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BOOK OF ABSTRACTS

“Recent Advances in Zoology in the Era of Climate Change (RAZECC-2026)”

RAZECC-2026
May 04-05,2026

Department of Basic and Applied Zoology
Shaheed Benazir Bhutto University,
Sheringal, Dir (U), KP, Pakistan

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1st International Conference on "Recent Advances in Zoology in the Era of Climate Change"

(RAZECC-2026)
May 04-05, 2026

Organized by

Department of Basic and Applied Zoology
Shaheed Benazir Bhutto University, Sheringal, Dir (U), KP, Pakistan

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MESSAGE

It is my proud privilege and honor to welcome you all to the 1st International Conference on “Recent Advances in Zoology in the Era of Climate Change (RAZECC-2026). The objective of this conference is to provide a unique opportunity for Researchers, Scientists, and Professionals from around the world to come together and share their innovative ideas, experiences, and cutting-edge research in RAZECC-2026,being held at Shaheed BB University, Sheringal, Dir Upper, Khyber Pakhtunkhwa, Pakistan. The RAZECC-2026 will be an important network opportunity for faculty members, researchers, and students. RAZECC-2026 is the result of the endless efforts of the organizing committee. The committee worked hard to organize the conference, including local arrangements, inviting papers, and evaluation/screening submitted papers. Therefore, I congratulate them on hosting such a wonderful event. I am also thankful to the reviewers for sparing valuable time for the review process and selection of top-quality papers for publication at the conference. I am hopeful that the contribution of the local and foreign scholars and researchers at this forum will strengthen the academia, industry, and R&D organizations’ future and ongoing endeavors. I am confident that this collection of quality abstracts and invited talks will provide you with an insight into recent trends and research directions in the fields ofLife Science and Zoology.

May this conference be a complete success for everyone

Prof. Dr. Muhammad Shahab
Patron in Chief, RAZECC-2026

PREFACE

The 1^{st} International Conference on “Recent Advances in Zoology in the Era of Climate Change (RAZECC-2026) will be held on May 04-05, 2026, at Shaheed Benazir Bhutto University, Sheringal, Dir Upper, Khyber Pakhtunkhwa, Pakistan. It has been organized by the Department of Basic and Applied Zoology, Shaheed Benazir Bhutto University, Sheringal, Dir Upper, Khyber Pakhtunkhwa, Pakistan. “Recent development in Zoology under climate change” was selected as the theme of the conference. The conference attracted 300s abstracts to be presented at the conference, with more than 100 participants with different affiliations, including researchers, academicians, and nascent students. From these s, 150 s are then shortlisted to be published in this proceeding. The abstracts offered in this proceeding are expected to stimulate new perceptions in talking to promote science andtechnology culture in Pakistan.The main objective of the conference is to provide a dedicated platform for researchers, young, inspired scientists, and academicians to share the knowledge that is yet to be revealed in the field of Zoology and Life Sciences at this 2-day event scheduled for May04-05, 2026. The conference will comprise keynote addresses, oral presentations, and poster presentations. The event will bring together a unique mix of experts, researchers, graduate and undergraduate students, as well as decision-makers from both academia and industry across the country to exchange their knowledge, expertise, and research innovations to build an excellent “Life Science and Zoology” conference. We hope that youwill find this 1st International Conference on “Recent Advances in Zoology in the Era of Climate Change (RAZECC-2026) perceptively inspiring andthat the RAZECC-2026 is a prestigious event organized with a motivation toprovide an international platform for scientists and scholars around theworld to share their research findings in the various research fields.

Dr. Asmat Ullah Khan
Conference Chair

ACKNOWLEDGMENT

It is our pleasure to welcome you to the 1st International Conference on “Recent Advances in Zoology in the Era of Climate Change (RAZECC-2026). We thankthe authors for giving the content of the program in the form of postersand oral presentations. We are also grateful to the keynote speakers fromacademia and research centers. These valuable speeches will guide us to a superior appreciationof the fields of Life Science and Zoology. We also thank our sponsors, includingthe Higher Education Commission (HEC), Islamabad, Pakistan Scientific andTechnological Information Center (PASTIC), and Shaheed Benazir Bhutto University, Sheringal, Dir Upper, Khyber Pakhtunkhwa, Pakistan. Without their support, it would not be possible to hold this international conference. We are thankful to all organizers, who worked hard day and night tomake this event successful.

Dr. Asmat Ullah Khan
Conference Chair

ABOUT PASTIC

The Pakistan Scientific & Technological Information Centre (PASTIC) is a constituent organization of the Pakistan Science Foundation (PSF) operating under the Ministry of Science and Technology (MoST), Government of Pakistan. PASTIC is a premier national institution specialized in the collection, organization, management, and dissemination of Scientific and Technological Information (STI) to support research, development, and industrial innovation across the country.

The PASTIC National Centre is located at the Quaid-e-Azam University Campus, Islamabad, with a nationwide network of six Sub-Centres in Karachi, Lahore, Peshawar, Quetta, Faisalabad, and Muzaffarabad, ensuring broad regional outreach.

Since its inception, PASTIC has played a pioneering role in supporting Pakistan's research community, particularly during the early stages of national S&T infrastructure development. Its services historically included the supply of scientific and technical literature, abstracts and indexes, bibliographies, translations, patent information, science reference library services, technological information transfer, computer-based information dissemination, and reprographic and publication services.

For further details visit: www.pastic.gov.pk

PASTIC Objectives

- Development of National Scientific & Technological Information (STI) resources (databases)
- Dissemination of Scientific & Technological Information through contemporary reference information tools
- Collaboration & Cooperation with institutional libraries/repositories for resource sharing
- Promotion of R&D based industrial development
- Printing of S&T/R&D Publications
- Capacity/skill development of researchers, information professionals, innovators & entrepreneurs
- Development of collaborations with national and international information networks

PASTIC Functions

S&T Publications

- *Technology Roundup:* Publish bi-monthly bulletin by repackaging of latest global Trade and Technology information.

- Abstract Books of Conferences: PASTIC supports publication/printing of Abstract Books of Conferences organized by various S&T universities (on request).

PASTIC Online databases

- Pakistan Science Abstracts (PSA): Abstracts of research published in Pakistani S&T Journals & Conference Proceedings etc.
- National Digital Archive (NDA): Full text digital repository of National Journals
- PakCat: Union Online Public Access Catalogue (OPAC) of books available in Scientific & Technological Libraries of Pakistan
- Digital Repository of Indigenous S&T literature
- Directory of Scientific Periodicals of Pakistan: An index of scientific periodicals (e.g., Journals, Magazines etc.) published in Pakistan.
- Database of R&D Projects executed in Pakistan
- Database of Books published by Pakistani authors
- National Scientists Directory (NSD)
- Industry related databases (e.g., Industries, Industrial challenges etc.)

Promotion of Commercializable Technologies & Industrial Products

Organize STEM and IT Expo for promotion of local R&D, SMEs, technologies/products/services, as well as empowering youth and general public on new and faster ways of delivering and accessing information.

National Science Reference Library Facility

A state-of-the-art Traditional Library facilitating the researcher through following services: Reference & Referral Services; Reader Service; Internet Service, Journal Listings; Photocopying & Scanning Services.

Skill Development/Capacity Building

Organize Seminars/Workshops /Trainings/ for capacity building of:

- Young Researchers on Data analysis, Reference Management etc.
- Women Entrepreneurs on E-marketing &E-business skills
- Library Professionals on Library automation & digitization
- Journal Publisher/Editors on E-Journal management & publishing
- Researchers and Innovators on Intellectual Property Rights, Media Information Literacy

CONFERENCE PROGRAM

Venue: Main Library, SBBU, Sheringal, Dir (U), KPK, Pakistan

Time	**Day I,04th May 2026 (Main Library)**	
08:00 – 09:00	Registration	
	Inauguration ceremony	
09:00 – 09:10	Recitation of the Holy Quran	
09:10 – 09:20	Conference Overview/Welcome Speech **Dr. Asmat Ullah Khan**/Conference Chair	
09:20 – 09:30	Opening Remarks, Vice Chancellor **Prof. Dr. Muhammad Shahab**, SBBU, Sheringal	
09:30 – 09:45	Address by **Prof. Dr. M. Akram Shaikh** (Director General, PASTIC)	
09:45 – 10:15	Keynote Address 1	
10:15 – 10:30	**Morning tea**	
Day I: 04th May 2026 (Hall I)		
10:30 – 11:00	Keynote Address 2	Paper 1
11:00 – 11:30	Keynote Address 3	Paper 2
11:30 – 12:00	Keynote Address 4	Paper 3
12:00 – 12:30	Keynote Address 5	Paper 4
12:30 – 13:00	Keynote Address 6	Paper 5
13:00 – 14:00	**Lunch and prayer break**	
14:00 – 14:30	Keynote Address 7	Paper 6
14:30 – 15:00	Keynote Address 8	Paper 7
15:00 – 15:30	Keynote Address 9	Paper 8
15:30 – 16:00	Keynote Address 10	Paper 9
16:00 – 16:30	Keynote Address 11	Paper 10
16:30 – 17:00	Keynote Address 12	Paper 11
Day II: 05th May 2026, Main Library, (Hall I)		
08:00 – 09:00	**Registration**	
09:00 – 09:15	Keynote Address 13	Paper 12
09:15 – 09:30	Keynote Address 14	Paper 13
09:30 – 09:45	Keynote Address 15	Paper 14
09:45 – 10:00	Keynote Address 16	Paper 15
10:00 – 10:30	**Morning tea**	
10:30 – 11:00	Keynote Address 17	Paper 16
11:00 – 11:30	Keynote Address 18	Paper 17
11:30 – 12:00	Keynote Address 19	Paper 18
12:00 – 12:30	Keynote Address 20	Paper 19
13:00 – 14:00	**Award and Closing Ceremony** (Venue: Main Library)	
14:00 – 15:00	**Farewell Lunch and prayer break**	

INTERNATIONAL INVITED SPEAKERS

Francisco C. Ferreira
Department of Entomology, Texas A&M University, United States
Erika Martin Braga
Programa de Pós-Graduação em Parasitologia – ICB – UFMGBelo Horizonte – Minas Gerais – Brazil
Sajid Iqbal
Wenzhou Medical University, Wenzhou, China
Zul Kamal
Key State Laboratory for Basic and Applied Research of Bayu Prescriptions and Herbs, College of Chinese Materia Medica, Chongqing University of Chinese Medicine, Puguobao Road, 402760, Chongqing, P.R China

NATIONAL INVITED SPEAKERS

Mohammad Attaullah
Department of Zoology, University of Malakand
Rehman Mehmood Khattak
Department of Zoology, University of Peshawar, KPK, Pakistan
Furhan Iqbal
Institute of Zoology, Bahauddin Zakariya University, Multan, Pakistan
Hussain Ahmed
Department of Zoology, University of Buner, KPK, Pakistan
Sanaullah Khan, Maryam Naseem
Institute of Zoological Sciences, University of Peshawar
Syed Ishtiaq Anjum
Department of Zoology, KUST, Kohat, KPK, Pakistan
Irfan Zia Qureshi
Department of Zoology, Quaid-i-Azam University, Islamabad
Sajid Malik
Quaid-i-Azam University, Islamabad
Muhammad Shoaib Alam
Department of Zoology, Faculty of Basic Sciences, University of Agriculture, D.I. Khan, KPK, Pakistan

INTERNATIONAL INVITED SPEAKERS

Tick-mammal-pathogen interactions in urban areas in New Jersey, USA

Francisco C. Ferreira

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The northeastern United States (US) is a hotspot for tick-borne diseases. Adding to analready complex vector landscape under intense anthropic changes, in 2017, largepopulations of the invasive Haemaphysalis longicornis, the Asian longhorned tick, were detected in New Jersey is now found to be widespread across 21 states. In its nativerange in northeastern Asia, H. longicornis is considered an important vector of dead lypathogens to humans, companion animals, and livestock. For this talk, I will summarize studies we conducted to identify the primary hosts of H. longicornis in New Jersey. Wesurveyed synanthropic small and medium-sized mammals in three different sites insuburban areas. Specifically, we collected approximately 9,000 tick specimensbelonging to nine species from 11 different species of mammals sampled between Mayand September 2021. We found that H. longicornis feeds more frequently on rodentsthan previously thought, and that this invasive tick is likely exposed to importantenzootic and zoonotic pathogens. I will present detailed information about the seasonaldynamics and feeding patterns of six tick species common in the northeastern US.Overall, the newly invasive H. longicornis was the most numerous tick species, both onmultiple host species and in the environment, raising significant questions regarding itsrole in the epidemiology of tick-borne pathogens, especially those affecting livestock,companion animals, and wildlife. In the second part of the presentation, I will show dataon further pathogen detection studies conducted to take advantage of the immensenumber of samples collected during this widely collaborative study. We utilized standardmethods for the detection of multiple piroplasm parasites in H. longicornis collectedwhile questing in our study areas. Then I will show results from a metagenomics studyexploring the diversity and host specificity of Borrelia burgdorferi infecting the highdiversity of mammals we sampled.

Global Impact of Malaria with Emphasis on Latin America

Erika Martin Braga

Programa de Pós-Graduação em Parasitologia – ICB – UFMGBelo Horizonte – Minas Gerais – Brazil
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Malaria remains one of the most significant public health challenges worldwide, affecting millions of people each year and imposing substantial socio-economic burdens on endemic regions. Caused by Plasmodium parasites and transmitted through the bites of infected Anopheles mosquitoes, malaria is prevalent in tropical and subtropical regions, particularly in Africa, Asia, and Latin America. Although global malaria incidence has declined due to extensive control measures, including insecticide-treated nets, antimalarial drugs, and vector control programs, the disease continues to pose a major threat, particularly in

resource-limited settings. In Latin America, malaria transmission is largely confined to the Amazon Basin, which spans multiple countries, including Brazil, Colombia, Peru, Venezuela, and Bolivia. The region experiences a lower malaria burden compared to sub-Saharan Africa; however, persistent transmission, socio-economic disparities, and environmental changes challenge eradication efforts. The primary malaria-causing species in Latin America are *Plasmodium vivax* and *Plasmodium falciparum*, with *P. vivax* accounting for the majority of cases. *P. vivax* presents unique challenges due to its ability to remain dormant in the liver and cause relapses, complicating treatment and eradication efforts. Malaria disproportionately affects indigenous and rural communities in Latin America, where access to healthcare and preventive measures is often limited. Socioeconomic factors, including poverty, poor infrastructure, and limited education, contribute to the persistence of malaria in these populations. Furthermore, illegal mining, deforestation, and migration patterns have exacerbated malaria transmission by expanding breeding grounds for mosquitoes and increasing human exposure to vectors. Climate change further influences the distribution and intensity of malaria transmission, as rising temperatures and altered rainfall patterns create favorable conditions for mosquito proliferation in previously non-endemic areas. Efforts to control malaria in Latin America have been driven by national malaria elimination programs and regional initiatives, such as the Pan American Health Organization's (PAHO) Malaria Elimination Plan. These programs focus on early diagnosis, treatment, vector control, and community engagement. Notable successes have been observed, with countries such as Argentina, Paraguay, and El Salvador achieving malaria elimination status in recent years. However, malaria resurgence remains a threat due to drug resistance, insecticide resistance, and weakened public health infrastructure. The emergence of resistance to antimalarial drugs, particularly artemisinin-based combination therapies, poses a significant concern for effective malaria treatment and elimination efforts. In conclusion, malaria remains a significant public health challenge globally, with Latin America facing unique regional complexities in its elimination efforts. While substantial progress has been made, sustained interventions and investments are necessary to overcome existing challenges and prevent malaria resurgence. With continued global and regional cooperation, malaria elimination in Latin America is within reach, contributing to the broader goal of eradicating malaria worldwide.

Personalized Cancer Therapy Based on ROS/Mitochondrial Phenotyping: A Strategy for Screening Natural Compounds from Traditional Chinese Medicine

Cheng Ma, Sajid Iqbal

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Traditional Chinese medicine (TCM) has thousands of years of history and remains a valuable repository. Numerous TCM-derived natural compounds show anti-cancer activity by regulating ROS and mitochondrial function. However, patient responses vary significantly. Cancer cells exhibit substantial heterogeneity in baseline ROS, mitochondrial membrane potential, and antioxidant capacity. Mitochondrial ROS is a double-edged sword—low levels promote proliferation; high levels induce death—and this balance differs across tumors. This variability may determine sensitivity to ROS-modulating compounds, yet it has not been exploited for personalized therapy. Here, we propose a phenotype-driven strategy to match patients with suitable TCM compounds based on their ROS/mitochondrial profile. This pan-cancer approach has three phases. First, establish a ROS/mitochondrial phenotype database by profiling cancer cell lines and patient samples across multiple cancer types (lung, breast, colon, oral). Parameters include intracellular ROS, mitochondrial superoxide, $\Delta\Psi m$, and glutathione. Second, screen natural compounds from TCM formulas against each phenotype subgroup to identify sensitivity patterns. Third, validate the strategy in xenograft models and a small patient cohort. We hypothesize that high-ROS tumors are sensitive to pro-oxidant compounds pushing ROS beyond a lethal threshold, while low-ROS or high-antioxidant tumors respond better to compounds inhibiting antioxidant defenses or targeting mitochondria. The expected outcome is a "ROS/mitochondria phenotype–compound matching matrix" to guide personalized TCM prescriptions. This strategy shifts TCM from "one-size-fits-all" to precision medicine, using ROS/mitochondrial phenotyping as a simple biomarker applicable to multiple cancer types.

Keywords: Chines Traditional medicine; Cancer therapy; personalized medicine; Mitochondria; Pan-cancer

Plant-Derived Exosomes in Animal Health: A Novel Approach to Managing Inflammation and Infections in the Era of Climate Change

Zul Kamal[1,2]*, Yi Chen[1]

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The search for novel, biocompatible, and sustainable therapeutic agents has led to growing interest in plant-derived exosome nanoparticles (PDENs) as promising nanotherapeutics for animal and human health. These naturally occurring extracellular vesicles, isolated from edible plants, carry bioactive lipids, proteins, nucleic acids, and metabolites that may modulate immune responses and support disease management. Their unique properties, including high biocompatibility, low immunogenicity, oral stability, and scalable production, make them attractive candidates for veterinary and zoological applications. Recent studies suggest that PDENs from sources such as ginger, grapes, and broccoli can reduce inflammatory responses by downregulating pro-inflammatory cytokines and promoting tissue repair. They also show antimicrobial activity and may enhance the effectiveness of conventional antimicrobials by interfering with pathogen growth and biofilm formation. In animal health, these properties may be especially valuable for managing inflammation and infections that are increasingly influenced by climate change, such as heat stress-associated immune dysfunction, emerging infectious pressures, and environmental stress-related disease susceptibility. This highlights the therapeutic potential of PDENs as green nanotechnology platforms for future applications in veterinary medicine and zoological health. Their natural origin, multifunctional activity, and environmentally friendly profile make them a compelling candidate for innovative strategies to improve animal disease management under changing climatic conditions.

Keywords: Plant-derived exosomes; exosome-like nanoparticles; animal health; inflammation; infections; climate change; nanotherapeutics

NATIONAL INVITED SPEAKERS

Insect pests and recent trends in their eco-friendly management

Mohammad Attaullah

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Insects are the most diverse group of animals on earth, constituting more than 70% of all animals in the Animal Kingdom. They play essential roles in ecosystems, such as pollination, decomposition, and feeding other wildlife. Insects also produce useful products such as honey, silk, etc. On the other hand, insects cause severe economic losses to fruits and vegetables and transmit deadly diseases. To protect food, agriculture, and human health from the economic damage of insects, various control strategies are applied. Chemical Controls are mainly in practice in which wide formulations of synthetic chemicals/pesticides are used to control these pests to enhance the productivity of fruits, vegetables, and to protect stored products. The use of broad-spectrum and non-selective pesticides has caused the problem of health and environmental contamination, insecticide resistance, and non-target impacts. The use of nuclear techniques, particularly irradiation, presents a promising alternative. The development of sustainable control technologies against insect pests is strongly needed to protect both agriculture and public health.

EUthyroid2 – The next step towards the elimination of iodine deficiency and preventable iodine-related disorders in Europe and Beyond

Rehman Mehmood Khattak

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The iodine status is a primary determinant of thyroid disorders, and thyroid disorders are a major cause of morbidity in Europe and beyond, particularly in women. Iodine-deficiency disorders (IDD) affect the entire life cycle, and women and children are especially vulnerable. Therefore, the EUthyroid consortium was founded in 2015 to pave the way towards a euthyroid European population. Based on the first EUthyroid project, which evaluated existing IDD prevention programmes in Europe and established critical infrastructures to conduct valid monitoring studies, EUthyroid2 is planned to fill important gaps in IDD prevention programmes in Europe and beyond and will help overcome the current situation of at least partly ineffective IDD prevention programmes. Given these challenges, the major objective of EUthyroid2 is to improve the low awareness with respect to iodine deficiency-related risks in adolescents and young women. The aim is to find best practice models for accessing and informing them to increase their awareness and to improve their iodine status to lay the foundation for their own thyroid and general health, and that of their offspring. Implementation studies and community-based randomised-controlled trials are in progress to increase IDD-related awareness in adolescents and young women up to the age of 24 before pregnancy. EUthyroid2 will perform intervention studies in the educational system and in ambulatory care as two settings. Setting 1: Educational systems Secondary schools, high schools, and vocational schools represent the first setting targeting pupils aged 13 to 17 years. Within each region, at least five schools of each type are recruited for study participation, aiming for 150 pupils at each school (corresponding to, e.g., 25 pupils per class and two classes per grade). The interventions will be implemented within all classes. For the process evaluation, the RE-AIM framework will be implemented, allowing to assess Reach, Effectiveness, Adoption, Implementation, and Maintenance of the intervention. Age-specific intervention materials are culturally adapted and include storybooks, main messages, and train-the-trainer materials for teachers, lesson material, written information for pupils with a visualization/infographic approach, country-specific iodine cookbooks, and online videos enhancing the information. Setting 2: Ambulatory care; The second type of multi-region and multi-centre intervention studies will be performed in the medical setting. Eligibility criteria for study entry will be non-pregnant women aged 18 to 24 years who will give informed consent, focusing on those who are planning pregnancy in the said age range.

Aluminum chloride-induced amyloid β accumulation and endoplasmic reticulum stress in rat brain are averted by melatonin

Furhan Iqbal[1], Banthit Chetsawang[2]

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Accumulation of aluminum (Al) in the brain is known to be a toxic insult that results in neurodegenerative diseases, and melatonin is known to have a neuroprotective role. The present study was designed to investigate the neuroprotective effects of melatonin for aluminum chloride ($AlCl_3$)-induced neurotoxicity in rats. Twelve-week-old male Wistar rats were orally administered 175 mg/kg AlCl3 with or without 5 mg/kg melatonin intraperitoneal pretreatment. Group 3 intraperitoneally received 5 mg/kg melatonin, and group 4 rats were orally treated with saline solution for 8 weeks. A series of behavioral tests, biochemical analysis, and expression of Ad-associated proteins in the brain were determined after 7 weeks of all treatments. Our results indicated that $AlCl_3$ treatment tends to induce memory and cognitive impairment. However, melatonin treatment attenuated amyloid beta (Aβ) (1–42) level by decreasing β-secretase, augmenting low-density lipoprotein receptor-related protein 1, and neprilysin protein expression. Moreover, $AlCl_3$ -induced endoplasmic reticulum (ER) stress and oxidative stress were attenuated by melatonin supplementation. In conclusion, these findings demonstrate a protective role of melatonin against Aβ peptide accumulation, ER stress, and oxidative stress in the $AlCl_3$ -treated AD model. Hence, the melatonin supplement might be an alternative way to alleviate the development of AD.

Relaxin (a regulatory peptide) enhances cryotolerance, fertility potential, and plasma reproductive hormones of Nili Ravi buffalo (*Bubalus bubalis*) during the low breeding season

Hussain Ahmed

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Buffalo is a chief component of livestock by providing milk, meat, and draught power. In this respect, it plays a significant role in the agricultural economy of many developing countries. It has been described that buffalo semen is affected by season, prevailing temperature, and relative humidity. Continuing summer heat/ ambient temperature distresses the functioning of Leydig cells, followed by testosterone synthesis, causing a reduction in the secretory ability of the epididymis and seminal vesicles. These structures are thermo-sensitive and androgen-dependent. Summer heat impairs spermatogenesis, which could lead to diminished sperm motility, increased secondary sperm abnormalities, testicular hypoplasia, and even tissue degeneration. This study investigated the beneficial effects of relaxin on cryotolerance of buffalo spermatozoa and reproductive hormones during the low breeding season. Collected semen was diluted in five aliquots with relaxin addition (0.25 mg/mL, 0.50 mg/mL, 0.75 mg/mL, 1 mg/mL, and control [0 mg/mL). After gentle dilution (37 °C), cooling (4°C, 2 h), equilibration (4°C, 4 h), and packaging in straws (polyvinyl French, 0.5 mL), frozen (cell freezer), and thawed (37 °C, 30 s) for analysis. Blood samples were collected at different time intervals, i.e., -60, -30, and 0 min (pre-dose) and 30, 60, 90, 120, and 150 min (post-dose) from the jugular vein. This study manifests that adding relaxin (1 mg/ mL) in freezing medium ameliorates sperm motility, functionality (%), and seminal plasma TAC (µM/L) compared to control during the low breeding season. Furthermore, we found that relaxin supplementation at 1 mg/mL significantly improves seminal plasma ATP concentrations (nmol/million) compared to control, 0.25 mg/mL, and 0.50 mg/mL, and fertility (control and 0.75 mg/mL). Further, relaxin injection significantly improves plasma T, LH, and IGF-1 levels (150 and 120 min vs. -60 and -30) and FSH, KP, and GnRH concentrations (150 min vs. -60, predose, during low breeding season. Taken together, this study revealed that relaxin ameliorates motility, functionality, and fertility of buffalo. Moreover, relaxin injection (1 mg/mL) improves essential reproductive hormone levels in buffalo, signifying its importance in the field of reproductive physiology. Further studies are required to determine the exact mechanism of action of relaxin in enhancing semen quality, fertility, and reproductive hormones.

