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DOI: https://doi.org/10.54393/mjz.v5i03.132

MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
ISSN (P): 2790-4377, (E): 2790-4385
Volume 5, Issue 3 (July-Sep 2024)

Technological Advances in Wildlife Monitoring and Conservation

OPEN ACCESS

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ARTICLE INFO

How to Cite:

Nawaz, A. (2024). Technological Advances in Wildlife Monitoring and Conservation. MARKHOR (The Journal of Zoology), 5(03), 01. https://doi.org/10.54393/mjz.v5i03.132

Wildlife as one of the major categories of research and ecosystem management within the ecosystems, have a key role in maintaining structure, function and balance of ecosystems. However, the wildlife, its interaction and associated processes with environment and ecosystem equilibrium have received inadequate focus due to the constraints of conventional tracking and detection techniques. But in recent years, the technology of wildlife monitoring has shown enhanced improvement and change with the advancement of automatical and information technologies into the science.

The significant emergence of AI in wildlife monitoring has particular importance as it helps to gather vast amount of data which can be used for computer vision and interpretation. Acoustic tracking be described as the identification and documentation of sounds produced by wildlife and their subsequent analysis for numerous purposes including the determination of species presence, distribution and behavior. The AI algorithms (Support Vector Machines (SVM), Random Forest (RF) classifiers) are capable of being trained over big data samples of audio recordings to accurately identify and categorize wildlife sounds including calls, songs and vocalizations [1].

Technologically, from early radio telemetry used in research to modern satellite tracking, more animals could be tracked in increasingly fathomable manners. Remote sensing with satellites is useful in developing complex ecological niches and predicting possible dangers to the animals. For example, satellite photographs have greatly assisted the identification and monitoring of illicit logging practices in rainforests and this has helped in interventions and formulation of wildlife protection and tracking strategies.

Also, recent tracking devices like GPS collars and satellite tags have enabled researchers to new outlooks in animal behavior and movement. Automated sensors, for example digital cameras and recording devices like microphones, can give high frequency of species recordation without influence on the species' behavior. Molecular tools like DNA barcoding and genomic sequencing are helping in understanding the genetic well-being of the wildlife species and assessing the effect of inbreeding and genetic drift going on in the species.

The advent of different thermal, optical and environmental sensors has helped in collecting information on wildlife habitat, and activities. The thermal infrared or TIR is very useful in tracking wildlife species since plants and animals emit light in TIR at their normal body temperature; this is the reason why, in contrast to the visual band they are detectable at night or in conditions of limited visibility.

In conclusion it was clearly evident that technological advances in monitoring wildlife and its conservation can make a great contribution to the traditional tracking methods. The future of wildlife preservation depends on capacity to strike a balance between creativity and sustainability by using technology not just to protect our natural habitats but also to reuse this in future.

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DOI: https://doi.org/10.54393/mjz.v5i03.119

MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
ISSN (P): 2790-4377, (E): 2790-4385
Volume 5, Issue 3 (July-Sep 2024)

Review article

OPEN ACCESS

Utilizing Medicinal Plants for Disease Treatment in Aquaculture: An Approach to Improve Fish Health

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ARTICLE INFO

Keywords:
Botanical Approaches, Medicinal Plants, Aquaculture, Fish health, Bioactive Compounds

How to cite:
Iqbal, T., Salma, U., Umair, M., Iqbal, H., Asmara, ., Khalid, T., & Hyder, S. (2024). Utilizing Medicinal Plants for Disease Treatment in Aquaculture: An Approach to Improve Fish Health: Medicinal Plants in Aquaculture. MARKHOR (The Journal of Zoology), 5(03). https://doi.org/10.54393/mjz.v5i03z.119

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Received Date: 15th August, 2024
Acceptance Date: 27th September, 2024
Published Date: 30th September, 2024

ABSTRACT

Botanicals were being used more and more in aquaculture to promote fish health as well as for disease prevention. In this review, we discuss the efficacy of botanicals in aquaculture worldwide and go through their modes of action that might play a key role in these activities. Similarly, some plants with well documented broad-spectrum antimicrobial, immunomodulatory activity, and antioxidant properties. These can be advantageous as supplements in fish feed to stimulate the immune function of fish. Plant extracts may exert positive effects on animal health through different modes rather than relying solely on a single mode. Using herbs as dietary additives has been shown to enhance immunity defense mechanisms. Recently, botanical treatments have been incorporated into aquaculture, resulting in increased growth rates and disease resistance, thus giving rise to more sustainable practices. Work was still being done in this area to find new bioactive compounds, understand how they work, and identify delivery systems that will ensure the compound reaches cells where needed. They can be incorporated with sustainable approaches, such as the aquaponics system, and possibly remain organic accredited, all while decreasing chemical residue on food products and sustaining environmental wellness. These emerging botanical approaches promise environmentally sustainable strategies for disease management in aquaculture, supporting the consumer shift to demand safe and sustainably produced seafood. The advantages that botanical treatments offer indicate them as essential tools for the development of a sustainable and eco-friendly aquaculture industry.

INTRODUCTION

Aquaculture is the farming of fish and aquatic plants as a crop that contributes to meeting global needs for seafood while also presenting an attractive alternative in response to overfishing. Medicinal plants that offer natural and environmentally sound ways to enhance fish health and manage diseases [1]. The use of medicinal plants to maintain the health of fish is a practice with firm cultural traditions spanning all five continents of the globe. Astragalus was used in traditional Chinese medicine, and neem and turmeric in India as a part of Ayurveda. Plants like *Vernonia amygdalina* and *Moringa oleifera* were employed for their antibacterial properties in Africa. Tribes of the South American Amazon used cat's claw and Lapacho for immune-enhancing and antimicrobial activities. Even in Medieval Europe, natural remedies like garlic and thyme were used for fish disease. All these old traditional practices have had significant contributions to the definition of modern botanical approaches in aquaculture research [2]. The use of medicinal plants in aquaculture has gradually grown from conventional practices through a systematic process into scientifically supported applications. In the early 20th century, research started

DOI: https://doi.org/10.54393/mjz.v5i03.119

identifying bioactive compounds in these plants as being antimicrobial, anti-inflammatory, and even immune-boosting. Since then, ethnobotanical surveys and phytochemical analyses have proved that such traditional methods are effective and have initiated the development of commercial botanical products tailored for use in aquaculture [3]. Common Diseases in Aquaculture: Bacterial Infections In Aquaculture: Bacterial infections constitute the major bottleneck in the fish farming enterprise, resulting in significant economic losses and adversely affecting fish production and fish health. Since the disease is highly transmissible, early detection and management are crucial. Aeromonas infections, caused mainly by *Aeromonas hydrophila*, affect several freshwater fish species, such as catfish, carp, and tilapia ulceration and abdominal distension are among the symptoms [4]. The *Vibrio* causes serious infections in marine fish like salmon, shrimp, and groupers, leading to skin lesions, ulcers, and high mortality rates. Therefore, despite the increased use of many antibiotics, including florfenicol, plant-derived alternatives to antibiotics are considered in bacterial diseases because of their antibacterial activities and hence offer sustainable options [5]. Columnaris disease is caused by *Flavobacterium columnare* and affects mostly catfish, carp, and tilapia. The symptoms include white or yellow lesions on the skin that ultimately lead to mortality. Traditional treatments include antibiotics such as potassium permanganate and oxytetracycline, although Indian almond leaves containing antibacterial tannins are a natural treatment to prevent bacteria from multiplying and assist in promoting fish health [6]. Streptococcosis, due to bacteria like *Streptococcus iniae* and *Streptococcus agalactiae,* afflicts warm-water fish, including tilapia and rainbow trout. Meningitis, exophthalmia, and erratic swimming are evident signs of the infection, which may be lethal if treated late. The use of medicinal plants, particularly thyme with potent antibacterial properties, is now being explored for such uses [7]. Infections with *Pseudomonas* and more specifically *Pseudomonas fluorescens* in freshwater fish, including trout, catfish, and carp, may lead to various symptoms, including lesions and fin rot. The control measure is that plant-based alternatives such as ginger extract is being valued for their antibacterial properties [8]. Botanicals in aquaculture consist of plant extracts and essential oils that possess antimicrobial properties to control bacterial infections, greatly reducing reliance on antibiotics and minimizing the risk of antibiotic-resistant strains. Moreover, numerous immunostimulants exist in medicinal plants that promote fish health and their disease resistance [9]. Parasitic Infestations in Aquaculture: The common health problems in fish, related to parasites, include slow growth rates and high mortality rates, which create a substantial impact on aquaculture. Organisms responsible for these diseases include protozoans, helminths, and crustaceans [10]. Ichthyophthiriasis, the disease produces white spots on the skin, gills, and fins labored respiration, and increased mucus production. Nevertheless, natural drugs that include garlic and neem extracts are currently being tested for eco-friendlier approaches in decreasing the intensity of this parasitic disease, therefore reducing synthetic chemical applications [11]. Trichodina species infect freshwater as well as marine fish, triggering copious amounts of mucus secretion with inflamed gills, frayed fins, and rapid respiration. Indian almond extracts, betel leaf extract, etc. Right now, oral use may start appearing as an alternative effective sustainable treatment in the years to come [12]. Monogenean parasites are often seen in freshwater fish such as carp goldfish and tilapia, causing gill rot, excessive production of mucus over the surface body wall along with flared opercula (gills), and erratic swimming behavior that could ultimately lead to death. Chemical treatments such as natural botanical solutions like pomegranate or garlic also have antiparasitic properties that can be used to maintain fish health [13]. The application of botanical treatments in aquaculture has a potential future as an environmentally friendly replacement for current practices used with other fish species [4]. Symptoms of nematode infestation include a swollen abdomen and visible worms in fish. Treatments include Anthelmintics like natural approaches are being investigated through garlic and wormwood [14]. It causes copepod infestations due to parasites such as *Argulus* and *Lernaea* species that live by eating the skin of fish like salmon, trout, and carp, resulting in sores on their skin and according to thin shed tissue with lots of mucus touching environmental substances. Nevertheless, natural extracts such as neem and turmeric are promoted for sustainable aquaculture management with less reliance on synthetic chemicals in controlling parasitic diseases [15]. Bioactive compounds reduce the use of synthetic chemicals for minor skin lesions and parasites, protecting. The environment is biodegradable and contributes to the health of fish. The use of these natural alternatives for fish health management is still on its way to being optimized, and ongoing against parasitic infestations that persistently trouble the aquaculture industry [16]. Fungal Infections in Aquaculture: Fungal infections are not ordinarily transmitted from fish to fish, but they can also be severely devastating. These infections should be called secondary; as primary effects are almost certainly necessary to cause sufficient damage for most bacterial pathogens [17]. Saprolegniasis, caused by fungi is mostly a problem in cool-water fish but occasionally can be associated with warm-water species as well. Affected fish show lesions and malaise a white plaque on the skin signifies infection. Treatment may be possible with botanical solutions for their anti-fungal properties [18]. Branchiomycosis,

DOI: https://doi.org/10.54393/mjz.v5i03.119

commonly known as Gill rot in fish is caused by *Branchiomyces parasitic* fungi. The infection can cause death, respiratory distress, and gill necrosis. Ways to reduce infection involve more benign uses, such as maintaining a proper balance of water quality, reducing organic matter, and treating the pond with copper sulfate. Recently, medicinal agents having antifungal action utilizing botanical spices like turmeric and neem extracts are being employed against infections in the infected fish [18]. The utilization of medicinal plants involved in these treatments could enhance the immune responses of fish and provide an earth-friendly alternative to chemical strategies for fungal conidia control [19]. Viral Diseases in Aquaculture: Viral diseases have a devastating impact on the aquaculture industry, leading to mortality and financial loss. Once viruses are established, they become some of the most elusive infections to cure, making prevention more crucial than ever [19]. Infectious Hematopoietic Necrosis is a potentially catastrophic disease caused by IHNV, most commonly affecting salmonids like salmon and trout. Affected fish exhibit white gills, a swollen abdomen, and high mortality rates, particularly in young fish. Although there are no successful antiviral treatments, prevention relies mainly on vaccination and good husbandry biosecurity practices. The efficacy of botanical drugs against viral infections, such as garlic and echinacea extracts [20, 21]. Infectious Pancreatic Necrosis (IPN) is caused by the IPNV virus, this disease affects salmonids, particularly juvenile fish. Symptoms include abdominal enlargement, darkened body coloration, and 100 percent mortality. As with IHN, there are no effective antiviral treatments, so prevention focuses on vaccination, biosecurity, and water quality management. Botanical treatments, possibly with immunostimulant properties to augment fish health, such as extracts from *Astragalus* or licorice, are under investigation [22]. Viral Hemorrhagic Septicemia (VHS) is caused by the VHSV virus, this disease can infect several species of freshwater, brackish water, and marine fish. Common signs of ill health include hemorrhages, bulging eyes, anemia, and high mortality. Because there are no commercially available effective antiviral treatments, control of VHS relies on biosecurity and more passive measures, including green tea and ginseng are two plant extracts that have been studied for their antiviral properties against VHSV [23]. Spring Viremia of Carp [SVC], with interference from the SVCV Virus, most commonly affects juvenile carp. Symptoms of infected fish include abdominal swelling, changes in skin color, high mortality, overall poor condition, and pop-eye. Similar to most viral diseases, control involves biosecurity, vaccination, and good management as there are no antivirals available that have demonstrated efficacy [24]. These treatments through botanicals may play a role in improving fish health and providing viral disease control. Further research is needed to fully investigate and utilize medicinal plants in the aquaculture industry [25].

Table 1: Plant Extracts Were Increasingly Being Used to Naturally Treat Bacterial, Fungal, and Parasitic Infections in Fish

S.No.	Plant Name	Constituents	Fish Disease Name	Type of Disease	Effect on Fish Body Part	Signs and Symptoms	Plant-Based Treatment	References
1	Garlic	Allicin, Sulfur Compounds	Aeromonas Infection	Bacterial	Skin and gills	Lesions, hemorrhages	Immersion baths with garlic extract	[26, 27]
2	Echinacea	Alkamides, Polysaccharides	Ichthyophthiriasis (Ich)	Parasitic	Skin, fins	White spots, lethargy	Feed supplementation with Echinacea powder	[28, 29]
3	Neem	Azadirachtin, Limonoids	Columnaris Disease	Bacterial	Skin, Mouth	Cottony Growth, Ulcers	Addition of neem leaf extract to fish feed	[30]
4	Turmeric	Curcuminoids	Aeromonas Infection	Bacterial	Skin, Internal Organs	Hemorrhages, Inflammation	Dietary Inclusion of Turmeric Powder	[31]
5	Ginger	Gingerol, Shogaol	Vibrio Infection	Bacterial	Digestive Tract	Bloating, Loss of Appetite	Immersion Baths with Ginger Extract	[32]
6	Aloe Vera	Polysaccharides, Anthraquinones	Flavobacterium Infection	Bacterial	Skin, Scales	Fin rot, Reddened Skin	Topical Application of Aloe Vera gel	[33]
7	Licorice	Glycyrrhizin, Flavonoids	Saprolegnia Infection	Fungal	Skin, Gills	White Cotton-like Growth, Lethargy	Bath Treatment with Licorice Extract	[34]
8	Basil	Eugenol, Linalool	Aeromonas Infection	Bacterial	Skin, Respiratory System	Lesions, Respiratory Distress	Feed Supplementation with Basil Powder	[35, 36]
9	Chamomile	Apigenin, Chamazulene	Trichodiniasis	Parasitic	Gills, Skin	Excess Mucus, Erratic Swimming	Immersion Baths with Chamomile tea	[37, 38]
10	Lemon Balm	Citronellal, Rosmarinic Acid	Ichthyophthiriasis (Ich)	Parasitic	Skin, Scales	White Spots, Rubbing Against Objects	Feed Incorporation of Lemon Balm Extract	[39]
11	Papaya	Papain, Chymopapain	Gill Flukes	Parasitic	Gills	Respiratory Distress, Excess Mucus	Bath Treatment with Papaya Leaf Extract	[40]
12	Thyme	Thymol, Carvacrol	Aeromonas Infection	Bacterial	Skin, Digestive Tract	Lesions, Bloating	Feed Supplementation with Thyme Oil	[41]
13	Sage	Rosmarinic acid, Thujone	Aeromonas Infection	Bacterial	Skin, Digestive Tract	Lesions, Digestive Issues	Feed Supplementation with Sage Extract	[42]
14	Rosemary	Rosmarinic Acid, Camphor	Vibrio Infection	Bacterial	Digestive Tract	Loss of Appetite, Internal Inflammation	Immersion Baths with Rosemary Extract	[42, 43]

