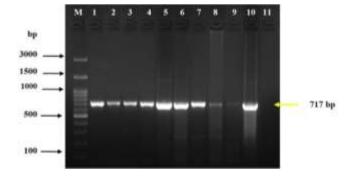


Figure 11: Beak and feather sickness viral polymerase chain reaction. DNA ladder of 100 bp, Lane M. The samples in lanes 1 through 10 are good. Positive control in lane 11.

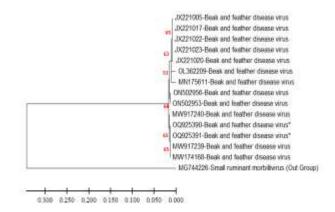


Figure 12: Phylogenetic tree of virus of beak and feather disease in Iraq (*). In MEGA11 software, the maximum likelihood technique based on the Tamura-Nei model and bootstrap analysis with 1000 resampling were used to create the phylogenic tree. As input data, incomplete fusion protein gene sequences with concatenated ends were employed, Gene bank accession numbers OQ925390 and OQ925391, host of Budgerigar in Iraq and world.

Discussion

The circovirus is most common viral disease in chicken, pigeon (28,29) and also very dangerous in other species of bird specially parrot (30,31). In this study infected of circovirus in parrot reveal of high morbidity in age of 9 month of age and 6-month age than in 3 month and one year which was observed from pests on the wings zonal red discoloration, symmetric feather dystrophy, pulpitis with feather destructive hyperkeratonics heaths (32-34).

Where found from this study in the cytological diagnosis of circovirus of the presence and observation of having a lot of samples mixes cell inflammation It is one of the indications of chronic infection from the virus with the distinctive sign of presence of intracytoplasmic inclusion bodies with keratinized squamous epithelium, This is in addition to being of lymphoplasmacytic inflammation which reveal Which indicates chronic infection of old age with the circovirus and it's all related to research (35,36). In this study, the pathological characteristics of the circovirus in microscopic diagnosis were found marked follicle atrophied of bursa of Fabricia, cluster of inclusion bodies with hepatocyte degeneration and necrosis, in addition to severe necrosis of epidermal to constriction of feather, pulp cavity and presence of large botryoid inclusion bodies, this was confirmed by Al-Noayme and Vucicevic (37,38).

There must be focus when founding of intracytoplasmic botryoid inclusions bodies suggest of PBFDv which epitheliotropic infections and follicles as well as with bursa of Fabricia and hepatocyte, that is reported as authors Vucicevic and Cunningham (38,39). In addition to cytology

and histopathology, the PCR technique was used to obtain DNA from 20 positive samples of feather pulp for the investigation of the nucleotide sequence and phylogeny of PBFDv. in which indicated in other authors Cunningham and Julian (39,40). That is the first description in this study isolated of circovirus in parrot in Iraq from budgerigar with sequencing tree and recorded in Gen bank accession number of two strain OQ925390 and OQ925391, these two strains are similar to countries such as Brazil and Poland (40,41). Absence of regulation the mandatory quarantine on import of parrot from countries that are not free from circovirus in parrot (42) because the dissemination and accelerated genetic diversification playing role in the pathological spread of strains among the countries of the world (43). There were specific bands may be due to binding of universal primer set targeting in Poland and Brazil (41,44). There are many factors that influence of circovirus diseases in parrot and mutation such as severe breeding of parrot, wild birds and trade international in exotic parrot (45).

Another interesting in this research not only for investigation or present of PBFDv but also indicated The effect of trade markets and interfaces of PBFDv spreading in Mosul between population city (46,47),therefore we need further studies with large number of psittacine forms in other species in different geographical regions to elaborate hosts and the incidence risk infections of PBFDv in Iraq because absence of regulate quarantine for import and exchange of pet birds all species from nearly countries that are present of PBFDv (48,49).

Conclusion

The disease was identified in this study for the first time in Nineveh, Iraq, by the characteristic pathological changes in the histological section and the signs that appear in the feathers. Several bodily organs, in particular the Fabricia bursa and the roots of the feathers, include botryoid inclusion bodies. Two novel strains were discovered for the first time in Nineveh, Iraq, and a strong correlation was found between them and strains from Poland and Brazil. Budgerigar parrot infection rates were also high, and other parrot species were widely affected.

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Conflict of interest

The content of this research study was not improperly influenced by the author's personal or financial relationships with any individuals or organizations.

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التحليل الأول لمرض المنقار والريش للببغاء في محافظة نينوى، العراق

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الخلاصة

الببغاوات عرضة للعدوى الفيروسية المعروفة باسم مرض منقار الببغاء والريش، الفيروس المسبب هو عضو في عائلة الفيروسات الدائرية تحت الجنس التصنيفي الفيروس الدائري. لم تكتشف أي دراسات

سابقة وجود الفيروس الحلقي في البيغاوات في محافظة نينوى. لذلك هدفت هذه الدراسة إلى التحقق من وجود الفيروس في البيغاوات باستخدام علم الأمراض الخلوي، وعلم الأنسجة، وتقنيات تفاعل البوليميراز المتسلسل، والتسلسل الجيني من بصيلات الريش، وأظهرت النتائج بصابة ٤٧٪ من طيور البيغاء بالمرض، مما أظهر هشاشة الريش، فقد جسدها وتحطم ريشها من بصيلاتها والتشخيص الخلوي والنسيجي أظهر وجود التهاب خلوي مختلط وأجسام متضمنة حشويه مع ضمور في بصيلات الريش مع تنخر وتنكس في خلايا الكبد. تم اكتشاف لفيروس الدائري بواسطة تقنيات تفاعل البوليميراز المتسلسل ٧١٧ نقطة أساس. تم تسلسل وتوثيق استهداف جين VP1 capsid من ٢٠ عينة إيجابية في بنك الجينات تحت أرقام الانضمام OQ925390 و OQ925391. يكشف مخطط شجرة النشوء والتطور أن تسلسل الجين VP1 مطابق ١٠٠٪