Torch pathogens-induced histopathological changes in placental tissues and associated post-obstetric complications

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Maternal infections caused by the ToRCH complex, comprising *Toxoplasma gondii* (*T.gondii*), Rubella Virus (RV), Cytomegalovirus (CMV), and Herpes Simplex Virus (HSV), are significant contributors to Bad Obstetric History (BOH). These infections can vertically transmit through the placental barrier, leading to complications in fetal development. This study investigates the histopathological changes induced by ToRCH pathogens in placental tissues and their association with post-obstetric complications in Pakistani women. A total of 83 women were enrolled in the study and divided into two groups: the Experimental group and the Control group. The Experimental group consisted of women with BOH and was further subdivided into two categories: Group A and Group B. Group A comprised women who experienced a recent miscarriage at the time of sample collection, while Group B consisted of women who gave birth normally at the time of sample collection. In contrast, the Control group included women with normal delivery and a normal obstetric history. To investigate ToRCH infections, venous blood samples were analyzed for ToRCH antibodies using enzyme-linked immunosorbent assay (ELISA). Additionally, small pieces of placental tissue collected after miscarriage or delivery were subjected to nucleic acid (NA) detection by conventional polymerase chain reaction (PCR) using ToRCH pathogen-specific primers. Histopathological examination of placental tissue was also performed to identify microscopic changes. The data obtained were analyzed using descriptive statistics (percentages) and chi-square tests to determine significant associations. The study revealed an overall seroprevalence of ToRCH pathogens of 57.83 % in the study population, with 53.01 % detected in the Experimental group and 4.81 % in the Control group. In the Experimental group, IgM and IgG antibodies were detected in 14.45 % and 37.34 % of participants, respectively, with 1.20 % testing positive for both. Notably, the Control group showed no IgM positivity, while IgG was detected in 4.81 %. Nucleic acid (NA) detection revealed ToRCH pathogens in 54.21 % of placental tissues from the Experimental group only. Specifically, antibodies for *T.gondii* and RV were most prevalent (19.27 % each), while NA detection was highest for RV, CMV, and HSV (14.45 % each). In contrast, the Control group showed minimal antibody detection, with only 2.40 % positivity for *T.gondii* and CMV each. Statistical analysis revealed significant differences in ToRCH infection (antibodies and NA) between study groups for CMV only ($p > 0.05$). Discrepancies between ToRCH antibodies and NA positivity were observed in 75.90 % of cases, with 71.08 % occurring in the Experimental group and 4.81 % in the Control group. Histopathological examination revealed chorionic villitis (CV) in 16.86 % of the Experimental group; specifically, 10.84 % in Group A and 6.02 % in Group B. CV was detected in women infected with *T. gondii* (in 4 cases), RV (in 6 cases), CMV, and HSV (both in 2 cases, each). The most common previous BOH complications were recurrent spontaneous abortions (RSAs) (47.05 %), with 21.87 % of these cases showing CV positivity. The study's key conclusion is that ToRCH pathogens are prevalent in the study population, and CV was identified as the primary histopathological change associated with BOH complications (mainly RSAs). These results underscore the importance of investigating ToRCH infections in women experiencing BOH complications.

Beekeeping in Pakistan: History, Potential, and Future Challenges

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Beekeeping has a long history in Pakistan, deeply rooted in traditional practices and local agricultural systems. Historically, honey collection was practiced in rural and tribal areas, primarily for medicinal and dietary purposes. Over time, beekeeping has evolved into a commercial enterprise, especially with the introduction of modern beekeeping techniques in the mid-20th century. The country's diverse flora, ranging from the Himalayan forests to the plains of Punjab and Sindh, provides an excellent environment for honey production. The indigenous species, *Apis dorsata* and *Apis cerana*, have traditionally been harvested, while the introduction of *Apis mellifera* has revolutionized honey production, leading to increased yields and economic viability. Pakistan holds significant potential for expanding its beekeeping industry. The country is among the leading honey producers in South Asia, with thousands of beekeepers engaged in the trade. Honey production contributes to rural livelihoods and supports agricultural pollination, enhancing crop yields. The government, along with various non-governmental organizations (NGOs), has initiated training programs and subsidies to promote beekeeping. Additionally, the export market for Pakistani honey, particularly in the Middle East, offers lucrative opportunities. The growing demand for organic and natural honey further strengthens the potential of this sector. Despite its promising prospects, beekeeping in Pakistan faces several challenges. Climate change and environmental degradation threaten bee populations, affecting honey production. The excessive use of pesticides in agriculture has led to the decline of pollinators, while deforestation and urbanization have reduced the natural habitats of bees. Additionally, the industry struggles with issues such as a lack of quality control, inadequate research, and limited government support. Many small-scale beekeepers lack access to modern equipment, training, and financial assistance, making it difficult for them to scale up their operations. Moreover, counterfeit and adulterated honey in the market undermines consumer trust and affects exports. In conclusion, beekeeping in Pakistan has a rich history and holds immense potential for economic growth and environmental sustainability. However, challenges such as environmental threats, lack of quality control, and insufficient government support must be addressed to unlock its full potential. By implementing strategic policies and promoting sustainable practices, Pakistan can strengthen its position in the global honey market while preserving its natural ecosystems.

Harmonizing Nanotechnology with Physiology: Exploring Synergies

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Nanotechnology has emerged as a transformative force in biomedical sciences, offering innovative solutions for diagnostics, drug delivery, regenerative medicine, and biosensing. However, its full potential can only be realized by achieving seamless integration with human physiology. This paper explores the synergies between nanotechnology and physiological systems, emphasizing how nanoscale interventions can be tailored to align with the body's biochemical, mechanical, and immunological processes. A key aspect of this integration is the biocompatibility of nanomaterials, which must be engineered to evade immune rejection, minimize toxicity, and ensure long-term functionality within the body. Advances in nanoparticle surface modifications, biomimetic coatings, and hybrid bio-nano interfaces have significantly enhanced their ability to interact harmoniously with physiological environments. For instance, lipid-based nanoparticles for drug delivery can cross biological barriers efficiently, mimicking natural vesicular transport mechanisms to improve therapeutic outcomes while reducing systemic side effects. Regenerative medicine has also greatly benefited from nanotechnology, particularly in tissue engineering and stem cell modulation. Nanostructured scaffolds, inspired by the extracellular matrix, provide an optimized microenvironment for cell adhesion, proliferation, and differentiation, accelerating wound healing and organ regeneration. Moreover, the controlled release of bioactive molecules from nanocarriers can guide cellular behavior, promoting targeted tissue repair with minimal invasiveness. Despite these advancements, challenges remain in fully harmonizing nanotechnology with human physiology. Potential issues such as nanoparticle accumulation, long-term biostability, and unintended physiological interactions must be rigorously addressed. Interdisciplinary collaborations between nanotechnologists, physiologists, and clinicians are crucial to overcoming these hurdles and translating laboratory innovations into safe and effective clinical applications. By leveraging the intricate interplay between nanotechnology and physiological systems, we can unlock new frontiers in medicine, ensuring that nano-enabled interventions not only enhance human health but also work in concert with the body's natural mechanisms. This exploration of synergy marks a significant step toward precision medicine, bio-integrated nanodevices, and advanced therapeutic strategies that redefine the future of healthcare.

Cerebral palsy in Pakistan: Challenges, Prevalence, and Intervention

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Cerebral palsy (CP) is one of the most common childhood neurological disorders worldwide, and Pakistan is no exception. CP is a non-progressive motor disorder caused by brain damage before, during, or shortly after birth, leading to lifelong disability. In Pakistan, the prevalence of CP is difficult to determine due to a lack of comprehensive data; however, it is estimated to be significant, given the high incidence of perinatal complications, inadequate maternal healthcare, and consanguineous marriages. The healthcare system in Pakistan faces multiple challenges in addressing CP, including late diagnosis, limited rehabilitation facilities, scarcity of trained professionals, and social stigma. The causes of CP in Pakistan are multifactorial and include prenatal, perinatal, and postnatal factors. Among the primary risk factors are birth asphyxia, premature birth, infections, and neonatal jaundice, which are exacerbated by inadequate prenatal care and lack of medical expertise in rural areas. Socioeconomic barriers prevent timely diagnosis and treatment, as many families cannot afford specialized care. Furthermore, limited awareness about CP leads to social discrimination, often leaving affected children without appropriate medical and educational support. To improve the situation of CP management in Pakistan, several steps need to be taken. First, awareness campaigns should be launched to educate the public and healthcare professionals about CP, its causes, and its management. Second, government policies should prioritize maternal and child healthcare by improving antenatal and neonatal services to prevent conditions leading to CP. Third, an expansion of rehabilitation centers and inclusive education programs is crucial to providing children with CP equal development opportunities. Lastly, financial assistance programs and community-based rehabilitation (CBR) strategies should be introduced to support affected families, particularly in rural areas. In conclusion, CP remains a significant public health concern in Pakistan, with numerous challenges hindering effective management. Addressing the issue requires a multi-sectoral approach, involving healthcare improvements, educational reforms, and increased social acceptance. With proper intervention, children with CP can achieve a better quality of life and contribute meaningfully to society.

Impact of Dietary and Waterborne Cadmium Exposure on Growth, Physiology, and Bioaccumulation in Rohu (Labeo rohita) Fingerlings

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The environmental degradation and contamination caused by heavy metals are a major worry in the twenty-first century. Growing urbanization and industrialization lead to heavy metal contamination of various environmental components. The rate of mobilization and transportation of heavy metals has increased dramatically since 1940. Cadmium (Cd), a most prominent heavy metal and an ecological stressor, can change many physiological processes, including growth, body composition, serum parameters, and bioaccumulation in fish organs. *Labeo rohita* is a freshwater species with great economic value; thus, the evaluation of its physiological responses to cadmium is essential.

Hence, the current study (45 days) aimed to assess the impact of cadmium sub-lethal concentration on growth performance, body composition, biochemical parameters, and bioaccumulation in *Labeo rohita*. One hundred ninety-five fingerlings (W 8.82±0.94 g, L 8.39±0.77 cm) were randomly assigned to five experimental groups in triplicate (13 fish/aquarium, 70 L water capacity) and exposed to two sub-lethal concentrations (1/3rd and 1/9th of LD50/LC50) of cadmium chloride through both dietary and waterborne routes. W1 exposed to 1/3rd of LC50 through the waterborne route exhibited significantly ($p<0.05$) the poorest in terms of weight and length gain, SGR and SLR, FCR and PER, HSI and VSI, feed intake, total feed consumption, average feed consumption, and survival rate. The control group (without Cd) showed the best growth performance. Waterborne exposure of Cd at 1/3rd of LC50 also led toward significantly adverse ($p< 0.05$) body composition with the lowest crude protein and crude lipid levels and blood biochemistry with abnormal AST, ALT, ALP, glucose, total protein, bilirubin, Gamma GT albumin, globulin, A/G ratio, BUN, creatinine, cholesterol, triglyceride, HDL, LDL, VLDL, and serum electrolyte levels as compared to other sub-lethal concentrations and routes. W1 exposed to 1/3rd of LC50 through a waterborne route showed a significantly elevated ($p<0.05$) bioaccumulation of Cd in gills, muscles, and brain. Therefore, the findings of this study highlighted that waterborne exposure of Cd at 1/3rd of LC50 leads to afflicted growth, impaired body composition, adverse blood biochemistry, and the highest bioaccumulation of Cd in the gills, muscles, and brain of *Labeo rohita* fingerlings.

ORAL PRESENTATIONS

Exploring the Diversity of Bats (Order: Chiroptera) of Tehsil Gadezai, District Buner, Khyber Pakhtunkhwa

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Bats are the only flying mammals belonging to the order Chiroptera, which is the second-largest order of mammals after rodents. Bats are cosmopolitan species present on all continents of the world except Antarctica. More than 1440 species of bats are present worldwide; however, Pakistan has about 57 species, while Khyber Pakhtunkhwa (KP) contains 29 species of bats. They are long-lived mammals, aging up to 30 years, and give birth to only one or two pups. The current study was the first attempt carried out from June 2021 to October 2021, in order to explore the diversity of bat fauna of Tehsil Gadezai, District Buner, KP, Pakistan, based on morphological characteristics. Both nocturnal and diurnal surveys were conducted for the observation and collection of specimens. In the current study, hundreds of bat specimens were observed at their roosting sites; however, 19 specimens were collected from different localities of the study area. The collected specimens were identified up to the species level using a key to the bats of South Asia by C. Srinivasulu *et al.* (2010), resulting in the identification of *seven species* belonging to *four genera* and *three families*. Among the seven identified species, family *Vespertilionidae* was represented by four species (*Scotophilus heathi, Scotophilus kuhlii, Pipistrellus javanicus,* and *Pipistrellus coromandra*), two species (*Rhinolophus Lepidus* and *Rhinolophus ferrumequinum*) were representatives of the family *Rhinolophidae,* while one species (*Pteropus giganteus*) belonged to the family *Pteropodidae.* The family *Vespertilionidae* was found to be the most common in terms of species number and diversity, followed by the family *Rhinolophadae and the* family *Pteropodidae,* respectively, in the study area. Although the mentioned bat species were reported for the first time from the study area, more detailed investigations are needed to explore new species of bats on the basis of morphological characteristics and genetic analyses.

Keywords: Buner, Biodiversity, Order Chiroptera, Vespertilionidae, Rhinolophadae, Pteropodidae

A Proteogenomics-Driven Strategy for the Discovery of Novel Nano-Vaccine Candidates Against Zoonotic Mastitis Pathogens

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Bovine mastitis, a major zoonotic-associated disease, continues to impose substantial economic losses on the dairy industry while posing risks to public health through compromised milk quality and pathogen transmission. The excessive and unregulated use of antibiotics in livestock has further intensified antimicrobial resistance (AMR), highlighting the urgent need for alternative preventive strategies. This study aims to develop a proteogenomics-based framework for the identification of novel, immunogenic, and cost-effective vaccine candidates targeting mastitis-associated bacterial pathogens. Clinical isolates from mastitis-infected dairy cattle will be obtained and characterized using standard microbiological techniques. Genomic analysis will be integrated with proteomic profiling to identify conserved, surface-exposed, and highly expressed proteins. These candidate antigens will be screened using immunoinformatics and bioinformatics tools to predict antigenicity, stability, and immune response potential. Promising targets will then be incorporated into a nano-vaccine delivery system to enhance immunogenicity and targeted immune activation. The proposed approach is expected to identify high-confidence vaccine candidates and establish a robust pipeline for precision vaccine design. The integration of proteogenomics with nanotechnology offers improved specificity, reduced development costs, and enhanced vaccine efficacy. This study provides a novel and sustainable strategy to combat mastitis and reduce antibiotic dependency, thereby contributing to the global fight against AMR and improving livestock health management in resource-limited settings.

Keywords: Antimicrobial Resistance, Immunoinformatics, Mastitis, Nano-vaccine, Proteogenomics, Reverse Vaccinology

In Silico Screening of *Cannabis sativa* Bioactive Compounds Targeting Ornithine Decarboxylase for Anti-Leishmanial Drug Development

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Leishmaniasis remains a significant health burden, particularly in developing nations, due to scarce and costly as well as mostly detrimental treatment regimens. The increasing number of drug-resistant *Leishmania* strains necessitates an immediate demand for new treatments that can be safe and more effective. Ornithine Decarboxylase (ODC) is an essential enzyme in the production of polyamines required to survive and proliferate, and so it represents a good target in the development of anti-leishmanial drugs. This experiment examined some cannabinoids, terpenoids, polyphenols, and flavonoids as potentially active inhibitory bioactive compounds of *Cannabis sativa* (*C. sativa*) against ODC using the in-silico molecular docking method. The ODC and these bioactive compounds' interaction revealed that out of all the compounds open to investigation, the affinities of rosmarinic acid, parthenolide, and caryophyllene had the highest binding affinity (-8.8 kcal/mol, -7.9 kcal/mol, and -8.7 kcal/mol, respectively), indicating the presence of strong molecular interactions with the ODC active site. Moreover, the potential of *C. sativa* compounds as the potential contributors to ODC inhibitors was also strengthened by such cannabinoids as cannabielsoin (CBE), delta-8 tetrahydrocannabinol (D8-THC), and tetrahydrocannabivarin (THCV). Such findings suggest that phytochemicals of *C. sativa*, in particular, caryophyllene, possess good inhibitory properties against ODC and can be deployed in the management of drug-resistant *Leishmania* strains. More in-vitro and in vivo research needs to be carried out to determine the efficacy of the bioactive compounds in drug development, pharmacokinetics of these compounds, and their safety in the future.

The Effect of Fermented Food Intake on Gut Microbiota and Cognition among College Students

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The gut–brain axis serves as a bidirectional signaling avenue connecting dietary habits, gut microbiota, and cognitive functions. Fermented foods contain valuable microorganisms and bioactive metabolites, which are increasingly considered to be able to act as precursors for brain modulation. The present study was aimed at exploring the association between consumption of fermented food, composition of gut microbiota, and cognitive performance among undergraduate students. Method: Cross-sectional analytical study on 150 university students (ages of 18–25 years). Dietary consumption of fermented foods was assessed by a validated food frequency questionnaire, while cognitive performance was assessed using the Digit Span Test and Stroop Test. 16S rRNA gene sequencing was performed to analyze gut microbiota diversity. Higher intake of fermented foods was significantly related to greater microbial diversity ($p < 0.001$) and better cognitive performance, specifically working memory and executive function ($p < 0.001$). Beneficial bacterial genera (Lactobacillus and Bifidobacterium) showed positive correlations with cognitive scores. These findings indicate that these cognitive benefits of fermented food may partly arise from the modulation of gut microbiota. Causality needs to be confirmed by more longitudinal and interventional studies.

Keywords: Fermented foods; Gut microbiota; Cognition; Gut-brain axis; Psychobiotics; University students

The Ecology of Sandy Shores of Karachi

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The present study addresses issues related to the ecology of the sandy shores of Karachi due to climate change. The investigations are based on samples taken from January 2024 to December 2004. The present probe is basically composed of studies on two selected beaches (Clifon and Sndspit). Firstly, the physical and chemical features of these selected sites are described. The distribution of macrofauna at different shore levels from these sandy shores has been compared. A general discussion and conclusion integrating the results of this and other studies, and suggestions for further studies are provided. Temperature gradients (Sediments and Water) are indicative of a typical seasonal pattern ranging between 210C to 310C, reaching the highest values (310C) in summer. The salinity gradients range between 36%o and 41%o. The interstitial salinities also did not differ greatly from one another, the range being 34.5%o to 39%o. The reduced effect of waves brings high turbidity at Clifon, but at Sandspit the turbidity is remarkably low due to the high wave action. In general, the percent moisture content ranged from 2 to 35 % and was highest in the lower zone as compared to other zones. Moisture content was high in sediments of Clifon due to fine sand, which is highly porous in nature. The percentage of organic matter content in sediments shows high values (14.3%) at Clifon as compared to Sandspit (2.5%). Data on macrofaunal density of these two beaches reveal significant differences that could be attributed to the varying morphodynamic conditions of the sites. In terms of biomass, crustaceans appear as the most dominant group (30%), followed by gastropods (25%), polychaetes (15%), and bivalves (10%). The others comprise only 20% of the total individuals. At Clifon, abundant groups composed of crustaceans (24%), polychaetes (20%), bivalves (14%), gastropods (10%), echinoderms 9(%), and others included (23%) of the total fauna. Monthly population density of total macrofauna fluctuated from 334/m2 to 131/ m2 and from 1056/ m2 to 813/ m2 at Sandspit and Clifon, respectively. At both sites, the maximum population density is observed in December. The monsoon period seems to have a profound effect on the number of individuals, as a marked decrease in the number of individuals is observed in the monsoon period at both sites.

Integrating Whole-Genome Sequencing and Immunogenomics for Surveillance of Foot-and-Mouth Disease Virus in Pakistan

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Foot-and- mouth disease (FMD) is a major transboundary highly devastating livestock disease worldwide, causing economic losses exceeding USD 20-30 billion. It is endemic in many parts of Asia, Africa, and the Middle East, while several regions such as Europe, North America, and Australia are considered FMD-free due to their strict control measures. In Pakistan, recurring outbreaks continue to pose a significant threat to livestock health and economic stability,

particularly in the provinces of Sindh and Punjab. This disease is caused by the highly mutable virus Foot-and-Mouth disease virus (FMDV), characterized by rapid genetic variation and the circulating multiple serotype, notably serotype A, O & Asia-1. Recurring outbreaks results in substantial reduction in milk production and deterioration of meat quality, severely impacting in livestock sector. The effectiveness of conventional diagnostic approaches, including ELISA and RT-PCR, is increasingly challenged by ongoing viral evolution and antigenic drift. Moreover, the climate change, may further influence animal movement, viral persistence & transmission patterns, host susceptibility and the emergence of new variants, complicating disease control effort. However, there is lack of region- specific genomic surveillance data in Pakistan, which limits effective control and management strategies. To address these challenges, this study proposes an integrated genomics- based framework for the surveillance and characterization of circulating FMDV strains. Field sample will undergo RNA extraction followed by whole genome sequencing to generate high-resolution genetic data. Comparative genomic analysis will have employed to identify conserved region for the development of improved molecular diagnostics and rapid detection tool. Additionally, immunogenomics approaches will be applied to identify potential antigenic targets for more effective, region specific vaccine design. This study is expected to identify region specific circulating FMDV strains and conserved genomic regions suitable for reliable diagnostic. It will support development of rapid, region-specific detection tool and provide insights and will help to identify potential vaccine targets for improved control strategies.

Effects of industrial effluents on the gonado-somatic index of a fish

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Fish is aquatic or marine animal that has backbone, gills and fins . Fish is a cold blooded vertebrate. There are 8000 freshwater fish species of the most important factor which helps are world. In fishes reproduction behavior is one in their management. The egg laying capacity of a fish is called fecundity, while the between males and ability of sperm to fertilize an egg is called fertility. GSI varies females during the reproduction cycle and it is influenced by the seasonal changes of the abiotic bounds such as temperature and photoperiod. Many chemicals employed harmful effects on the reproduction of aquatic populations. Temperature is a major environmental factor affecting the reproductive cycle and spawning fishes. Gonadosomatic index (GSI) is used as an important measure for expression of gonadal development and reproductive effort in fishes. The sampling was done from the CRBC (Chashma Road Bank Canal) Punjab province, Pakistan. Total of 12 mature fishes (5 males and 7 females) were collected in autumn, winter, spring and summer seasons of 2019-2020 in D.I.Khan. The gonads were washed. The measurement of gonadal weight and

Gonadosomatic Index (GSI) was also done. After wards the gonads were placed in 10 percent formalin for further analysis.

Evaluation of Diethylnitrosamine-Induced Liver Toxicity, Expression Regulation of Selected Proteins and Pharmacological Effect of Green Silver Nanoparticles in Male Rats

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Hepatocellular carcinoma (HCC) is the prevalent primary hepatic malignancy and is a major cause of cancer-related deaths in the world. HCC can be induced in experimental animals with the use of Diethyl nitrosamine (DEN), a potent hepatocarcinogen causing oxidative stress, DNA damage and chronic liver injury. The present study was conducted to evaluate DEN–induced liver toxicity and protective effects of biogenic silver nanoparticle (AgNPs) in male albino rats. A total of twenty rats, with an average body weight of approximately 150-200 g were divided into four groups; Control, DEN-treated, DEN+AgNPs-treated, and AgNPs treated group. Liver function tests (LFTs), histopathology, DNA isolation and western blot analyses were performed. A significant reduction showed in the body weight of experimental groups. A significant increase was observed in ALT and ALP of DEN-treated rats; however, no changes were found in the level of bilirubin, serum albumin, and total proteins compared to the control group. DEN+AgNPs showed a significant decrease in Alkaline Phosphatase. Histopathological examination showed the disruption of liver architecture by the formation of nodules with focal loss of the central vein in DEN-treated rats. However, AgNPs treatment reduced the hepatic lesions and showed recovery against the DEN. Furthermore, DNA damage was observed in the DEN-treated group, however intact genomic DNA found in DEN+AgNPs and AgNPs groups. The expressions of mechanistic target of rapamycin (mTOR), Poly (ADP-ribose) polymerase-1 (PARP-1) and TLR4 were downregulated, while GSK3-β was upregulated in liver tissue of DEN-treated rats. Altogether, the current results showed that AgNPs have protective effects against DEN induced liver toxicity in male rats.

Keywords: Diethylnitrosamine, Hepatotoxicity, AgNPs, Western blot

Molecular Mechanism of Doxorubicin-Induced Nephrotoxicity in Rats and the Protective Effects of the *Colebrookea oppositifolia*

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Doxorubicin is one of the most reliable anthracycline anticancer drug. However, the potent cytotoxic effects of Dox limits its clinical use. Clinical administration of Dox results in nephrotoxicity mediated by oxidative stress and mitochondrial dysfunction. Plant-derived extract may offer protective or modulatory effects against Dox-induced renal apoptosis. This study intended to evaluate the

therapeutic potential of *colebrookea oppositifolia* against the Dox-induced renal apoptosis in rats. Rats were randomly allocated into five different experimental groups each group contains five rats except the extract only group which consist of four rats. The rats were treated with Dox and *Colebrookea oppositifolia* plant extract on weakly and daily bases respectively. Once the experimental trial was completed, the rats were dissected and organs were isolated and preserved for further analysis. Apoptosis related gene p53, p21, BAX, Caspase-3, and Caspase-9 was assessed using PCR and Western Blot. Dox significantly upregulated all selected genes compared to control ($p < 0.05$), confirming activation of apoptotic pathways and cell-cycle arrest. Co- treatment with extract partially mitigated Caspase-3, Caspase-9, and p21 expression in a dose-dependent manner, while BAX and p53 exhibited higher expression at co-treatment with 400 mg/kg extract, suggesting an adaptive or stress-mediated response. The extract-only group showed moderate expression, higher than control but lower than Dox-treated groups, indicating physiological modulation without toxicity. Western blot analysis showed marked upregulation of p53, p21, BAX, Caspase-3, Caspase-9, and PARP1 in the DOX-only group. Cotreatment with *Colebrookea oppositifolia* extract attenuated this upregulation in a dose-dependent manner, while the extract-only group exhibited basal expression comparable to controls with no significant variances. Additionally, *Colebrookea oppositifolia* recovered Dox-induced oxidative stress by restoring renal enzymatic antioxidant activities (CAT, SOD, POD, and LPO) in a concentration-dependent way. Altogether, DOX upregulated apoptotic genes and proteins, while cotreatment with *Colebrookea oppositifolia* attenuated this in a dose-dependent manner. *Colebrookea oppositifolia* also recovers the renal enzymatic activities, signifying its nephroprotective and modulatory potential against DOX-induced renal impairment.

Molecular detection of Acinetobacter baumannii and Bartonella spp. in the DNA of head lices collected from human subjects in Punjab and Khyber Pakhtunkhwa provinces

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Human head lice, *Pediculus humanus capitis,* are obligate, permanent ectoparasites of eutherian mammals. *Pediculus humanus capitis* is known to transmit bacteria like *Acinetobacter baumannii,* and *Bartonella* spp. that can cause nosocomial and community-acquired infections. These bacteria were never screened in human head lices in Pakistan. Hence, the present study was aimed to report the molecular prevalence of *Acinetobacter baumannii* and *Bartonella* spp. in the DNA of human head lices samples that were collected from human subjects in Punjab and Khyber Pakhtunkhwa (KPK) during August to October 2024. Genetic diversity of pathogens and risk factors associated with the infection were also determined. A total of 97 human head lices were collected from Muzaffargarh district in Punjab (n = 42) and four districts in

KPK: Buner (n =19), Upper Dir (n = 7), Mardan (n = 13) and Sawat (n = 16). Polymerase Chain Reaction amplified a 350 base pairs fragment specific for *rpoB* gene of *Acinetobacter baumannii* in 02 out of 97 (02%) head lices while *Bartonella* spp. was not detected in all screened head lices. Subsequent Sanger sequencing followed by the BLAST analysis confirmed the presence of *Acinetobacter baumannii.* Partial Phylogenetic analysis that was based on partial *rpoB* gene sequence revealed that Pakistani *Acinetobacter baumannii* isolates were genetically similar and they resembled the isolates reported from Malaysia, USA and Egypt. Prevalence of *Acinetobacter* varied significantly among the lices collected from various sampling sites ($P < 0.001$) and between the months during which the lices were collected ($P < 0.001$). Epidemiological factors like age, sex, developmental stages of lices and the hygienic conditions of the human hosts were not associated (for all $P > 0.05$) with the prevalence of *Acinetobacter baumannii.* In conclusion, to the best of our knowledge, this is very first study from Pakistan that is reporting a low prevalence of *Acinetobacter baumannii* in the DNA of human head lices. As the bacterium was detected in lices from only one district included in this study, it is recommended that the human head lices from other provinces should also be screened to get more information about their prevalence as well as bacterial-host interactions that well help in designing control measures against these infections.