DOI: https://doi.org/10.54393/mjz.v5i03.119

15	Peppermint	Menthol, Menthone	Ichthyophthiriasis (Ich)	Parasitic	Skin, Gills	White Spots, Irritation	Feed Incorporation of Peppermint Oil	[44, 45]
16	Ginseng	Ginsenosides, Polysaccharides	Saprolegnia Infection	Fungal	Skin, Scales	Cottony Growth, Lethargy	Topical Application of Ginseng Extract	[46, 47]
17	Oregano	Carvacrol, Thymol	Aeromonas Infection	Bacterial	Skin, Digestive Tract	Lesions, Digestive Issues	Feed Supplementation with Oregano Oil	[48]
18	Cinnamon	Cinnamaldehyde, Eugenol	Vibrio Infection	Bacterial	Digestive Tract	Loss of Appetite, Internal Inflammation	Immersion Baths with Cinnamon Extract	[49]
19	Dandelion	Taraxasterol, Flavonoids	Flavobacterium Infection	Bacterial	Skin, Gills	Fin Rot, Reddened Skin	Feed Supplementation with Dandelion Extract	[50]
20	Marigold	Lutein, Zeaxanthin	Ichthyophthiriasis (Ich)	Parasitic	Skin, Scales	White Spots, Irritation	Bath Treatment with Marigold Extract	[51]
21	Yarrow	Sesquiterpene Lactones, Flavonoids	Ichthyophthiriasis (Ich)	Parasitic	Skin, Gills	White Spots, Irritation	Immersion Baths with Yarrow Extract	[52, 53]
22	Wormwood	Artemisinin, Sesquiterpenes	Gill Flukes	Parasitic	Gills	Respiratory Distr-ess,Excess Mucus	Bath Treatment with Wormwood Extract	[54]

Medicinal Plants and Their Properties

Anti-Bacterial Plants in Aquaculture: Plant extracts can inhibit bacterial growth with strength similar to synthetic antibiotics and offer an environmentally friendly option as a natural antibacterial for aquaculture purposes [55]. Garlic has proven to possess potent antibacterial qualities largely because of its abundant influx of bioactive compounds such as allicin, ajoene, and diallyl sulfides. The production of allicin when garlic was crushed can suppress the growth and reduce mortality rates due to disease by bacteria such as Aeromonads hydrophilic and Vibrio species in fish [56]. Turmeric was rich in curcuminoids, while allicin and sulfur compounds present in garlic indicate a high antibacterial effect [57]. These natural ingredients can also be supplemented as nutraceuticals in fish feed or used as water-soluble extracts for improving immunity and other metabolic functions in fish [58]. Antiparasitic Plants in Aquaculture: In inference, plant-derived antiparasitic extracts offer a promising and safe new alternative to chemical treatments in aquaculture. This natural approach can reduce parasite burdens and increase fish health status as well as survival rates without environmental damage because they were not chemically synthesized [59]. Neem water extraction can be applied or mixed with fish feed and directly applied to the affected areas. Organic fungicide neem also has bacteriostatic and antifungal activity. Papaya seeds, particularly those containing papain and chymopapain, were highly regarded as anti-parasitic treatments for aquacultured livestock, especially against nematodes and tapeworms [60, 61]. Anti-Fungal Plants in Aquaculture: These plants not only work as a defense mechanism against fungus but also provide better conditions to increase fish health and productivity [62]. Ginger can be administered either as a liquid or mixed with fish feed. Regarding its advantages as an aquaculture feeding regime, it has broad-spectrum anti-inflammatory action and digestive properties. The essential oils, including eugenol and linalool, have given basil a reputation as an antifungal plant. These were strong fungicides against saprolegnia species' fish and egg pathogens [63]. Antiviral Plants in Aquaculture: The use of antiviral plants in aquaculture echinacea boosts the immune system, especially against cat herpes virus, and prevents viral replication. It can be administered as an extract or blended into fish feed, delivering benefits such as enhancing innate immunity and adaptation and reducing inflammation [64]. Licorice, another powerful medicinal herb, contains glycyrrhizin and flavonoids, which have effective antiviral properties such as herpes and influenza. It can be taken orally or diluted to inhibit virus replication and stimulate the defense mechanism [65].

CONCLUSIONS

Aquaculture, when combined with plant-based techniques, provides a unique and sustainable way to improve fish health, improve productivity, and reduce environmental impact. Research continues to show the therapeutic potential of plants was effective in the removal of pollutants and chemical stressors that clean up aquatic ecosystems, improving their overall health. Whether in salmon farming in Europe, or tilapia farming in Africa, these techniques continue to offer benefits such as increased disease resistance, improved growth, and reduced reliance on antibiotics. Aquaculture practices incorporating plant-derived products were more sustainable and resilient disease control strategies. As research expands and practices that can support the profession evolve, phytotherapy was poised to reshape the future of aquaculture worldwide.

Authors Contribution

Conceptualization: US
Methodology: TI
Formal analysis: TK
Writing, review and editing: TI, MU, HI, A, TK, SH, MMJ, MUG

All authors have read and agreed to the published version of the manuscript.

DOI: https://doi.org/10.54393/mjz.v5i03.119

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

This research has been partially funded by ORIC KMU as Master Thesis approved by ASRB

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DOI: https://doi.org/10.54393/mjz.v5i03.109

MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
ISSN (P): 2790-4377, (E): 2790-4385
Volume 5, Issue 3 (July-Sep 2024)

Original Article

OPEN ACCESS

Conservation Status and Biodiversity of *Pelecanus onocrotalus* (The Great white pelican bird) at Manchar Lake, District Jamshoro, Sindh, Pakistan

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ARTICLE INFO

Keywords:
Avifauna, Diversity, Latitude, Fluctuation, Resident, Species

How to cite:
Memon, Q., & Sheikh, K. (2024). Conservation Status and Biodiversity of *Pelecanus onocrotalus* (The Great white pelican bird) at Manchar Lake, District Jamshoro, Sindh, Pakistan: Conservation Status and Biodiversity of *Pelecanus onocrotalus* . MARKHOR (The Journal of Zoology), 5(03). https://doi.org/10.54393/mjz.v5i03.109

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Received Date: 14th May, 2024
Acceptance Date: 1st September, 2024
Published Date: 30th September, 2024

ABSTRACT

Diversity amongst birds occurs in so many forms, which captivates their appearances and peculiar distributions. Avifaunal species enhance the beauty of wetlands with their ecological and economic benefits. Manchar Lake is a wetland of much importance, as it serves as a distinguished habitat for a variety of bird species, many of these species might be yet unknown to the scientific community. **Objective:** To explore the distribution, diversity, and conservation status of the great white pelican (*Pelicanus onocrotalus*). For this purpose, South Asia's one of the largest natural lakes, Manchar located in Jamshoro, Sindh, Pakistan was investigated. **Methods:** *Pelecanus onocrotalus* was identified as a migratory species. Notably, a few specimens have been recorded for the first time all through the summer. According to the IUCN red list of endangered species, the conservation status of the great white pelican was recorded as least concern. The prevalence of the species was recorded highest from October to March and lowest from April to September. **Results:** A total of 50 specimens of *Pelecanus onocrotalus* (Great White Pelican) belonging to the genus Pelecanus and the family Pelecanidae were recorded. **Conclusions:** This fact-finding study aims to document the emergence of bird diversity and its association with the lake surroundings and other species. The research findings will be valuable for wildlife departments and future researchers as a useful literature resource.

INTRODUCTION

The great white pelican (*Pelecanus onocrotalus*) is a large water bird species known for its distinctive large beak with a pouch for catching fish. It is a migratory bird that breeds in Eastern Europe and Asia, and winters in Africa, the Middle East, and the Indian subcontinent [1]. *Pelecanus onocrotalus* primarily feeds upon fish, requiring between 0.9 to 1.4 kg of fish daily [2]. It often forages in groups, utilizing cooperative feeding techniques to corral fish, and can fly over 100 km in search of food [3]. In Pakistan, the great white pelican is found in various wetlands and lakes, including the Manchar Lake in Sindh province [4]. Wetlands provide essential habitats for a wide range of species, including many water birds, and play a vital role in ecological balance by supporting diverse plant and animal life [5-6]. Manchar Lake is the largest freshwater lake in Pakistan and one of the largest in Asia [7]. It is an important habitat for a diverse range of bird species, including migratory birds. The lake is oval-shaped and varies in size from 30 to 200 square miles depending on the season. It is a brackish water and semi-natural wetland that supports rich and diverse aquatic vegetation [8, 9]. The Manchar Lake regularly hosts over 20,000 water birds in winter and is a breeding and wintering area for a wide variety of water birds [10-12]. It is also an important roosting site for the Night Heron (*Nycticorax nycticorax*). However, the lake has faced severe pollution problems from toxic waste discharged through drains, which has reduced the number of migratory birds from 20,000-30,000 to much lower levels and made the water unsuitable for humans and fish [12]. Despite its ecological importance, there is limited research on the conservation status and biodiversity of the Great White Pelican and other bird species at Manchar

DOI: https://doi.org/10.54393/mjz.v5i03.109

Lake. A study conducted by WWF-Pakistan in 2011 recorded 128 bird species from the Manchar Lake area, including the great white pelican [13, 14]. The study also provided information on the conservation status and feeding habits of the recorded species [13]. Climate change leads to alterations in water bodies like lakes, rivers, and wetlands, which are crucial habitats for pelicans. Changes in precipitation patterns, increased evaporation rates, and the frequency of extreme weather events can reduce the availability and quality of these habitats [15]. Pelicans primarily feed on fish, and climate change can affect fish populations and distribution. Warmer water temperatures, altered water chemistry, and changes in aquatic vegetation due to climate change can impact fish breeding, leading to a decline in food sources for pelicans. Pelican breeding cycles and nesting success are closely linked to environmental conditions. Unpredictable weather patterns, such as unseasonal floods or droughts, can disrupt breeding seasons, leading to lower reproductive success and higher chick mortality rates [16]. Pelicans play a crucial ecological role in wetland ecosystems by acting as both predators and prey within the food web. They help control fish populations and contribute to nutrient cycling through their feeding habits and guano, which enriches the soil. Additionally, pelicans can influence the structure of aquatic communities by dispersing plant seeds and affecting the dynamics of other wildlife in their habitats. The population of water birds at Manchar Lake in Pakistan varies significantly between the summer and winter seasons. During the Asian Water-bird Census in 2000, 31,852. Water birds were recorded at the lake, dropping to only 1,183 birds by 2011-16. This drastic decline was attributed to heavy pollution impacts on the lake's water quality in recent years [17-20]. To ensure the long-term conservation of the Great White Pelican and other bird species at Manchar Lake, it is crucial to conduct regular monitoring and assessment of their populations and habitats. This information can help inform management decisions and guide conservation efforts to protect this important wetland ecosystem [13, 14]. Climate change leads to alterations in water bodies like lakes, rivers, and wetlands, which are crucial habitats for pelicans. Changes in precipitation patterns, increased evaporation rates, and the frequency of extreme weather events can reduce the availability and quality of these habitats. Pelicans primarily feed on fish, and climate change can affect fish populations and distribution. Warmer water temperatures, altered water chemistry, and changes in aquatic vegetation due to climate change can impact fish breeding, leading to a decline in food sources for pelicans. Pelican breeding cycles and nesting success are closely linked to environmental conditions. Unpredictable weather patterns, such as unseasonal floods or droughts, can disrupt breeding seasons, leading to lower reproductive success and higher chick mortality rates. include habitat restoration, pollution control, and community engagement to foster sustainable conservation practices [5].

METHODS

Manchar Lake is located approximately 18 kilometers from Sehwan town, at coordinates 67º 38 '39.46" East and 26º25'40.41" North, based on the global positioning system. Surveys were conducted over a period from May 2023 to April 2024. Observations took place daily, from early morning (06:30–09:30 hours) to late evening (17:30–19:00 hours), aiming to see the maximum number of white pelican birds (Pelecanus onocrotalus).

- **Equipment:** Binoculars: Bushnell, Model 133450.
- **Observers:** Each survey was conducted by two observers independently.
- **Coverage:** Observations were made within a 150-meter strip on each side of the lake's bank, making each survey strip 300 meters wide.
- **Observation Points:** A stationary point was selected to keep a continuous watch.
- **Observation Methods:** Counting Methods, Direct Counting.
- **Close View:** Individual birds were counted as 1, 2, 3, 4, 5, and 6, etc., using binoculars for a close view.
- **Distant View:** Flocks were visually divided into smaller groups and counted. Groups were summed to obtain the final count.
- **Multiples Counting:** Birds were counted in multiples such as 3, 6, 9, 12, 15, or 2, 4, 6, 8, 10, etc., depending on their distribution. This method was applied to both evenly and unevenly distributed water-birds.
- **Flight Counting:** Birds in flight along the coast moving to roost sites were counted repetitively in large flocks.
- **Data Analysis:** Shannon-Weaver Diversity Index.

The Shannon-Weaver diversity index (also known as Shannon-Wiener index or Shannon index) was used to determine the distribution and biodiversity of white pelicans in the ecosystem. The steps involved were:

- **Selection of Sample Population:** A sample population of white pelicans was selected within the chosen area.
- **Species Count:** The species were counted within the sample population.
- **Richness Assessment:** The richness of species in the population was assessed to calculate the Shannon index, an important measure of bird biodiversity. The methods and steps described were adapted from Howes, J. and Backwell, D. (1989) for accurate and consistent data collection on white pelican populations at Manchar Lake.

Let's use our sample data (1 species, 50 total individuals) and calculate D i.e. to calculate the Shannon-Weaver Diversity Index (H') for the winter count of Pelecanus

DOI: https://doi.org/10.54393/mjz.v5i03.109

onocrotalus, need to follow these steps:

- **Determine the proportion (pi) of each species:** Since we have only one species, the proportion (pi) will be 1.
- **Calculate the natural logarithm (ln) of each proportion (pi):** Again, since pi is 1, ln(pi) will be 0.
- **Multiply each pi by its ln (pi):** This will result in 0 for each species as ln(1) is 0.
- **Sum the results:** The sum of pi * ln (pi) will be the Shannon index (H').

$H' = -\sum(pi \times \ln(pi))$ H′=−∑(pi×ln(pi)

Since we have only one species (*Pelecanus onocrotalus*) and pi is 1, the Shannon index H' for this single-species count is: H′=−(1×0)=0H′=−(1×0)=0 Therefore, the Shannon-Weaver Diversity Index (H') for the winter count of *Pelecanus onocrotalus* is 0. This result is expected because the Shannon index is higher when there is greater species diversity, and in this case, there is only one species present. Shannon Diversity Index, ni refers to the number of individuals of species (Table 1).

Table 1: Shannon Index (Bird Diversity) For Winter Count

Species	*ni* (population size)	Pi	ln(pi)	pi * ln(pi)
Pelecanus onocrotalus	40	1	0	0

Methodology of observations:

1. **Direct Field Observations:** Observations were conducted in early morning and late evening, likely to coincide with peak bird activity, and counted birds using binoculars (10x50 magnifications).
2. **Capture Techniques:** Birds were captured for closer study using fishing nets. Bird sounds were recorded and analyzed to help in identification and counting. Local children assisted in capturing and possibly identifying birds, utilizing their familiarity with the area and the species.

Howes, J. and Backwell, D. (1989): This reference suggests a methodology focusing on systematic counting and observing, ensuring reliable data collection through standardized techniques. The use of binoculars aligns with general search of birds, it practices to reduce disturbance at the same time as it maximizes the identification of the bird in question. This method helped in accurate monitoring of *Pelecanus onocrotalus* at Manchar Lake, contributing valuable information to the ornithological research and conservation status. During study 25 specimens of the great white pelican bird from Manchar Lake have been captured and identified.

Laboratory Analysis and Identification

Duration of research: Captured specimens were kept under observation for a few weeks in the Vertebrate zoology laboratory to take a closer look of the detailed work.

Body parameters Measurement and identification: Measurement of body parameters were taken such as weight of body, length of body, length of wings, length of tail Length of Head, neck, and tail feathers, width of web, and other relevant morphological features.

Physical characters: Identification of distinctive characteristics was done, such as plumage patterns, coloration, and other relevant traits that would aid further details in species identification.

Morphological parameters: Study of external structures adding further details in the identification of species such as; shape of feathers, eye coloration, leg coloration and length was done.

Species Identification Keys: Identification keys have been prepared by using international literature, ensuring accuracy and consistency. The references encompass: Harrison, 1966; pioneering work on chook identity and category. Boyd, 1987; contributions to ornithological studies and species differentiation. Dunning, 1992; Reference on avian frame measurements. Ali, 1993; Comprehensive guide on Indian birds, which include migratory species. Jonsson, 1996; Field guide specializing in hen identification. Rookmaaker & Pieters, 2000; Studies on bird morphology and taxonomy. Clements, 2007; renowned checklist of bird species. McCaffery *et al.*, 2010: Research on bird populations and their habitats. Bird Life International, 2019: Authoritative resource on bird conservation and species status. Jobling, 2010: Dictionary of bird names and their meanings. Amat *et al.*, 2011: Study on the behavior and ecology of water birds. Hancock & Kushlan, 2010: Detailed work on herons, which share habitats with pelicans.

RESULTS

The conservation status and biodiversity of *Pelecanus onocrotalus* at Manchar Lake likely involved several methodologies based on common practices in ecological and ornithological studies. Systematic surveys were conducted to count the population of Great White Pelicans and other water birds at Manchar Lake. This involved point counts, transect surveys, or aerial surveys to estimate bird populations and distribution. Evaluations of habitat quality, including water levels, vegetation cover, and food availability, were conducted to understand the environmental conditions affecting pelican populations. Studies on the white pelican birds at Manchar Lake, conducted from May 2023 to April 2024, utilized direct field observations based on the methodology of Howes and Backwell-1989. The huge occurrence of *Pelecanus onocrotalus* (The Great White Pelican) at Manchar Lake highlights the importance of this habitat not only as a permanent residence but also as a migratory refuge (Figure 2), belonging to family pelecanidae. Measurement of body parameters recorded are as: Body Length 140 to 182 cm (55

DOI: https://doi.org/10.54393/mjz.v5i03.109

to 72 in); Bill Yellow, measuring 29 to 36 cm (11.5 to 18.5 in); gular pouch is dull to pale yellow; Wingspan length 226 to 360 cm (7 ft 5 in to 11 ft 10 in); Weight: 8 to 16 kg (20 to 33 lb); Sexual dimorphism was seen. Female pelican having Bill Length of 28.9 to 40.0 cm (11.4 to 15.7 in); Body Length about 147 cm (55 in); Weight was recorded 5.5 to 9.5 kg (12 to 21 lb); Plumage was predominantly white with a yellowish base on the fore neck and a faint pink tinge on the neck; Primary Feathers were black, with white shafts at the bases and occasionally paler tips and narrow fringes; Secondary feathers were black with a whitish fringe.24. Legs and Feet were fleshy-yellow legs; short, strong legs and webbed feet facilitate aquatic life. It was observed during study that this bird, the great white pelican is well- adjusted for aquatic environments, by using its strong webbed feet it propels through water and also assists in powerful takeoff. During the powerful flight *Pelecanus onocrotalus,* holds its head near and aligned with the body for the maximum duration of flight. The breeding sites of the great white pelican are South Eastern Europe through Asia and Africa, commonly in swamps and shallow lakes. Figure 1 shows the scientific key used to identify the species in the field which includes four steps. Pelicans are large birds, typically characterized by their long necks, stout bodies, and large wingspans. The size can vary significantly among species, with the Australian pelican being one of the largest, reaching up to 1.83 meters in length and a wingspan of about 3 meters-24. One of the most distinctive features of pelicans is their long, down curved bills and large gular pouches. The pouch is used for catching fish and can hold a significant amount of water. Note that the bill length can vary, with some species like the Australian pelican having bills that can grow up to 0.5 meters long-24. Most pelicans have predominantly light-colored plumage, although the brown and Peruvian pelicans are exceptions with darker feathers. During the breeding season, the bare facial skin and throat pouch of pelicans often change color, becoming more vibrant-24. Pelicans are typically found near water bodies such as lakes, rivers, and coastal areas. Observing the habitat can help narrow down the species, as different pelicans may prefer specific environments 24. By combining these keys—size and shape, bill characteristics, plumage and habitat you can effectively identified pelicans and distinguish between them as different species within the genus Pelecanus (Figure 1).

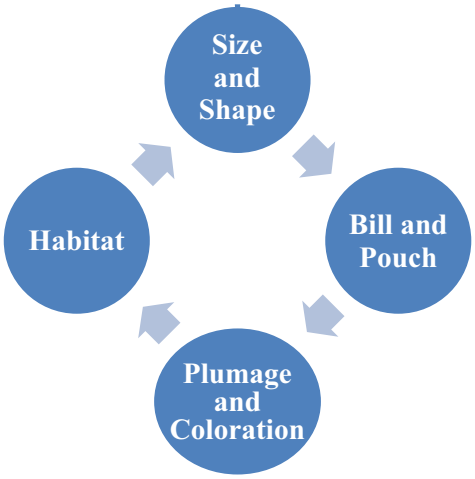

Figure 1: The Scientific Key

A total number of specimens collected that were fifty in total, out of which 10 specimens were collected in summer and forty were collected in the summer season (Table 2).

Table 2: Number of specimens collected during summer and winter season

Pelecani-formes	English name	Scientific name	Status	Occurrence	Count Summer /winter	
	White Pelican	*Pelecanus onocrotalus*	Winter visitor	Scarce	10	40

The prevalence of collected specimens in graphical form (Figure 2).

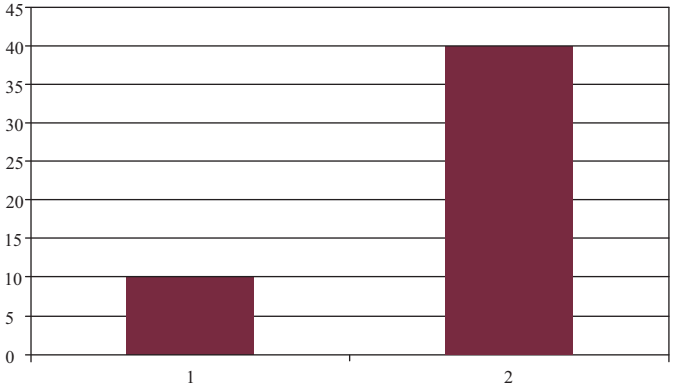

Figure 2: Prevalence of Collected Specimens in Summer and Winter

Detailed information on the white pelican bird (Table 3).

Table 3: Detailed account of Great white pelican bird

Scientific Name	*Pelecanus onocrotalus*
Common Name	White pelican
Family	Pelecanidae
Order	Pelecaniformes
Status on IUCN Red List	Least concern
Status as Migratory/Resident	Migratory
Diet	Carnivorous

The availability of great white pelicans at Manchar Lake during the winter and summer seasons (Figure 3).

DOI: https://doi.org/10.54393/mjz.v5i03.109

Figure 3: Occurrence of Pelican Birds at Study Area (Manchar Lake)

DISCUSSION

The study on the conservation status and biodiversity of *Pelecanus onocrotalus* (Great White Pelican) at Manchar Lake in Sindh, Pakistan, provides valuable insights into the ecological importance of this wetland ecosystem. [7,10,13,14] Manchar Lake is a significant habitat for a wide variety of water birds, including the Great White Pelican, which uses the lake as a breeding and wintering area. The lake regularly supports over 20,000 water birds during the winter season, making it globally important site for avifaunal biodiversity [5-6]. The study highlights the importance of Manchar Lake as a roosting site for the Great White Pelican. The presence of these birds indicates the lake's ability to provide suitable habitats and resources for their survival and reproduction. However, the study also identifies several threats to the biodiversity of Manchar Lake, including water diversion for rice cultivation, which has led to a decrease in the lake's size in recent years [3, 11]. Additionally, livestock grazing and the cutting of shrubs for fuel purpose challenges to the conservation of the lake's ecosystem [4]. To address these threats and ensure the long-term protection of *Pelecanus onocrotalus* and other avian species, the study emphasizes the need for effective management strategies and conservation measures. These may include regulating water usage for agriculture, controlling livestock grazing, and promoting sustainable practices in the surrounding areas [15]. Furthermore, the study emphasizes the importance of continuous monitoring and research to understand the trends in species composition and abundance at Manchar Lake. Satellite remote sensing and GIS techniques can be valuable tools for studying the spatial distribution of bird species and tracking changes in the lake's land cover over time [18]. In conclusion, the study on the conservation status and biodiversity of Pelecanus onocrotalus at Manchar Lake highlights the ecological significance of this wetland ecosystem and the need for comprehensive conservation efforts to protect the Great White Pelican and other avian species that rely on it [19]. By addressing the identified threats and implementing sustainable management practices, stakeholders can ensure the long-term preservation of Manchar Lake's rich biodiversity.

CONCLUSIONS

This comprehensive and rigorous approach ensured that the birds were thoroughly studied, with meticulous attention to detail in measuring and identifying morphological characteristics. The use of established international literature provided a robust framework for accurate species identification and furthered the scientific understanding of the white pelican population at Manchar Lake. It is a significant habitat for a wide variety of resident and migratory birds including the white pelican, which is an economically important species for the region. The liver oil of the white pelican is utilized for treating joint pain, and its flesh is highly valued for its medicinal properties, commanding high prices in the market. Despite these uses, the white pelican is classified as Least Concern (LC) according to conservation status. The lake supports a rich diversity of avifaunal species, encompassing both carnivorous and omnivorous birds.

Authors Contribution

Conceptualization: QM
Methodology: QM, KS
Formal analysis: QM, KS
Writing-review and editing: QM, KS

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

This research has been partially funded by ORIC KMU as Master Thesis approved by ASRB

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DOI: https://doi.org/10.54393/mjz.v5i03.128

MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
ISSN (P): 2790-4377, (E): 2790-4385
Volume 5, Issue 3 (July-Sep 2024)

Original Article

OPEN ACCESS

Antibacterial Efficacy of Pure Aloe Vera, Methanol Extract and Gentamicin Against Pathogenic Bacteria

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ARTICLE INFO

Keywords:
Aloe Vera, Antibacterial, Extract, Gentamicin

How to cite:
Keerio, R. A., Bughio, S., Buriro, R., Bilawal, M., Jamali, M. A., Tariq, M., Jamali, I., Jamali, N. A., & Jamali, F. (2024). Antibacterial Efficacy of Pure Aloe Vera, Methanol Extract and Gentamicin Against Pathogenic Bacteria: Antibacterial Efficacy of Pure Aloe Vera Against Pathogenic Bacteria. MARKHOR (The Journal of Zoology), 5(03), 17-21. https://doi.org/10.54393/mjz.v5i03.128

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Received date: 7th August, 2024
Acceptance date: 25th September, 2024
Published date: 30th September, 2024

ABSTRACT

The Aloe vera plant has been utilized for thousands of years for traditional medicinal reasons, including the prevention of bacterial development. **Objectives:** To investigate the antibacterial effectiveness of pure Aloe vera and its methanol extract, comparing their efficacy to that of the commonly used antibiotic gentamicin. **Methods:** 50 broiler meat samples were collected from the butcher markets of Tandojam. Isolation and identification of the microorganisms, the minimum inhibitory concentration (MIC) was done on 96 well plates. For this purpose, similar concentrations of pure Aloe vera, its methanol extract and gentamicin were to evaluate their minimum inhibitory concentration against *Staphylococcus aureus* and *Escherichia coli*. The minimum inhibitory concentration was tested based on the transparency and turbidity of the medium. **Results:** Out of 50 meat samples identified 19 (38%) were found positive for *Staphylococcus aureus* and 24 (48%) were positive for *Escherichia coli*. The mean susceptibility value of *Staphylococcus aureus* was noticed at 10, and 20µg/µl for pure Aloe vera, 20, 10, 5µg/µl for methanol extract and 20, 10, 5, 2.5, 1.25µg/µl for gentamicin. While the mean concentration at which *Escherichia coli* growth inhabited was 20 µg/µl and 20, 10 µg/µl, and 20, 10, 5, 2.5, 1.25, 0.625, 0.312 µg/µl for pure Aloe vera, its methanol extract and gentamicin, respectively. **Conclusions:** It was concluded that all treatments i.e., pure Aloe vera, its methanol extract, and gentamicin inhibited the growth of isolated bacterial organisms, but the methanol extract stopped the growth of isolated organisms at lowered concentration in comparison to pure aloe vera.

INTRODUCTION

Poultry meat is believed to be the most common vehicle for the transmission of foodborne diseases, followed by red meat. S. *aureus* is a bacterium that causes several infections in animals including mastitis, arthritis, and urinary tract infections. Food poisoning caused by *Staphylococcus aureus* is the third most common cause of food-borne disease worldwide [1]. It is the pathogen's ability to produce enterotoxins that makes it deadly. This kind of food poisoning is most frequently associated with fresh and prepared meals, namely meat products [2]. *Escherichia coli (E. Coli)* has also important dissemination and association as a foodborne pathogen. This pathogen can cause intestinal and extra-intestinal infections. It has been reported that human foodborne illnesses are caused by the consumption of meat and meat products contaminated by *E. coli* [3]. On the other hand, *E. coli* is an indicator of fecal contamination in poultry meats. Some strains of *E. coli* are highly pathogenic in humans as well as in animals [4]. The Aloe vera plant has been utilized for thousands of years for traditional medicinal reasons, including the prevention of bacterial development [5]. It has been stated that *Aloe vera* gel has antibacterial action

DOI: https://doi.org/10.54393/mjz.v5i03.128

over gram-negative and gram-positive bacteria and that it has been shown to effectively kill or substantially decrease the growth of *S. aureus* and *E. coli* bacterias [6]. It has been shown that there are variations in the antibacterial properties of different Aloe vera extracts [7]. As previously shown, the methanol extract of *Aloe vera* has strong antimicrobial action, as evidenced by its ability to limit the maximal growth of S. aureus and *E. coli* when compared to alternative solvents such as ethanol and distilled water [8]. It has been stated that the methanolic, ethanolic, and acetone extract showed antimicrobial activity over E. coli and Bacillus subtilis. The methanolic extract of Aloe vera presented pronounced activity against S. aureus than other extracts [9]. Gentamicin is a broad-spectrum antibiotic that has been used to kill gram-positive and gram-negative bacteria strains with significant colonies [10]. It can be applied topically to treat local infections such as infectious impetigo, seborrheic dermatitis, and superficial ocular infections. It has been used to treat endocarditis, meningitis, and systemic biliary tract disorders. Gentamicin, however, exacerbates serious toxicity issues, particularly ototoxicity, nephrotoxicity, and neuromuscular blockade, when administered parenterally at high concentrations over a long period [11]. The above studies indicated that the Aloe vera possessed antibacterial effects to treat various food-borne bacterial infections. To the best of our knowledge, now, some researchers have detected the antimicrobial effects of methanol extract with Aloe vera. Therefore, considering the importance of food-borne diseases in humans, this study was intended to evaluate the antibacterial efficacy of pure Aloe vera its methanol extract and compared it with gentamicin which is a commonly used antibiotic to treat food-borne diseases caused by Staphylococcus aureus and Escherichia coli isolated from broiler meat.

This study aims to investigate the antibacterial effectiveness of pure Aloe vera and its methanol extract, comparing their efficacy to that of the commonly used antibiotic gentamicin.