Genomic-Based Screening of Prevalent Mastitis Strains Towards Chimeric Vaccine Production And Adjuvant Formulation

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The rising burden of antimicrobial resistance (AMR) among pathogens causing bovine mastitis has become a critical challenge for both animal health and the dairy economy of Pakistan. Excessive and inappropriate use of antibiotics has led to the emergence of resistant bacterial strains, significantly reducing treatment efficacy and increasing economic losses in the dairy sector. Moreover, currently available commercial vaccines fail to provide adequate protection, as they are not tailored to local circulating strains. This study aims to develop a chimeric, strain-specific vaccine targeting mastitis-causing bacterial pathogens prevalent in Sindh, Pakistan. Milk samples were collected from dairy farms across the region, and mastitis-associated bacteria were isolated using CHROMagar™ Mastitis medium. Identification was performed through 16S rRNA gene sequencing. Following this, selected isolates will be subjected to whole genome sequencing to identify potential antigenic targets. Subsequently, an immunoinformatics-based approach will be employed to predict B-cell epitopes and design a multi-epitope chimeric vaccine candidate. In silico immune simulation will be conducted to evaluate the immunogenic potential of the designed construct. Preliminary findings indicate that *Staphylococcus aureus*

and *Escherichia coli* are the most prevalent mastitis-associated pathogens, followed by *Klebsiella pneumoniae* and *Streptococcus dysgalactiae*. Additionally, less commonly reported species, including *Staphylococcus chromogenes*, *Staphylococcus simulans*, *Staphylococcus sciuri*, *Staphylococcus saprophyticus*, and *Staphylococcus haemolyticus*, were also identified, highlighting the diverse microbial landscape of mastitis in the region. This study is expected to provide a foundation for the development of a region-specific chimeric vaccine and offers a promising alternative to conventional antibiotic-based control strategies.

Molecular Characterization of Oculocutaneous Albinism in Pakhtun Families Reveals Recurrent Variants in TYR and OCA2

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Oculocutaneous albinism (OCA) is a rare, genetically heterogeneous disorder characterized by impaired melanin biosynthesis and distribution in the skin, hair, and eyes. The severity of dermatological and ophthalmological manifestations varies depending on the underlying genetic defect. Five unrelated consanguineous families with multiple individuals affected by OCA were recruited from Khyber Pakhtunkhwa, Pakistan. Whole-exome sequencing (WES) was performed for the probands, followed by Sanger sequencing to confirm variant segregation. Variants were prioritized using the in-house VARDIGS pipeline and evaluated through population databases (gnomAD, ClinVar, HGMD), computational prediction tools (SIFT, PolyPhen-2, CADD, MutationTaster), and interpretation platforms (Franklin, VarSome, GeneBe). Final classification was performed according to ACMG guidelines. Clinical evaluation revealed hypopigmented skin and hair, translucent irides, nystagmus, photophobia, and reduced visual acuity in affected individuals. Molecular analysis identified four recurrent pathogenic and one likely pathogenic variants in *TYR* and *OCA2*. Three variants were detected in *TYR*, including a frameshift variant c.216delA (p.Val74TrpfsTer46), observed for the first time in Pakistani patients, a missense variant c.132T>A (p.Ser44Arg), and a nonsense variant

c.346C>T (p.Arg116Ter). Two variants in *OCA2* including a missense variant c.1211C>T (p.Thr404Met) and a frameshift splice region variant c.2430delC (p.Phe810LeufsTer7) were identified in *OCA2*. Sanger sequencing confirmed co-segregation of all variants with the disease phenotype in respective families. In silico analyses consistently supported their pathogenicity with the exception of last *OCA2* variant. This study reports five previously described variants, including three pathogenic in *TYR*, one pathogenic and one likely pathogenic *OCA2* variants in consanguineous families of Pakhtun ethnicity, thereby expanding the mutational spectrum of OCA in the Pakistani population. The recurrence of these variants underscores the significant contribution of *TYR* and *OCA2* to OCA in this region and highlights the impact of consanguinity. These findings have important implications for genetic counseling, carrier screening, and informed family planning.

Keywords: Oculocutaneous albinism, whole-exome sequencing (WES), *TYR*, *OCA2*, consanguinity, Pakistan, genetic counseling

Clinical and Molecular Characterization of Stargardt Disease: Documentation of ABCA4 Variants in Two Consanguineous Families

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This study aimed to characterize the clinical and molecular features of Stargardt disease type 1 (STGD1) by identifying pathogenic *ABCA4* variants and evaluating their genotype-phenotype correlation and co-segregation in affected families. In the observational study, ophthalmological screening was performed for two recruited families from Khyber Pakhtunkhwa, with Stargardt disease phenotypes. Whole-exome sequencing was performed in index patients of both families. Variants were prioritized through inhouse VARDIGS pipeline and validated via Sanger sequencing. Co-segregation analysis was performed in available family members and variants were classified based on ACMG (2015) guidelines. Subsequently, *in-silico* analysis was conducted to evaluate potential structural and functional significance of the identified variants. The ophthalmological tests in affected individuals revealed STGD1 features

including photophobia, poor vision, central vision loss, reduced visual acuity, presence of yellowish flecks, macular dystrophy, and refractive errors (myopia). WES of index patients identified two homozygous *ABCA4* missense variants, c.2588G>C (p.Gly863Ala) and c.1957C>T (p.Arg653Cys), which segregated with disease in available family members and were consistent with typical STGD1 features. This study characterizes STGD1 in consanguineous families from Khyber Pakhtunkhwa, confirming segregation of *ABCA4* variants and supporting genetic counseling, while contributing to genetic and clinical understanding of *ABCA4* associated disease.

Keywords: Stargardt disease 1, Consanguineous families, Whole Exome Sequencing, *ABCA4* mutations, genotype-phenotype correlation

Melissopalynological analysis of honey samples from district Attock Punjab, Pakistan

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Mellisopalynology serves as a robust tool for authenticating honey and elucidating its botanical and geographical origin. This study aimed to characterize pollen diversity and evaluate spatial variation in pollen spectra of honey samples collected from District Attock Punjab, Pakistan. A total of 18 honey samples representing distinct localities were subjected to centrifugation and standard acetolysis followed by microscopic examination at 400× and 1000× magnifications. The analysis revealed 13 distinct pollen types belonging to 8 major plant families namely Rhamnaceae, Brassicaceae, Fabaceae, Myrtaceae, Asteraceae, Poaceae, Lamiaceae, and Rosaceae, reflecting the prevailing flora of the region. Among these *Ziziphus* spp. (Rhamnaceae) was identified as the dominant pollen type, indicating its significant role and monofloral tendencies in regional honey production. *Acacia* spp. and *Eucalyptus* spp. were recorded as secondary pollen types, whereas *Brassica* spp., *Trifolium* spp., and members of Poaceae were categorized as minor pollen contributors. The observed variation in pollen spectra among samples highlights spatial heterogeneity in floral resources and foraging patterns of *Apis mellifera*. The findings provide a scientific basis for honey authentication and contribute to the understanding of plant pollinator interactions under changing environmental conditions.

Keywords: Mellissopalynology, Honey Authentication, Botanical Origin, Prevailing flora, Pollen spectra, *Apis mellifera*, Floral Diversity, Attock (Pakistan), Monofloral, Plant Pollinator

Antibacterial activity and biochemical characterization of Withania coagulans fruit extract against grass carp-borne *Klebsiella pneumoniae*

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Aquaculture is a crucial and sustainable source of animal protein because of the major concerns to world food security posed by rapid population increase, unstable climates, and the gradual loss of wild fish resources. However, the development of antibiotic-resistant *Klebsiella pneumoniae* poses a serious risk to public safety and fish health, making the investigation of efficient, environmentally friendly, plant-based antimicrobial substitutes necessary. The antibacterial effectiveness of *Withania coagulans* fruit extract against *K. pneumoniae* isolated from grass carp was assessed in this study. Methanol, ethanol, and acetone were used to prepare Methanolic, Ethanolic and Acetonic extracts. The agar well diffusion experiment was used to assess the antibacterial properties of these extracts. All of the extracts showed significant inhibitory activity against *K. pneumoniae*, but the methanolic extract was more effective than the others. Additionally, a variety of bioactive phytoconstituents, including alkaloids, flavonoids, phenolic compounds, terpenoids, and withanolides—all of which are well known for their antibacterial potential—were found by gas chromatography-mass spectrometry (GCMS). These substances may have antibacterial effects by virtue of disrupting bacterial cell membranes, inhibiting vital enzyme systems, and interfering with vital metabolic processes. All things considered, the results indicate that *W. coagulans* fruit extract has promising antibacterial qualities and could be an environmentally safe substitute for managing bacterial infections in aquaculture while also helping to reduce antibiotic resistance.

Keywords: Aquaculture; *Klebsiella pneumoniae*; *Withania coagulans*; Antibacterial activity; Grass Carp

Exploring the Abundance and Diversity of Lizard Fauna in District Karak, Khyber-Pakhtunkhwa (KP), Pakistan

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Lizards are universal animals that exist in a broad range of forms. Although the revolutionary process decreased their number, they still managed to survive due to their high adaptability to the environment. The current study aimed to explore the species abundance and diversity of lizard fauna in District Karak, Khyber

Pakhtunkhwa (KP), Pakistan. A total of 75 specimens were collected from six (6) different locations in District Karak; Warana Ahmad Abad, Hamidan, Takht-e-Nasrati, Karak city, Sabirabad and Mitha Khel. Various techniques and approaches were used for capturing and trapping including nets, cages, strings, pitfall traps, funnel traps, and manual collection. Seven species were identified using taxonomic keys*: Ophisops elegans* (28%), *Acanthodactylus cantoris* (19%), *Laudakia* pakistanica (13%), *Uromastyx hardwickii* (12%), *Laudakia stellio* (11%), *Eutropis dissimilis* (9%), *and Varanus bengalensis* (8%), belonging to four families: Lacertidae (Oppel, 1811), Agamidae (Gray, 1827), Scincidae (Oppel, 1811) and Varanidae (Merrem, 1820). The most abundant species observed were *O. elegans* and *A. cantoris* while *V. bengalensis* was the least abundant. Shannon's diversity index (H') was applied to assess species diversity. The observed values of Shannon index (H') for all species in all six locations were less than 1, indicating low diversity, and the evenness values showed a weak equilibrium between the effectiveness of sampling species. As well as the Margalef's index results indicated a richer habitat for *O. elegans* and *A. cantoris* at certain locations. Observations revealed that District Karak has low lizard diversity, compared to other regions. This study provides the baseline and framework for further research to explore the unsurvey locations of District Karak.

Keywords: Lizard diversity, Abundance, Shannon's diversity index (H'), Margalef's index, District Karak, Khyber Pakhtunkhwa

Prevalence and diversity of blood parasites of lizards in tehsil Sheringal district Dir Upper KP Pakistan

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Lizard are cold-blooded creatures having a worldwide distribution. There are 7,176 species of lizards worldwide, including 103 species and subspecies in Pakistan, making them the most diverse and widely distributed group of reptiles. A total of 32 lizard representing three species, one genus and one family from three different sampling sites along an elevational gradient from Tehsil Sheringal doag Dara Dir Upper from April 2023 to July 2024. The sampling sites were divided into low elevation (below meters), mid elevation (1801-2100 meters), and high elevation (Above 2100 meter). Lizards were captured using glue trap and hand capture method. A small amount of blood was drawn from the coccygeal vein using sterile insulin needle and thin smear was prepared from each lizard on spot. The smear was airs dried, fixed in methanol, stained with geimsa solution and were observed under microscope for the presence of hemoparasites. Overall, 34.3% (n=11/32) prevalence was observed. Parasite prevalence varied among the three species. With highest prevalence observed in *Laudakia tuberculata*. Fifty percent of the captured lizard (08/16) were positive for the blood parasites followed by *laudakia agrorensis* (2/7 28.5%) while the lowest prevalence was observed in *laudakia pakistanica* (1/9 11.1%). The

parasite community was composed of two different parasites *Hepatozoan* and *Schellakia.*

Keywords: reptile**,** lizard, heamoparasite, Hepatozoon, schellackia

Biodegradation of Polyester Polyurethane Separately by Five Fungi and Three Consortia

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Human being has prepared multiple plastic items for luxuries and comforts that have occupied a great extent of our habitat. Polyurethane is a polyester type of plastic used in shoes, furniture and foams. Polyester polyurethane is non-degradable and resistance to decomposition. It has led to the environmental pollution like damage to terrestrial ecosystem, aerial ecosystem and aquatic ecosystem. Scientists are looking for the possible solutions to the polyester polyurethane born environmental problems. After thorough review of recycling methods, biodegradation was considered as safe, environment friendly and appropriate mean of polyester polyurethane deterioration. The present work aimed to study the biodegradation of polyester polyurethane separately by five monoculture fungi and three consortia. This study aims to isolate five different types of fungal strains from dumping sites and to test the biodegradation potentials of fungal strains against polyester polyurethane as monocultures (MC) and their three consortia. Moreover to compare the biodegradation efficacy of three consortia of the fungal strains and their monocultures for the efficient biodegradation of polyester polyurethane. The results revealed that the monoculture MC-A and MC-E were effective in polyester polyurethane biodegradation in comparison to other monocultures i.e. MC-B, MC-C and MC-D. The degradation of PU by MC-A and MC-E is clearly seen in the pictures and the microscopic images. ATR-FTIR results showed that in the MC-A and MC-E deformation of bonds at wavelength 3320.02 cm-1 is shifted to 3306.72 cm-1 and 3320.48 cm-1 wavelength. MC-E is effective than MC-A follower by MC-B, MC-C and MC-D respectively first, second, third, fourth and fifth in their biodegradation potentials. Moreover Consortium C-1, C-8 and C-9 showed biodegradation capacities in the series C-1 followed by C-8 and the C-9. The ATR-FTIR results of C-1 and C-8 showed displacement of peak from 3328.02 cm-1 wavelength to 3316.14 cm-1 wavelength, 3304.77 cm-1 wavelength and 3283.62 cm-1 wavelength. The comparision of weight bearing capacities of biodegrading polyester polyurethane indicated that MC-A treated polyester polyurethane bear 0.22 grams, MC-E 0.24 grams, C-1 0.09 grams, C-8 0.08 grams and C-9 0.12 grams. It revealed that among the monoculture fungi MC-A was effective th reducing weight bearing capacity of polyester polyurethane while C-8 was effective among the three consortia. This study will be helpful to recycle polyester polyurethane and no environmental pollution will be caused by polyester polyurethane.

Assessing the Nutritional Status and Disparities Based on International References Among School Children in District Mardan

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In developing countries like Pakistan, Malnutrition including underweight, overweight, and obesity remains a major health concern among the public. Children of school age (6-12) are more particularly affected. Pakistan faces double burden of malnutrition reflects the coexistence of undernutrition and overnutrition. International references such as International Obesity Task Force (IOTF), World Health Organization (WHO), Centers for Disease Control and Prevention (CDC) are most widely used to assess nutritional status and disparities among them. To assess the nutritional status among school children (6-12) in District Mardan and to examine age-wise and gender-based disparities among them by using antropometric measures and international references for BMI. A cross-sectional study was conducted in the year 2025 in District Mardan. A total of 1000 healthy children data was collected from various private and government schools in the district. A multistage cluster sampling technique was used. BMI was calculated by recording Anthropometric measurements (height and weight). IOTF and CDC cut-offs were used to classify nutritional status. According to IOTF classification, prevalence of underweight was higher than overweight and obesity among all age groups, having higher frequencies at age 8 and 12. Thinness (particularly thin I) was common, with severe thinness more prominent in girls in older age. Based on CDC percentiles, a significant proportion of children fell below the 3rd percentile, showing severe underweight especially at age 12. Overweight and obesity was also recorded, with considerable prevalence at higher percentiles (85th- 97th), particularly among boys. Overall, findings from this study highlight a coexistence of undernutrition and overnutrition along with gender-based differences in district Mardan. The study reveals that malnutrition exists among school children in district Mardan., with underweight being more prevalent than overweight and obesity. Strategic nutritional interventions and policies are needed to address all forms of malnutrition and gender and socioeconomic disparities.

Keywords: Malnutrition, BMI, IOTF, CDC, Anthropometry

Thermal stress-induced changes in gill morphology and oxygen consumption in native snow trout (Schizothorax plagiostomus) under simulated climate warming

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Freshwater fish in high-altitude regions are increasingly threatened by rising water temperatures due to climate change, yet very few studies have focused on native Himalayan species like snow trout. In this study, we exposed

Schizothorax plagiostomus (average weight 120 g) to gradually increasing water temperatures (18°C to 28°C over 10 days) in a controlled laboratory setup to simulate a future warming scenario. We measured oxygen consumption rates daily using a closed respirometer and examined gill tissue under a light microscope at the end of the experiment. Results showed that oxygen consumption increased by 42% when temperature reached 24°C, but dropped sharply by 35% at 28°C, indicating respiratory distress. Gill examination revealed lamellar fusion, epithelial lifting, and curling of secondary lamellae in 80% of exposed fish, while control fish (maintained at 18°C) showed normal structure. These changes suggest that even moderate warming beyond 24°C may exceed the thermal tolerance of snow trout, potentially reducing their survival in natural streams. This is the first report linking thermal stress directly to gill damage in *Schizothorax plagiostomus*. Our findings provide a simple, low-cost biomarker (gill morphology + oxygen consumption) for monitoring climate impacts on cold-water fish populations in the Hindukush-Himalayan region.

Keywords: Snow trout, thermal stress, gill histology, oxygen consumption, climate warming

Effects of Deforestation and Hunting on Bird Species Decline in Darazinda, Sherani Tribe Area

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In a world of rapid anthropogenic land use, changes and declining biodiversity, there is an urgent need for understanding the state of biodiversity to aid management and conservation. In order to successfully manage land use in ways that is least harmful for biodiversity, it is vital to be able to identify habitats that are of most importance for biodiversity. In the present study the causes of extinction were noted in Darazinda. In our world, diversity of birds decreasing with the passage of time due to so many known and unknown factors, it has been discovered that the most important one is the deforestation. In the world total bird diversity is 9040 species in which 132 species are extinct now while hundreds have been declared threatened and endangered due to anthropogenic activities. Same phenomena were observed in the present research in which the bird diversity alarmingly decreased with the passage of time and most of the birds face imminent extinction. The estimated data shows that there has been a disturbing 36% decrease in the bird population during the previous 10-15 years and the main factor has all through been deforestation. The research declared that if the deforestation and hunting are not stopped through strict measures then in the coming 10-15 years the burgeoning population explosion will add to the above and it is feared that diversity will be further decrease by 92% and most of the species will soon be extinct from the area of Sherani tribe. The total bird species in our area were 32 among including vultures which have vanished from the area while a few economically important birds are near extinction. The population of economically important birds has decreased more than the other

birds and this sudden decrease can be blamed on deforestation but it was hunting as sport which played the key role in their alarming decrease, for they are a delicacy for the hunters and their delicious meat is eaten with relish. The population of some of the birds has suffered terrible decline and this includes Black Partridge whose numbers have decreased almost 80%, Alectorus chukor met a decrease 98%, Gray Partridge lost 70% its population and See-See Partridge fell to 40% decrease which is calamitous. To conserve bird diversity and also to maintain the population of the economically important birds, we must stop deforestation and hunting simultaneously. This is only possible if we adopt proper legislation to curb deforestation and hunting by punishing those who augment it and are thus responsible for it.

Abundance of the Striped Hyena (Hyaena hyaena) and Factors Influencing Its Population in District Dera Ismail Khan, Pakistan

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Striped hyena belongs to the hyaenidae family and it is listed as Near Threatened by IUCN. Despite of its low population, it is widely distributed in different areas of the world . A news reported the existence of stripped hyena in the peripheral area of district D I Khan. This research deals with the abundance of striped hyena and factors affecting the population of striped hyena in the district D I Khan. These factors are human hyena conflict, public unawareness, mythical conception and predation. Questionnaire survey methods were used to collect information on these factors. The study indicate that stripped hyena frequently found in the rural areas. It is also evaluated that it is a strictly solitary species. Grazing of livestock of the local in the habitat of hyena become major reason of conflict. There is no mythical conception among the community regarding this animal. The conservation outreach programs should be formulated to conserve and maintain the population of the striped hyena which would be helpful for long term conservation of the striped hyena in D I Khan.

Role of Physicochemical Water Properties in Shaping Aquatic Insect Distribution and Diversity

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Water has various properties like density, temperature, pH, velocity, viscosity etc. These properties are very essential for the distribution of the insects in the pond. These properties are called the physiochemical properties of water. These are combination of both physical and chemical properties of the insects. I collected insects along with the water from the pond and then took them to lab for checking the physiochemical properties and the distribution of the insects. This study was conducted to evaluate the influence of some water quality parameters on the aquatic insects Sampling of the aquatic insects was conducted weekly in the wet season, from May through June, the insects' abundance seems to be influenced by substrate, physiological and morphological adaptation, current velocity and turbidity. The physico-chemical properties and Margalef index of water quality shows that the quality of a water body has a role to play in the abundance, diversity and distribution of aquatic insect.

Keywords: Physicochemical properties, insects, pH, dissolved oxygen, temperature, velocity

Prevalence of Warble Fly Infestation in Goats of Paharpur, Pakistan

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Livestock Sector occupies n unique position in the socio-economic development of country. Parasitism is one of major veterinary problems affecting livestock in many parts of the world. Hypodemiatosis is an cattle of Pakistan. Warble fly infestation is a major disease of economic significance affecting livestock throughout the world. The study was conducted to important disease after ticks in see the prevalence of warble ily in goals of paharpur A total of 300 goats wore observed. The goats were palpated on the back and Hank region to chock the presence of warbles. The collected larvas were stored in 70% ethanol with 2-3 drops ol glycerin for further analysis. Out of 300 45 goats were positive, 1 8 were male and 27 were female, white 33 goats of age group I and 12 were from age group 2. From Zones of paharpur Zone I is 19 goats were of Damani breed,17 of teddy while 9 of I3eetai breed more prevalent with 18 positive goats.

Keywords: warble fly, goats

A Preliminary Survey of Butterfly Species (Nymphalidae, Papilionidae, Pieridae) in Dera Ismail Khan

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This study was conducted to provide baseline fauna for the butterflies ot D.l.Khan region. The butterflies were collected ruing January April 2019 from 5 different localities of D.l.Khan areas. The collected specimens yielded 10 species from families Namphalidae, Papilionidae and Pieridae cover 43%, 14%, and 43% of the butterflies. Family Namphalidae included species Argynnis hyperbius, Cynthia cardiu, Ariadne merione, Junonia orithya. Family Papilionidae: Catopsilia Pomona, Colias croceus, Colotis etrida, C. protractus, Eumera hecab, Pieris ajaka, P. brassicae, P. rapae and P. napae respectively. While Pieridae included Papilio demoleus and P. polytes only. From the current research it can be concluded that area of D.l.Khan is best for the butterflies is badly affected but may be favour if avoid these activities.

Keywords: Species, Butterfly, Fauna, Family, Identification

Impact of Sugar Mill Effluent on Morphological Indices and Organ Condition of Labeo rohita from Teer Garh Mam Drain

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The Impact of water toxicology on fish were evaluate by morphological examination of different internal organs of fish body from Teer garh mam drain, like gills, liver and kidney, by fish morphometric indices the mean wet weights and total length of Labeo rohila from sampling sites. Significantly ($p<0.05$) higher values were recorded for fish body weight and length of Labeo rohita caught upstream (125.01±34.06* and 18.09±2.67 respectively) than those collected downstream from effluent discharge point (Al-Moiz sugar mill waste discharging site) (87.15±27.98 and 20.14±3.84 respectively). Morphological examination of fish samples upstream showed reddish gills while those of fish downstream were pale, morphological examination of fish samples upstream showed reddish gills while those of fish downstream were pale and Morphological examination of fish samples upstream showed brownish red colored kidney while those of downstream were yellowish. There is need for the adoption of proper treatment of the toxic effluents prior to their discharge into the water bodies.

Keywords: Morphometric indices, gills, liver, kidney, upstream, downstream, Teer garh main drain, Labeo rohita

First Report of 14 Dragonfly Species (Order Odonata) from District Dera Ismail Khan, Pakistan

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The main objectives of the current study were to Explore the fauna of dragon flies in district Dera Ismail khan. Present study was carried on exploring the Fauna of Dragonflies. Collection of dragonflies was carried during April to july 2021, from the whole District D.I. Khan Total 200 dragonfly’s specimen was collected from different areas. Among 14 species, 8 genera of dragonflies were identified under two families i.e., Libellulidae and Aeshnidae of order Odonata. Which are the Orthetrtrum chrysis, Orthetrum cancellaham cancellaham, Orthetrum gluacumluzinicum, Orthetrum Sabina ,Pantala flavescence, Palpopleura sexmaculata sexmaculata, Palpopleura sexmaculata octamaculata, Trithemis /estiva, Crocothemis nigrifrons, Anax immiculifrons, Bradinopyga geminate, Trithemis kirbyi and Acisoma panorpoides panorpoides. This is the first report of dragonflies from District D.I. Khan, from this study site, same type of study on large scale is recommended to fully explore dragonfly’s fauna of region. It was addition to science and this addition will be valuable for exploring new areas of biological control in Pakistan. It is concluded that there is an abundance of dragonfly fauna from District D.I. Khan.

Keywords: Dragonflies, Insect, identification, Dera Ismail khan. Pakistan.

PCR based amplification of ITS-1 gene of Toxoplasma gondii among the donkey blood samples collected from various regions in Punjab

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Donkeys are among the common companion and working animals throughout the world and in some countries their meat and milk are used for human consumption. Donkeys are known to act as carriers of pathogens and are involved in the transmission of infectious agents to for other animals and humans. The present study was aimed to report the molecular prevalence of *Toxoplasma gondii* in the blood samples of two donkey breeds that were enrolled during September 2024 till November 2024. Genetic diversity of parasite and risk factors associated with the infection were also determined. A total of 272 blood samples were collected from nine districts in Punjab: Muzaffargarh (N = 82), Bahawalnagar (N = 54), Faisalabad (N = 30), Khanewal (N = 25), Rajanpur (N = 24), Dera Ghazi Khan (N = 19), Lodhran (N = 18), Pakpattan (N = 10) and Kasur (N = 10). Polymerase Chain Reaction amplified a 300 base pairs fragment specific for *ITS-1* gene of *Toxoplasma gondii* in 112 out of 272 (42%) donkey blood samples. Subsequent Sanger sequencing followed by the BLAST analysis confirmed the presence of parasite. Partial *ITS-1* gene sequence based phylogenetic analysis revealed that Pakistani *T. gondii* isolates were genetically diverse and they resembled the isolates reported from birds and mammals from Iraq, Australia, Pakistan, Brazil and Thailand. Prevalence of parasite varied significantly among the sampling sites ($P < 0.001$). Epidemiological factors e.g. age, sex, breed and ectoparasites presence or absence was not found associated ($P > 0.05$) with the prevalence of *Toxoplasma gondii*. Prevalence of *Toxoplasma gondii* varied significantly when compared between the donkeys having different coat colors ($P = 0.03$). In conclusion, to the best of our knowledge, this is very first study from Punjab that is reporting a very high prevalence of *Toxoplasma gondii* in donkeys blood samples. As the parasite was detected in all nine districts included in this study, it is recommended that the donkey blood samples from other provinces should be screened for this parasite and in those countries where donkey meat is part of food, poorly cooked meat should be avoided to restrict the zoonotic transmission of this parasite.