METHODS

Fifty samples of fresh broiler meat were obtained in an aseptic method. For primary culture, the materials were cultivated on nutrient agar. The isolates were purified by sub-culturing on blood and MacConkey agar and then incubated at 37°C for 24 hours. On the blood agar medium, sub-culturing was used to carry out additional purification. Based on morphological, biochemical, and cultural characteristics, organisms have been identified. For observing the staining reaction, gram staining was used. Similar concentration of pure Aloe vera, its Methanol extract and gentamicin were used i.e., 20 µg/µl, 10µg/µl, 5 µg/µl, 2.5 µg/µl, 1.25 µg/µl, 0.625 µg/µl, 0.312 µg/µl, 0.156 µg/µl, 0.78 µg/µl, and 0.039 µg/µl, to evaluate their MIC against *Staphylococcus aureus* and *Escherichia coli.* For detection of *Escherichia coli* broiler meat samples were subculture on MacConkey agar for the identification of the bacteria. Following that, Petri dishes were examined for dry, doughnut-shaped, dark pink-in-colour colonies with the whole border encircled by a dark pink region of precipitated bile salts, which were found to be present. Further validation was obtained via the use of Gram staining and biochemical reactions with isolates. Samples of broiler meat were subculture on blood agar to detect *Staphylococcus aureus,* which was found in the meat. Following that, colonies of yellow-golden colour were seen in Petri dishes, and additional confirmation was achieved via the use of gram staining and the biochemical response of isolates. Bacterial organisms were further confirmed by biochemical tests, which include the following. Oxidase test and Coagulase test followed by [12]. The Aloe vera plant leaves were obtained from the Tandojam plant nursery, which is located nearby. The plant leaves were cleaned with 70 percent alcohol before being processed. With the aid of a sterile knife, the leaves were incised, and the gel was removed from the leaves. Further, the gel was mixed to ensure homogeneity and then filtered through a muslin cloth before being autoclaved at 121°C at 15 pounds of pressure for 15 minutes to sterilize it. Then, as a stock solution, a sterilized filter was used (100 percent concentration).Fresh leaf gel will be oven-dried at 80°C for 48 hours to prepare methanol extracts. Afterwards, a pestle and mortar will be used to grind the dried gel into a powder, which will then be blended into a fine powder. Twenty grams of this powder will be steeped in 200 milliliters of methanol. After passing the mixture through Whatman filter paper number 1, the filtrate will be evaporated until it is completely dry. After drying, the extract will be further ground into a powder and mixed with distilled water. Gentamicin Stock solution was prepared by adding 10mg of Gentamicin powder in 10ml distilled water then dissolved thoroughly. This solution was autoclaved at 121oC for 15 minutes at 15lb pressure. Subsequently, the solution was kept under refrigeration at -40oC until further analysis. The antibiotic susceptibility test was carried out using *Aloe vera,* its methanol extract, and Gentamicin as the test substances. It was decided to use the micro broth dilution method on the Muller-Hinton (MH) medium for the antibiotic susceptibility test. For the MIC test, a dilution of 1:1000 was produced. To do this, 6 ml of Muller Hinton (MH) medium was mixed with 6 µl of bacterial culture in Tryptic Soy Broth (TSB). The minimal inhibitory concentration of Aloe vera, its methanol extract, and Gentamicin against *Staphylococcus aureus* and *Escherichia coli* was determined using 96-well plates. 100µl of concentration was added to all wells, then 80 µl of cultured was added to the first and second wells, with the first well remaining as

DOI: https://doi.org/10.54393/mjz.v5i03.128

the control of the micro-titer and 20 µl of *Aloe vera* being added to the second well, then 100µl of *Aloe vera* concentration was taken from the second well and added to the third well of the micro-titer, and so on respectively. The isolates were treated with Gentamicin at a concentration of 10mg/10ml (stock solution) to eradicate them. Following that, the methanol extract and Gentamicin were subjected to the same MIC testing method. The MIC plates were incubated at 37°C for a whole night. It was therefore necessary to record the turbidity break points in each well to determine the minimal inhibitory activity of Aloe vera, its methanol extract, and Gentamicin [13]. The turbidity/cloudy appearance in cultured wells was used to determine the breakpoints when bacterial growth was stopped.These breakpoints were referred to as the minimal inhibitory concentrations (MIC) for pure Aloe vera, its methanol extract, and Gentamicin. The collected data were tabulated and analyzed by using one-way ANOVA through the statistical 8.1 version.

RESULTS

A total of 50 broiler meat samples were collected and examined. Out of 50 milk samples, 19 (38%) were found positive for *Staphylococcus aureus*, 24 (48%) were found positive for *Escherichia coli*, and mixed colonies were found 7 (14%) positive in meat samples. The different concentrations of pure Aloe vera were used to check the susceptibility of isolated organisms. The results indicated the susceptibility of *Staphylococcus aureus* at 20 and 10µg/µl, while lower concentrations of pure Aloe vera were found resistant. Whereas *Escherichia coli* growth stopped at 20, µg/µl while concentrations lowered than this, the organism showed resistance. Statistical analysis showed a ($p<0.05$) difference in MIC between both isolated organisms against pure Aloe vera (Figure 1).

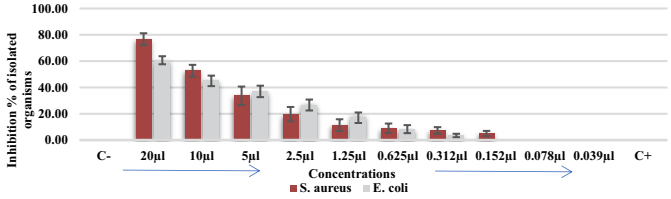

Figure 1: Growth Inhibition of *Staphylococcus Aureus* and Escherichia coli Towards Concentrations of Pure Aloe Vera (20 to 0.039µg/µl)

The various concentrations of methanol extract of Aloe vera were used. The susceptibility of *Staphylococcus aureus* at 20, 10, and 5µg/µl, while lower concentrations of methanol extract of Aloe vera were found resistant. Whereas *Escherichia coli* growth halted at 20, 10 µg/µl, while concentrations lowered than this, the organism showed resistance. Statistical analysis showed ($p<0.05$) a difference in MIC between both isolated organisms against methanol extract of Aloe vera (Figure 2).

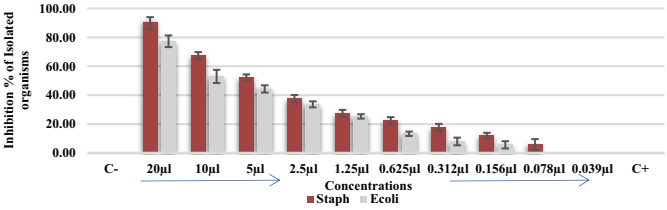

Figure 2: Growth Inhibition of *Staphylococcus Aureus* and *Escherichia Coli* Towards Concentrations of Methanol extract of Aloe vera (20 to 0.039µg/µl)

Different concentrations of gentamicin were used. The *Staphylococcus aureus* obtained susceptibility at 20, 10, 5, 2.5, 1.25 µg/µl, while lower concentrations of gentamicin were found resistant. Whereas Escherichia coli growth was hampered at 20, 10, 5, 2.5, 1.25, 0.625, and 0.312 µg/µl while concentrations lowered than this, the organism showed resistance. Statistical analysis showed a ($p<0.05$) difference of MIC between both isolated organisms against gentamicin (Figure 3).

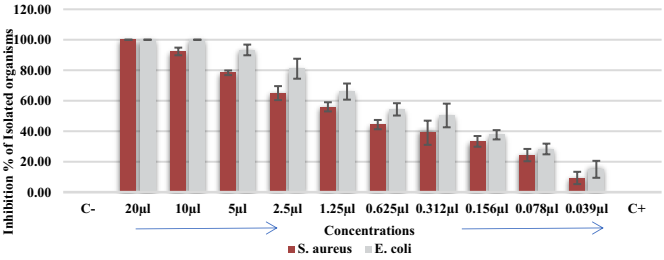

Figure 3: Growth inhibition of *Staphylococcus aureus* and *Escherichia coli* towards concentrations of gentamicin (20 to 0.039µg/µl)

DISCUSSION

The result of the current study is following the previously reported studies that showed bacteriological investigations from 604 samples of raw meat, 601 samples of frozen meat, and 645 samples of ready-to-eat meat, among other sources. S. aureus positive samples were found in all 39 cities, according to both qualitative and quantitative techniques S. aureus-positive samples, as well as *E. coli*-positive samples. The presence of Staphylococcus aureus was identified in 35.0 percent (647/1,850) of the samples. In addition, *E. coli* was found in 48 percent (634/1850) of tested samples. The quantities of the presence of *E. coli* in retail meat were found to be in the high range [14]. The isolated organisms were identified based on their physical characteristics, as well as their culture and staining responses. Furthermore, the biochemical responses of these organisms were used to establish their identity. Our present study agrees with the previous studies in which the author did prevalence studies of microorganisms associated with poultry meat. The most frequent organisms are recorded from poultry meat in a high rate of *Escherichia coli* with 66 (68.7%) and

DOI: https://doi.org/10.54393/mjz.v5i03.128

Staphylococcus aureus (29.2%) and other frequent mixed colonies were found 46 (47.9%) positive. Further, it was observed that *E. coli* and *Staphylococcus* remained the predominant cause of contaminated poultry meat [15]. It was observed in the present study that the minimum inhibitory concentration of pure Aloe vera at which the growth of *S. aureus* and *E. coli* were inhibited was 20µl and 10µl, respectively. The findings of the current study are also supported by a previous study in which it was reported that *Aloe vera* showed antibacterial efficacy against a wide range of bacteria gram-positive as well as gram-negative. The antibacterial agents of *Aloe vera* were suggested to kill or prevent the growth of *S. aureus, E. coli* [16]. The current study showed agreement with a previous study that *Aloe vera* has antibacterial activity because *Aloe vera* contains some antibacterial substances i.e, aloin, fumaric acid, and anthraquinone [17, 18]. The current study showed agreement with a previous study in which it was found that Methanol extract of *Aloe vera* has great antibacterial activity against both tested microorganisms as compared to other extracts i.e: ether and petroleum with a high zone of inhibition against gram-positive *Staphylococcus aureus,* and moderate zone of inhibition against gram-negative *Escherichia coli*. The Aloe vera gel extracts were shown to have moderate to high antimicrobial effects on both gram-negative and gram-positive bacteria when compared to the other extracts evaluated for antibacterial activity [19]. The recent study showed agreement with the previous findings in which it has been reported that gentamicin is a broad-spectrum aminoglycoside antibiotic which effective in a wide range of gram-positive and gram-negative pathogens by producing bactericidal action [10]. The finding of the present study has been supported by previous studies that Gentamicin is the most frequently used aminoglycoside antibiotic which showed antibacterial action against moderate to severe bacterial infections caused by sensitive agents, including *Escherichia coli* and *Staphylococcus aureus* which produce different moderate to severe bacterial infections like other aminoglycosides. Additionally, the present study is by previous study explained that gentamicin was found effective antibiotic with the MIC standards of 1.56 µg/mL, 6.25 µg/mL, 3.13 µg/mL, 6.25 µg/mL against *S. aureus,* P. aeruginosa, *E. coli* and B. cereus, respectively [20]. Although, this antibiotic is termed as bactericidal. Thus, in the present study, Gentamicin produced a significantly lower minimum inhibitory concentration as compared to pure *Aloe vera* and its methanol extract. Consequently, it is concluded from this study that *Escherichia coli* is more prevalent than *Staphylococcus aureus* in broiler meat samples. The average susceptibility of both organisms is 1.25 µg/µl and 0.312 µg/µl to Gentamicin through MIC. However, the antibacterial activity of Gentamicin is significantly higher than pure Aloe vera and its methanol extract.

CONCLUSIONS

It was concluded that all three treatments i.e., pure *Aloe vera*, its methanol extract, and gentamicin inhibited the growth of isolated bacterial organisms, but the methanol extract halted the growth of isolated organisms at lowered concentration in comparison to pure *Aloe vera. Escherichia coli* is more prevalent than *Staphylococcus aureus* in broiler meat. Growth inhibition of *Staphylococcus aureus* was observed at lower concentrations as compared to *Escherichia coli* following the usage of various treatments. Gentamicin exhibited MIC at a lowered concentration in comparison to other used treatments and *Escherichia coli* remained more susceptible to it.

Authors Contribution

Conceptualization: RAK, SB, RB
Methodology: RAK, MAJ
Formal analysis: MT, IJ, NAJ, FJ
Writing review and editing: SB, RB, MB

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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DOI: https://doi.org/10.54393/mjz.v5i03.137

MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
ISSN (P): 2790-4377, (E): 2790-4385
Volume 5, Issue 3 (July-Sep 2024)

Original Article

OPEN ACCESS

Taxonomic description of new species *Diplotriaena Sarmasti n.sp.* (Nematode: Filariidae) in Jungle Mynas (*Acridotheres fuscus*) Wagler, 1872 (Passeriformes: Sturnidae) from District Larkana, Sindh, Pakistan

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ARTICLE INFO

Keywords:
Jungle Myna, Nematode, Acridotheres Fuscus, Taxonomic Sorting

How to cite:
Soomro, B., & Memon, S. A. (2024). Taxonomic description of new species *Diplotriaena Sarmasti n.sp.* (Nematode: Filariidae) in Jungle Mynas (*Acridotheres fuscus*) Wagler, 1872 (Passeriformes: Sturnidae) from District Larkana, Sindh, Pakistan: *Diplotriaena Sarmasti n.sp.* in Jungle Mynas Wagler, 1872 (Passeriformes: Sturnidae). MARKHOR (The Journal of Zoology), 5(03), 22-27. https://doi.org/10.54393/mjz.v5i03.137

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Received date: 5th August, 2024
Acceptance date: 25th September, 2024
Published date: 30th September, 2024

ABSTRACT

Nematodes that recycle nutrients, govern pests, and offer data regarding the wellness of the soil, rendering them vital for both the environment and the soil. The crop yield, nematode management, gastrointestinal nematodes, predatory nematodes, entomopathogenic nematodes, nutrient cycling, soil quality and health indicators are all severely affected by worms. **Objectives:** To conduct zoological studies in District Larkana, Sindh, taxonomic sorting, or ecological research. Jungle mynas belong to the order Passeriformes, which are usually collected and preserved. **Methods:** The standard method employed for morphological analysis and ornithological research. Certain procedures are followed throughout the mynas' collection, preservation, and morphological examination to guarantee the credibility of this research while causing the least amount of injury to individual birds. The methods used differ depending on the study's goals and the ethical issues surrounding animal research. Mynas are a family of birds in the Passeriformes order. Frequently, investigations of them are conducted for zoological, taxonomic and ecological research studies. A new species of nematodes, *"Diplotriaena sarmasti n.sp"* documented in Jungle Mynas *"Acridotheres fuscus"* from the vicinity of Larkana, Sindh, Pakistan. **Results:** Overall, 25 nematodes (♂♂) were recovered from the body cavity of hosts. In the present study, nematodes reflected variations by their following characteristics viz: body dimensions, spicule shape and 23 to 24 pairs of caudal papillae. **Conclusions:** Therefore, these morpho-metrical changes are recommended as a new species and authoress devoted this species, *Diplotriaena sarmasti n.sp.*

INTRODUCTION

Nematodes have successfully adapted to virtually every ecosystem from freshwater to saltwater, from soils, from the Polar Regions to the tropics, and from the highest to the lowest elevations. They can be found in an extensive array of places, including mountains, deserts, and oceanic trenches. They are common in freshwater, marine, and terrestrial habitats, occasionally exceeding other animals in both individual and species counts. They are present in all regions of the Earth's lithosphere, including deep underground gold mines in South Africa, which lie 0.9–3.6 km (3,000–12,000 feet) below the surface of the planet [1]. They make up 90% of all creatures found on the ocean floor [2]. There are 4.4 × 1020 nematodes in the Earth's topsoil overall, or around 60 billion for every human, with the highest densities seen in boreal and arctic woods [3]. They play a significant role in many ecosystems due to their diversity of lifecycles, presence at different trophic levels, and numerical dominance, often surpassing a million individuals per square meter and making up almost 80% of all individual creatures on Earth [4]. In polar habitats, they are fundamental; there are 256 families among the approximately 2,271 genera [5]. Pathogens in the majority of plants and animals are among the numerous parasitic forms. About 35 nematode species are parasites of humans, and a third of the genera are found as vertebrate parasites [6]. Jungle myna (*Acridotheres fuscus*) Wagler is

DOI: https://doi.org/10.54393/mjz.v5i03.137

a myna found and distributed across the world, including Pakistan [7]. Characteristically, mostly found in cultivated areas such as forests, rice fields and near watery areas. They built their nest mostly in the palm trees and feed on a variety of food like several grains, fruits, insects, ticks and pests found in cattle and sheep. In many Asian countries, a variety of mynas, kept as pet birds. They have blackish heads and wings; grey fluff, bright yellow legs, and contain a forehead tuft on their heads and during flight, white patches are visible in their wings. The jungle mynas by nature are scavengers and omnivorous, and their stomach contains a variety of food contents, including beetles, termites, ants, caterpillars and fly larvae. Due to such type of feeding habits, they are more susceptible to parasitic infections. The Jungle Myna (*Acridotheres fuscus*) is a common bird species found in the tropical forests of Asia. Despite being considered an invasive species in some areas, the Jungle Myna plays a significant ecological role in its native habitats. The ecological importance of the Jungle Myna is a seed role as a seed dispersal agent. The Jungle mynas are known to feed on fruits, berries, and nectar, and in the process, they help disperse seeds of various plant species [8]. By scattering seeds, they contribute as well to the regeneration of forests. By ensuring the diversity of plant species, they aid in the regeneration of forests [9]. They demonstrate insectivorous behaviour as a means of controlling insects. Jungle Mynas help to control their populations by consuming insects, notably pests that harm crops [10]. Jungle Mynas promotes the ecological equilibrium by moderating insect populations, which keeps any one species from taking over the ecosystem [11]. Like other birds, Jungle mynas use their droppings as a natural fertilizer to move nutrients from one place to another [12]. Jungle Mynas droppings can improve soil quality, fostering the growth of crops and safeguarding the health of the ecosystem [13]. Using twigs, leaves, and other plant-based materials, they create nests that can serve as habitat for insects and small reptiles, among other vertebrates [14]. Jungle mynas' nesting habits provide the ability to alter their environment and produce microhabitats that encourage biodiversity [15]. A prevalent bird species in Asia's tropical jungles is the Jungle Myna (*Acridotheres fuscus*). The Jungle Myna, a member of the starling family, is a significant biological element within its natural setting. Jungle Myna's significance biologically includes: its food source, ecological niche, predator-prey dynamics, and ecosystem engineering. Their appetite for insects, such as caterpillars, grasshoppers, and beetles, aids in population control [16]. They modify local predator-prey dynamics by grazing on small animals like rodents, lizards, and snakes. Furthermore, these people were preyed upon by other creatures of all kinds, such as monkeys, snakes, and eagles, illustrating the complex connections found in ecosystems [17]. As omnivores, jungle mynas fill an ecological niche by devouring an abundance of foods, including fruits, seeds, and insects [18]. They are remarkably adaptive, blossoming in many different kinds of settings, from urban settings to jungles and enduring a wide range of environmental constraints [19]. For a variety of reasons, nematodes and parasitic infections frequently connect with avian hosts, or birds: Over millions of years, nematodes and birds co-evolved, resulting in intricate host-parasite relationships [20]. Due to their common past with nematodes, several bird species are more vulnerable to infection [21]. Birds are more likely to become infested because they frequently reside in areas where nematodes are present [22]. Infection may result from nematodes that birds obtain through their diet, such as insects or worms [23]. In light of their specialized antibodies, birds may be more exposed to nematode infections. The avian gastrointestinal tract provides a suitable environment for nematodes to establish themselves. A nematode parasite that infects chickens and other birds, causing gastrointestinal problems [24]. A nematode parasite that infects birds, causing respiratory problems [25]. A genus of nematodes that infect birds, causing gastrointestinal and respiratory problems [26].

This study aimed to present the first time, a new species of *Diplotriaena sarmasti n.sp* reported in the Jungle Myna District, Larkana, Sindh, Pakistan.

METHODS

In the present study, field observations were made on a total of seventy Jungle myna (*Acridotheres fuscus*) collected from the locality of Larkana, Sindh, Pakistan. Capture Techniques: The nets are frequently positioned where mynas are known to roost or feed. Fine nets were utilized to catch birds without hurting them physically. Traps: Researchers also employed automatic traps to capture several birds at once. Hand Capture: In certain situations, skilled people might be able to capture birds manually, especially if they have become accustomed to people. Handling: To prevent stress or harm, captured mynas are handled carefully. To minimize the effects on the health of the bird, appropriate field protocols are followed. Preservation of Mynas Euthanizing: According to ethical standards, a bird may be humanely put down if the researcher desires a specimen to be mounted or safeguarded for comprehensive morphological inspection. Taxidermy: Taxidermy can be done for specimens that are meant to be on display or in collections. This entails delicately skinning and mounting the birds and then properly preserving them (Freezing). Morphological Analysis Techniques and External Morphological Measurements: Researchers frequently measured multiple exterior traits, such as notably body mass, tarsus length, tail length, wing length, and bill length.

DOI: https://doi.org/10.54393/mjz.v5i03.137

Morphometric Analysis: To evaluate distinctions across populations or species, morphometric analysis uses quantitative metrics such as size and form. The data were then interpreted using statistical methods. Skeletal Analysis: In certain instances, the skeleton may be sanitized for determining osteological traits or analyzed post-mortem to perform further morphological analysis. Mynas may be frozen for genetic research to preserve its biological elements, notably DNA. Preservation Techniques and Alcohol Preservation: To keep smaller specimens from decomposing and to preserve their cellular features, they can be preserved in ethanol or isopropyl alcohol (Preservation Techniques). Alcohol Preservation: To stop disintegration and preserve cellular attributes, smaller specimens can be stored in ethanol or isopropyl alcohol. Drying: If preparing for a museum specimen, the bird may be dried and stored in a dry environment to prevent mold or rot. Overall, 25 nematode (♂♂) specimens were recovered from hosts. Recorded nematodes were killed in (70%) alcohol, cleared by applying the procedure of lactophenol and glycerol solution and alcohol- glycerol solution was used for their preservation. Their illustrations were completed under Camera Lucida by applying the procedure of (Garcia and Ash 1979) [27]. The collected mynas were anaesthetized and autopsied to examine helminth parasites. Photographic Techniques: For further investigation and reporting of apparent features, high-resolution photos may be gathered. Formalin fixation: A common method for preserving nematodes, involving fixation in a formalin solution (10% formalin in water) [28]. Glycerin fixation: A method used for preserving nematodes for microscopic examination, involving fixation in a glycerin solution [29]. Glycerin preservation: Nematodes can be preserved in glycerin (70-80% glycerin in water) for long-term storage [30]. Ethanol preservation: Nematodes can be preserved in ethanol (70-80% ethanol in water) for long-term storage [31]. By considering these ethical principles, researchers can ensure that their sample collection of nematodes is responsible, sustainable, and respectful of the environment, animals, and indigenous communities. Family: *Diplotriaenidae* [32]. Species: *Diplotriaena sarmasti n.sp.* Host: Jungle Myna (*Acridotheres fuscus*). Number of specimens examined (Twenty-five). Number of hosts found positive: (Five). Parasites recovered from the host. Locality: Vicinity of District Larkana. The author dedicated a new species in honors of her beloved Mother, Late Miss Fahmida Soomro.

RESULTS

Results for *Diplotriaena* Railliet" and "Henry, 1909" species recorded out of host are shown (Table 1).

Table 1: Shows Relative Characters of Genus "*Diplotriaena* Railliet" and "Henry, 1909" Species Recorded Out of Host

Parameters	Present Species (mm)	*D. niltavae*	*D. tristisi*	*D. bargusinica*	*D.almoraensis*
Body Length and Width	12. 43 x 0.31	24.0-24.4 x 0.496-0.512	32.94 x 0.455	31.0-46.4 x 0.72-0.77	39.2 x 0.32
Trident Dimension L and W	0.14 x 0.15	0.128 to 0.144	0.13	0.13 to 0.14	0.16
Numbers Caudal Papilla	23-24 Pairs	N.D	09	01	05
Dimension L and W Left Spicules	01.64	0.656 to 0.720	02.39	0.64 to 0.66	0.84 to 1.07
Dimension L and W Right Spicules	0.32	0.46 to 0.49	0.58	0.38 to 0.5	0.44 to 0.56
Parasite Host	*Acridotheres fuscus*	*Niltava grandis*	*Acridotheres tristis*	*Turdus roficollis*	*Parus major*
Parasite Locale	Physique Cavities	Physique Cavities	Physique Cavities	Physique Cavities	Physique Cavities
Areas/ Vicinities	Vicinity of District Larkana	(Uttarakhand, India)	(Burdwan)	(Bhutan)	Uttarakhand, India

Whereas, N. D denotes not detectable. Measurements are presented in millimetres (mm). Dimension: L denotes length and W denotes Width.

The body of the recovered nematode was elongated, thick, and highly muscular, with a length measurement of (12.43-12.47 mm) and a width of (0.31-0.26) mm wide and the anterior-posterior side was round in type. Two unequal-sized tridents, one with a rounded tip and the other with a pointed tip, were examined. They possessed unequal prongs in left and right trident, the length was measured (0.14 to 0.11 mm) and (0.15 to 0.07 mm) wide. (♂♂) made with the help of a camera Lucida a. Anterior section showing two prominent tridents of unequal size, with Scale bar: (0.2) mm (Figure 1).

Figure 1: Illustration of Nematode (*Diplotriaena sarmastini sp*)

DOI: https://doi.org/10.54393/mjz.v5i03.137

During examination it was observed that, their close-fitting right spicules projected outside to the posterior bearing slight curve with a measurement of (1.64 mm) in length and small in size than the left spicules jutted outside to posterior apex bearing large curve projected towards the downward with a measurement of (0.32 mm) in length. Posterior section showing two spicules, one larger other smaller, with scale bar (0.2) mm (Figure 2).

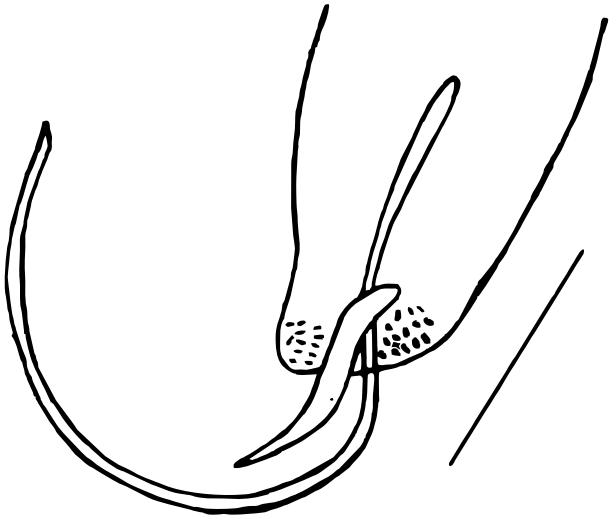

Figure 2: Posterior Section Showing Two Spicules, One Larger Other Smaller, with Scale Bar

A photography of the anterior section showing the tridents of Diplotriaena sarmasti n.sp(♂♂)(Figure 3).

Figure 3: *Diplotriaena Sarmasti n.sp.* (♂♂). Photography of Anterior Section

Photography of the posterior section showing the two spicules right spicule is shorter and the left spicule is larger (Figure 4).

Figure 4: Photography of the Posterior Section

DISCUSSION

Worldwide and at national level, a very insufficient species of Genus *Diplotriaena* has been described which include (*D. sternopastori*). *Myiophoneus caeruleus temmincki* differed from present species (*Diplotriaena sarmasti*) have large sized length and broader width; equal prongs of trident observed have been observed, larger left spicule and smaller right spicule. Study documented in Zoothra citrine of Uttara-khand differed from current species (*Diplotriaena sarmasti*) with large sized length, maximum body width; trident with equal prongs having pointed tips, unequal right and left spicules [33], *Niltava grandis* differed from the current species (*Diplotriaena sarmasti*) with greater body length, maximum width; equal trident prongs with small in length; the left spicule was large as compared with right spicule, *D. almoraensis* differed from the present species (*Diplotriaena sarmasti*) have large body lengths, maximum width; equal trident prong and large sized length; left spicule larger than right spicule, Turdus ruficollis of Bhutan which differed from present species (*Diplotriaena sarmasti*) having large sized body length and broader width; the tridents were small in length with unequal spicules [33]. *D. nagpurensis* documented in *Acridotheres tristis* of Nagpur which differed from the current species (*Diplotriaena sarmasti*) with large sized length, maximum body width; large tridents; unequal spicules. *D. tricuspis* in *Acridotheres tristis* of Nagpur which differed from the present species (*Diplotriaena sarmasti*) with large sized specimens; smaller tridents, and unequal both spicules. *D. bhamoensis* reported in *Ethiopsar albocinclus* which differed from the (*Diplotriaena sarmasti*) which have large sized body length and maximum body width. *D. graculi* verified in *Pyrrhocorax* of Calcutta which differed from (*Diplotriaena sarmasti*) which have large sized body length, maximum width and unequal spicules. *D. dubia* chronicled in *Pyrrhocorax* of Calcutta which differed from (*Diplotriaena sarmasti*) which have larger length with maximum body width; left spicule recoded as large in length and right

DOI: https://doi.org/10.54393/mjz.v5i03.137

spicule is small. *D. urocissae* reported in *Urocissa flavirostris* of Calcuta which differed from (*Diplotriaena sarmasti*) with large sized body and broader width; left spicule recoded large whereas right spicule small in length [34]. (*D. lagopusi*) documented in White-tailed ptarmigan (*Lagopus leucurus*) of Central and Northern Colorado, USA which differed from the current species (*Diplotriaena sarmasti*) which have large sized body length and broader width; small trident, unequal spicule [35], (*D. andersoni*) reported in White-tailed ptarmigan (*Lagopus leucurus*) from Canada which differed from the present species (*Diplotriaena sarmasti*) which have large sized body length and broader width and unequal spicule [35], Present recorded species (*Diplotriaena sarmasti*) differ from the aforementioned species in length, width, tridents, right and left spicules, showing complete disparity at the morphological. Therefore, identified as a new species, which is a new contribution in the field of parasitology and taxonomy.

CONCLUSIONS

It was concluded that the species documented in current studies reflects variation from earlier described allies of nematodes about the following characteristics, such as dimensions of body, shape of tridents and spicules, with the occurrence of 23 to 24 pairs of caudal papillae. Therefore, such morpho-metrical variations are measured as a new species, Diplotriaena sarmasti, contributing a new addition to the domain of taxonomy and parasitology.

Authors Contribution

Conceptualization: BS, SAM
Methodology: SAM
Formal analysis: BS
Writing review and editing: BS, SAM

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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DOI: https://doi.org/10.54393/mjz.v5i03.168

MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
ISSN (P): 2790-4377, (E): 2790-4385
Volume 5, Issue 3 (July-Sep 2024)

Original Article

OPEN ACCESS

Length-Weight, Length-Length Relationships and Condition Factor of *Hypophthalmichthys nobilis* Fingerlings from Bahawalnagar, Pakistan

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ARTICLE INFO

Keywords:
Hypophthalmichthys nobilis, Length-Weight Relationship, Condition Factor, Morphometric Analysis, Bighead Carp Fingerlings

How to cite:
Ishtiaq, A., Mehboob, S., Aziz, M. S., Shahid, A., Nawaz, A., & Ahmad, B. (2024). Length-Weight, Length-Length Relationships and Condition Factor of Hypophthalmichthys nobilis Fingerlings from Bahawalnagar, Pakistan: Morphometric Study of Bighead. MARKHOR (The Journal of Zoology), 5(03), 28-32. https://doi.org/10.54393/mjz.v5i03.168

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Received Date: 24th July, 2024
Acceptance Date: 26th September, 2024
Published Date: 30th September, 2024

ABSTRACT

Bighead carp (*Hypophthalmichthys nobilis*) is an economically important aquaculture species valued for its rapid growth and filter-feeding ability. **Objective:** To examine the morphometric parameters specifically the length–weight relationship (LWR), length–length relationships (LLRs), and condition factor of *Hypophthalmichthys nobilis* (bighead carp) fingerlings reared in hatcheries in Bahawalnagar, Pakistan. **Methods:** A total of 35 fingerlings were sampled. Morphometric measurements including total length (TL), standard length (SL), fork length (FL), head length (HL), and body weight (W) were recorded. The LWR was calculated using the formula $W = aTL^b$, and the LLRs were derived through linear regression between different length parameters. The condition factor (K) was determined using the Fulton's formula. **Results:** The mean ± SD values for TL and W were 10.21 ± 0.52 cm and 9.53 ± 1.45 g, respectively. The LWR showed a negative allometric growth pattern with constants a = –1.5671 and b = 2.52, and a strong correlation (r = 0.887). Strong positive correlations were observed in LLRs: TL vs. SL (b = 1.03), TL vs. FL (b = 0.74), and TL vs. HL (b = 0.85). The mean ± SD condition factor was 0.89 ± 0.06, indicating moderately good fish health. **Conclusions:** This study presented updated, population-specific data on morphometric relationships and the condition factor of *Hypophthalmichthys nobilis* fingerlings in Pakistan. These findings provide a valuable baseline for region-specific stock assessments and contribute to informed, sustainable management strategies for bighead carp in hatchery settings.