Keywords: *Toxoplasma gondii;* Molecular prevalence; Phylogeny; Donkey, Pakistan.

PCR based amplification of msp1bgene for the molecular detection of Anaplasma marginalein buffalo blood samples collected from Haroonabad in district Bahawalnagar.

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Nili-Ravi is a major dairy buffalo breed of Pakistan, widely raised in Punjab and valued for its high milk yield. Despite the fact that buffaloes are vital to rural livelihoods in Pakistan, they remain largely unscreened for vector-borne diseases in most part of the country. These infections pose a serious health threat to this breed, reducing their productivity and causing significant economic losses to farmers and the livestock sector. Present study was aimed to report the molecular prevalence and phylogenetic evaluation of *Anaplasma marginale* in buffalo blood samples (n = 250) that were collected from District Bahawalnagar in Punjab, Pakistan. Genomic DNA was extracted from blood and a 265 base pair fragment from *msp-1b* gene of *Anaplasma marginale* was target though PCR assay. Results revealed that 40/250 (16%) of the screened buffaloes were *Anaplasma marginale* infected. DNA sequencing and BLAST analysis confirmed the presence of this pathogen among the screened buffaloes. Phylogenetic analysis showed that sequences generated in this study were genetically similar with *msb-1b* gene of *Anaplasma marginale* isolated from ruminants in USA, Israel, India and Pakistan. Risk factor analysis indicated that the prevalence of *Anaplasma marginale* was not associated with age (P = 0.8), sex (P = 0.4), presence and absence of ectoparasite (P = 0.2) or the disease history (P = 0.4) of the screened Nili-Ravi buffaloes. In conclusion, we are reporting a relatively high prevalence of *Anaplasma marginale* in Pakistani buffaloes enrolled from Bahawalnagar district during present investigation. We recommend the screening of buffaloes in all areas of Pakistan that are still unexplored for the prevalence of this bacterium. This will help us in better understanding the associated risk factors and will contribute towards the effective control of anaplasmosis among the buffalo population in Pakistan.

PCR based amplification of 30KDa merozoite surface antigen protein gene for the molecular detection of Theileria annulata in dog blood samples collected from Charsadda district in Khyber Pakhtunkhwa Province

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Dogs, *Canis lupus familiaris*, are considered as one of the most popular domestic animals across the globe. Being in close association with humans, they are frequently exposed to the environment and hence to a variety of vectors, making them potential reservoirs for the transmission of several vector-borne pathogens to other animals and humans. Present study aimed to report the molecular prevalence and phylogenetic evaluation of *Theileria annulata* in dog blood samples (N = 165) that were collected from different areas in District Charsadda, Khyber Pakhtunkhwa (KPK) during December 2024 till May 2025. Molecular analyses revealed that 26/165 (15%) of the screened dogs amplified a 721 base pair fragment from *30KDa* merozoite surface antigen gene of *Theileria annulata*. DNA sequencing and BLAST analysis confirmed the presence of this parasite among the screened dogs. Phylogenetic analysis revealed that sequences generated in this study were genetically similar with the *30KDa* merozoite surface antigen gene sequences reported from large ruminants in India and Pakistan. Epidemiological parameters such as age, sex, breed and presence or absence of ectoparasites on the dogs were not found associated with *Theileria annulata* infection. In conclusion, we are reporting a relatively high prevalence of *Theileria annulata* in Pakistani dogs screened during present investigation. These findings highlight the need for large-scale studies across unexplored areas of Pakistan for the better understanding of epidemiology, genetic diversity and host-parasite interactions that will lead to the effective control of *Theileria annulata* infections among the local dog population.

Keywords: *Theileria annulata;* Molecular prevalence; Phylogenetic analysis; Dogs; Pakistan

PCR based detection of *Mycoplasma haemofelis* in dog blood samples collected from Charsadda district in Khyber Pakhtunkhwa

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Dogs, *Canis lupus familiaris,* are considered as one of the most popular domestic animals across the globe. Because of their close association with humans and frequent exposure to the environment, they are commonly exposed to various vectors and vector-borne infections, making them potential reservoirs for transmitting these pathogens to other animals and humans. Present study was aimed to report the molecular prevalence of *Mycoplasma haemofelis* in dog blood samples (n = 165) that were collected from different areas in District Charsadda, Khyber Pakhtunkhwa (KPK) during December 2024 till May 2025. Molecular analyses revealed that 11/165 (6.7%) of the screened dogs amplified a 393 base pair fragment specific for *16S rRNA* gene of *Mycoplasma haemofelis.* Risk factors such as age, sex, breed and presence or absence of ectoparasites on the dogs were not associated with *Mycoplasma haemofelis* infection. In conclusion, this study provides the first evidence of *Mycoplasma haemofelis* in dogs in Pakistan, despite this bacterium being uncommon in canine hosts. These findings underscore the need for broader investigations across currently unexplored regions to better understand its epidemiology, genetic diversity, and host–pathogen interactions, which will support improved surveillance and prevention strategies in the local dog population.

PCR based amplification of cytochrome b gene of Theileria annulata in sheep blood samples enrolled from six districts in Punjab

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Pakistan has huge sheep population with approximately 33.1 million heads during 2024-25. Despite this huge population, sheep have been insufficiently investigated in Pakistan for the presence of vector-borne pathogens. *Theileria annulata* is an apicomplexan parasite that primarily infects large ruminants, especially cattle and buffaloes, but it can also spill over and infect small ruminants such as sheep and goats. Present study was aimed to report the molecular prevalence of *Theileria annulata* in the sheep blood samples (n = 329) that were collected from six districts of Punjab: Khanewal (n = 100), Dera Ghazi Khan (n = 51), Layyah (n = 51), Taunsa (n = 50), Rajanpur (n = 45) and Muzaffargarh (n = 32) during August till December 2024. Molecular analysis revealed that 8/329 (2%) of the screened sheep amplified a 312 base pair fragment from cytochrome b gene of *Theileria annulata*. Parasite prevalence varied non-significantly between the sampling districts (P = 0.3) as well as among the sheep breeds (P = 0.1). Risk factor analysis indicated that sheep sex (P = 0.3), presence or absence of ectoparasite on sheep (P = 0.4), presence of dogs with the herd (P = 0.7) or herd size (P = 0.7) were not associated with *Theileria annulata* infection in the enrolled sheep. In conclusion, the present study reports the presence of *Theileria annulata* in accidental host, sheep, in three of the six districts from Punjab that were included in this investigation. We recommend large-scale studies in various geo-climatic regions of Pakistan to further explore the epidemiology, genetic diversity, host-parasite interactions that will lead to effective control of this apicomplexan parasite infection among the local sheep population.

Keywords: *Theileria annulata*; Molecular prevalence; Phylogeny; Sheep, Pakistan.

PCR based amplification of 18S rRNA gene of Theileria ovis in the blood samples of dogs collected from five districts in Punjab

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Dog are one of the two most ubiquitous and most popular domestic animals in the world. Being a companion of man, they are more exposed to the environent and hence vectors and may act as a reservior for variety of vector borne pathogens. The present study was aimed to report the molecular prevalence of *Theileria ovis* in the dogs with a note on epidemiology and genetic diversity of pathogen. A total of 275 dog blood samples were collected from five districts in punjab: Dera Ghazi Khan (n = 57), Vehari (n = 7), Layyah (n = 34), Bahawalpur (n = 38) and Muzaffargarh (n = 139) during March till October 2024. Polymerase chain reaction amplified a 520 base pairs fragment specific for 18S rRNA gene of *Theileria ovis* in 32 out of 275 (11.6%) dog blood samples. Subsequent sanger sequencing followed by the BLAST analyses confirmed the pesence of *Theileria ovis*. Phylogenetic analysis that was based on partial 18S rRNA gene sequence revealed that Pakistani *Anaplama* ovis isolates were genetically similar and they were clustered with *Theileria ovis* sequences that were deposited from Pakistan, Iran, Kyrgyzstan, Ghana and South Africa. Prevalence of parasite varied significantly among the sampling sites as well as among the dog breeds that were enrolled during present investigation. In conclusion, to the best of our knowledge , this is very first report regarding the

higher prevalence of *Theileria ovis* in Pakistani dogs. Limited number of dogs were screened in this study that demands that dog blood samples should be collected and screened from other unexplored areas of Pakistan to get more information about the prevalence of this parasite as well to learn about its epidemiology and host-parasite interactions that will help in designing control measures against this infection.

Molecular detection of Theileria ovis in sheep blood samples that were collected from six districts in Punjab

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Sheep population in Pakistan was approximately 37.2 million during 2023-24 and despite this huge population, sheep remained unexplored for the presence of majority of vector borne pathogens. Present study was aimed to report the molecular prevalence and phylogenetic evaluation of *Theileria ovis* in the sheep blood samples (N = 329) that were collected from six districts (Muzaffargarh, Rajanpur, Dera Ghazi Khan, Layyah, Taunsa and Khanewal) in Pakistan during August till December 2024. Molecular analyses revealed that 70/329 (21%) of the screened sheep amplified a 520 base pair fragment from 18S rRNA gene of *Theileria ovis*. DNA sequencing and BLAST analysis confirmed the presence of all three pathogens. Phylogenetic analysis of these pathogens showed their genetic similarities with the sequences deposited from worldwide countries. *Theileria ovis* prevalence varied significantly between the sampling district (P = 0.001). The highest infection rate was observed in sheep enrolled from Khanewal (33%) followed by Rajanpur (27%), Muzaffargarh (25%), Taunsa (16%), Dera Ghazi Khan (10%) and Layyah (8%). Similarly, the parasite prevalence varied significantly among the sheep breeds (P = 0.003). *Theileria ovis* infection was more common in large herds as compared to smaller ones (P < 0.001). In conclusion, we are reporting a higher prevalence of *Theileria ovis* in Pakistani sheep and animals from all six districts were infected indicating that the parasite is endemic. We recommend similar large-scale studies in various geo-climatic regions of Pakistan to further explore the epidemiology, genetic diversity, host-parasite interactions that will lead to effective control of this common parasitic infection among the local sheep population.

Impact of mealwormas a substitute for feather meal on growth and meat quality of juvenile golden mahseer

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The search for sustainable and cost-effective protein sources in aquaculture feeds has become increasingly important due to the rising cost of conventional

ingredients. This study evaluated the effect of partially replacement of dietary feather meal with mealworm (*Tenebrio molitor*) meal on the growth performance and meat quality of juvenile golden mahseer.A 90-day feeding trial was conducted under controlled laboratory conditions in aquaria at Aquaculture and Fisheries Laboratory at PMAS-AAUR using three experimental diets: Supplementary feed having feather meal as control (C), supplementary feed having 25% replacement of feather meal with mealworm meal (G1), and supplementary feed having 50% replacement of feather meal with mealworm meal (G2). Fish were fed daily at 5% of their body weight and water replacement was done to maintain the quality standard. Growth parameters (viz. body weight & body length) were observed fortnightly and the proximate analysis (viz. moisture, crude protein, crude fats and ash percentage) was done at the end of the feeding trail. The results indicated significant highest weight gain in the control group(4.43 g), followed by G1 (4.10 g) and G2 (4.00 g) whereas the body length was observed significantly higher in G1 (1.93 cm) followed by Control (1.7 cm) and G2 (1.64 cm). Proximate composition analysis showed clear variations among treatments; Moisture content was highest in the control group (19.55%) and lowest in G1 (11.24%). Crude protein remained constant across all groups (57.76%), Crude fat was higher in the control group (39.66%) as compared to G1 and G2 (37.36%) while Ash contents were higher in G1 (13.0%) than control and G2(12.5%) group.In conclusion, although mealworm replacement did not enhance weight gain compared to the control diet but25% replacement improved certain growth traits and influenced body composition. These findings suggest that mealworm meal can be used as a partial alternative protein source, with its effects more pronounced on body composition than on weight gain in juvenile golden mahseer.

Keywords: Composite analysis, Feather meal, Feed replacement, Golden mahseer (*Tor putitora*), Growth Performance, Mealworm (*Tenebrio molitor*),

Outbreak of lumpy skin disease in the cattle of district Buner Pakistan

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Lumpy skin disease (LSD) is infectious disease in cattle caused by a virus of the family Poxviridae, also known as Neethling virus. The present study was conducted on the outbreak of Lumpy Skin Disease in district Buner, Pakistan during July 2022 to December 2022. During the study a total of (n=3351) cases of animals (cattle) of Lumpy Skin Disease were reported in which (n=750) (22.4%) death cases were reported. The total recovered cases of Lumpy Skin Disease were (n=2600). During the outbreak there are around (41625) cattle were vaccinated in district Buner. The study area was divided into six different tehsils. The most affected tehsil during the outbreak was Mandanr. Tehsil Mandanr are highly effected in which (n=1071) cases were reported with mortality rate of (n=19.6%). The most rear effected tehsil was Gagra in which only (n=290) cases of LSD were reported with mortality rate (n=27%). During

the outbreak we visited the local community, DVM, Livestock office of district Buner, veterinary assistance, Doctors etc. In this outbreak many economic losses were reported of LSD among the farmers community. There is also banned meat and milk during the outbreak.

Partial replacement of black soldier fly grub with feather meal in diets of juvenile golden mahseer: effects on growth performance and proximate composition

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Aquaculture is growing rapidly to meet the increasing worldwide demand for protein; however, the high cost and limited accessibility of conventional feed components such as fishmeal and feather meal remain key challenges. Feather meal, despite its high crude protein content, is often limited by poor digestibility and imbalanced amino acid composition. Therefore, the present study was designed to evaluate the effectiveness of Black Soldier Fly (BSF) larvae as an alternative protein source in the diet of juvenile Golden Mahseer (*Tor putitora*). A 90-day feeding trial was conducted under controlled laboratory conditions using glass aquaria at Aquaculture and Fisheries Laboratory, PMAS-AAUR. Fish were divided into three feeding groups: Control group (C) fed with supplementary feed, group one(T1) fed with 25% replacement of feather meal with BSF meal, and group two(T2) fed with 50% replacement of BSF meal in feed. Fish were fed daily at 5% of their body weight, and growth parameters (body weight and total body length) were recorded on a fortnightly basis. At the end of the experiment, proximate composition (moisture content, crude protein, crude fat and total ash) of fish meat was analyzed to assess meat quality. The results showed that the highest weight gain was observed in T1 (3.87 g), followed by the C (3.80 g) and T2 (2.37 g). Final total length was highest in T2 (11.36 cm), indicating improved linear growth at higher replacement levels. Statistical analysis revealed a significant effect of dietary replacement on growth performance ($p < 0.05$), while the interaction between time and treatment was non-significant. Proximate composition analysis indicated that crude protein content remained highest and equal in C and T1 (57.76%), whereas Ash content was higher and equal in C and T2 (12.5%) as compared to other groups; whereas, crude fat declined with increasing BSF inclusion, ranging from 38.6% in Control to 36.6% in T2. Moisture content also showed a decreasing trend with higher BSF levels with 11.21% in control to 9.88% in T1 followed by 9.85% in T2. In conclusion, partial replacement of feather meal with BSF grub` at 25% replacement level significantly enhanced growth performance without compromising meat quality. However, higher replacement (50%) negatively affected protein content and growth efficiency. These findings highlight the potential of BSF grubs as a sustainable and cost-effective alternative protein source in aquaculture feeds, contributing to environmentally friendly fish production systems.

Keywords: Golden Mahseer (*Tor putitora*), Proximate Composite Analysis, Fish meal, Black Soldier Fly,Feather meal, Partial replacement.

Prevalence of drugs addiction in District Buner Pakistan

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Drug addiction, also called substance use disorder, is a disease that affects a person's brain and behavior and leads to an inability to control the use of a legal or illegal drug or medicine. The present study was conduct on prevalence of different Drugs Addiction in District Buner Pakistan during August 2022 to August 2023 During the study a total of 8056 drugs addicted Male and Female were reported. The number of Addicted Female are 28 and Male are 8028, in which 714 Ice addicted people reported, 7004 Marijuana, Heroin 113, and Wine are 225. The most effected tehsil are Daggar and Gagra. The total number of cases in Tehsil Gagra is 1917. The total number of cases in tehsil Daggar is 2455. The total reported cases in tehsil Chagharzai are 887. The total reported cases in Tehsil Gadezai are 926. The total reported cases in tehsil Mandanr are 853 and Total reported cases in tehsil Khudokhail are 1018. During our collection we collected 8056 cases in which the number of Female cases are 29 which percentage are 0.4% and male cases are 8027 which percentage are 96.6% in District Buner in which the percentage of male are on the top. During our serve we work on Drug addiction in which we collected specific and common types of Drug addiction which are common and most useable in our study area District Buner. In which include the types of Drug addiction are Heroin (1.40%), Ice (8.86%), Wins (2.8%) and most common and most use types is Charas (marijuana 86.94%).

Assessing the Nutritional Status and Disparities Based on International References among School Children in District Peshawar

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Keeping the global critical coexistence of underweight and overweight and obesity in mind this study was conducted to assess the nutritional status and disparities among school-going children in district Peshawar, using international standard references. A cross-sectional comparative study was conducted among 1,000 school children aged 6–12 years in District Peshawar. Data were collected through a stratified multistage cluster sampling technique from public and private schools in urban and rural areas, ensuring equal sex distribution. Anthropometric measurements (height and weight) were obtained using standardized procedures to calculate Body Mass Index (BMI). Nutritional status was assessed using CDC growth charts and IOTF BMI cut-offs. Data were analyzed using STATA (version 14.2), applying descriptive statistics, logistic regression, and Kappa statistics, with significance set at $p < 0.05$. Our preliminary finding showed double burden of malnutrition in school children. Underweight was more common in younger age groups, especially among boys, peaking at age 6 (40%, n=8). In contrast, overweight and obesity increased with age, particularly among children aged 10–12 years, with obesity reaching 18.75% (n=6) among 12-year-old boys. Girls showed lower underweight prevalence but comparable or slightly higher overweight in middle age groups, especially at age 8 (20.21%, n=19), while obesity remained low. Overall, a shift from Undernutrition in early childhood to over nutrition in later years was observed. Agreement between CDC and IOTF classifications was excellent for overweight ($\kappa = 1.000$) but poor for underweight and obesity ($\kappa = 0.000$). The study demonstrates the coexistence of under nutrition and over nutrition among school children in District Peshawar. These findings support targeted, age and gender specific intervention and guide policymakers in improving child health and reducing nutritional disparities.

Keywords: Malnutrition, School Children, BMI, IOTF, CDC, Double Burden of Malnutrition, Peshawar, Pakistan

DNA barcoding and phylogenetic analysis of fish fauna of river Barandu District Buner Pakistan

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DNA barcoding technique for fish identification is an effective, rapid and precise method as compared to the morphological method. *Cytochrome c oxidase* 1 gene- based DNA barcoding is frequently used in species identification and biodiversity studies. The recent study was designed to identify the fish fauna of river Barandu with the help of DNA barcoding and phylogenetic tree analysis resulting *COX1* gene sequences which were used in the construction of genetic diversity and evolutionary history of fish fauna of river Barandu district Buner Pakistan. A short template of DNA sequence of 650 base pairs (*COX1*) was amplified, sequenced and analyzed by using different bioinformatics tools. The pairwise distance and phylogenetic analysis by Maximum Likelihood (ML) tree based on Kimura 2 Parameter method was constructed by using MEGA11 software. The present study was conducted on molecular phylogenetic study of fish fauna of river Barandu district Buner Pakistan during July 2022 to July 2023. In the recent study we have reported 11 species belonging to eleven genera, five orders and six families of fish. The reported species are *Tor putitora*, *Schizothorax plagiostomus*, *Lebio rohita*, *Cyprinus carpio*, *Metacembelus armatus*, *Channa gachua*, *Puntius sophore*, *Barilius pakistanicus*, *Hypomesus japonicus*, *Oreochromis niloticus*, and *Schistura punjabensis*. The reported species were treated molecular characterized and constructed phylogenetic tree of each species.

Keywords: Fish fauna, COX1 gene, DNA barcoding, Phylogenetic analysis, river Barandu, Buner, Pakistan

Diversity and Distribution of Butterflies in Lower Mohmand Area of District Mohmand, Khyber Pakhtunkhwa, Pakistan

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Widely recognition of butterflies as bioindicators of environmental health and rare previous reports in Lower Mohmand region of Khyber Pakhtunkhwa, limits a comprehensive understanding of the region's biodiversity and its conservation requirements. Therefore, the present study was conducted to document butterfly species composition, abundance, and seasonal patterns in Lower Mohmand to support ecological monitoring and biodiversity conservation in this understudied region. A year-long field survey was conducted from January to December 2024 using standard entomological sampling techniques. Butterfly specimens were collected using hand nets and recorded through direct field observations in their natural habitats. Collected specimens were taxonomically identified up to species level using standard identification keys and relevant literature. To assess biodiversity patterns, several ecological indices were applied, including the Shannon–Wiener diversity index, Simpson's dominance index, Margalef's species richness index, and Pielou's evenness index. A total of 2,809 butterfly specimens were recorded during the survey, representing 11 species belonging to 11 genera and 4 families. The family Nymphalidae exhibited the highest species richness, comprising 6 species from 5 genera, indicating its ecological dominance in the study area. The family Pieridae was represented by 3 species from 3 genera, whereas Papilionidae and Lycaenidae were each represented by a single species. The observed distribution indicates moderate butterfly diversity in the region and reflects variations in habitat preference, resource availability, and environmental adaptability among different butterfly groups. The dominance of the Nymphalidae family and the observed patterns of species richness highlight the ecological importance of the area. These findings emphasize the need for habitat conservation and sustainable land-use practices to protect butterfly diversity and maintain ecological balance. The results contribute to regional biodiversity knowledge and may assist in conservation planning and environmental policy development in Pakistan.

Detection and Association of Rubella Virus in Pregnant Women with Bad Obstetric History in Mardan and Peshawar Pakistan

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Rubella virus (RV), causing German measles, is teratogenic and can lead to congenital rubella infection (CRI) and congenital rubella syndrome (CRS), resulting in lethal and devastating pregnancy outcomes. Having public health concerns and lacking awareness among masses particularly in women make it more toxic. Hence this epidemiological study was conducted to elucidate the probable association of rubella virus with women reproductive history. This study aimed to determine the prevalence of RV infection and associated risk factors among women with bad obstetric history (BOH), and to observe the ratio of miscarriages, abortions, and stillbirths in BOH cases. Samples of venous blood, cord blood, and placental tissues were collected from 50 pregnant and non-pregnant women with BOH at Mardan Medical Complex, Mardan and Khyber Teaching Hospital, Peshawar. Samples were processed for RV RNA in the molecular virology lab, Department of Zoology, University of Peshawar. Overall, 6% of samples were positive for RV RNA. Prevalence was highest (16.1%) in the 17–32 years age group. RV positivity was greater in housewives compared to working women, particularly those with 2 abortions (18.1%) and 4+ abortions (14.28%). Higher rates were also seen in illiterate women with 2 abortions (10%) and 4 abortions (25%), and in matriculate women with 2 abortions (20%), while graduates showed no positive results. Miscarriage-related prevalence was higher in illiterate women with 3 and 4 miscarriages (12.5% each). Rural women had higher RV positivity than urban women. Pregnant women with BOH show greater positivity toward RV infection. The findings suggest that multiple miscarriages and abortions in BOH cases may be associated with RV infection, with risk concentrated in younger, rural, less-educated housewives.

Assessing the Nutritional Status and Disparities Based on International References among School Children in District Charsadda; Ten Years Follow Up

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Childhood nutrition is increasingly characterized by a double burden in which undernutrition persists alongside rising overweight and obesity. Previous district-level data from Charsadda (2013–2015) showed coexistence of underweight and obesity; however, no recent follow-up has been conducted, and variability in BMI classification limits comparability. To determine the prevalence and ten-year trends of underweight, normal weight, overweight, and obesity among 6–12-year-old school children and to examine disparities and predictors of nutritional status. Data were obtained from two independent cross-sectional studies conducted in 2015 and 2025, including 1,200 and 1,000 school-going children aged 6–12 years, respectively, in District Charsadda. A stratified multistage cluster sampling technique was used to select children from 40 public and private schools in urban and rural areas. Height and weight were measured using standardized procedures, and BMI was calculated. Nutritional status was assessed using CDC growth charts and IOTF BMI cut-offs. Our preliminary findings showed that a total of 1,000 school children were assessed in 2025 and compared with baseline 2015 data. BMI was generally lower in 2025 across most age-sex groups, indicating a leaner body size profile over the ten-year follow-up. IOTF showed higher overweight/obesity in 2015, 2025 showed reduced obesity and increased Thinness I–II. CDC indicated decline in high BMI, with low BMI persisting in older boys. Agreement between IOTF and CDC was perfect for overweight classification but poor for underweight and obesity, showing that prevalence estimates vary depending on the reference used. Over the ten-year follow-up, obesity declined, yet undernutrition remained a major public health concern among school-going children in District Charsadda. Marked differences between BMI classification systems, particularly for underweight and obesity, underscore the need for a standardized BMI classification approach and targeted school-based nutritional interventions to address the persistent double burden of malnutrition.

Keywords: Child nutrition, malnutrition, BMI, IOTF, CDC, anthropometry

Hepatoprotective Role of Biogenic Silver Nanoparticles against Diethyl Nitrosamine-Induced Toxicity via Selected Apoptosis Regulators in Male Rats

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Liver cancer is one of the leading causes of cancer-related mortality worldwide, and its development is strongly associated with chemical carcinogens such as diethylnitrosamine (DEN), which induce progressive hepatic injury and malignant transformation. Hence, investigating novel therapeutic agents is essential for developing effective treatment strategies. Aim: This study was designed to assess the expression of apoptosis regulators in the liver tissues of a rat model treated with DEN, and to assess the protective effects of green silver nanoparticles (AgNPs). Male Sprague-Dawley rats (~200g body weight) were equally distributed into four groups, including control, DEN-treated, Co-treated (DEN+ AgNPs), and AgNPs-treated groups. Histopathology, DNA isolation, and western blot analyses were performed. A Significant decrease in the body weight of DEN-treated rats was observed compared to the control group. DEN-treated rats showed disruption of liver architecture with nodule formation and loss of the central vein. AgNPs co treatment showed recovery in body weight and attenuation of the histopathological lesions. Furthermore, damage in genomic DNA was detected with DEN treatment compared to the DEN+AgNPs and AgNPs groups. The expressions of Bax, p53, and Caspase-3 were downregulated, while Bcl-2 was upregulated with DEN treatment in the liver tissues of rats. The expressions of the targeted proteins were controlled with AgNPs treatment. The current results showed the regulatory role of AgNPs in apoptosis regulation in the liver tissues of DEN-exposed rats and its anticancer therapeutic potential.