INTRODUCTION

The sustainability of fisheries science and aquaculture management hinges on the accurate assessment of fundamental biological parameters, including fish growth rates, body composition, and reproductive performance. Simple measures to assess the growth pattern and general fitness in fish populations comprise Length-Weight (LW) relationship and condition factor, respectively, which provide efficient management strategies and stock assessment Naeem *et al.*, in 2010 [1]. Dietary interventions also substantially influence fish growth, feed utilization efficiency, and the overall sustainability of aquaculture systems, highlighting the importance of optimized nutritional formulations in promoting fish health and development Ismat *et al.*, in 2013 [2]. Morphometric analyses play a vital role to understand the fish biology, principally in observing the relations among various body lengths Naeem *et al.*, in 2011; Pervaiz *et al.*, in 2012 [1, 3]. Such morphometric data and their statistical relationships are crucial for fisheries managers and taxonomists in order to determine growth variation in fish and to identify and classify fish species. Furthermore, morphometric studies can uncover distinctions among different populations of same fish species [4, 5]. Assessing morphometric traits and condition factors is essential in evaluating fish health, growth patterns, and aquaculture potential. The condition factor is widely used as an indicator of the physiological status of fish in aquaculture systems [6]. Selective breeding has also been shown to significantly improve

DOI: https://doi.org/10.54393/mjz.v5i03.168

growth rates and overall quality in farmed fish species, reinforcing the importance of genetic and morphometric assessments [7]. Studies on length–weight and length–length relationships across various fish species, such as those conducted by González-Acosta *et al.*, in 2024 provide comparative benchmarks for evaluating regional fish populations. Additionally, morphometric variability influenced by geographic isolation and environmental conditions has been demonstrated in related species like *Labeo calbasu* stated by Hossain *et al.*, in 2010, underscoring the relevance of localized assessments like the present study [8, 9]. Because, in response to environmental factors, morphometric adaptations are more frequent in fish leading to morphological variation than in other vertebrate groups Loy *et al.*, in 2000 [10]. Length-Weight Relationship (LWR) usually indicated as $W = aTL^{b}$, where W represents weight and TL shows total length, aids to evaluate if a species displays allometric or isometric growth pattern Ding *et al.*, in 2023 [4]. Recent research has applied LWRs to estimate growth patterns in different fish species across different geographical regions, emphasizing their significance in management and ecological contexts Naeem *et al.*, in 2011; Rodriguez *et al.*, in 2023; Yousuf *et al.*, in 2023 [1, 11, 12]. Length-Length Relationships (LLRs) are generally implied in for estimation of population management and dynamics through size conversion (for instance calculated TL to SL), providing accuracy of assessment models for population estimation González-Acosta *et al.*, in 2024 [8]. Condition factor is generally used to evaluate the conformation of the fish and can help producers optimize feed management and the nourishment (energy state) of fish [6]. The length–weight relationship (LWR) and condition factor are widely used tools in fish biology for assessing growth patterns and overall fish health. Studies such as that by Ferdous Jerin *et al.*, in 2023 on threatened riverine catfish in Bangladesh highlight the importance of these parameters in conservation and stock monitoring [13]. Similarly, Khan *et al.*, in 2020 reported region-specific LWR data for *Cyprinus carpio* from the Indus River, underscoring the variability in growth dynamics across ecosystems [14]. According to King in 2013, accurate LWR and condition factor measurements are fundamental to fisheries biology, contributing to effective stock assessment, management strategies, and sustainability planning [15].

The aim of the study was to evaluate the length- weight (LWR), length–length relationships (LLRs), and condition factor of *Hypophthalmichthys nobilis* (bighead carp) fingerlings collected from Bahawalnagar, Pakistan to assess the growth patterns and well-being of carp findings.

METHODS

This study employed a descriptive design to analyze biometric and morphometric parameters (length-weight relationships, length-length relationships, and condition factor) of *Hypophthalmichthys nobilis* fingerlings. Data were collected through standardized measurements without experimental manipulation, following observational protocols for hatery-reared fish. A total of 35 fish fingerlings were procured via convenience sampling from a single hatchery batch at Fish Seed Hatchery, Minchinabad, Bahawalnagar, Pakistan during 2024. Fingerlings were selected based on being healthy, active specimens with no visible deformities or signs of disease (size range: 9:40-11:50 cm TL). To ensure uniformity, fish of similar size and age from the same hatchery batch were used, while excluding any deformed, injured or abnormal specimens. The fingerlings were transported in oxygen-filled plastic bags to the Aquaculture Research Lab, The Islamia University of Bahawalpur, Bahawalnagar Campus, Bahawalnagar, Pakistan . Body Weight (W) was recorded in grams; while various body lengths of *H. nobilis* including Total Length (TL), Standard Length (SL), Fork Length (FL) and Head Length (HL) were measured in centimeter. Condition factor (K) was evaluated following standard formula: $K=W/TL^{3}\times100$. Descriptive analyses such as mean, standard deviation and range; and correlation coefficient was calculated. Relationship of TL (X) with W and various studied morphometric lengths (Y) of Hypophthalmichthys nobilis was estimated using the commonly applied power function equation, as described by Le Cren [16] and Froese [5]:

$$W = aL^{b}$$

where W is weight (g), L is total length (cm), a is the intercept, and b is the slope. This was log-transformed to linear form for analysis

Where 'Y' is either W, SL, FL or HL; a is constant (intercept), and b is growth coefficient (or slope). To facilitate linear regression analysis, this equation was log-transformed into the following linear form:

$$\text{Log } Y = \text{Log } a + b \text{ Log } X$$

The value of b provides insight into the growth pattern of the fish. In LWR, if b = 3, growth is considered isometric, meaning weight increases proportionally with TL. When $b \neq 3$, growth is allometric, either positive when $b > 3$ (weight increases faster than length) or negative allometric pattern when $b < 3$ (length increases faster than weight), whereas for LLRs b-value is compared with 1.00. Statistical analysis was done by use of SPSS 26.0, p-value of less than 0.05 was considered statistically significant [5]. Several regional studies support the importance of analyzing length–weight relationships (LWR) and condition factors to assess the growth performance and health status of various fish species.

DOI: https://doi.org/10.54393/mjz.v5i03.168

RESULTS

The total length of the *Hypophthalmichthys nobilis* fingerlings (n = 35) ranged from 9.40 cm to 11.50 cm, and the corresponding body weight from 7.46 g to 14.15 g. The mean condition factor was observed 0.89±0.06 for the studied samples of *Hypophthalmichthys nobilis* fingerlings. Descriptive statistics for various morphometric measurements, body weight and condition factor of *Hypophthalmichthys nobilis* fingerlings from Bahawalnagar, Pakistan are illustrated as table 1.

Table 1: Descriptive Statistics for Various Body Lengths, Weight and Condition Factor of *Hypophthalmichthys nobilis* Fingerlings

Variables	Range	Mean ± SD
Total Length (TL)	9.40-11.50	10.21 ± 0.52
Standard Length (SL)	7.20-9.10	8.01 ± 0.43
Fork Length (FL)	1.90-2.60	2.22 ± 0.14
Head Length (HL)	2.10-2.70	2.27 ± 0.14
Body Weight (W)	7.46-14.15	9.53 ± 1.45
Condition Factor (K)	0.76-0.99	0.89 ± 0.06

SD= Standard Deviation

The assessed parameters of the length-weight relationship (LWR) were a = -1.5671 and b = 2.52. The coefficient of correlation (r) was 0.887, representing a strong positive relationship between length and weight. The b value suggests a negative allometric growth pattern in the studied Bighead carp population (Table 2).

Table 2: Length-Weight and Length-Length Relationships of *Hypophthalmichthys nobilis* Fingerlings

Equation	a	b	95% CI of a	95% CI of b	r
Log *W*= *a* + *b* Log *TL*	-1.5671	2.52	-2.0348 to -1.0994	2.06-2.98	0.887
Log *SL*= *a* + *b* Log *TL*	-0.1309	1.03	-0.2472 to -0.0145	0.91-1.14	0.953
Log *FL*= *a* + *b* Log *TL*	-0.4020	0.74	-0.7529 to -0.0512	0.39-1.09	0.603
Log *HL*= *a* + *b* Log *TL*	-0.4999	0.85	-0.8074 to -0.1923	0.54-1.15	0.701

a = intercept; b = slope; CI = confidence intervals; r = correlation coefficient

Significant linear relationships were observed between Total Length (TL) and other morphometric measurements of *Hypophthalmichthys nobilis* fingerlings (Table 2). The relationship between TL and SL showed a slope (b) value of 1.03, indicating an isometric relationship. The relationship between TL and Fork Length (FL) yielded a b value of 0.74, while the relationship between TL and Head Length (HL) had a b value of 0.85, both representing negative allometric pattern. All length–length relationships demonstrated strong positive correlations (r=0.603-0.953), suggesting consistent proportional growth among these body dimensions. These findings support the reliability of using alternative length measurements (SL, FL, and HL) for estimating TL in morphometric and stock assessment studies.

DISCUSSION

Ujjania *et al.*, in 2013 investigated *Labeo rohita* in Southern Rajasthan, reporting LWR patterns relevant for aquaculture and resource management [17]. Similarly, Salam *et al.*, in 2005 documented the LWR and condition factor of *Puntius chola* from freshwater bodies in Pakistan, emphasizing environmental influences on fish morphology [18]. Javaid *et al.*, in 2010 explored the relationship between body composition and condition factor in *Oreochromis nilotica*, reinforcing its significance in understanding growth efficiency [19]. Chakravarty *et al.*, in 2012 further extended these findings to marine species, highlighting species-specific variability in LWR, such as in *Trichiurus lepturus* and *Lepturacanthus savala* from the Visakhapatnam coast [20]. Collectively, these studies provide valuable comparative insights that support the present findings on *Hypophthalmichthys nobilis*. The observed length-weight relationship in the present study revealed a strong positive correlation (r = 0.887) between the total length and body weight of carp, indicating that length is a reliable predictor of weight in this species. Compared to earlier studies on carps, the b value reported here falls within normal biological range (2.5–3.5) as described further validating the accuracy of the measurements and analytical approach. The calculated b value (2.52) deviates from the ideal isometric value of 3.0, suggesting negative allometric growth. It indicated that the fish increases in length faster than in weight as it grows [21-23]. Such growth patterns are common in juvenile or early growth stages when linear growth is more pronounced. Several factors could influence the allometric nature of growth, including food availability, sex, and environmental conditions of the fish [24, 25]. While the allometric interpretations (b ≠ 3) in this study are biologically plausible, we acknowledge that the estimated b-values may be sensitive to measurement errors given the modest sample size (n=35). Small variations in length measurements can disproportionately influence slope estimates in power-law relationships [5]. These findings should therefore be interpreted as population-specific estimates for this hatchery cohort, with validation recommended through larger-scale studies. While the sample size (n=35) allowed detection of strong morphometric relationships (e.g., r > 0.88 for LWR), the study's hatchery-specific convenience sampling limits generalizability to wild populations or other rearing conditions. These findings provide preliminary biometric baselines for *Hypophthalmichthys nobilis* fingerlings, but larger, multi-location studies are needed to validate broader applicability. The strong positive correlations (P<0.001) among LLRs specify that Standard Length (SL), Fork Length (FL) and Head Length (HL) increase in a

DOI: https://doi.org/10.54393/mjz.v5i03.168

proportionate and predictable manner in relation to among Total Length (TL) of *Hypophthalmichthys nobilis*. In a previously reported study on *Ompok bimaculatus*, Ishtiaq *et al.*, in 2021 documented isometric pattern between total (TL) and Standard Length (SL) [23]. The slope value (b=1.02) for the TL–SL relationship in the present study also proposes isometric growth pattern, indicating that total length and standard length of *Hypophthalmichthys nobilis* fingerlings grow at the same rate. This outcome suggests that SL can aid as a reliable substitution for TL in fishery assessments or field studies where measuring TL may be challenging due to specimen preservation methods or fin damage. In contrast, the relationships of TL vs FL and TL vs HL yielded lower *b* values being less than 1.00, representing a negative allometric pattern as also documented by other studies [24, 25]. Another carp (Cyprinus carpio) have also reported negative allometric growth pattern for the relationship between TL and HL [16]. The finding suggests that HL (head length) and FL (fork length) of *Hypophthalmichthys nobilis* increase at a lower proportion when compared to TL (total length) of the fish. These differences are not unusual and can result from developmental stages, species-specific body shapes, and/or environmental factors like habitat structure, food availability and water quality. Some authors, including Datta *et al.*, in 2013 reported the condition factor 'K' of the studied fish species above 1.0 indicating robustness or wellbeing of the fish [22]. Whereas the calculated condition factor (K) value for *Hypophthalmichthys nobilis* fingerlings, in this study, remained below than the ideal value of 1.0. This study analyzed the length–weight relationship and condition factor of *Labeo bata* from the Ranikot stream in Sindh, Pakistan, to assess its growth pattern and health status [26].

CONCLUSIONS

This study shown a strong LWR in *Hypophthalmichthys nobilis* fingerlings with negative allometric growth pattern. LLRs also represented strong correlations, representing consistent morphometric proportions. Additionally, condition factor (K) of the studied *Hypophthalmichthys nobilis* population was calculated as 0.89, indicating that the carp fingerlings were in moderately good condition but slightly below the optimal health benchmark. Findings of the present work are useful for fisheries management, stock assessment and growth analysis. Future studies on a larger sample size and the inclusion of habitat or seasonal-based variations may help refine these relationships. Generalizability of the results might also be constrained due to the small size of the sample (n = 35). The results are based on the data collected in one hatchery, and this data cannot show the variability of a wild population. It is suggested that future research that involves big sample size and various locations be conducted to confirm these results.

Authors Contribution

Conceptualization: AI
Methodology: SM, MSA, AS, AN, BA
Formal analysis: SM, MSA, AS, AN, BA
Writing, review and editing: AI, SM, MSA, AS, AN, BA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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DOI: https://doi.org/10.54393/mjz.v5i03.151

MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
ISSN (P): 2790-4377, (E): 2790-4385
Volume 5, Issue 3 (July-Sep 2024)

Original Article

OPEN ACCESS

Prevalence and Chemotherapy of Canine Monocytic Ehrlichiosis in Lahore, Pakistan

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ARTICLE INFO

Keywords:
Ehrlichia canis, Microscopy, Immunochromatography, Ticks, Rifampicin

How to cite:
Iftikhar, M., Ahmed, S. S., Ijaz, M., Khan, A. Y., Muneeb, M., Hussain, M., Ahmad, D., Zaman, S., & Hizbullah, . (2024). Prevalence and Chemotherapy of Canine Monocytic Ehrlichiosis in Lahore, Pakistan: Prevalence and Chemotherapy of Canine Monocytic Ehrlichiosis . MARKHOR (The Journal of Zoology), 5(03), 33-39. https://doi.org/10.54393/mjz.v5i03.151

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Received Date: 27th July, 2024
Acceptance Date: 21st September, 2024
Published Date: 30th September, 2024

ABSTRACT

Preventing animals from vector-borne diseases is of prodigious concern in the current era. **Objective:** To estimate the prevalence of Canine Monocytic Ehrlichiosis (CME) (*Ehrlichia canis*) in Lahore, Pakistan. **Methods:** For initial diagnosis, microscopy was performed. Later on, anti-*E. canis* antibodies were detected through immunochromatography (ICA) based *Ehrlichia canis* Ab tests (Quicking biotech ®). A total of 100 dogs showing clinical signs of CME were tested, and an overall seroprevalence rate of 29% was noticed. The success rate of microscopy was observed to be 3% in the present study. **Results:** Occurrence of CME was not statistically associated with age, sex, and breed ($p>0.05$), while body condition ($p<0.001$) and tick infestation ($p=0.004$) were significantly associated with CME. Moreover, blood parameters, including Hb, RBC count, WBC count, and Total platelet count, were decreased ($p<0.001$) in the infected animals. Rifampicin was found to be more effective in diminishing clinical signs and normalization of blood parameters as compared to Doxycycline. **Conclusions:** Dogs in Lahore are at great risk of acquiring CME due to increased tick population and lack of awareness among owners. For rapid diagnosis and timely treatment against the infection, veterinarians can use ICA-based rapid test kits as an effective tool. In addition, Rifampicin is found to be more effective than Doxycycline, and may be preferred for the treatment of Canine Monocytic Ehrlichiosis.