Keywords: Diethylnitrosamine, Hepatotoxicity, AgNPs, Apoptosis, Western blot

Evaluation of Silver Nanoparticles as a Therapeutic Agent Against DEN-Induced Lung Toxicity Via Modulation of TLR4, ERK1/2, and PI3K Pathways

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Lung cancer is the most prevalent, rapidly spreading, and highly invasive, malignancy that kills many people. Diethyl nitrosamine (DEN) is a well-known carcinogen that causes cancer in a variety of organs in rats. Aim: The aim of the study was to investigate therapeutic applications of biogenic silver nanoparticles (AgNPs) as well as the detrimental effects of DEN on the regulation of TLR4, ERK1/2, and PI3K expression in lung tissues of male rats. A total of twenty rats (weight 150-250 grams) were randomly assigned to four experimental groups: Control, DEN-treated (90 mg/kg, intraperitoneally), DEN+AgNPs, and AgNPs alone (100 mg/kg, administered orally). After five weeks, the rats were dissected, and the blood samples were collected for complete blood count (CBC) analysis. Lung tissues were fixed in 10% buffered formalin for histopathological examination. Frozen lung tissues were used to extract DNA for the DNA ladder assay. Additionally, western blotting was performed to assess the expression levels of TLR4, ERK1/2, and PI3K. A significant reduction was observed in body weight of rats due to DEN treatment which was overcame with AgNPs treatment. Furthermore, in comparison to the control group, there was significant increase in MCV and MCH in the DEN group, Hb, MCV, and MCH in the DEN+AgNPs group, and Hb and MCV in the AgNPs group. Moreover, lung tissues of DEN-treated animals showed a modest proliferation of alveolar cells compared to control group. Genomic DNA isolated from the lung tissues of DEN treated animals showed smear, however, genomic DNA isolated from AgNPs treated groups was found normal. Down regulation of TLR4 and upregulation of ERK1/2 and PI3K was found in DEN-treated rats. Treatment with AgNPs reversed the expression of TLR4, ERK1/2, and PI3K. Altogether, these results showed that AgNPs have therapeutic potential against DEN-induced toxicity in a male rat model.

Keywords: Diethyl nitrosamine, pulmonary toxicity, Silver nanoparticles, Western blot

Effect of Heavy Metals on the Proximate Meat Quality in Cirrhinus mrigala Collected from Warsak Dam, Pakistan

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The aim of the present study was to investigates health risk hazards heavy metals in various organs (Gills, Liver, Intestine, Skin and Muscles) of *Cirrhinus mrigala* and its impacts on the meat quality collected from Warsak Dam Khyber Pakhtunkhwa, Pakistan. The selected heavy metals were (Cadmium, Zinc, Nickel, Copper and Lead) respectively. The data was analyzed by using SPSS software for the analysis of heavy metals and meat quality data. The heavy metals distribution was found variable in different organs of the fish samples. Results indicate that there was a variation among the concentration of heavy metals in various organs of the *C. mrigala* collected from Warsak Dam. Maximum concentration of heavy metals was detected in the Gills of C. mrigala as compared to other organs such as Liver, Intestine, Skin and Muscles respectively. The current results reviled that the warsak Dam is badly affected by the accumulation of heavy metals through various pathways. The fish *C. mrigala* comprising verities of heavy metals which transferred to human being by consumption. The study also highlights the detrimental effects of heavy metals pollution on C. mrigala and its impacts on meat quality. These finding underscore the urgent need for the effective pollution control measured and habitat restoration efforts to safeguard the health and sustainability of aquatic ecosystems and their inhabitants including *C. mrigala*.

Keywords: Heavy metals, Cirrhinus mrigala, Water quality, Warsak Dam, Concentration

Mutational analysis of Monkeypox virus P2L-KPNA2 binding interface and identification of small molecule inhibitors to rescue the host immune system

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Mpox, a re-emerging zoonotic disease caused by the Mpox virus (MPXV), has become an increasing global health concern due to recurrent outbreaks and the emergence of tecovirimat-resistant strains. The viral P2L protein plays a central role in immune evasion by suppressing host antiviral responses through inhibition of karyopherin-α2 (KPNA2)-mediated nuclear import of interferon regulatory factor-3 (IRF3). In this study, a comprehensive comparative and structural analysis of P2L protein variants was conducted using the reference sequence NP_536451.1 to investigate the functional consequences of newly emerged mutations. Multiple sequence alignment identified significant amino acid substitutions across 13 isolates, including recurrent multi-mutation variants (P11S, E25D, A55T, D88N, D112F, and D123G). Computational stability analyses indicated that several substitutions, particularly P11S, E25D, D88N, and D112F, reduce protein stability, while structural modeling revealed notable conformational alterations and changes in transmembrane topology. Molecular docking analyses demonstrated that selected mutant variants, especially P11S, E25D, and D88N, exhibited enhanced binding affinity toward KPNA2 through increased hydrogen-bond and salt-bridge interactions, suggesting a potential gain-of-function mechanism in viral immune evasion. Furthermore, structure-based virtual screening and molecular dynamics simulations identified two promising lead compounds (CID: 73062 and CID: 10494) that formed stable complexes with both wild-type and mutant P2L proteins. Collectively, these findings provide mechanistic insight into mutation-driven modulation of P2L function and establish a computational framework for the development of next-generation antiviral strategies targeting evolving MPXV variants.

Keywords. Mpox virus, KPNA2, Immune evasion, Virtual Screening, Molecular Dynamics Simulations

Species Richness and Abundance of Lepidoptera (Butterflies) in Tehsil Charbagh of District Swat

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Butterflies are one of the most beautiful and fine-looking creatures, act as ecological indicators of a healthy ecosystem. They are important pollinators and bio-indicators of climate change. The present study was the first attempt to uncover the butterfly fauna of various ecological habitats in the tehsil Charbagh of district Swat, Khyber Pakhtunkhwa, Pakistan. After an active collection of 7 months from March to September 2022, a total number of 517 individual butterflies were collected with the help of hand sweep nets from 3 distinct ecological habitats, including mountains, gardens, and open fields. Morphological identification revealed 23 different species belonging to 21 genera and 5 families. Peak butterfly collection was recorded in the month of May due to moderate temperatures and optimum humidity. In July, the study observed a notable decline in butterflies' abundance due to high temperature and humidity. Family *Pieridae* was the most abundant family in the area, followed by *Nymphalidae*. Species such as *Pieris daplidice* (16.3%) and *Pieris rapae* (19.1%) were the most prevalent species in the area. Butterfly fauna was highly abundant in Gardens and open fields due to the abundance of nectar-producing flowers and host plants for caterpillars, while the lowest diversity was observed in the mountains due to harsh environmental conditions unfavorable to butterflies. The activities of butterflies were significantly higher on sunny days as compared to cloudy days. Our findings highlight the importance of habitat conditions and climatic factors in influencing butterfly populations and provide critical insights for conservation strategies in the region.

Keywords: Entomology, Lepidoptera, Abundance, Diversity, Swat

Assessment of bacterial and protozoal contamination in drinking water and its public health implications in district Hangu, Pakistan

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Safe drinking water is essential for human health, yet microbial contamination remains a major public health concern in many developing regions. The present

study was conducted to evaluate the presence of bacterial and protozoal agents in the drinking water of District Hangu. A total of 60 water samples were collected from different sources and initially analyzed for physicochemical parameters. Subsequently, samples were processed for microbial contamination using the membrane filtration method. Gram staining and biochemical tests were employed for bacterial identification, while direct microscopy was used for protozoal detection. The bacteriological analysis revealed the presence of *E. coli* (n=8; 13.33%), *Bacillus* (n=6; 10%), *Shigella* (n=5; 8.33%), *Enterobacter* (n=4; 6.67%), *Staphylococcus aureus* (n=4; 6.67%), *Klebsiella* (n=2; 3.33%), *Citrobacter* (n=2; 3.33%), *Proteus* (n=2; 3.33%), *Pseudomonas* (n=2; 3.33%), and *Salmonella* (n=1; 1.67%). Protozoal analysis further revealed the presence of cysts of *Entamoeba histolytica* and *Giardia lamblia*, indicating fecal contamination of drinking water sources. *Entamoeba histolytica* cysts were more frequently observed, particularly in urban water samples, likely due to inadequate sanitation, leakage in water supply systems, and improper waste disposal. In contrast, *Giardia lamblia* was detected at a much lower frequency, suggesting lower prevalence or reduced environmental persistence under local conditions. The presence of these protozoa is of significant public health concern, as they are associated with gastrointestinal infections such as amoebiasis and giardiasis. The study concludes that majority drinking water sources in District Hangu are contaminated with both bacterial and protozoal pathogens and do not meet the recommended safety standards of the WHO, emphasizing the urgent need for improved water treatment, sanitation, and regular monitoring to ensure safe drinking water.

Keywords: Bacteria, Protozoa, *Shigella*, *Enterobacter*, *Entamoeba histolytica*, *Giardia*, *Staphylococcus aureus*, *Klebsiella*, Chlorination, WHO World Health Organization

New Species and a New Record of Genus Elathous (Elateridae: Dendrometrinae) from Pakistan

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The current study described the taxonomy, biogeography and dissection of the new click beetles belongs to order Coleoptera, family Elateridae with the aim empathetic of its anatomy and morphological perspective. Click beetles are identified by the specific clicking mechanism, which helps them flying into the air when sited on their back. Persevered specimens were wisely dissected using standard entomological techniques to distinctly visable external as well as internal the reproductive organ like (Male aedeagus, Pygophore, paramers) were observed and identified. Dissections of Click beetles is most important for identication of insect, morphology, reproductive, taxonomical features and it form important part of entomological laboratortical study. The present study has

been described the new addition of click beetle *Elathous sindhensis* sp.n. (Coleoptera: Elateridae) from Sindh province of Pakistan is described. Photographs of adult, through the manual bar scale as well as with Charge-coupled device (CCD) Sterozoom Microscope of Meiji (3.5x to 90 x) often (7x-45x). Contradictory with table, key with Nearctic and Palearctic areas of *Elathous* species and scattering map of various localities of Sindh Pakistan. This study give the novelty, new record of Genus *Elathous*.

Prevalence and Diversity of Haemoparasites in Domestic Chickens (Gallus gallus domesticus) of Dir Lower, Pakistan: Baseline Data for Climate-Responsive Poultry Health Management

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Domestic chickens (*Gallus gallus domesticus*) are vital to food security and rural economies in Pakistan, yet vector-borne haemoparasites pose an emerging threat that may intensify under climate-driven shifts in vector ecology. This study provides the first systematic assessment of haemoparasite prevalence and diversity in backyard poultry across eight localities in Tehsil Lal Qilla, District Dir Lower. A total of 134 chickens were sampled during spring and summer seasons. Thin blood smears were prepared, fixed in absolute methanol, stained with Giemsa, and examined microscopically. An overall haemoparasite prevalence of 32.09% (43/134) was documented. Five parasite genera were identified: *Plasmodium* spp. (10.45%) was most prevalent, followed by *Aegyptianella* spp. (7.46%), *Anaplasma* spp. (6.72%), *Haemoproteus* spp. (3.73%), and *Leukocytozoon* spp. (3.73%). No statistically significant difference in prevalence was detected between male (31.48%) and female (32.5%) birds ($\chi^2 = 2.211$, df = 1, p = 0.690), nor between spring (30%) and summer (33.78%) seasons ($\chi^2 = 0.218$, df = 1, p = 0.641). Although seasonal variation was not pronounced within the study period, the diversity of vector-borne genera highlights the susceptibility of free-range poultry to climate-mediated changes in vector abundance. Rising temperatures may extend transmission windows, elevating disease risks for smallholder poultry systems. These baseline findings underscore the need for continued surveillance and molecular characterization of avian haemoparasites to inform climate-adaptive poultry health strategies.

Keywords: *Gallus gallus domesticus*, Haemoparasite Prevalence, *Plasmodium*, Seasonal Variation, Climate Change, Dir Lower.

PCR based prevalence of Hepatozoon canis in the DNA of ticks infesting dogs in two provinces of Pakistan

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Hepatozoon canis is an apicomplexan protozoan parasite that is transmitted to canids and by hard ticks belonging to *Rhipicephalus* and *Amblyomma* genera. As the dogs are common companion and pet animals throughout the world, they are more exposed to the vectors and vector borne diseases. The present study was aimed to report the molecular prevalence of *Hepatozoon canis* in the DNA of *Rhipicephalus sanguineus* (n = 88) and *Hyalomma anatolicum* (n = 16) that were infesting dogs in two provinces of Pakistan. Genetic diversity of the parasite and risk factors associated with the infection were also determined. A total of 104 ticks were collected from six districts: Dera Ghazi Khan (47%), Upper Dir (n = 10), Swat (n = 10), Buner (n = 13), (n = 13) and Kohat (n =11). Overall, PCR amplified a 460-520 base pair amplicon from the 18S Peshawar rRNA gene of *Hepatozoon canis* in 18 out of 104 (17%) screened ticks. Specifically, 19% of *Hyalomma anatolicum* and 17% of *Rhipicephalus sanguineus* were infected. Subsequent Sanger sequencing followed by the BLAST analysis confirmed the presence of the parasite. Partial 18S rRNA gene based phylogenetic analysis revealed that sequences generated in this study were genetically diverse and they were clustered with the 18S rRNA gene sequences reported from various animals in Hungry, Iran, Venezuela, Romania, Spain, Italy India, Israel, Taiwan and Czech Republic. Prevalence of *Hepatozoon canis* was not limited to a particular tick species or their sex, feeding or developmental stages. Parasite prevalence also varied non significantly between the breed of the host dogs. In conclusion, we are reporting the high prevalence of *Hepatozoon canis* in *Rhipicephalus sanguineus* and *Hyalomma anatolicum* infesting dogs in two provinces of Pakistan confirming the endemic nature of this parasitic infection. This study will add to the existing information regarding *Hepatozoon canis* prevalence in hard ticks from Pakistan and will assist in planning and implementation of canine vector borne disease control in Pakistan.

Molecular Insights into Drug Repurposing and Drug Target Proteins Identification for Yersinia enterocolitica

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Infectious diseases caused by bacteria, such as *Yersinia enterocolitica*, pose significant global health challenges. *Y. enterocolitica*, a Gram-negative bacterium, is known for causing gastrointestinal diseases in humans. The objective of this study was to identify potential drug targets to combat *Yersinia enterocolitica* infection. Using subtractive genomics, pan-genome analysis was performed on 23 complete genomes of *Y. enterocolitica*, resulting in 3025 core genes. Using VacSol, 2267 non-host homologous proteins were identified, and 29 essential proteins were identified using the Database of Essential Genes (DEG). Moreover, from these essential genes, nine cytoplasmic proteins were acquired based on topology, modeled, and analyzed for their druggability using Swiss Model, AutoDock Vina, and Galaxy Refine tools. Furthermore, these proteins were docked with 30 fluoroquinolone compounds, which revealed several promising interactions. Notably, the Two-component system response regulator OmpR showed strong interactions with ciprofloxacin, whereas the large ribosomal subunit protein uL13 demonstrated significant binding with nalidixic acid. Phosphoheptose isomerase and protein-export protein SecB exhibited potential with norfloxacin and ciprofloxacin, respectively. These findings indicated that these proteins are viable targets for drug development against *Y. enterocolitica*. The increasing antibiotic resistance of *Y. enterocolitica* highlights the urgent need for novel therapeutic strategies, making these insights crucial for future drug development.

Keywords: *Yersinia enterocolitica*; Subtractive genomics; Pan-genome analysis; Drug targets; Fluoroquinolone compounds; Protein-protein interactions; Antibiotic resistance

Epidemiological patterns of dengue NS-1 (nonstructural) protein and associated manifestations of the infection in the Peshawar Region of Pakistan

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Dengue Virus is arthropod born virus belonging to family *Flaviviridae*. This virus can lead to various clinical manifestations like mild flu, fever, sever life threatening complications like dengue shock syndrome and dengue hemorrhygic fever. This study was conducted in the serology lab of Hayatabad Medical Complex Peshawar for the detection of dengue virus NS1 protein. The patients with clinical signs and symptoms of dengue fever like illness, presented to the outpatient’s department or admitted in the dengue isolated ward from August to October 2022, were sampled. Total of 799 samples were collected from suspected dengue cases. The data obtained included gender, clinical manifestations, area, and admission date. Among patients, 66.1% were male and 33.9% were female. 82.6% of the patients had body aches, 1.3% had bleeding, 1.4% had skin rashes and all patients had fever and 88.4% had NS1 positive. This study concluded that males and people of age 30 are at higher risk of dengue. Fever and body aches are the most common symptoms in dengue patients. Moreover, there were three deaths reported with low platelet count. This study provides insight into the prevalence of DenV infection in the population of Peshawar, Pakistan. Strict measures and intervention strategies are needed to halt the spread of DenV in Pakistan.

Keywords: Dengue; Body aches; Bleeding; Skin Rashes; Dengue Hemorrhagic Fever

Epidemiology and Molecular Characterization of Leihmaniasis in Khyber Pakhtunkhwa, Pakistan

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The present study was conducted to investigate the prevalence of *Cutaneous leishmaniasis* in different are areas of Khyber Pakhtunkhwa. Prevalence was studied area-wise (Urban or rural), Month wise, gender wise and ethnicity wise from January 2016 to December 2019. Result shows that prevalence was higher in Rural regions as compared to urban regions except 2013 and 2018 where the prevalence was slightly higher in urban regions as compared to rural regions. But the chi-square test shows that p-values are non-significant at 0.05 level of significance. The percent prevalence in urban areas was 11.28, 6.04, 10.7, 14.33, 16.03, 11.03 and 1.79 during the year 2013, 2014, 2015, 2016, 2017, 2018 and 2019 respectively. While in rural areas the prevalence percentages were 10, 11.11, 17.97, 17.5, 9.55 and 6.68 during the above-mentioned years respectively. The difference between the prevalence in rural and urban regions was not significantly different. Results that display month wise prevalence of Cutaneous leishmaniasis was different in months of year from 2013 to 2019. In year 2013 it was significantly high in the month of April (20.26%) and lowest in months of July (0%) and August (0%). In 2014, 2016 and 2017 the month wise prevalence presented non-significant p-values. While significant results were recorded during the years 2013, 2015, 2018 and 2019 for month wise prevalence. During these years the prevalence was high in months of April, May and August. In the present study age-wise prevalence of Cutaneous leishmaniasis was also investigated which shows that the incidence was higher in the age groups 1-15 years and 16-30 years. But the results were non-significant in years 2013 and 2014. The prevalence was significantly high in age groups 16-30 from 2016-19. Gender wise prevalence was also evaluated during the present study. The result shows that prevalence was significantly high in male as compared to female during the years 2014, 2016 and 2019. The prevalence of Cutaneous leishmaniasis recorded from 2013-2019 were 10.58%, 9.22%, 13.07%, 16.29%, 16.73%, 10% and 5.28% respectively. The p-value was 0.0000 which highly significant. Ethnicity wise (population) wise prevalence of Cutaneous leishmaniasis showed high prevalence in Afghan refugees (15.20%) as compared to local population (11.48%). The p-value was 0.0131. The over-all month wise prevalence shows highest prevalence in the month of March (18.28%) and lowest prevalence (4.54%) in the month of August. P-value was highly significant. Over all age wise prevalence exhibited highest percentage (18.21%) in age group 16-30 and lowest prevalence (5.86%) in age group above 60 with p-value of 0.0000 which is highly significant. The overall gender wise prevalence was also assessed with a high prevalence (14.02%) and low prevalence (9.07%) in female. The p-value was highly significant (0.0000) at 0.05 level of significance. Samples were randomly collected from the leishmanial infected patients which included smears on filter paper, smears on slides, tissue biopsy. The samples were cultured on NNN media, half number showed growth and half were either contaminated or negative. Samples, from confirmed *Cutaneous leishmaniasis* patients, were selected randomly to conduct comparison of sensitivity, and specificity of various diagnostic assays like culture microscopy kDNA PCR, ribosomal gene PCR and ITS 1 PCR.

Freshwater Gastropod Diversity in District Swat, Pakistan: A Baseline Study and Climate Change Perspectives

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The diversity of freshwater gastropods is a hallmark of aquatic health and was previously unreported in District Swat, Khyber Pakhtunkhwa, Pakistan prior to this study. Field sampling and systematic collection of snail specimens from spring water, the River Swat and rice fields across six tehsils (n = 1,135) produced the first record of freshwater gastropod fauna in the Swat area. Conchological analysis identified three genera of gastropods: *Physa acuta* (54.18%); *Indoplanorbis exustus* (26.43%); and *Bellamya bengalensis* (19.38%), representing families Physidae, Planorbidae and Viviparidae, respectively. The calculated diversity indices for freshwater gastropods were statistically significant according to the Shannon-Wiener (H = 1.001) and Simpson's (D = 0.599) metrics, thus establishing an important baseline for global biodiversity. Species richness was found to have maximum values in tehsils with water temperatures of 27–28 degrees C which correlates with established maximum thermal tolerances for freshwater gastropods of 15–31 degrees C. Climate change impacts are accelerating within the Hindu Kush-Himalayan (HKH) region, including glaciers retreating, altered monsoon seasons, and increasing temperatures of freshwater bodies; therefore, the value of this biodiversity baseline requires immediate attention. Future studies should focus on: (i) long-term monitoring of overall gastropod community shifts due to climate change; (ii) altitudinal distribution modeling throughout a 600–6,000 m elevation gradient throughout Swat; (iii) understanding interactions between parasites and climate because of the potential for I. exustus to serve as a vector for schistosomiasis; and (iv) utilizing RCP projections to develop species distribution models to predict future trends of biodiversity in the watersheds of KPK. Emphasis should also be placed on incorporating freshwater gastropod diversity into climate-adaptive conservation planning for mountain river ecosystems within Pakistan.

Keywords: Diversity, Invertebrates, Freshwater Snails, Gastropods, Mollusca, Climate change, Pakistan

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Heavy-metal induced physiological changes and tissue loading in Jhelum River catfish (Wallago attu)

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Heavy-metal inputs to freshwater rivers are a persistent threat to aquatic biodiversity and can compromise the health and market value of edible fish,

particularly in regions where industrial, municipal, and agricultural discharges are poorly controlled. This study evaluated spatial differences in metal bioaccumulation and associated multi-biomarker responses in the Asian catfish Wallago attu from the Jhelum River (Punjab, Pakistan). Fish were collected from February through April 2024 from an upstream reference area and a downstream section impacted by anthropogenic inputs (N=30; 15 fish per site). Ten elements (Zn, Cd, Pb, Cr, Co, Ni, Mn, Fe, Cu, and As) were quantified in the gills, liver, and muscle, alongside hematological indices, serum biochemistry, and tissue biochemical/oxidative-stress endpoints (including ROS, lipid peroxidation, and antioxidant/enzymatic biomarkers). Site comparisons were supported by inferential statistics and multivariate analyses (Pearson correlations and PCA). Metals were detected in all tissues, with consistently higher concentrations downstream and a clear tissue distribution trend (gills>liver>muscle); Fe and Cu dominated the overall metal burden. Downstream fish exhibited stronger toxicological signatures, including elevated oxidative stress, altered tissue enzymatic profiles (e.g., increased transaminases/phosphatases and reduced AChE activity), hypoproteinemia patterns in tissues and serum, increased bilirubin, dyslipidemia, and higher nitrogenous wastes. Hematological shifts indicative of physiological stress were also evident downstream (reduced erythrocyte-related indices and lymphocyte metrics, with increased leukocyte-related indices). Overall, the integrated biomarker and bioaccumulation indicate greater contaminant pressure and health impairment in downstream W. attu, supporting the need for stricter effluent control and routine monitoring to protect riverine ecosystem integrity and fish safety.

Assessment of Heavy Metals and Essential Elements Bioaccumulation and Toxicities in Labeo gonius from Khushab Lake (Pakistan): A Multi-Marker Approach

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The continuous increase in the concentration of heavy metals in bodies of water due to human activity poses a serious threat to the sustainability and overall health of the ecosystem. This study investigated the essential and toxic elements bioaccumulation in various tissues (gills, muscles, liver, and brain) of the Minor carp (*Labeo gonius)* from two sampling sites of highly polluted Khushab Lake, a tributary of River Jhelum (in the Pakistan’s Punjab province), along with a less exposed reference site (Chashma Lake-less polluted site). The impacts of pollution on the health profile of the fish were assessed using multiple biomarkers for analysis. The maximum accumulation of essential and toxic elements was reported in liver tissues, followed by gills. Copper (Cu) and iron (Fe) were the highest accumulated essential elements in liver and gills tissues, while magnesium (Mg) and calcium (Ca) were the highest accumulated in muscles and brain tissues. Hematological and serum biochemical profile of *L. gonius* showed intense abnormalities at Khushab Lake as compared to reference site. Histopathological investigations indicated severe alterations in the liver tissues along with significant changes in other tissues. Principal component analysis (PCA) showed strong correlations among essential and toxic elements in various tissues of fish. The results revealed that aquatic life in Lake Khushab is at the greatest risk to its health. Therefore, mitigating measures should be adapted by key stakeholders including environmental protection agency (ensure disposal of pre-treated wastes and effluents) and fisheries department (limnological assessment and stocking of fish at regular intervals).

Ecological distribution and morphological characterization of ladybird beetles in the Tehsil Khwaza Khela

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Millions of species of organisms are present on the Earth. But ladybirds are one of the beautiful and fine-looking creatures on the surface of the earth. Ladybird beetles are the most important group of insects that belong to the family Coccinelinae of the order Coleoptera. The purpose of the study was to find out the diversity, ecological distribution, role, and morphological identification of ladybirds in Khwaza Khela, Swat, Pakistan. The collection was carried out in four months from April to July 2023. The specimens were collected from different habitats like open fields, gardens, and grasslands. The collected specimens were preserved and identified with the help of taxonomic keys, online websites, and available literature. A total of 300 specimens were collected, preserved, and identified. Identification revealed that the collected specimens belonged to two subfamilies (Subfamily Coccinelinae and Chilocorinae) of the family Coccinellidae in the area. The subfamily Coccinelinae shows the highest number of individuals collected, followed by the subfamily Chilocorinae. The maximum number of specimens was collected in May and June, while the minimum number of specimens was collected in April and July. Phytophagous ladybirds were not found in the area during collection. Ladybirds were highly active in sunny weather, while less active in cloudy weather, and almost absent during the rain. Ladybird beetles are very beneficial because they are predators of soft-bodied insects like aphids and play a key role in pest control. The ecological conditions were favorable for ladybirds as they are found in sufficient amounts in tehsil Khwaza Khela, Swat.