INTRODUCTION

Canine Monocytic Ehrlichiosis (CME) poses a significant threat to the dog population of the world. It is caused by *Ehrlichia canis*, an obligate, intracellular, and pleomorphic gram-negative proteobacterium of the genus Anaplasma and family *Anapalasmataceae* [1]. *E. canis* is transmitted through a Brown tick named *Rhipicephalus sanguineus* of the Ixodidae family prevalent worldwide [2]. *E. canis* multiplies in the salivary glands, hemocytes, and midgut of the tick after feeding on the blood of an infected animal [3]. *E. canis* was first identified in Algeria in 1935 by Donatien and Lestoquard. Later, the disease was reported in Tunisia [4]. Infections due to *E. canis* have been commonly reported from tropical and subtropical areas of the world [5]. In Pakistan, *Ehrlichia* infections are commonly reported in dogs. Literature revealed that the prevalence of *E. canis* in Lahore was 48%, with an overall prevalence of 28% in Punjab, Pakistan [6]. Canine Monocytic Ehrlichiosis obtained global attention in 1970 due to the high mortality rate of German Shepherd dogs owned by the army during the Vietnam War [7]. *E. canis* primarily attacks immune cells, in particular monocytes, resulting in a cytoplasmic membrane-bound cluster of bacteria known as morulae [8]. This disease is multi-systemic and affects multiple organs and has three forms, including acute, subclinical, and chronic [9]. During the acute form, major signs in dogs are pyrexia, anorexia, mild anemia, lymphadenopathy, epistaxis, splenomegaly, and ophthalmic lesions. Moreover, the complete blood count of an infected dog

DOI: https://doi.org/10.54393/mjz.v5i03.151

shows drastic changes, including severe thrombocytopenia and mild leukopenia during the acute phase [10]. In subclinical CME, thrombocytopenia of the mild category, along with a notable decline in leucocytic count, is observed [11]. The chronic form of disease is more lethal and involves severe pancytopenia, anemia, weakness, paralysis, and death [12]. Microscopic examination of stained thin blood smear, PCR, and ELISA are the technical aids for confirmatory diagnosis [13]. Inclusions of this rickettsial organism are present in the cytoplasm of monocytes, detected when blood is stained with Romanovsky stains. Inclusions are present as dense granules that are identified after staining. Multiplication of organisms observed in macrophages and endothelial cells can be identified by microscopy of stained blood smears [14]. The inclusion bodies vary in shape and size. They can be round or oval, and the size of the smaller spherical structure can be 0.2µm -0.4µm, and of the larger ovoid structures can range between 2.5µm -3µm in diameter. The success rate of morulae identification through blood smear is very low, and it is a difficult and time-consuming route [15]. Microscopy of blood smears stained with Giemsa stain appeared to be an insensitive technique in the subclinical form of CME. Although PCR, IFA, and ELISA are more specific and sensitive but they could be expensive as well as time-consuming, moreover require specialized personnel to process samples. Immunochromatography is a rapid and comparatively cheaper diagnostic technique that could prove convenient in field practices such as veterinary clinics. Sensitivity and specificity of immunochromatography-based tests are reported to be 79.2% and 95.3% respectively [16]. Multiple clinical and experimental trials have shown that dogs with acute infection recovered subsequently to specific treatment with effective antibiotics administered for the recommended period. Even with the recommended dose of antibiotics, some dogs are found to be subclinical carriers if treated for a shorter duration. The drug of choice for treatment of Ehrlichiosis is Doxycycline at the dose rate of 5mg/kg twice daily (BID) for 4 weeks [17]. Rifampicin, known to be an inhibitor of DNA-dependent RNA polymerase, specifically its B subunit, has been found effective in the clearance of subclinical, clinical, as well as chronic CME. Rifampicin 15mg/kg twice daily (BID) for 21 days was found effective in dogs that did not respond after Doxycycline therapy [8]. Evaluation of therapeutic efficacy is also a significant challenge. Usually diminishing of clinical signs and normalization of hematological parameters are considered as indicators of the therapeutic efficacy [17].

This study aimed to assess the current status of CME in Lahore, Pakistan, to develop a better and alternative route for rapid diagnosis, along with a more effective line of treatment.

METHODS

A cross-sectional observational study was conducted from April 2020- September 2020. Blood samples were collected as per recommendations and permission from the Ethical Review Committee of the University of Veterinary and Animal Sciences (UVAS), Lahore. The current study was carried out at the Pet center (UVAS), government hospitals, and private veterinary clinics in Lahore. A total of 100 pet dogs' samples were collected. Sample size was calculated using an expected prevalence of 33%, a 95% confidence level, and a 10% margin of error. Dogs showing any of the following signs, i.e., fever, lethargy, hematuria, anemia, vomiting, and epistaxis, were included in this study. Inspected dogs were classified into 3 groups concerning age: a)<1 year, b)1-3 years, and c)>3 years. Data regarding owner, animal, management, environment, etc., were recorded on a predesigned questionnaire at the time of sample collection. Sample collection was done in two ways. Convenience sampling was used. Initially, thin blood smears (triplet) were made by using ear tip venipuncture and were air-dried on the spot. Then, blood samples were drawn from the cephalic vein into 3 ml gel-clot activator and EDTA-coated vacuum vials separately. The samples were transported to the Medicine laboratory, UVAS, in an ice box. The sera were centrifuged and preserved (-40°C) for further serological assay. The blood smears were fixed in a 10% ethanol solution, and Giemsa stain was used for staining of the blood smears. After 15 min, the stain was wiped out with water, and the smears were air-dried and observed at 100X oil immersion lens. Then smears were examined for the presence of intracytoplasmic inclusion bodies. For serological diagnosis of CME, a commercially available Quicking *Ehrlichia canis* Ab test kit (Quicking biotech. Cat No. W81132) was used according to the manufacturer's instructions. The result was interpreted as follows: Positive: Appearance of both C band and T band, no matter T band was clear or faint. Negative: If only a clear C band appeared. Invalid: When no color band appeared in the C zone, no matter whether the T band appeared. For hematology, 10 dogs were equally divided into two groups based on serological diagnosis, i.e., group A: CME-infected dogs, group B: healthy dogs. Blood samples were analyzed (total erythrocyte count; TEC, total leukocyte count; TLC, hemoglobin; Hb, platelet count; PC, and packed cell volume; PCV) using a hematological analyzer (Model no. DW-3680/DW-36). Hematology was performed at day 0, 7, 14, and after completion of the treatment protocol at day 21. Chemotherapy was conducted on 10 dogs positive for Ehrlichiosis based on microscopy and serological testing, and were divided into 2 equal groups, A and B. Doxycycline

DOI: https://doi.org/10.54393/mjz.v5i03.151

@ 5 mg/kg body weight twice daily (BID) and Rifampicin @ 15 mg/kg body weight twice daily (BID) PO were used to treat dogs of group A and B, respectively, for 21 days. The efficacy of the drug used was measured on the basis of the disappearance of clinical signs and hematological parameters noticed at day 0, 7, 14, and 21, after initiation of treatment. Data analysis was conducted using appropriate statistical methods. Serological prevalence was calculated using the Thrusfield formula. Therapeutic trials against ehrlichiosis were evaluated using the chi-square test. Hematological data were analyzed using the Student's t-test, while repeated-measures ANOVA was employed to assess hematological parameters in therapeutic trials. All statistical analyses were performed using SPSS version 20.0, with a p-value of <0.05 considered statistically significant.

RESULTS

Out of 100, dog samples were found positive (29%) for *Ehrlichia canis*. Prevalence was marginally found higher (p=0.848) in dogs of age 1-3 years, followed by the group of age >3 years and <1 year, respectively. *E.canis* infection was noted to be numerically more prevalent (p=0.739) in male dogs as compared to the female (Table 1).

Table 1: Prevalence of CME According to Age and Sex of Animal

Variables	No. of samples	Positive	Negative	p-Value
Age				
<1 Year	17	4 (23.53%)	13 (76.47%)	0.848
1-3 Years	62	19 (30.64)	43 (69.36%)	
>3 Years	21	6 (28.57)	15 (71.43%)	
Sex				
Male	63	19 (30.16%)	44 (69.48%)	0.739
Female	37	10 (27.02%)	27 (72.8%)	

Presence of ticks and emaciated body condition were noted as significant determinants for CME (p=0.004; p<0.001, respectively)(Table2).

Table 2: Association of Tick Infestation and Body Condition with Prevalence of CME

Variables	Category	No. of samples	Positive	Negative	p-Value
Tick Infestation					
Ticks infestation	Yes	57	23 (40.35%)	34 (59.65%)	0.004
	No	43	6 (13.95%)	37 (86.05%)	
Ticks history	Yes	72	27 (37.50%)	45 (62.50%)	0.003
	No	28	2 (7.14%)	26 (92.86%)	
Body Condition					
Body Condition	Healthy	52	4 (7.69%)	48 (92.31%)	<0.001
	Emaciated	48	25 (52.08%)	23 (47.92%)	

Clinical signs such as fever (p=0.642), vomiting (p=0.222), splenomegaly (p=0.786), and lethargy (p=0.462) were noted as non-significantly associated with CME, whereas epistaxis (p=0.001) and anemia (p=0.025) were noted to be significantly associated. Thrombocytopenia was shown by 23 (p=0.025) dogs positive for ehrlichiosis and was noted to be statistically significant (Table 3).

Table 3: Association of Clinical Manifestations and CME

Variables	Category	Positive (%)	Negative (%)	p-Value
Fever	Present	17 (30.90)	38 (69.10)	0.642
	Absent	12 (26.67)	33 (73.33)	
Vomiting	Present	09 (39.13)	14 (60.87)	0.222
	Absent	20 (25.97)	57 (74.03)	
Mucous Membranes	Pink	08 (17.78)	37 (82.22)	0.025
	Pale	21 (38.18)	34 (61.82)	
Epistaxis	Present	11 (61.11)	07 (38.89)	0.001
	Absent	18 (21.95)	64 (79.05)	
Splenomegaly	Present	07 (25.00)	19 (75.00)	0.786
	Absent	22 (29.73)	52 (70.27)	
Lethargy	Lethargic	08 (24.24)	25 (75.76)	0.462
	Active	21 (31.34)	46 (68.66)	

p-value<0.05 indicates significance.

Hematological parameters are severely affected by *E.canis*. There is a significant decrease in HB, PCV, RBCs, and Thrombocytes (Table 4).

Table 4: Blood Parameters of Diseased and Healthy Dogs

Parameters	Diseased dogs	Healthy dogs	p-Value
WBCs	8.48 ± 1.67	10.76 ± 0.46	<0.001
RBCs	2.94 ± 0.46	6.48 ± 0.61	<0.001
Hb	7.40 ± 1.34	15.80 ± 1.92	<0.001
PCV	28.00 ± 1.58	41.80 ± 2.38	<0.001
Thrombocytes	44 ± 7.86	313 ± 23.55	<0.001
Monocytes	5.74 ± 0.86	6.44 ± 0.96	0.261
Lymphocytes	20.08 ± 0.84	23.76 ± 3.48	0.051
Granulocytes	66.40 ± 3.13	68.80 ± 3.49	0.286

p-value<0.05 indicates significance

Characteristic *E.canis* morulae were found in the stained microscopic blood slides of only 3 positive dogs. While 29 dogs tested positive serologically using Quicking *Ehrlichia canis* Ab tests (Quicking biotech, Cat No. W81132). Results of Immunochromatographic Assay (ICA) for detection of *Ehrlichia canis* antibodies in canine serum samples using the Quicking *Ehrlichia canis* Ab test kit were shown (Figure 1).

DOI: https://doi.org/10.54393/mjz.v5i03.151

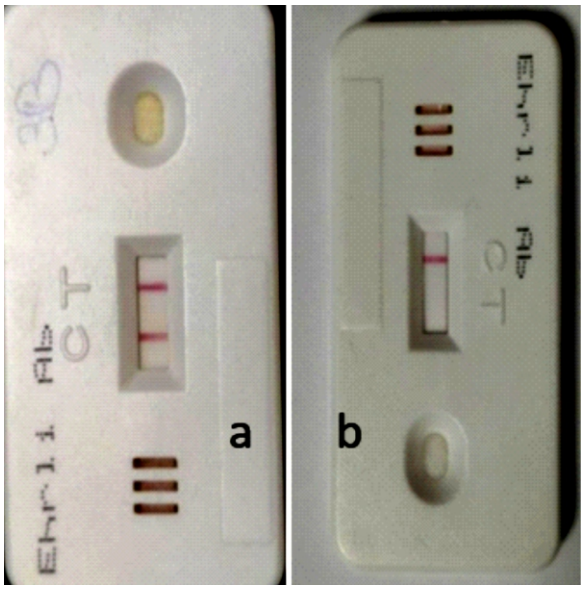

Figure 1: a) Rapid Test Kit Showing Positive Test Result and (b) Rapid Test Kit Showing Negative Test Result

Results of the Rifampicin-treated group showed that all dogs in the group had normalized temperature, controlled vomiting, and epistaxis by day 14. Anorexia and blood parameters were normalized by day 21. Blood parameters, including Hb, RBCs, platelets, and PCV, were noted to be in normal range by day 21, showing 100% efficacy in the elimination of infection. Results of the Doxycycline-treated group showed that 4 dogs out of 5 in the group had normalized temperature, controlled vomiting, epistaxis, and anorexia by day 21. Blood parameters of 4 dogs got normalized, falling in the lower limits of the normal ranges, while 1 dog remained with abnormal hematology after the completion of the protocol. Blood parameters, including Hb, RBCs, platelets, and PCV, were noted to be in normal range by day 21, depicting efficacy of 80% (Table 5).

Table 5: Comparison of Blood Parameters of Dogs Treated with Doxycycline and Rifampicin

Parameter	Groups	Day 0	Day 7	Day 14	Day 21	CI 95 %	p-Value
Hb	Group A	7.56 ± 0.89	7.920 ± 0.83	9.180 ± 1.11	10.78 ± 1.65	10.94-12.33	<0.001
	Group B	7.60 ± 1.517	9.960 ± 1.11	11.740 ± 0.56	13.06 ± 0.39		
	Healthy	15.22 ± 1.92	15.440 ± 1.51	15.620 ± 1.57	15.56 ± 1.53		
RBCs	Group A	3.10 ± 0.69	3.860 ± 0.68	4.680 ± 1.02	4.98 ± 1.11	4.75-5.38	<0.001
	Group B	3.04 ± 0.18	4.080 ± 0.22	4.920 ± 0.26	5.88 ± 0.24		
	Healthy	6.48 ± 0.61	6.640 ± 0.57	6.680 ± 0.52	6.44 ± 0.38		
Platelets Count	Group A	44.20 ± 5.89	151.40 ± 36.07	279.40 ± 97.11	279.80 ± 59.88	258.19-297.43	<0.001
	Group B	44.60 ± 7.86	166.00 ± 6.96	362.00 ± 20.29	323.00 ± 33.04		
	Healthy	410.00 ± 46.16	420.40 ± 39.93	431.80 ± 34.29	421.20 ± 29.97		
PCV	Group A	29.60 ± 2.07	33.00 ± 3.39	35.20 ± 4.09	36.80 ± 4.38	37.22-40.58	<0.001
	Group B	30.00 ± 2.91	36.60 ± 1.67	41.60 ± 1.67	43.00 ± 0.71		
	Healthy	45.60 ± 4.56	44.40 ± 3.51	45.20 ± 3.96	45.80 ± 3.35		

Abbreviations: Hb; Hemoglobin, RBCs; Red blood cells, PCV; Packed cell volume. Values differ significantly where p-value<0.05

DISCUSSION

Canine Monocytic Ehrlichiosis is reported throughout the globe [1] in tropical as well as subtropical areas [5]. Increased occurrence of CME is reported because its vector population density is high throughout the world [18]. In Pakistan, prevalence and molecular characterization of E.canis have been reported [6], but to date, there is no data available regarding the efficacy of the treatment protocol. Therefore, this is a novel study regarding serological prevalence and association of risk factors with dynamics of the disease in dogs of District Lahore, Pakistan. Overall prevalence of CME in this study, on the basis of immunochromatography-based rapid test kits, was 29%. Current findings are supported by the previous studies, which reported PCR-based prevalence to be 30% in India [19] and ELISA-based prevalence to be 30.98% in Iran [20]. In contrast, a higher prevalence of 48% in Lahore [6] and 57.7% in India [21] has been reported. On the other hand, a 9.6% prevalence has been documented on the basis of Immunochromatography in Iran [16]. The plausible reason for variation in the prevalence of CME in different regions of the world may be associated with geographical climate, socioeconomic status of the population, vector load, and infection status. Analysis of risk factors showed that it is socioeconomic and climatic factor that plays a vital role rather than the immunological status of the animal [22]. A higher number of male dogs tested positive for the disease as compared to female dogs in the current study, but this association was noted to be non-significant, indicating no relationship between prevalence of disease and sex of animal, as supported by similar studies in which more male

DOI: https://doi.org/10.54393/mjz.v5i03.151

dogs were reported positive for the disease than female dogs [6]. On the other hand, Selim *et al.* reported a higher prevalence of disease in female dogs as compared to male dogs [23]. Adult animals were infected at a higher rate as compared to young animals in the current study, which is also supported by [24]. This increased infection rate in adult animals could be explained by increased exposure to the vector over time. Contrary to this, Botros *et al.* reported a higher incidence of CME in young animals than in adults [25]. Canine Monocytic Ehrlichiosis is a tick-borne ailment, and that is why the rate of infection in dogs with tick infestation and a history of previous tick infestation is higher. In the current study, animals with tick infestation tested positive at a higher rate as compared to those without ticks. This finding is also supported by [10]. In this study, animals with poor body condition had a higher infection rate as compared to animals with good body condition. This finding is also supported by [24]. Microscopic identification of *E. canis* is comparatively problematic, as the success rate of this technique is very low. In the current study, only 3 samples out of 29 positive samples and overall, 100 samples tested were found positive on the basis of microscopy, with a success rate of only 3% which is supported by [26] who reported a success rate of microscopic examination to be 2.33%. Serological diagnosis through immunochromatography assay (ICA) based rapid test kits provided better results and are a better and quicker tool to diagnose the disease at early stages, and these results are supported by [16]. The values of RBCs, WBCs, PCV, Hb, and Thrombocytes were significantly decreased in Ehrlichia-infected dogs in the current study. Anemia is characterized by decreased RBCs, HB, and PCV. The findings of the current study were supported by Parashar *et al.* who screened 46 infected dogs and found similar results as in the current study [27]. Thrombocytopenia, the characteristic feature of CME, can be explained by low production of thrombocytes due to aplastic anemia, annihilation of thrombocytes as a result of an immune response, sequestration, and depletion of the cells as a result of an inflammatory response. Severe thrombocytopenia can be a predictor of clinical as well as subclinical ehrlichiosis. This statement is also supported by Moonarmart *et al.* who reported that every 10000 decrease in thrombocytes increases the 15% probability of an animal being positive for ehrlichiosis [10]. Rifampicin, a DNA-dependent RNA polymerase inhibitor, is considered an alternative to Doxycycline in the treatment of ehrlichiosis in dogs. In the current study, Rifampicin was found to be more effective in diminishing clinical manifestations along with normalization of hematological parameters as compared to Doxycycline against canine ehrlichiosis. All 5 animals treated with Rifampicin showed complete recovery after completion of the treatment protocol. These rectifications of clinical signs in response to Rifampicin were supported by Schaefer *et al.* who reported complete recovery of 2 Ehrlichia-infected dogs after completion of the Rifampicin regimen [28]. Akhtardanesh *et al.* also reported similar results of all dogs treated with Rifampicin tested negative and clinical signs diminished after a week [29]. However, Doxycycline is considered as drug of choice against ehrlichiosis in dogs, but in the current study, 4 dogs out of 5 recovered when treated with Doxycycline. These findings are well supported by Mylonakis *et al.* who reported failure in clearance of infection when treated with Doxycycline against CME [8].

CONCLUSIONS

The prevalence of Canine Monocytic Ehrlichiosis in Lahore, Pakistan, is found to be 29%. Rifampicin is found to be more effective than Doxycycline (drug of choice for Ehrlichiosis), and may be preferred for treatment of Canine Monocytic Ehrlichiosis.

Authors Contribution

Conceptualization: MI[1]
Methodology: MI[1], SSA, AYK
Formal analysis: MI[1], MI[2], AYK, MM
Writing review and editing: SSA, AYK, MM, MH, DA, SZ, H

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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DOI: https://doi.org/10.54393/mjz.v5i03.151

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DOI: https://doi.org/10.54393/mjz.v5i03.117

MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
ISSN (P): 2790-4377, (E): 2790-4385
Volume 5, Issue 3 (July-Sep 2024)

Case Report

OPEN ACCESS

Diagnosis and Treatment of Infectious Coryza in a Peacock (Pavo cristatus): A Case Report

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ARTICLE INFO

Keywords:
Infectious Coryza, Peacock Treatment, Respiratory Infection, Gentamicin Therapy, Antibiotic Sensitivity

How to cite:
Rehman, F. U., Ahmad, K., Awais, M., Mushtaq, S., Parmina, ., Mohsin, M., Ullah, Z., Khan, A. M. A., Raza, B., & Asif, M. (2024). Diagnosis and Treatment of Infectious Coryza in a Peacock (Pavo cristatus): A Case Report: Infectious Coryza in Peacock. MARKHOR (The Journal of Zoology), 5(03), 40-44. https://doi.org/10.54393/mjz.v5i03.117

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Received Date: 28th July, 2024
Acceptance Date: 11th September, 2024
Published Date: 30th September, 2024

ABSTRACT

Infectious Coryza (IC) is a contagious respiratory bacterial infection in peacocks. The current study evaluated the outcomes of treating infectious coryza in a peacock. The adult peacock at the age of 2 years was presented with a history of anorexia, difficulty breathing, sticky eyes, nasal and ocular discharge, and semi-solid swelling under the eyes. Clinical examination confirmed respiratory depression, a swollen head and face, gasping, occluded eyelids, sticky purulent discharge from the eyes and nostrils, moist rales, depression, and recumbency. Treatment included the use of injection of gentamicin, meloxicam, and multivitamins therapy, along with surgical debridement of facial edematous swelling. The peacock successfully recovered after 5 days of treatment. It was concluded that infectious coryza was a treatable disease, and an antibiotic sensitivity test was highly recommended for selecting a suitable antibiotic against bacterial infections.

INTRODUCTION

The peacock is the male peafowl. The female peafowl is technically called a peahen, but many refer to the female peafowl simply as peafowl. The peafowl chick is called a peachick [1]. Infectious Coryza is an infectious and contagious respiratory bacterial disease affecting several avian species, particularly poultry, peacocks, and other game birds. The disease initially presents as acute to sub-acute but can progress to a chronic state as it spreads through the flock [2]. IC is caused by the bacterium *Haemophilus paragallinarum.* This disease is characterized by clinical signs such as respiratory distress, purulent nasal and ocular discharge, swollen head and face, gasping, dullness, occluded eyelids, depression, and anorexia [3]. *Haemophilus paragallinarumis* is a Gram-negative, non-

DOI: https://doi.org/10.54393/mjz.v5i03.117

motile, and polar-staining bacterium. The Indian Peafowl belongs to the family Phasianidae (also known as pheasants) and their order is Galliformes [4]. The chicken (Gallus gallus) is the natural host for *Haemophilus paragallinarum*. This bacterium affects birds of all ages. The disease is mostly transmitted via drinking water contaminated with nasal exudates containing infection. Infection may also spread via direct contact and airborne resources such as infected dust and water droplets [5]. The current study was designed to evaluate surgical and medical treatments for IC, including microbiological and histopathological assessments. Case Report: An Indian Blue Peacock of 2 years came to the outdoor surgery clinic at the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. The patient's history included labored breathing, anorexia, nasal and ocular discharge, sticky eyes, and semi-solid swelling under the eyes. Clinical signs of the infection were scored and are presented in table 1 [6].

Table 1: Clinical Signs of Infectious Coryza

Disease Name	Grade	Clinical Signs
Infectious Coryza	Grade 0	No signs
	Grade 2	Conjunctivitis with Partial Closure of Eye, Swollen Infraorbital Sinus
	Grade 3	Conjunctivitis with Closure of Eye, Obvious Swelling of Periorbital and Paranasal Sinus
	Grade 4	Conjunctivitis with Complete Closure of Eye, Sticky Eyes, Marked Swelling of Periorbital and Paranasal Sinus, Swollen Head, Facial Edema, Gasping and Moist Rales

A clinical examination and signs include swollen head and face, respiratory distress, occluded eyelids, gasping, sticky purulent ocular and nasal discharge, depression, moist rales, and recumbency (Figure 1).

Figure 1: The Swollen Face of Infected Peacock

A surgical approach was recommended to remove the caseous mass from under the eye area. The peacock was restrained, the hair under the eye was clipped, and the surgical site was scrubbed with gauze dipped in 5% methanol (Methylated Spirit®, Oval Pharma®, Pakistan). Local anesthesia using 2% lignocaine with epinephrine (Medicain®, Huons Co. Ltd®, South Korea) with a dose of 1mg/kg was administered around the affected area to reduce pain. An elliptical incision was made, and the caseous mass was removed. Extra skin was also excised and removed. Bleeding was controlled using a tincture of benzoin (Tinct Benzoin Co®, Oval Pharma®, Pakistan) (Figure 2). The tincture of benzoin has adhesive, dehydrating, and antiseptic properties.

Figure 2: Removal of Caseus Mass

In figure 3, they removed mass indicating the extent of tissue excised during surgical intervention. The sampling was done by taking swabs from eyes, nostrils, and mouth with the help of a platinum loop for microbial isolation, identification, culture, and antibiotic sensitivity test. Antibiotic sensitivity testing was done using the disc diffusion method. A bacterial sample was collected and swabbed onto a Petri dish containing chicken serum, followed by cross streaks of Staphylococcus aureus as a Nicotinamide Adenine Dinucleotide (NAD) provider for the medium. The Petri dish was then incubated at 37°C for 24 hours. Antimicrobial discs containing gentamicin were placed on the plate, and the zone of inhibition was measured after 18 hours of incubation. Gram staining was done. A sample of the caseous mass collected was also used for histopathological examination. All samples were sent to the University Diagnostic Lab (UVAS). Pending the laboratory results, the peacock was treated with intramuscular injection of gentamicin (Gentafar®, Farvet Lab, Netherland) at 4.4 mg/kg of body weight, and meloxicam (Diclostar®, Star Lab, Pakistan) at 0.2 mg/kg three times in a day, and orally with orange juice, vitamin A, and multivitamin drops [7].

DOI: https://doi.org/10.54393/mjz.v5i03.117

Figure 3: Removed Mass

In figure 4, gram's staining of swab sample showing *Haemophilus paragallinarum* under a light microscope with magnification 1000x and scale bar 10 µm.

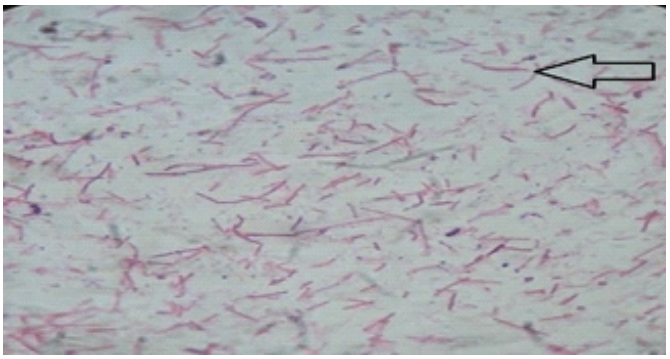

Figure 4: Gram Staining of *Haemophilus paragallinarum* in Swab Sample at 1000x Magnification

In figure 5, Hematoxylin and eosin (H and E) staining of the trachea reveals significant pathological changes under 100x magnification with a 10 µm scale bar. Panel A illustrated the disruption of cilia, indicating damage to the respiratory epithelium. Panel B showed infiltration of neutrophils, highlighting an acute inflammatory response, while Panel C demonstrated edema, reflecting fluid accumulation in the tissue.

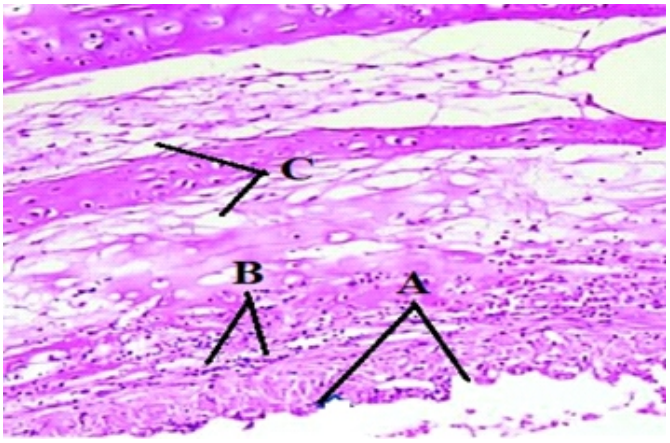

Figure 5: H and E Staining of Trachea: Disruption of Cilia (A), Neutrophil Infiltration (B), and Edema (C) at 100x Magnification (Scale Bar: 10 µm)

In figure 6, the antibiotic susceptibility test for *Haemophilus paragallinarum*, the causative agent of infectious coryza, evaluates the effectiveness of gentamicin in inhibiting bacterial growth. The test involves inoculating a culture medium with the bacterial strain and applying varying concentrations of gentamicin. After incubation, the zones of inhibition are measured to determine the antibiotic's efficacy.

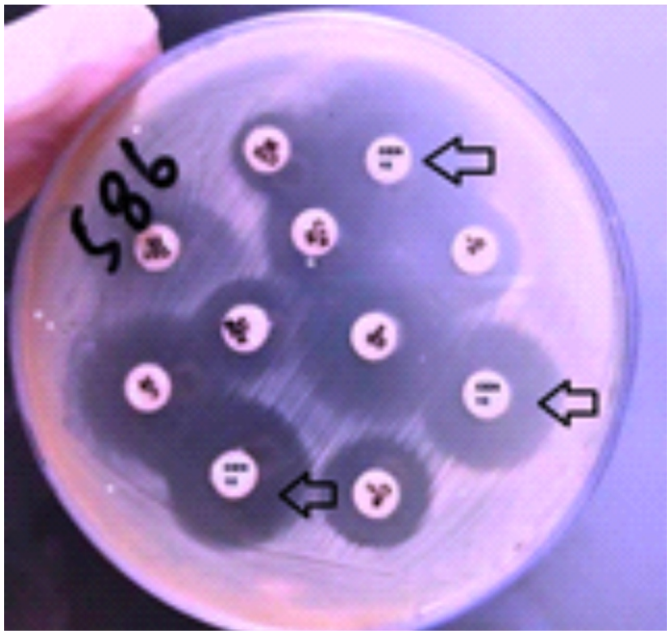

Figure 6: Antibiotic Susceptibility Test for Infectious Coryza against Gentamicin

DISCUSSION

In the current case study, the clinical examination of the peacock confirmed oculonasal discharge, conjunctivitis and discharge, facial edema, respiratory noise, mucoid rales, swollen infraorbital sinus, and exudates in the conjunctival sac in chickens. The laboratory examination revealed the presence of *Haemophilus paragallinarum* on blood agar with Gram staining. The Gram-negative rods were observed beneath a light microscope. These findings were consistent with the results of [8]. In another study of chickens, the documented clinical signs included respiratory distress, gasping, swollen head and face, occluded eyelids, purulent nasal and ocular discharge, anorexia, moist rales, depression, and recumbency [9]. The clinical signs of IC revealed extensive inflammatory lesions, which can result from complications because of *Avibacterium spp*. Other bacteria such as *Klebsiella*. Pneumonia supports the severity of infectious coryza with the mortality rate in chickens from 0.7% to 10% [10]. Other studies showed clinical signs including secretion of purulent and watery nasal and eye discharge, swelling of the infraorbital sinus and face, edema of the head, air

DOI: https://doi.org/10.54393/mjz.v5i03.117

vasculitis, conjunctivitis, fetid odor from the conjunctival sac filled with exudates, dyspnea, anorexia and inflamed wattles [11]. The treatment included the antibiotic gentamicin, which was continued for 5 days after confirmation of the antibiotic sensitivity test, and the Non-Steroidal Anti-Inflammatory Drug (NSAID) meloxicam along with multivitamins, used for 5 days at a frequency of three times a day. The signs began to reduce in 3-4 days, and the peacocks recovered fully after a week. Similar results were reported in a study of peacocks [6]. In another study, after five days of treatment, there was a significant regression of facial swelling and total absence of respiratory distress in peacocks [12]. The bacterium was sensitive to gentamicin, neomycin, erythromycin, lincomycin, pectinomycin, oxytetracycline, ciprofloxacin, tylosin, and azithromycin [13]. Histopathological examination of the trachea revealed that the respiratory tract was disintegrated, with disruption of epithelia and cilia, edema, and infiltration of neutrophils and macrophages. These results were according to [14]. The histopathological lesions including deciliation and tracheitis were observed in the lungs and trachea of affected birds which showed marked interstitial pneumonic changes. These changes are associated with complex and chronic cases of IC [15]. The infraorbital sinus was found with heterophilic granulocytes and infiltration of macrophages. Also, there was severe head edema and hemorrhages of the nasal cavity. The trachea showed lodging, adhesion, and partial exfoliation of cilia. The mucosal edema in the lamina propria and inflammatory cell infiltration were also found [16].

CONCLUSIONS

It was concluded that infectious coryza was a curable disease. The effective treatment could include surgical debridement and excision of facial purulent and edematous swelling, along with using antibiotic gentamicin, non-steroidal anti-inflammatory drug meloxicam, and multivitamins as supportive therapy. It can result in the successful recovery of the peacock from infectious coryza disease. An antibiotic sensitivity test could be very useful in the selection of a suitable antibiotic for bacterial infections.

Authors Contribution

Conceptualization: FUR
Methodology: MA[1]
Formal analysis: MM
Writing, review and editing: FUR, KA, SM, P, MM, ZU, AMAK, BR, MA[2]
All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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