Keywords: Entomology, Coccinelinae, Coleoptera, Ladybird beetle, Diversity

Occurrence and diversity of ectoparasites and endoparasites in Anthropoides virgo in southern Khyber Pakhtunkhwa, Pakistan

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This study examined ectoparasite and endoparasite diversity and occurrence in *Anthropoides virgo* (demoiselle crane) across southern Khyber Pakhtunkhwa, Pakistan. Of 323 birds examined, 19 (5.88%) harbored ectoparasites and 4 (1%) had endoparasites. Infestation peaked in April (10.53%), followed by June (7.14%) and March (5.56%), with lower rates in February (4.08%), May (3.77%), and July (3.70%). Rural areas showed the highest infestation (7.84%) versus urban (5.26%) and semi-urban (3.19%). Juveniles had slightly higher infestation (6.20%) and infection (2%) than adults (5.67% and 1%). District-wise, Lakki Marwat recorded higher infestation (6.33%) than Bannu (4.65%). Local hotspots included Baharat, Bannu (20%), Mira Khel (11.1%), and Ismail Khel (10.0%), while several sites had no parasites. Females showed higher infestation (2.04%) and infection (1.02%) than males (1.57% and 0.79%). Among endoparasites, *Capillaria* spp. was most prevalent (68.42% of infected birds; 13/19), followed by *Eimeria* spp. (42.11%; 8/19) and Coccidia spp. (36.84%; 7/19), corresponding to overall prevalence rates of 4.02%, 2.48%, and 2.17%, respectively. Among ectoparasites, *Colpocephalum* spp. dominated (83.33%; 5/6), while *Dermanyssus gallinae* and other mites accounted for 16.67% (1/6). Relative to total sample size, *D. gallinae* prevalence was 1.55% and other mites 0.31%. Although overall parasite prevalence in *A. virgo* was relatively low compared to domestic birds, the diversity of parasites suggests this species may act as a reservoir for transmission. Seasonal peaks, rural habitats, and juvenile age were key risk factors, highlighting the need for ongoing monitoring, ecological management, and preventive measures for crane conservation.

Keywords: *Anthropoides virgo*; ectoparasites; endoparasites; prevalence

Formulation and evaluation of Artemisia essential oils-based Nano emulsions against malarial vector mosquitoes (*Culex pipiens*)

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The present study describes a new herbal synergistic formulation, consists of nano-emulsions prepared from the essential oils of four local *Artemisia* species, i.e. *Artemisia scoparia (AS)* and *Artemisia absinthium (AB), Artemisia annua (AN)* and *Artemisia vulgaris (AV)* growing in Dir Upper, KP, Pakistan. The present formulation having toxic effects against larval stages of malarial vector *mosquito "Culex pipiens".* The possible toxicity against malarial vector is due to presence of potent chemical constituents in the leaf's essential oils of the selected medicinal plant species. The essential oil (EO) from the fresh leaves of each plant were obtained through hydro-distillation using Clevenger type apparatus. Their oil in water nano-emulsions were prepared by combining each EO with olive oil (in 70:30 ratio) to form the disperse phase and homogenizing it at 4000 rpm for six minutes with continuous phase having Tween-80, polyethylene glycol and Bovine serum albumin (1% each) in distilled water. The emulsion was sonicated for 45 minutes at room temperature to achieve nano dispersion with final concentration of 100 ppm. Emulsion microscopy suggested with a mean droplet size of all nano emulsions between from 20-35 nm with no flocculation, even distribution and spherical morphologies. The thick dark interface was observed around the oil droplets due to the combination of EO, olive oil and standard protein. The Nano emulsions were stable for one month as no change in the droplet sizes and distributions was observed after keeping in refrigerator at ambient temperature. The mosquitocidal activities of these nano emulsions were determined against *Culex pipiens* larva. The results suggested that all the nanoemulsions were extremely potent as 50 % mortality was achieved within 1 hour of incubation while 100 % mortality was observed within 12 hours of incubation for the Nano emulsions. Additionally, the cells viability tests against NIH 3T3 fibroblasts cells suggested that the tested dose of 2-4 % from 100 ppm concentration of each sample was safe as they did not reduce the cell viability in Alamar blue assay. In conclusion, this embodiment describes a successful innovation of nanoemulsion from plant essential oils and their implementing in killing the *Culex pipiens* larva at commercial scale.

Keywords: Artemisia essential oils (EOs); Nanoemulsions (NEs); *Culex pipiens* larva, Larvicidal effects, GC-MS analysis.

Climate Change-Driven Flood Disaster of 2025 in District Buner, Pakistan: Impacts on Humans, Wildlife, Agriculture, Infrastructure, and Local Livelihoods

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The current study was conducted on the recent floods disaster in August 2025 of district Buner Pakistan related to climate change, cloud burst and its impacts on human, wildlife, livestock, agriculture and local business. The data was collected from different sources, like questionnaires, wildlife departments, Agriculture department and livestock departments. During the survey we have reported different effects of climate changes on livestock, Wildlife, Agriculture and local business. The most effected tehsils were Gadezi (Bishuny, Malakpur, Qadar Nagar, Pirbaba, Bhaty Kaly, Bhai Kaly, Sultanwas, Ghaze khany), Tehsil Daggar (Kot Gokand, Bar Gokand, Kuz Gokand, Bagra, Topdara, Hisar Daggar), and Tehsil Gagra (Daggar hospital, Sunigram, Takhtaband, Kalpani, Bajkata, Kulyary, Matwani). We have reported 252 deaths, 290 injured, 55890 acre agriculture land effected, 4405 Livestock perished, 180 house damaged, 980 shops/ markets damaged, 15 mosques damaged, and 250 vehicles damaged.

Keywords: Disaster, Climate change, cloud burst, district Buner, Pakistan

Fish diversity of Tanda Dam, district Kohat, Khyber Pakhtunkhwa

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The present investigation was conducted to assess the ichthyofaunal biodiversity of Tanda Dam, a significant freshwater reservoir located in Kohat District, Khyber Pakhtunkhwa Province, Pakistan. Fish sampling was carried out monthly from December 2023 to June 2024, covering both winter and summer seasons. A total of four freshwater fish species were identified and taxonomically confirmed: *Cyprinus carpio*, *Mastacembelus armatus*, *Sperata sarwari*, and *Ictalurus punctatus*. These species belong to three distinct orders—Cypriniformes, Synbranchiformes, and Siluriformes—encompassing four families (Cyprinidae, Mastacembelidae, Bagridae, and Ictaluridae) and four genera (*Cyprinus*, *Mastacembelus*, *Sperata*, and *Ictalurus*). Specifically, *C. carpio* belongs to the family Cyprinidae, *M. armatus* to Mastacembelidae, *S. sarwari* to Bagridae, and *I. punctatus* to Ictaluridae. The presence of both indigenous and exotic species reflects the ecological status and potential anthropogenic influences on the dam. This study provides the first baseline data on the fish diversity of Tanda Dam, offering valuable information for future research in systematic ichthyology, fisheries stock assessment, and conservation planning.

Keywords: Fish biodiversity; Tanda Dam; *Cyprinus carpio; Sperata Sarwari;* Fisheries management

Diversity and impacts of horsefly flies (Family Tabanidae) infestation in family bovidae (cows and buffaloes) of tehsil Matta district Swat

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Tabanidae are blood feeding insects that pose a significant threat to cattle population worldwide. These blood sucking insects are known for their painful bites, which can cause discomfort, blood loss, and potential disease transmission. Tabanids are a major concern for cattle former with their presence causing significant economic losses and animal suffering. They are vary in size from small to large. Tabanids also play role in plant pollination and act as a vector of different disease. The current study was conducted in Matta tehsil which is located in swat district of Khyber pakhtunkhawa, Pakistan. To find the diversity and impact of tabanids on cattle population different habitats were selected such as grassland, wetlands and planted areas. The study duration was four months from april 2024 to august 2024. The impact of tabanids on cattle health, productivity, and wellbeing was assessed through surveys and field observation. Result showed that tabanids caused significant blood loss, reduced milk production, and increased stress in cattle. For identification total number of n=345 specimens were collected from different members of cows and buffalo.

For specimens collection and identification different keys and available literature was used. Specimens were collected using sweep nets and hand collection. Stereo microscope were used for identification. A total number of n=5 species of tabanids were identified which are *Tabanus bromius*, *Tabanus sudeticus, Tabanus autumnalis, Hybomitra solsitialis and Haematopota pluvailis*. Tabanids are highly destructive insects and it's the need of time to control their infestation. Effective tabanids control stretegies were investigated, including the use of insecticides, repellent and trapping methods. Result showed that a combination of these methods was most effective in reducing horsefly population and to reduce their impact on cattle.

Keywords: Entomology, Housefly, Infestation

Molecular identification of the fish fauna of Jhallar Algad river, North Waziristan, Khyber Pakhtunkhwa, Pakistan

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The term *ichthyodiversity* encompasses the variety of fish species, as well as the genetic diversity (alleles or genotypes) within fish populations, the diversity of life forms within fish communities, and the range of species inhabiting different aquatic ecosystems. Fish diversity contributes significantly to the global economy, ecosystem balance, food security, and the production of various by-products. The present study aimed to assess the ichthyodiversity of the Jhallar Algad River, located in the Mir Ali region of North Waziristan, Khyber Pakhtunkhwa, Pakistan. This investigation represents the first scientific documentation of fish fauna in this river and provides essential baseline data on the status of its fish assemblages. Fish sampling was conducted from February to July 2022. Data analysis revealed the presence of six freshwater fish species: *Crossocheilus diplocheilus*, *Crossocheilus latius*, *Schizothorax plagiostomus*, *Garra gotyla*, *Barilius vagra*, and *Labeo rohita*. All recorded species belong to a single order, Cypriniformes, and a single family, Cyprinidae, representing five genera: *Crossocheilus*, *Schizothorax*, *Garra*, *Barilius*, and *Labeo*. The most dominant species was *Crossocheilus diplocheilus*, with 17 specimens collected. The observed diversity and abundance of fish fauna in the study area indicate that the river provides favorable ecological conditions for fish propagation. Furthermore, it is recommended that the fisheries department introduce additional cultured species to enhance the aquatic biodiversity and productivity of this water body.

Keywords: Ichthyodiversity; Cyprinidae; *Crossocheilus diplocheilus;* Jhallar Algad River; Baseline survey

Impact of marble mining on wildlife species of district Buner, Pakistan

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Mining is important for human sustenance and an essential division in the national economy. On the other hand, its effects on the environment and biodiversity cannot be taking too lightly. Which are effective to attract government's attention. Environment and wildlife are focus to the harmful impacts of mining and its associated activities. The current study was conducted on the impact of marble mining on wildlife of district Buner Khyber Pakhtunkhwa Pakistan from September 2022 to August 2023. During our study large number of species of mammals and birds are found extinct due to marble mining, blasting and transportation of stones from mining sites to the factories. District Buner consists of about total number of 566 mining sites which increase the economic importance of district Buner. On the other hand it has also negative impact on wildlife and habitat of wild animals of the study area. In the study area we identified 11 species of mammals and 11 species of birds with different order and families. The mammalian species are Wild cat, Porcupine, Pangolin, Monkey, Jackal, Hyena, Hare, Grey goral, Fox, Bat, and Common leopard. While the bird's species are Crow, Crane, Quail, Chukar, Owl, Sparrow, Pigeon, Bulbul, Myna, Kingfisher, See-see partridge. Key words: mining, wildlife, blasting, impact, habitat destruction, Buner.

Knowledge, attitude and practices of people about ticks and tick-borne diseases in Kohat

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Ticks and tick-borne diseases (TBDs) are major veterinary and public health threats in Pakistan, including Kohat, Khyber Pakhtunkhwa, where no prior knowledge, attitude, and practices (KAP) study had been conducted. This questionnaire-based KAP study assessed 238 livestock farms/households across Gumbat and Kohat tehsils. Most respondents (92.8%) could differentiate ticks from other arthropods. Although rural respondents, many of whom were illiterate or had low education, recognized that ticks transmit diseases, most could not name specific TBDs. Educated urban respondents identified anaplasmosis? (Note: the text says "anemia" but likely means anaplasmosis or babesiosis; I retain "babesiosis" as given) and babesiosis. Tick infestation awareness was high (89%), while 10.9% (non-livestock keepers) were unaware. Traditional herbal methods were commonly used; 80.6% applied acaricides, and 32.3% practiced manual tick removal—a risky practice for zoonotic pathogen transmission, reflecting illiteracy. Other methods included towels, pesticide sprays, and washing animal bodies. These inappropriate practices may increase TBD transmission and economic losses. Findings highlight critical knowledge gaps and the need for educational interventions to promote effective tick prevention and control among farmers.

Keywords: Ticks. TBDs, livestock, control measures of TBDs

Larvicidal activity of Silybum marianum and Melia azedarach against mosquito and Bactrocera dorsalis larvae

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The growing resistance to synthetic insecticides and rising environmental concerns highlight the need for eco-friendly pest control alternatives. This study evaluated the larvicidal potential of leaf extracts from Silybum marianum and Melia azedarach against Culex mosquitoes (disease vectors) and Bactrocera dorsalis (an agricultural pest). Ethanolic extracts were prepared via cold maceration for 15 days, concentrated, and tested on larval stages (25 larvae per replicate) at concentrations of 25–400 mg/ml following WHO guidelines. Mortality was recorded over 72 hours and analyzed using Probit analysis. Both extracts showed significant, concentration- and time-dependent activity. Against Culex, S. marianum (400 mg/ml) caused 65.78% mortality after 72 hours, and M. azedarach (200 mg/ml) caused 58.67%. Both were more potent against B. dorsalis: S. marianum achieved 100% mortality at 400 mg/ml and 81.33% at 50 mg/ml ($p < 0.05$). M. azedarach reached 82.67% mortality at 400 mg/ml ($p = 0.0035$). Microscopy revealed larval deformities and midgut damage. This first comparative study demonstrates strong larvicidal activity of both plants, with S. marianum showing exceptional efficacy against B. dorsalis, supporting its potential as a novel botanical insecticide for integrated pest management.

Keywords: Silybum marianum; Melia azedarach; Larvicidal activity; Culex mosquitoes; Bactrocera dorsalis

Fish Parasite as Bioindicator of Water Quality of River Swat and River Kabul

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Evident from the previous studies the medium level of stress among inland species of Pakistan with potential to experience even greater attributed to water pollution this study was planned to report for the first time parasite population in selected fish species of economic importance. Previously having no studies in this area of river Kabul and River Swat in District Charsadda therefore this study was conducted. This study describes physicochemical parameters of water and find parasitic load on three economically significant fish species of both rivers. Between May to August 2024, 82 fish samples of three fish species namely, *Cyprinus carpio, Cirrhinus marigala* and *Tor putitora* along with water samples for physicochemical parameters were collected from ten sites of river Kabul and river Swat. Ecto-parasites were examined on the skin and fins with magnifying glass. Wet mount method was used for the examination of internal parasites. The water quality parameters such as temperature, pH, Alkalinity, Electrical conductivity, Turbidity, Total hardness, Total dissolved solids and Chloride were recorded through established standardized methods.

Parasitological examination of fish samples showed seven species of parasites out of total 82 fish samples. Among the isolated parasites five were ectoparasite and two were endoparasites in which three were crustacean (*Lernaea barnimiana, Argulus, and unidentified*) two were protozoans (*Apiosoma, Parasagitta elegans*) one was Monogenea (*Gyrodactylus*) and one was Cestode (*Diphyllobotrium latum*). The physicochemical parameters at site of infestation of river Kabul, temperature (27.4 °c), pH (8.2), Alkalinity mg/l (96.51), E. Conductivity µS/cm(286), Turbidity NTU(8.0), Total hardness mg/l (140.29), Total dissolved solid mg/l (179), Chloride mg/l (13.30) and river Swat was Tem(23.6), pH (7.64), Alkalinity (40.6), E. Conductivity (117), Turbidity (5), T. Hardness (104.77), T. Dissolved solid (76.05), water Neutral pH, Turbidity and total hardness are linked to parasites prevalence. Our data demonstrate that the physicochemical parameters of water exacerbated due to water pollution have significant effect on the occurrence of parasites. The prevalence level if increases further, this may affect the fishes to such a level that fish may not be fit for edible purposes.

In vitro efficacy of selected plant extracts against fish bacteria Escherichia coli and Aeromonas hydrophila

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Plant and animal culture in fresh, brackish, and marine waters is known as aquaculture. Aquaculture has grown rapidly over the past decade and is now recognized as a major food-producing industry. Bacterial species such as *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* can cause diseases in fish and pose a risk of human infection through consumption or handling of contaminated fish. The rise of antimicrobial resistance has increased research interest in medicinal plants and their phytochemical compounds. This study aimed to assess the efficacy of *Azadirachta indica* (neem) and *Citrus limon* (lemon) leaf extracts against *Aeromonas hydrophila* and *E. coli* strains. The agar well diffusion method was used to evaluate antimicrobial activity on Mueller-Hinton Agar (MHA). Ethanolic extracts of neem and lemon at four concentrations (10, 20, 30, and 40 mg/mL) were tested. Ciprofloxacin (0.25 µg) served as a control. Experiments were performed in triplicates. Data analysis revealed remarkable inhibitory activity of both ethanolic extracts. *A. indica* extract at 10 mg/mL produced the largest zone of inhibition against *E. coli* (6.44 mm), while against *A. hydrophila*, the largest zone (7.03 mm) was observed at 20 mg/mL. *C. limon* extract at 30 mg/mL exhibited the largest zone against *E. coli* (8.17 mm), and against *A. hydrophila*, the highest activity (8.06 mm) was also at 30 mg/mL. The study concludes that lemon and neem leaf extracts have considerable inhibitory activity against fish-associated bacteria. Further studies are needed to identify and purify the bioactive compounds and to test these plants against other fish bacterial species.

Keywords: Aeromonas hydrophila; Escherichia. Coli; Lemon; Neem

Prevalence and molecular detection of Hepatozoon spp. In horses from Dir lower, Dir upper (Khyber Pakhtunkhwa) and Islamabad Pakistan

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Equine blood parasite infections are becoming a major issue for animal health and productivity in Pakistan. One of these parasites is *Hepatozoon* spp., infects horses (*Equus Caballus*) and is primarily transmitted through ingestion of infected ticks. This study was conducted to establish the prevalence of *Hepatozoon* spp. in horses from Lower Dir, Upper Dir, and Islamabad, as well as to investigate potential risk factors. Blood samples were drawn from horses during the winter and preserved in EDTA tubes. DNA was extracted from the samples, and *Hepatozoon* spp. were detected using polymerase chain reaction (PCR) targeting the 18S rRNA gene. The PCR results were examined using gel electrophoresis, and samples with clear bands were refereed positive. The overall infection prevalence was 27%. Geographically, Upper Dir has the highest prevalence (46%), followed by Islamabad (29%), and Lower Dir (19%). In breed wise prevalence Thoroughbred horses showed the highest prevalence (100%), followed by Baluchi (39%), Punjabi (36%), Desi and mixed breeds (29%), Arabian (18%), Morna (12%), and Hindki (8%). There was no significant relationship detected between infection and age (P = 0.3), gender (P = 0.6), or treatment history (P = 0.8). There were no ectoparasites detected during sampling. Although some infected horses showed clinical indications such as lethargy, decreased job performance, and mild anaemia, many cases were asymptomatic. These findings demonstrate the existence and spread of *Hepatozoon* spp. in equine populations throughout the study areas. The higher incidence in rural locations may be due to increased tick exposure and limited veterinarian treatment. The study emphasises the importance of effective tick control measures, enhanced management practices, and routine screening. Further molecular research is needed to accurately identify species and gain a better understanding of the epidemiology of *Hepatozoon* infections in Pakistan.

Keywords: *Hepatozoon, Hemoparasites,* Equine, prevalence, Pakistan, Molecular detection

Evaluation of Concurrent Malaria and Dengue Infection among Febrile Patients at Khyber Teaching Hospital, Peshawar, Pakistan

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Among the highest prevalent mosquito-borne illnesses, especially in tropical and subtropical areas are dengue and malaria. These illnesses frequently have similar symptoms, including fatigue, fever, and thrombocytopenia, making diagnosis more difficult. Evaluating the prevalence, demographic trends, and clinical features of dengue, malaria, and their co-infection among feverish patients in

Peshawar, Pakistan, was the purpose of this study. A cross-sectional study with 500 febrile patients was carried out between May and October 2024, while NS1 antigen rapid tests were used to detect dengue, peripheral blood smear microscopy was used to diagnose malaria. Using standardized questionnaires, demographic and clinical data were gathered and evaluated. One-way ANOVA and independent samples t-tests were used to determine statistical significance. Results show that out of the 500 patients, 248 (49.6%) tested positive for malaria, 208 (41.6%) for dengue, and 8 (1.6%) for co-infection. Notably, dengue infection was significantly more prevalent in males (68.8%), and co-infections were more prevalent in outdoor workers and those from lower socioeconomic backgrounds. Malaria was prevalent in younger people, but dengue was more common in slightly older people. Co-infected patients had lower hemoglobin and platelet levels than single-infection cases, and they had more severe clinical symptoms, such as hemorrhagic manifestations and systematic complications. Despite being rare, co-infections have a significant clinical impact. The greater prevalence of malaria in the area compared to dengue is highlighted by this study, which also emphasizes the significance of focused public health initiatives and integrated diagnostic approaches, especially for those at risk in endemic areas.

Keywords: Dengue Fever, Malaria, Co-infection, Vector-Borne Diseases, Epidemiology, Tropical Medicine, Aedes aegypti and Anopheles spp., Climate Change and Disease Ecology

Tricomposite Polymer Dressings: Fabrication and Characterization for Wound Healing

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Microbial infections complicate the treatment of surface wounds and continue to pose a significant health and clinical complication. Infection of a wound with bacteria delays healing, and may even cause potentially fatal systemic infections, as well as increasing the issue of antibiotic resistance. The World Health Organization states that each year millions of individuals have infected wounds and the number of deaths caused by burn-related complications is in the thousands, which is preventable. Due to the emergence of multi-drug-resistant bacteria, there is an increasing demand for in-depth and more efficient wound work. To address this, We have designed polymer-based wound dressings that contain Clindamycin, a known antimicrobial agent. The polymer structure forms a porous and supportive structure that assists in moisture retention, oxygen diffusion, as well as tissue healing. When Clindamycin was incorporated into this structure, the drug was slowly released, which gave the dressings a long duration of protection against a vast range of bacteria and decreased the likelihood of developing side effects. Experimentally, the dressings have proven to inhibit the growth of gram-negative and gram-positive bacteria. Furthermore, The scaffolds have exhibited better *In-Vitro* and *In-Vivo* results. Clindamycin

based polymer dressings are a potential combination of infection control and tissue support in the management of complex and infected wounds. This approach could provide a safer and better alternative to traditional wound treatments, particularly during the times of increasing antibiotic resistance.

Keywords: Wound healing, Silver sulfadiazine, Polymer dressing, Antimicrobial activity, Biocompatibility

Association of single nucleotide polymorphism, rs8099917 in IL28B gene with the incidence of ischemic stroke in subjects enrolled from Multan: a case control study

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Ischemic stroke is a complex pathological condition in which blockage of the cerebral blood vessels results in tissue necrosis and focal neuronal deficits that results in brain damage. Ischemic stroke is a multifactorial condition that is shaped by interactions between vascular dysfunction, environmental influences and inherited susceptibility. *IL-28B*, also known as *IFN-λ3*, encodes interleukin-28, a cytokine that is produced by immune cells and plays an important role in host defense by regulating both innate and adaptive immune responses against chronic viral infections. Role of *IL-28B* and their genetic variations is not confirmed as it is rarely studied in stroke-related inflammation. This study aimed to investigate the genotype and allelic frequency at the single-nucleotide polymorphism (SNP) rs8099917 in *IL-28B* and to correlate them with the incidence of ischemic stroke. A total of 156 clinically confirmed ischemic stroke patients and 156 age and sex-matched controls were enrolled from Multan District between September 2024 and February 2025. Genotyping of the rs8099917 was performed by using the Tetra-primer Amplification Refractory Mutation System-polymerase chain reaction (T-ARMS PCR). Analysis of results revealed a significant association of the polymorphic “TT” genotype at rs8099917 in *IL-28B* with the incidence of ischemic stroke. Cases had more frequent polymorphic “T” allele while wild “G “ allele was more frequent in controls indicate that T allele might be a genetic risk factor for ischemic stroke. Additionally, smoking was found associated with an increased risk of the disease, whereas age and sex showed no significant association with stroke incidence. In conclusion, we are reporting that genotypes at rs8099917 in the *IL-28B* may play an important role in the susceptibility to ischemic stroke in the studied Pakistani population.

Molecular and phylogenetic analysis of family Phasianidae fauna of Totalai game reserve, district Buner, Pakistan

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The present study focuses on the molecular and phylogenetic characterization of avian species belonging to the family *Phasianidae* in Totalai Game Reserve, District Buner, Khyber Pakhtunkhwa, Pakistan, conducted over a period from September 2024 to September 2025. During this investigation, four species were identified: *Francolinus francolinus* (Black Partridge; Brisson, 1766), *Francolinus pondicerianus* (Grey Partridge; Gmelin, 1789), *Alectoris chukar* (Chukar; Gray, 1830), and *Coturnix japonica* (Japanese Quail; Temminck & Schlegel, 1848). These species represent four distinct genera within the family *Phasianidae*. Molecular analysis was performed using genomic DNA extracted from blood samples with the Qiagen Mini Kit, following standard protocols. Target gene regions, including mitochondrial *COI* (Cytochrome c Oxidase Subunit I) and *12S rRNA*, were amplified via polymerase chain reaction (PCR) using specific primers. The amplified products were subjected to Sanger sequencing at Macrogen Inc., Seoul, South Korea. The resulting chromatogram files (ab1) were processed and analyzed using BLAST for sequence identification and similarity assessment. Subsequently, phylogenetic analyses were conducted using MEGA version 12, where multiple sequence alignments and phylogenetic tree construction were performed to infer evolutionary relationships among the studied taxa and their closely related species. The findings highlight the significance of integrating molecular tools with traditional taxonomy for accurate species identification and evolutionary interpretation of avian fauna in the Totalai Game Reserve. This study contributes to a better understanding of the genetic diversity and phylogenetic relationships within *Phasianidae* species in the region.

Keywords: *Phasianidae*, phylogenetic analysis, *COI* gene, *12S rRNA*, Sanger sequencing, avian diversity, Pakistan

Quantification of heavy metals accumulation in Cirrhinus mrigala of Baran dam and fish farms of district Bannu

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Fish bio accumulate metals like Ni Cu, Cr and Cd after exposures. The Heavy metals concentration were examined in *Cirrhinus mrigala*, soil, water collected from Baran dam and fish farms in district Bannu Khyber pakthunkhwa Pakistan. Basic aims of the research to quantify Ni, Cu, Cr and Cd in muscles, gills and skin of *Cirrhinus mrigala* by using atomic absorption spectrophotometer (AAS). The average mean of Ni in gills, skin and muscles of *Cirrhinus mrigala* was (Ni > Cr > Cd > Cu). Mean value of Cu and Cr was (0.000.0018ppm), (0.151± 0.0156ppm) in Baran dam. And mean average of baran dam were in order (Ni> Cu> Cd> Cr>. The overall mean average in order (Cu > Ni> Cd> Cu) in the soil of Baran Dam. The highest were of Ni 0.182 ±0.280 mg/L and the lowest Cu 0.079 ± 0.0093 mg/L in fish farms of district Bannu (Ni > Cd > Cu > Cr, 0.182 mg/L, 0.079 mg/L, 0.086 mg/L). In fish farms the quantification in gills of *Cirrhinus mrigala* were 0.233mg/L, 0.088mg/L, and 0.08 mg/L (Ni > Cd > Cu >

Cr) similarly in fish farms the quantification in skin of *Cirrhinus mrigala* were 0.141mg/L, 0.08 mg/L, and 0.095 mg/L (Ni > Cd > Cu > Cr). In muscles the quantification were 0.172mg/L, 0.09 mg/L, and 0.064 mg/L (Ni > Cd > Cu > Cr). In fish farm of Bannu the quantity of Ni (5.909 ±0.0099), Cu (2.485 ±0.0070), Cd (0.252 ±0.0019) and Cr (0.00) mg/L (Ni >Cu > Cd > Cr). In Conclusion Heavy metals quantification were greater in fish farms as compare to Baran dam because of water source runoff, feeds, management and soil source. Physicochemical parameters in Baran Dam recorded a pH of 7.73, electrical conductivity of 712 µS/cm, and TDS of 356 ppm, while in fish farms, the values were 7.91, 789 µS/cm, and 294 ppm, respectively. The average temperature was 26°C in Baran Dam and 24°C in fish farms. All parameters were suitable for fish culture and. In conclusion all the heavy metals quantify were within permissible limits (WHO).

Keywords: Baran dam, Fish form, Heavy metal

PCR-based amplification of 18S rRNA gene of Theileria ovis in blood samples of dogs collected from Charsadda district, Khyber Pakhtunkhwa

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Dogs (*Canis lupus familiaris*) are one of the most widely kept companion animals and are frequently affected by ectoparasite infestations due to their close contact with humans and environmental exposure. This makes them potential reservoirs and sentinel hosts for various vector-borne pathogens including those of zoonotic concern. *Theileria ovis* is primarily a tick-borne hemo-protozoan parasite of small ruminants; however, dogs may become infected indirectly through exposure to infected ticks that commonly infest multiple host species. The present study investigated the molecular prevalence and the phylogenetic associations of *Theileria ovis* in dogs that were enrolled from District Charsadda in Khyber Pakhtunkhwa. A total of 165 blood samples along with epidemiological data were collected during December 2024 until May 2025 and they were screened for the presence of *Theileria ovis* by using PCR that targeted the 18S rRNA gene of this parasite. Out of 165 blood samples 25 (15.15%) yielded a ~520 bp amplicon specific for *Theileria ovis*. Sanger sequencing and BLAST analysis confirmed the presence of T*heileria* ovis. Phylogenetic analysis showed that the sequences obtained in this study clustered closely with the 18S rRNA gene of *Theileria ovis* previously reported from ruminants in Pakistan and India. No significant association was observed between *Theileria ovis* infection and risk factors such as age (P = 1), sex (P = 0.3), breed (P = 0.7) or the presence of ticks on screened dogs (P = 0.4). In conclusion, for the first time, we are reporting a relatively high molecular prevalence (15.15%) of *Theileria ovis* in dogs from Charsadda district in Khyber Pakhtunkhwa Pakistan. These findings underscore the need for broader epidemiological surveys across the country to better understand the distribution, genetic diversity, transmission

dynamics and potential zoonotic implications of *Theileria ovis* in canine populations.

Molecular detection of avian Haemosporidian parasites in Dir upper Khyber Pakhtunkhwa Pakistan

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The class Aves is a diverse group of vertebrates having worldwide distribution. More than 20000 species have been reported globally. Birds are prone to many types of blood parasites and some of them can have considerable effect on their population. In the mountainous areas of northern Pakistan, very little work has been done on these parasites. The current study provides an insight to the molecular detection of avian Heamatozoon parasite in district Dir Upper KP Pakistan. A total of (n=136) birds representing, 23 families and 31 species were sampled from two different sampling sites, Barawal (n=114) and Sheringal (n=22) from January 2024 to March 2025. Blood was collected from brachial veins, making a thin blood smear, and collect blood in EDTA tube then blood smear was observed under microscope, and the collected blood was used for the DNA extraction. The DNA extracted was used for PCR (polymerase chain reaction). A general 5.9% (n=8) parasite prevalence was observed in bird species. Three different blood parasites (*Plasmodium, Heamoproteous*, and *Leucocytozoon speies*) were observed through the PCR analysis. The *Plasmodium* species (n=2), *Heamoproteous* species (n=3) and *Leucocytozoon* species (n=3) were observed positive. The risk factor shows the body weight of *Plasmodium (*p= 0.001) highly significant, *Heamoproteou* (p=0.486) non-significant and *Leucocytozoon* (p=0.06) was also non-significant. This is the first molecular study on blood parasites of birds from Dir (Upper), KP Pakistan.

Keywords: Molecular detection, Avain heamosporidian, Dir upper

Exploring the species diversity, distribution, host plant, seasonal occurrence and morphology of ladybird beetle (Coleoptera: Coccinellidae) fauna of Malakand region Pakistan

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The current study was conducted on the Ladybird beetle fauna of Malakand region Pakistan during April 2022 to April 2023. Malakand region consists of seven districts namely, District Buner, Swat, Shangla, Malakand, Lower Dir, Upper Dir and Chitral. During the study thirty-six different species of Ladybird beetle under twenty-two genera and three subfamilies (Subfamily Chilocorinae, Subfamily Coccinellinae and Subfamily Epilachninae) were collected. These species are *Brumoides suturalis* (Fabricius, 1789), *Exochomus nigripennsis* (Erichson, 1843), *Coccinella septempunctata* (Linnaeus, 1758), *Coccinella transversalis* (Fabricius, 1781), *Adalia bipunctata* (Linnaeus, 1758), *Adalia*

tetraspilota (Hope, 1831) *Cocinella novemnotata* (Herbst, 1793)*, Menochilus sexmaculatus* (Fabricius, 1781)*, Propylea dissecta* (Mulsant, 1850)*, Propylea japonica* (Thunberg, 1781)*, Coelophora bissellata* (Mulsant, 1850)*, Angelies cardoni* (Weise, 1900)*, Illies confusa* (Mulsant, 1850)*, Illeis cincta*(Fabricius, 1798) *Coccinella undecimpunctata* (Linnaeus, 1758)*, Oenopia conglobata* (Linnaeus, 1758)*, Oenopia mimica*(Weise, 1902)*, Oenopia sauzuti* (Mulsant, 1866)*, Aiolocaria hexaspilota* (Hope, 1831)*, Hippodamia (Adonia) variegate* (Goeze, 1777)*, Calvia punctata* (Mulsant, 1850)*, Psyllobora bisoctonotata* (Muslant, 1850)*, Psyllobora vigintduopunctata*(Linnaeus, 1758)*, Psyllobora vigintioctopunctata* (Linnaeus, 1758)*, Lemnia bisselata* (Mulsant, 1850)*, Harmonia dimidiata* (Fabricius, 1781)*, Harmonia axyridis* (Pallas, 1773)*, Cryptogonus nepalensis*(Bielawski, 1972)*, Henosepilachna vigintioctopunctata* (Fabricius, 1775)*, Henosepilachna septima* (Dieke, 1947)*, Afidentula manderstjernae* (Mulsant)*, Parexochomus nigromaculatus* (Goeze, 1777)*, Propylea quattuordecimpunctata* (Linnaeus, 1758)*, Subcoccinella vigintiquatuorpunctata* (Linnaeus, 1758)*, Hippodamia tredecimpunctata* (Linnaeus, 1758) and *Micraspis inops* (Mulsants, 1866). During the research, we also studied the distribution, diversity, morphology, seasonal occurrence, morphology and host plants of Ladybird beetle fauna of Malakand region. During the sample collection the *Coccinella septempunctata* (Linnaeus, 1758) and *Harmonia demediata* were found abundant and *Illies confusa* and *Calvia punctata* were found rare in the study area. We added this species *Illies confusa* (Mulsant, 1850), for the first time to NCBI database. These species *Parexochomus nigromaculatus* (Goeze, 1777), *Illeis cincta* (Fabricius, 1798), *Angelies cardoni* (Weise, 1900), *Propylea japonica* (Thunberg, 1781)*, Propylea quattuordecimpunctata*(Linnaeus,1758)*,Psyllobora vigintduopunctata*(Linnaeus, 1758), *Subcoccinella vigintiquatuorpunctata* (Linnaeus, 1758)*, Hippodamia tredecimpunctata* (Linnaeus, 1758) and *Micraspis inops* (Mulsants, 1866) are new to our study area.

Keywords: New record, Checklist, Ladybird Beetle, fauna, Coleoptera, Malakand, Pakistan

Quantifying the Ecological Impacts of Dam Construction on Freshwater Biodiversity and Water Quality

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Dam construction is a major anthropogenic disturbance to freshwater ecosystems, yet before–after ecological assessments remain limited in data-sparse regions. This study evaluated changes in fish biodiversity and water quality following the construction of Zameer Gul Dam in semi-arid area of Pakistan using a dataset spanning pre-dam (2010–2013) and post-dam (2024–

2025) periods. Fish assemblages and key water quality parameters were compared between pre- and post-dam periods across upstream, dam-site, and downstream zones using standardized sampling and statistical analyses. Fish species richness declined from 26 to 17 species (34.6% reduction) following dam construction. The endangered Tor putitora showed a significant decline (83.2%, $p<0.001$), while several native species were no longer detected post-dam. Commercially important cyprinids decreased by 68–76% ($p<0.001$), whereas the exotic Oreochromis niloticus emerged downstream. Water quality also deteriorated, with dissolved oxygen decreasing by 45.6% and temperature significantly increasing downstream ($p<0.001$). No significant changes we re-observed at upstream reference sites ($p > 0.05$). The construction of Zameer Gul Dam was associated with substantial declines in fish biodiversity and deterioration of water quality downstream. These findings highlight the ecological risks associated with dam development in semi-arid regions and underscore the need to incorporate environmental flow management and biodiversity conservation measures into water resource planning.

Keywords: Before–after assessment; dam impact; fish biodiversity; *Tor putitora*; water quality; habitat fragmentation; Pakistan

Sustainable mitigation of heavy metal toxicity in Alpine and Riparian habitats: Integrating Bioremediation into Zoological conservation frameworks

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As the global climate undergoes unprecedented shifts, the mobilization and bioavailability of heavy metals such as lead (Pb), cadmium (Cd), and mercury (Hg) have emerged as critical threats to zoological biodiversity. Rising temperatures and erratic precipitation patterns, characteristic of the current climate change era, significantly alter soil chemistry and hydrologic cycles, often leaching sequestered pollutants into sensitive ecosystems. This explores the synergy between environmental biotechnology and zoological preservation, specifically focusing on how bioremediation serves as a vital tool for protecting animal health in increasingly contaminated landscapes. Heavy metals are notorious for their persistence and ability to bio accumulate through trophic levels. In the context of the rugged, high-altitude ecosystems of Khyber Pakhtunkhwa, where the 1st International Conference on "Recent Advances in Zoology" is hosted, these pollutants pose a direct risk to indigenous fauna. Toxic accumulation leads to severe physiological stress, impaired reproductive success, and genetic mutations in both aquatic and terrestrial species. Traditional physicochemical remediation methods are often prohibitively expensive and ecologically disruptive. Consequently, this study advocates for the deployment of indigenous microbial and plant-based bioremediation agents. These biological catalysts effectively sequester, stabilize, or detoxify metallic contaminants without further damaging the habitat. By utilizing hyper-accumulating microbes and phytoremediation techniques, we can proactively reduce the toxicological burden on local wildlife populations already stressed by shifting climates. Integrating these "green" technologies into conservation management allows for the restoration of essential habitats, ensuring that the genetic integrity and population dynamics of vulnerable taxa are preserved. This research emphasizes that bioremediation is not merely a waste management solution but a fundamental conservation strategy. Aligning with the core themes of this conference, it underscores the necessity of interdisciplinary approaches to safeguard the future of zoology against the compounding challenges of anthropogenic pollution and global climatic instability.

Climate Driven Shifts is Faunal Dynamics: Integrating Zoological Research for Conservation and Adaptation

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Climate Change is altering ecosystems at unprecedented rates, driving shifts in species distribution, phenology, behaviour, and physiology. These changes pose critical challenges for biodiversity conservation and require urgent integration of zoological research with climate science. This highlights the recent advancement in zoology which addresses the impacts of climate change on animal population, emphasizing adaptive mechanisms, emerging ecological interactions and evidences based on conservation strategies. A synthesis of current research was conducted across key zoological sub disciplines, including ecophysiology, behavioural ecology, molecular zoology, and conservation biology. Case studies were reviewed to assess climate induced range shifts, thermal tolerance plasticity, disease dynamics in wildlife and disruption of predator-prey, and pollinator network. Recent advance findings demonstrate that many species exhibit rapid behavioural and physiological adaptations to thermal stress, yet vulnerability remains high for habitat specialist and ectothermic fauna. Molecular tools are revealing cryptic genetic diversity crucial for resilience, while satellite tracking and AI based assessment of migratory and range shifts. Climate change is also amplifying zoonotic spill over risks through altered host vector interactions. Integrated approaches combining habitat restoration, assisted migration and climate smart protected areas show promise for mitigating biodiversity loss. Zoology in the climate change era demand interdisciplinary collaboration to predict, monitor, and manage faunal responses. Advancing genomic, ecological and technological tools will be essential to support adaptive conservation policy and safeguard animal biodiversity under future climate scenarios.

Keywords: Climate change, zoology, species adaptation, biodiversity conservation, thermal physiology, wildlife ecology, ecosystem resilience

Prevalence And Antibiotics Resistance Patterns Of Salmonella enterica Serovars Typhi And Paratyphi In Suspected Individuals At Hayatabad Medical Complex, Peshawar, Pakistan

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Typhoid and paratyphoid fevers remain pressing public health challenges in Pakistan, largely driven by poor sanitation, inadequate vaccination coverage, and the growing emergence of antibiotic-resistant *Salmonella enterica* strains. The descriptive cross-sectionsl study was conducted to determine the prevalence and antibiotic resistance profiles of *Salmonella* serovars Typhi and Paratyphi among patients clinically suspected of enteric fever at Hayatabad Medical Complex (HMC), Peshawar, Pakistan. A total of 246 blood samples were collected and processed using standard culture techniques alongside Widal testing and anti-microbial susceptibility testing using CLSI guidelines. The overall culture positivity rate was 39.8% (98/246). Of these isolates, 77.6% (76/98) were identified as *S. Typhi* and 22.4% (22/98) as *S. Paratyphi*. Antibiotic susceptibility testing revealed high levels of resistance: 41% (40/98) of isolates were multidrug-resistant (MDR), while 23% (23/98) were extensively drug-resistant (XDR), exhibiting resistance to fluoroquinolones and third-generation cephalosporins. Statistical analysis using the Chi-square test demonstrated significant associations ($p < 0.05$) between blood culture positivity and factors such as age, source of drinking water, and sanitation practices. The high prevalence of MDR and XDR strains poses serious challenges to effective treatment and necessitates urgent public health interventions.

Keywords: *Salmonella enterica*, Typhoid Fever, Antibiotic Resistance, MDR and XDR Strains, Hayatabad Medical Complex, Public Health Surveillance, Pakistan

Blood profile of individuals attending hematology laboratories in district Buner, Khyber Pakhtunkhwa, Pakistan

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Blood is a key component of the human being with numerous functions including transportation, immunity, temperature regulation, removal of wastes etc. Complete blood count (CBC) is a common blood test that gives information about five major parts of blood: three types of cells (RBCs, WBCs, and PLTs and two values (Hb and HCT). This study was conducted with the objective to explore the blood profile of individuals attending hematology laboratories in district Buner, KP, Pakistan from October 2022 to September 2023. A total of 96 (n = 96, male 35, and female 61) individual's data was collected during this study. This study comprising 36.46 % male, and 63.54 % female individuals. Data was collected in terms of height, weight, BMI and CBC of individuals.

BMI data was further divided into four categories (underweight, normal, overweight and obese). It was recorded that 10.42 % (10/96) of district Buner attending hematology labs were underweighted. Whereas 30.21 % (29/96) individuals belonged to overweight category of BMI. Moreover, 8.33 % (8/96) individuals of district Buner were classified as obese. Remaining 51.04 % (15/96) individuals were considered as normal based on categories of BMI. This study observed that 13.54% (13/96) individuals showed lower RBC and 2.08% (2/96) individuals showed higher RBC in comparison to standard values (4.0–5.4 × 10^6/µL). This study reported that 19.79% (19/96) of individuals showed higher WBC in comparison to standard values (4.0–10.8 × 10^3/µL). It was observed that 3.15 % (3/96) individuals showed lower PLT count and 11.48% (11/96) individuals showed higher PLT count in comparison to the standard values (150–400 × 10^3/µL). Further, 39.58 % (38/96) individuals displayed lower HGB and 47.91 % (46/96) HCT in comparison to the standard values (HGB 12.0–16.0 g/dL, and HCT 37.0–47.0%). There was fluctuation in CBC values of the individuals studied in this study in comparison to standard values. The ultimate reason for this fluctuation may be lack of proper exercise and awareness, nutritional deficiency, improper and outdated use of instruments, lack of expertise of user, and even poverty. It is suggested to carry out study covering large population to screen out the overall picture in terms of RBCs count in this region.

Climate Change and Insect Pest Dynamics: Adaptive Strategies for Sustainable Crop Protection

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Anthropogenic climate change is fundamentally altering the population dynamics of agricultural insect pests through shifts in temperature regimes, precipitation patterns, and atmospheric CO_2 concentrations. Current integrated pest management frameworks struggle to accommodate these rapid changes. This review synthesizes recent mechanistic research with particular attention to geographic context and practical management implications. Thermal physiology underlies most documented shifts in pest behavior and abundance. Laboratory-derived development models consistently underestimate generation times under field conditions where temperatures fluctuate. In *Helicoverpa armigera* populations across subtropical cotton systems, warming of 1.5°C is expected to advance peak densities by 10–18 days and permit an additional generation where late-season temperatures currently limit reproduction. Experimental studies on *Plutella xylostella* at 30–34°C demonstrate increased fecundity accompanied by reduced early-instar survival, illustrating physiological trade-offs essential for accurate population projections. These responses vary substantially among populations, making region-specific calibration necessary. Elevated CO_2 influences pest pressure indirectly by altering host plant

nutritional quality. Reduced foliar nitrogen in cereals and brassicas grown at 550 ppm causes compensatory feeding in several lepidopteran larvae. Effects on phloem-feeding insects remain inconsistent across studies. The combined influence of CO_2-driven plant chemistry, temperature-mediated herbivore physiology, and disrupted natural enemy synchrony represents a critical knowledge gap. Geographic range expansions, while real, require careful interpretation. *Tuta absoluta* established throughout suitable South Asian habitats within two years of its 2016 arrival, consistent with climate envelope predictions. Other species show more limited spread, constrained by host availability and biotic interactions beyond simple climatic suitability. Effective adaptation demands recalibrating phenological models at five-year intervals, screening biological control agents for thermal tolerance, and accounting for temperature effects on monitoring tool performance.

Keywords: biological control; climate-pest interactions; *Helicoverpa armigera*; integrated pest management; *Plutella xylostella*; *Tuta absoluta*

Epidemiological, Morphological and Molecular Characterization of Head Lice (Pediculus humanus capitis) based on Cox1 Gene Sequencing in District Dir Lower, Khyber Pakhtunkhwa Pakistan

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Head lice (*Pediculus humanus capitis*) infestations pose a significant public health concern, leading to itching, secondary infections, and psychological distress. These ectoparasites, transmitted through direct contact, exhibit genetic diversity across six phylogenetic clades. The present study aimed to investigate epidemiological, morphological and molecular characterization of head lice (*Pediculus humanus capitis*) based on Cox1 gene sequencing in District Dir Lower, Khyber Pakhtunkhwa Pakistan. Specimens were collected using specially designed anti-lice combs, and live lice were preserved in 70% ethanol for morphological and molecular analyses. A structured questionnaire was administrated to collect data on sociodemographic factors, hygiene practices, and infestation history. Morphological identification involved specimen clearing, mounting, and morphometric analysis using ImageJ software. DNA was extracted using the GeneJET kit, followed by PCR amplification of the Cox1 gene. PCR products were confirmed through agarose gel electrophoresis, purified, and sequenced. Sequence data were analyzed using Chromas and CLUSTALW, while phylogenetic relationships were inferred using the Maximum Likelihood method in MEGA X. Statistical analyses, including chi-square and t-tests, were performed to identify significant risk factors associated with infestation. A total of 500 individuals were examined, revealing an overall infestation prevalence of 11%. Infestation was more common in females than in males, though the difference was not statistically significant. Significant associations were observed between lice infestation and family size, parental

education, socioeconomic status, and rural residency. Personal hygiene habits such as hair length, bathing frequency, and sharing combs were also identified as significant risk factors. Clinical symptoms, particularly itching and allergic reactions, were strongly linked to infestation, while the use of anti-lice shampoo and seeking medical consultation were associated with lower risk. Morphometric analysis confirmed sexual dimorphism, with females being significantly larger than males in body length and width. Molecular characterization of the Cox1 gene identified notable interspecific variations across global populations. Phylogenetic analysis based on Cox1 gene sequences revealed that Pakistani head lice samples clustered into two distinct clades: Clade A and Clade C. Evolutionary divergence estimates ranged from 0.017 to 0.186, highlighting genetic diversity among *Pediculus humanus capitis* populations. This study identified sociodemographic and hygiene factors as key influences on head lice infestation. Morphometric analysis confirmed sexual dimorphism, with females being larger than males. Molecular characterization of the Cox1 gene revealed existence of two clades in Pakistan. These findings contribute to a better understanding of head lice biology and may aid in developing effective control strategies.

Identification of Therapeutic Targets and potential inhibitors against Streptococcus gordonii

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Streptococcus gordonii belongs to viridans group streptococci and is Gram-positive cocci. It is primarily involved in development of biofilms on tooth surfaces also known as dental plaque but can result in development of infectious endocarditis *i.e.* inflammation of inner lining of heart. As treatment of this disease is very expensive and various bacteria have shown resistance against various commonly used antibiotics, it is now important to find the novel therapeutic targets and drugs to cure and treat this disease. In this study, we predicted pan-genome of 11 strains of *Streptococcus gordonii* by using EDGAR web tool. A total of 1,255 core proteins were identified; later subtractive proteomics was used to extract set of 20 essential proteins for bacteria. Considering human as host, 15 of these proteins were considered as essential and non-host homologs and were subjected to virtual screening against ZINC drug like molecules library. Following virtual screening, the top 10 compounds for each protein were chosen based on highest binding affinity, docking Score and number of residue interactions. Since some of these targets have already been identified and validated in other organisms, our suggested approach speeds up the selection of *Streptococcus gordonii* putative proteins for broad-spectrum therapeutic development.

Keywords: *Streptococcus gordonii,* Infectious endocarditis, Pan-genomics, Subtractive proteomics

Smart Entomology: Integrating Insect Behavioral Ecology with AI-Driven Precision Pest Management

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Insect pest management has traditionally depended on scheduled insecticide applications that ignore pest behavior and uneven field distributions. Recent advances in insect behavioral ecology and machine learning now enable more targeted approaches. This review explores how understanding pest sensory biology can improve AI-based management systems. Behavioral research provides essential mechanistic insights. Herbivore-induced plant volatiles such as (E)-β-ocimene and methyl salicylate attract natural enemies while deterring pests. In whiteflies *Bemisia tabaci*, olfactory responses to host cues have been characterized well enough to guide trap optimization. Fall armyworm *Spodoptera frugiperda* shows temperature-dependent changes in pheromone tracking and oviposition that matter for predictive models. Without this behavioral foundation, detection tools often fail to enable effective control. Machine learning offers practical decision support. Convolutional neural networks achieve over 90% accuracy in identifying pest species from trap images, though performance drops when local variants are absent from training data. Random forest models integrating weather, crop stage, and trap counts have reduced insecticide use by 20–40% in field trials across South Asia and Africa without yield loss. Significant technical challenges remain. Trap placement errors as small as 15 meters can alter pest counts by over 30%, directly affecting model reliability. Sensor inconsistencies and irregular sampling create noise that requires careful data protocols. Progress depends on addressing three needs: standardized semiochemical formulations for regional pest populations, validated reinforcement learning frameworks for biocontrol, and movement data for non-lepidopteran species.

Keywords: *Bemisia tabaci*; herbivore-induced plant volatiles; machine learning; precision agriculture; semiochemicals; *Spodoptera frugiperda*; trap monitoring

A Microscopic Investigation of Avian Malaria and Other Haemoparasites in Motacillidae and Passeridae Birds from Dir (Upper), Khyber Pakhtunkhwa, Pakistan

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Birds are found everywhere, but they also carry many blood parasites. In the mountainous areas of northern Pakistan, very little work has been done on these parasites. This study was carried out to check the diversity and occurrence of blood parasites in two passerine families, Motacillidae and Passeridae, in Dir (Upper), Khyber Pakhtunkhwa, Pakistan. A total of 101 wild birds were captured and blood smears were prepared, stained with Giemsa, and examined under a microscope. Out of 101 birds, 12 (11.9%) were found infected with blood parasites. The Motacillidae family (N = 66) showed higher infection (13.6%) compared to the Passeridae family (N = 35, 8.6%). Three types of blood parasites were observed. *Haemoproteus* was the most common (6 birds), followed by *Plasmodium* (2 birds) and microfilariae (2 birds). Mixed infection with both *Haemoproteus* and *Plasmodium* was seen in 2 birds. The white wagtail (*Motacilla alba*, N = 51) was the most infected species, with 6 positive birds. Among sparrows (*Passer domesticus*, N = 22) had 3 infections. Season also influenced infection rates. Most infections (9 out of 12) were recorded in summer, while only 3 were recorded in winter. The intensity of infection ranged from low to high, with some birds showing very heavy parasitemia. No significant difference was found between summer and winter infections ($p = 0.454$). This is the first study on blood parasites of Motacillidae and Passeridae birds from Dir (Upper), Pakistan. It shows that these birds carry a range of parasites, with *Haemoproteus* being the most common. Further studies using molecular methods are needed to identify parasite species and to understand how these infections affect bird health in this region.

Keywords: Avian malaria, *Haemoproteus*, *Plasmodium*, Microfilariae, Motacillidae, Passeridae, Dir Upper

Denv-Serotypes Distribution and Management of Aede Mosquitoes through Synthetic Pyrethroids at Dengue Hotspots Dir Lower

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In Tehsil Adenzai, District Dir Lower Khyber Pakhtunkhwa this study thoroughly examines the epidemiological distribution of dengue virus infections particularly NS1-positive cases, and evaluates the insecticidal effectiveness of synthetic pyrethroids against the Aedes mosquito vector in 2024. Blood samples were taken from 87 conformed dengue patients who were admitted to nearby hospitals. Rapid NS1 antigen detection kits were used to test these samples to identify dengue virus and confirm its presence. To identify high-risk groups and geographic hotspots structured questionnaires and hospital records were used to collect patient demographic data, including age, gender, area, and occupation and in addition to clinical diagnosis. The results showed that adult males aged 20 to 59 were the most often affected with a significant concentration of cases in the Bahram Shilman and Ooch regions. DENV-1 and DENV-2 were the most common strains circulating in the area according to serotyping. Laboratory bioassays were performed on field-collected Aedes mosquitoes using six insecticides Allethrin, Cypermethrin, Resmethrin, Sumithrin, Tau-fluvalinate and Permethrin to assess the efficacy of mosquito control. Each insecticide knockdown impact and mortality rate was noted following exposure for 24, 48, 72, 92 and 120 hours respectively. With a 92% fatality rate, Cypermethrin outperformed the other tested insecticides followed by Resmethrin and Tau-fluvalinate. The lowest fatality rate was seen with permethrin indicating potential resistance or diminished efficacy in the local mosquito population According to the study's findings, preventing dengue transmission in endemic areas like Tehsil Adenzai requires an integrated strategy that includes ongoing epidemiological surveillance and the selective application of potent pesticides like Cypermethrin. The results give public health officials important information to enhance epidemic response plans in the area and optimize vector control methods.

Keywords: Dengue serotypes, mosquito mortality, insecticides, vector control, Dengue Virus

Microbial Management of American Foulbrood in Honey Bees, Apis Mellifera Colonies at District Dir Lower

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Paenibacillus larvae, the causative agent of American foulbrood (AFB), poses a significant threat to honeybee (*Apis mellifera*) colonies, leading to serious economic losses in apiculture due to colony collapse. This study was conducted from July 2024 to December 2024 to assess the seroprevalence of AFB and evaluate the efficacy of biological treatments against *P. larvae* in the Dir Lower district of Khyber Pakhtunkhwa, Pakistan. A total of 254 honeybee colony samples were collected from seven localities: Kamrani-Bandagai, Khall-Tormang, Bagh Dushkhail, Samarbagh-Khema, Zolam-Arang, Talash-Nasafa, and Timergara-Khongi. Laboratory analysis was carried out at the Apiculture Rearing Museum and Pathology Laboratory at the University of Agriculture, Peshawar. Pathogen cultures of *P. larvae* were obtained from the National Agricultural Research Centre (NARC), Islamabad. Out of the 254 colonies, 100 tested positive for AFB, yielding an overall prevalence of 39.4%. The highest seroprevalence was recorded in Khall-Tormang (55%), followed by Timergara-Khongi (44%) and Zolam-Arang and Talash-Nasafa (both 50%), indicating moderate-to-high infection levels. Kamrani-Bandagai (33.3%), Samarbagh-Khema (33.3%), and Bagh Dushkhail (25%) showed relatively lower infection rates, suggesting localized outbreaks. In addition to prevalence analysis, this study evaluated the efficacy of three biological control agents: probiotics (*Lactobacillus spp.*), antagonistic microorganisms (*Bacillus subtilis*), and entomopathogenic nematodes (EPNs; *Steinernema spp.*). Among the treatments, probiotics exhibited the highest efficacy, reducing *P. larvae* density by 80% within 12 hours. Antagonistic microorganisms achieved 75% reduction over the same period, while EPNs were most effective in reducing pest abundance (80%) but had relatively lower direct impact on pathogen density. These findings highlight the urgent need for localized disease management strategies and underscore the potential of probiotics and microbial antagonists as sustainable and effective alternatives to chemical treatments in controlling AFB. The integration of such biological agents into apicultural practices may enhance honeybee health and ensure long-term productivity in regions like Dir Lower.

Keyword: American Foulbrood, probiotics, antagonistic microorganisms, entomopathogenic nematodes, honeybee health, Dir Lower, Khyber Pakhtunkhwa

Diversity and Abundance of Odonata Fauna in Tehsil Wari, District Dir Upper, Khyber Pakhtunkhwa, Pakistan

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The Present research work was undertaken to explore the richness and relative abundance of Odonata fauna of the Tehsil Wari, district Dir Upper, Khyber Pakhtunkhwa, Pakistan. The fieldwork was conducted from May 2023 to September 2023 in various areas like Nehag Dara, Wari Town, Jelar Dara, Akhagram and Karo Dara. Specimens were collected through handmade aerial nets, male specimens were killed in killing jars containing ethyl acetate-soaked tissue papers, and females were released back into the field. The specimens were identified in the laboratory of the Department of Zoology under the stereomicroscope by following the taxonomic dichotomous key of the Odonata fauna of Pakistan. The survey yielded 160 specimens, of which 143 belonged to the suborder Anisoptera, and 17 belonged to Zygoptera. Dragonflies' specimens were identified into two families, six genera and ten species. Among recorded Odonata, the family Libellulidae appeared as the most dominant, represented by nine species, followed by Aeshnidae, represented by one species. Similarly, 17 specimens of damselflies were identified into two families, three genera and four species, in which the family Coenagrionidae was dominant, represented by three species, followed by Platycnemididae with one species. Details of the collected species and their status in the study area are provided.

Key words: Odonata, Anisoptera, Zygoptera, Dichotomous Keys,

Species Composition and Abundance of Anisoptera Fauna in Sheringal Valley, Dir Upper Khyber, Pakhtunkhwa, Pakistan

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The present study was conducted to explore the species composition and abundance of Anisoptera fauna of Sheringal valley, Dir Upper, Khyber Pakhtunkhwa, Pakistan. Field visits were carried out in the summer season from May to October, 2023, weekly in different localities such as Sheringal, Sawni, Shahoor, Gwaldi, Narkoon, Samang, Kandaw, Ganshal, AcharBala, Doon, and Doag Dara of the study area from 9:00 am to 4:00 pm in clear sunny conditions. Specimens were collected using a handmade aerial net. The fieldwork yielded a sum of 192 specimens, sorted into 38 female and 154 male specimens. Specimens were identified to the species and subspecies levels using dichotomous taxonomic keys for the Aniosptera fauna of Pakistan. The 154 male specimens were recognised into 11 species under 7 genera and 2 families. Families Libellulidae was declared as the most dominant, represented by 6 genera, 10 species, followed by Aeshnidae, represented single genus and species. Among the genera orthetrumwas declared as the most dominant genus represented by 5 species, followed by *Pantala*, *Crocothemis*, *Trithemis*, *Sympetrum*, *Brachythemis*, and *Anax*, each represented by a single species. Among the species, *Pantala flavescens* was declared as the most abundant and widespread species, comprising 26 specimens, while *Anax immaculifrons* was declared as the least abundant and rare species of the study area, represented by 3 specimens.

Key words: Odonata, Anisoptera, Libellulidae, Aeshnidae, Dichotomous Keys, Sheringal Valley

To Ascertain Species Composition and Abundance of Anisoptera Fauna in Timergara, District Dir Lower, Khyber Pakhtunkhwa, Pakistan

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The current research was undertaken to record the species composition and abundance of Anisoptera fauna of Timergara, Dir Lower, Khyber Pakhtunkhwa, Pakistan. The specimens were collected by handmade aerial nets. The study area was surveyed in clearly sunny conditions on a daily and weekly basis from 9:00 am to 4:00 pm from June 2024 to November 2024. Diverse habitats such as streams, riverbanks, rice paddies, and marshes, water channels in the crop fields, spring waters, storage water tanks and stagnant water were searched in Balamabat, Kandaroo, Walai Kandow, Shatai Dara, Khazana, Timergara, Haji Abad, Koto and Malakand. The specimens were identified in the laboratory of the Department of Basic and Applied Zoology, SBBU, under a stereomicroscope following the taxonomic literature of Fraser (1933-1936), Zia (2010) and Chaudhry (2010) to the species and subspecies level. The fieldwork yielded a sum of 146 male specimens and 23 female specimens. The male specimens were recognized into 03 families, 10 genera and 15 species. Among the families, Libellulidae was declared as the most dominant and widespread family represented by 8 genera and 13 species, followed by Gomphidae and Aeshnidae, each of which was represented by a single genus and a single species. Similarly, *Orthetrum* was declared as the most common genus of the study area, represented by 5 species and 80 specimens. The most abundant species was *Orthetrum pruinosum neglectum,* followed by *Orthetrum triangulare triangulare,* while *Anax immaculifrons* and *Onychogomphus bistrigatus* were declared as rare species of the area. The study area is a hotspot for various unexplored entomofauna, which require exploration.

Keywords: Entomofauna, Specimens, Stereoscope, Taxonomic literatures

Epidemiological Profile of Cutaneous Leishmaniasis and Related Risk Factors in Dir Upper Khyber Pakhtunkhwa, Pakistan

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CL or leishmaniasis is a skin infection caused by a minute parasite called Leishmania, which causes itch. The bite of an infected female sand-fly transmits it. The disease can affect animals, including human beings. In Pakistan, two kinds of Leishmania, that is, Leishmania major and Leishmania tropica, are known to cause CL. Prevalence of CL in different localities of District Upper Dir, Pakistan, and factors associated with the prevalent cases were studied. It was a confirmed case study conducted between June 2023 and February 2024 in six areas: Barawal, Sheringal, Dir, Kalkot, Thal, and Wari, in which a total of 1313 cases were confirmed. Prevalence was highest in Barawal (31.12%), and the lowest prevalence was found in Wari at 5.41%. The vast majority of the CL cases presented with dry type lesions, with 85.15%, and the face was observed to be the most common site for such sores in 40.77% of the cases. These were upper limb parts (39.91%), and 17.72% of the sites occurred at the lower limbs. The study further found some associated risk factors for CL. The provision of space control where the sandfly thrives, and elimination should be carried out. Rodent control in homes is essential, and policies should be implemented for wild and stray dogs since they contribute to the facilitation of CL. Briefly, this research provides valuable information regarding how prevalent CL is in District Upper Dir. It postulates that controlling sandflies, rodent management, and dog regulation may prevent this severe skin disease.

Key Words: Prevalence, Cutaneous leishmaniasis, vector-borne, Leishmania, phlebotomine, Leishmania tropica, Leishmania major, DIR Upper, Anthroponotic, Zoonotic, Lesion

Detailed Analysis of Malaria Epidemiology in District Dir Lower and Malaria-Endemic Union Council Bagh Dushkhail Talash, Dir Lower, Khyber Pakhtunkhwa, Pakistan

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Malaria remains a pressing public health concern in District Dir Lower, Pakistan. This study provides an in-depth analysis of the epidemiological patterns of malaria over the past year, examining seasonal trends, demographic vulnerabilities, geographical distribution, and risk factors associated with the disease. Data collected from August of the previous year to July of the current year reveals significant seasonal variation in malaria prevalence, with the highest rates occurring in July (23.27%) and the lowest in December (1.72%). Peaks in malaria cases were observed during the monsoon months of July and August, with a gradual decline towards the end of the year. The analysis indicates that the highest prevalence of malaria was among children aged 0-15 years (36.17%), while adults aged 31-45 years also showed a notable prevalence (25.58%). Prevalence rates among older adults (>45 years) were comparatively lower (18.01%). These findings highlight the greater vulnerability of younger age groups to malaria. Geographically, Tehsil Khall emerged as the area with the highest malaria prevalence (31.12%), followed by Tehsil Timergara (24.57%). Other tehsils such as Tehsil Balambat, Tehsil Lal Qilla, and Tehsil Adenzai showed lower prevalence rates. These geographical disparities suggest the need for targeted malaria control strategies in high-prevalence areas. Plasmodium vivax was identified as the predominant species, accounting for 85.15% of malaria cases, while mixed infections were less common (14.85%). The predominance of Plasmodium vivax highlights the necessity for species-specific control measures. Among pregnant women, 53.45% were infected with Plasmodium vivax, underscoring the need for targeted interventions for this vulnerable group. Case categorization revealed that the majority of cases were in individuals under 15 years with vivax malaria (40.78%), and persistent cases comprised 17.72% of the total. The presence of persistent cases indicates ongoing transmission and a need for sustained treatment and control efforts. Risk factor analysis identified several significant contributors to malaria transmission. Recent travel was reported by 76.94% of infected individuals, indicating its role as a major risk factor. Outdoor activities (72.33%) and the presence of domesticated animals (88.76%) were also associated with higher malaria prevalence. Additionally, rural residence and specific housing materials, such as stone/mud walls and wood ceilings, were linked to increased risk of infection. Overall, the findings of this study emphasize the complex nature of malaria epidemiology in District Dir Lower. The significant seasonal, geographical, and demographic patterns observed underscore the need for targeted control measures and enhanced public health interventions. Addressing identified risk factors and improving surveillance will be crucial for effective malaria management and reduction in the region.

Keywords: Malaria, epidemiology, risk factors

Effect of commercially available probiotics Lactobacillus rhomnosus GG on growth performance and body composition (proximate analysis) of Pangasius hypophtalmus

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The current study was conducted to investigate the impression of probiotics *Lactobacillus Rhomnosus* gg (PREPRO) regarding the growth performance and proximate analysis of Pangasius *hypophthalmus* (fingerling). *Pangasius hypophthalmus* is a famous emerging edible freshwater fish with a rich nutrient profile. The purpose of the study was to increase *Pangasius hypophthalmus* meat production as well as the quality of the meat. A total of 140 *Pangasius hypophthalmus* (fingerlings) were chosen, and they were stocked in glass

aquariums (n = 12 fish each) with four distinct diet groups: T1 (control), T2 (10^6 CFU/g), T3 (10^9 CFU/g of *L. rhamnosus),* and T4 (10^{12} CFU/g of *L. rhamnosus)* as a feed additive. Statistical analysis was done by implementing one-way analysis of variance (ANOVA), and Duncan's multiple range tests (DMRT) were used to identify significant differences within the treated groups. After the 60-day experimental trial, the meat quality of the experimental groups was considerably ($P < 0.05$) better than that of the control group. Best meat quality in terms of crude protein (47.81 ± 0.21) was observed in T3, and high Ash content, crude fat, and total moisture (15.40 ± 0.3, 39.10±0.05, 11.70 ±.10) in control as compared to other treatment groups. The probiotics *Lactobacillus Rhomnosus* gg (PREPRO) enhanced the meat quality and growth performance of *Pangasius hypophthalmus.*

Keywords: Probiotics, Meat Quality, Growth Performance

Comparative analysis of proximate composition and heavy metals (Fe & Zn) of commercially important wild & farm fish species meat of Dera Ismail Khan

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Fish is a nutritional powerhouse, depicting a peculiar array of macronutrients, and plays a pivotal role in the dietary requirements of human beings. This study was conducted to assess the proximate composition (crude protein, ash, fats, fiber, NFE, moisture, dry matter) and heavy metals concentration (Fe & Zn) in different fish species i.e *Labeo rohita, Wallago attu, Sperata sarwari, Cyprinus carpio, Bagarius bagarius* & *Rita rita* of the farm and wild fish. For this purpose, 27 samples were collected from different areas of the River Indus as well as from local fish markets in Dera Ismail Khan, KPK. According to the results of the current study, values of each parameter vary within & between species. However, it was observed that the content of crude protein, fats, ash, dry matter & fiber was greater in farm-raised samples. Significant difference ($P<0.05$) was observed between several proximate parameters and heavy metals bioaccumulation. In contrast, the concentration of moisture and NFE (nitrogen-free extract) was estimated to be maximum in wild-caught samples. In terms of heavy metal analysis, accumulation of iron was greater in muscle tissues of wild specimens, whereas zinc level was identified to be almost the same in both wild & cultured fish species. However, bioaccumulation of both elements was determined to be within permissible limits. Hence, it is not wide of the mark to conclude that farm-raised fish species are much better than wild ones from a nutritional standpoint. Subsequently, this area has deficient data about the proximate composition of fish, so current factual information can be considered as a solid foundation for the evaluation of additional advanced work in the future.

Keywords: Wild fish, Farm fish, Proximate composition, Heavy metals, Comparative analysis

Distribution of Monogeans (Gyrodactylus and Dactylugyrus in fish of Gomal Zam dam, district south Waziristan, Khyber Pakhtunkhwa, Pakistan

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A parasite is considered to be a lower organism that may associate with another organism. They depend on the host species, which may metabolize them directly or indirectly. There are various types of parasites in aquatic animals; the *gyrodactylus, dactylugyrus,* and crustacean parasites may be more damaging to fish. These parasites directly affect the fish's gills and kill the host fish. That may cause a huge economic loss, morbidity, and mortality in fish. Therefore, current studies aimed to assess the distribution of monogeans (*Gyrodactylus* and *Dactylugyrus*) in fish of Gomal Zam dam, district South Waziristan, Khyber Pakhtunkhwa, Pakistan. A total of 210 fish samples were collected from four different sites, and the parasitic infection on gills, scales, and fins was assessed. A total of 115 parasites were reported, which belong to three genera, on 20 infected fish. Two species of monogenic parasites were reported that is Dactylogyrus, Gyrudectylus where as one parasitic specie were recorded that is Leach specie. The highest number of parasitic records was found to be Leach (39.13%), Dactylogyrus (33.04%), and the lowest in Gyrudactylus (27.82%). The p-value (Pearson Chi-Square) was 0.000, which is significant for the length of fish. The Monogenean parasite was found on gills, scales, and fins, whereas the crustacean parasites were found only on skin and scales. The parasitic infection in common carp fish was higher than in other commercial fish species. The highest mean intensity (7.16%) and abundance (0.92%) were found in the medium fish length group. Physicochemical water parameters of the dam protect the aquatic ecosystem, and there is less parasitic infection in the fish. This research work will further support research on commercial fish in the field of fisheries and the development of the fisheries sector in Pakistan.

Extraction and isolation of keratin from sheep wool and broiler poultry feathers, and its comparative quantitative analysis

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The high protein content of renewable sources of keratin, such as animal feces, human hair, and chicken feathers, makes them important. The waste from wool fabric and chicken feathers is very high in keratin; yet, when the short fibers from these materials are thrown away, they can accumulate in water bodies and cause other environmental problems. In order to compare their keratin yield for prospective uses in biological materials, medications, and dietary supplements, this study attempts to create an effective process for extracting recoverable

keratin from wool and chicken feathers. The goal of this study is to maximize the keratin extraction process from chicken feathers and wool waste. We identified the best techniques for precipitating keratin by using chemical analysis. Sodium sulfide was used for the extraction process for feathers, and NaOH for Wool. A statistical examination demonstrated a noteworthy distinction in keratin production between feathers and wool ($p<0.05$). The findings show that keratin content in chicken feathers is higher than in wool. The percentage yield produced from Feathers is comparatively higher than Wool**.** According to the study's findings, sodium sulfide works best as a reducing agent to extract keratin from chicken feathers, whereas NaOH works best for wool. Keratin may be recovered effectively with these techniques; feathers yield more keratin than wool. The studies look into how natural keratin might be used in the pharmaceutical and cosmetics sectors. Potential uses of several technologies for purifying and separating keratin from organic waste have been highlighted. By using other chemical processes, we can get much better results.

Keywords: quantification, wool, poultry feathers, purification, and keratin extraction

Assessment of pets owner knowledge, awareness, and practices related to pets care and their zoonotic diseases in Dera Ismail Khan, Pakistan

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Dog and cat owners hold the belief that their pets should be treated as close companions. They serve as pets. Pet owners transmit zoonotic diseases to each other when they encounter their pets. This research examines pet contact-associated zoonosis knowledge, attitudes, and practices in Dera Ismail Khan and assesses dog or cat owner understanding regarding pet care. A total of 384 respondents made up the pet owner participant group. Random sampling was used to select participants for a questionnaire-based study on knowledge, attitude, and practice (KAP) from both rural and urban areas of Dera Ismail Khan, Pakistan. The demographics, pet care habits, religious convictions, and knowledge of zoonotic diseases of pet owners in Dera Ismail Khan are all examined in this study. The 75% responded are 18 and 30 years old, with 61% being female and 97% being literate, while earning more than 90% of doctorate degrees. Pet owners chose cats as their most popular choice with 55%, while dog owners made up 30%, and bird owners made up 23%. Fewer than 15 percent of survey respondents visited a veterinarian once a month, although data showed that hand washing occurred after pet contact by 73.8 percent of participants, and 76.3 percent utilized safety equipment for pet waste handling. Only 23.8% of the respondents understood the appropriate spaying age for pets, but 47.5% gave their pets food prepared at home. A significant 88.5% of the population recognized zoonotic illnesses, while social media (23.4%) and schools (47.4%) functioned as the main sources of information. While numerous people were unaware of the symptoms or protective measures. Diagnostic test data for four diseases revealed rabies had the highest infection rate (30%), while scabies had the most reported cases (19/80), emphasizing the need for targeted disease control measures. To advance responsible pet keeping alongside zoonotic disease hygiene, the authors stress enhancing public awareness campaigns alongside both veterinary care and multicultural healthcare messaging methods.

Keywords: attitude, knowledge, pet, pet's owner, practice, zoonosis, Dera Ismail Khan

Assessment of winter fish consumption patterns and fish nutritional profile of district Dera Ismail Khan

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The current research was conducted at District Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan. To assess the fish consumption patterns and also to identify the nutritional profile of fish species most frequently consumed in District Dera Ismail Khan. For this purpose, a questionnaire-based survey was conducted at District Dera Ismail Khan in winter from November 2024 to February 2025. Data were collected from 300 people of the district belonging to different tehsils of District Dera Ismail Khan through a questionnaire. So, according to the survey results obtained, the socio-demographic profile of the Dera Ismail Khan population represented a younger age structure and varied income levels, providing a specific context for understanding fish consumption patterns. While some relationships, such as the influence of age and potentially employment, align with broader research, there was a lack of statistical significance for gender and education. The consumption habits in Dera Ismail Khan reveal a strong overall acceptance of fish, with a notable seasonal preference for winter and a reliance on local shops for purchase. While the prevalence of consumption is high, the frequency and amount consumed annually suggest a moderate role in the overall diet. The population of Dera Ismail Khan demonstrates a strong general belief in the nutritional and health benefits of fish. Further, a proximate analysis was carried out of those species that are most commonly consumed in order to find out their nutritional profile. This represented that *Labeo rohita* (rohu) was a valuable food source in terms of nutrient profile as compared to other locally consumed fish, providing high-quality protein, omega-3 fatty acids, vitamins (like B12 and D), and essential minerals, contributing to heart health, brain function, and overall well-being.

Keywords: Consumption patterns, Nutritional profile, Household fish consumption, Proximate analysis, *Labeo rohita*, Protein, Omega-3 fatty acid.

Ameliorative Potential of Pumpkin Seeds Against Lead-Induced Toxicity in Poultry Chicken (Gallus gallus domesticus)

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This study aimed to evaluate the protective effects of pumpkin seed supplementation against lead-induced hepatorenal toxicity in Gallus gallus domesticus. Young chickens were divided into four groups:control (GO), lead-exposed (G1), pumpkin seed-treated (G2), and combined lead and pumpkin seed-treated (G3). Lead acetate (0.4 mg/kg body weight) and pumpkin seed powder (300 mg/kg body weight) were administered for four weeks. Liver and kidney function tests (ALT, AST, ALP, bilirubin, urea, creatinine), lipid profiles (LDL, HDL,cholesterol, triglycerides), and antioxidant enzymes (CAT, GPx, GR,SOD) were analyzed. Histopathological evaluations of liver and kidney tissues were conducted. Data were analyzed using SPSS 25, with $P<0.05$considered significant. Lead exposure increased ALT(14.6±0.01IU/L),AST(2210.7±10.31 IU/L), ALP(1970.4±1.07 IU/L), bilirubin(0.7±0.00mg/dL),and LDL(56.9±0.91mg/dL).Pumpkin seed supplementation reduced AST (110.1 ± 8.84 IU/L), ALP (1360.5±1.00 IU/L), and LDL (22.9±1.04mg/dL), and improved antioxidant enzyme activities. Pumpkin seed supplementation mitigates lead-induced oxidative stress and improves hepatorenal function, presenting a natural strategy to reduce heavy metal toxicity in poultry

Epidemiology of Hepatitis B and C Viruses in the General Population of District Dera Ismail Khan, Pakistan

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This study aimed to determine the prevalence and epidemiology of hepatitis B (HBV) and hepatitis C (HCV) in the general population of Dera Ismail Khan, Pakistan, and to identify demographic and biochemical associations. A cross-sectional study was conducted involving 1,244 individuals randomly selected across various age groups and genders. Blood samples were collected and analyzed using immunochromatographic testing (ICT) and enzyme-linked immunosorbent assay (ELISA) to detect HBV surface antigen (HBsAg) and HCV-specific antibodies. Biochemical analysis of alanine transaminase (ALT) was performed using Microlab 300(Merck, USA). Statistical analysis was conducted using SPSS version 25 to evaluate associations between demographic variables and disease prevalence. The overall prevalence of HBV and HCV was 25.72%. HBV was detected in 228 (29%) individuals, with a higher prevalence in males (34.31%) compared to females (19.20%) and the 16-30years age group (43.17%). HCV was identified in 92 (20.08%) individuals, with males (22.05%) more affected than females (17.43%), and the 46-60 years age group showing the highest prevalence (27.82%). ALT levels were significantly elevated in

infected individuals, with mean values of 111.63 ± 101.38 in males and 143.50 ± 52.50 in females. The study highlights the substantial burden of HBV and HCV, emphasizing the need for enhanced public health interventions, including vaccination, early screening, and community education, to mitigate the disease.

Development of a Bacteriophage Biobank against Major Pathogenic Bacteria of Poultry, and Poultry Born Human Pathogenic Bacteria

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Anthropogenic climate change is reshaping host-pathogen dynamics and disease epidemiology in animal systems, particularly in poultry, intensifying the spread of infections and antimicrobial resistance (AMR). These problems necessitate robust, non-antibiotic control strategies, positioning bacteriophage-based biocontrol as a targeted and adaptive advancement in zoology under climate-driver stressors. In this context, the present study addresses the rising AMR threat by establishing a bacteriophage biobank targeting poultry pathogens and poultry-borne human bacterial infections. Eighteen bacterial strains were collected from laboratory sources and selected as hosts for phage isolation. Environmental sampling was conducted using sewage water from poultry farms and surrounding areas in Rawalpindi and Islamabad. Multiple bacteriophages were initially isolated against eleven bacterial strains, followed by purification of six distinct phages exhibiting lytic activity. All bacteriophages were cryopreserved for further analysis. Phage characterization included temperature and pH stability testing, host-range determination, lytic efficiency, and one-step growth kinetics. At present, three bacteriophage samples have been sent for whole genome sequencing to Macrogen, South Korea. The sequencing results will enable genetic profiling and functional insights into the isolated phages. Preliminary findings highlight the diversity and specificity of isolated bacteriophages, underscoring their potential as effective biocontrol agents against poultry pathogens, The development of this phage biobank provides a promising platform for reducing antibiotic resistance, controlling bacterial infections in poultry, and minimizing zoonotic transmission risks to human. Further analysis will determine their suitability for therapeutic and large-scale application.

Lipid profile of smokers and non-smokers inhabitants of district Buner, Khyber Pakhtunkhwa, Pakistan

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Smoking is a process in which tobacco is burnt and smoke is inhaled by diverse ways i.e. cigarette, cigar, biri and pipe. It gives sense of inclination and pleasure to the smoker. It is complex external and internal stimulus consisting of visual, tactile, mechanical (mouth movement), gustatory, olfactory and irritating factor. This study was collected from October 2023 to August 2024 in order to evaluate blood profile of smokers and non-smokers inhabitants of district Buner. A total of 51 (n = 51) individuals data was collected during this study. Out of 51, 26 individuals (n = 26) were smokers, and 25 (n = 25) were non-smokers. Blood profile of smokers and non-smokers was evaluated in terms of different parameters viz-a-viz cholesterol (mg/dl), triglycerides (mg/dl), high density lipoproteins (HDL, mg/dl), low density lipoproteins (LDL, mg/dl) and very low density lipoproteins (VLDL, mg/dl). Data was collected from different laboratories located in district Buner including Amreek Clinical Laboratory, Shifa Clinical Laboratory, Chughtai Clinical Laboratory and Karachi Clinical Laboratory. Our study reported that cholesterol levels in blood plasma of smokers (226.65 mg/dl) was higher in comparison to the non-smokers (161. 36 mg/dl) inhabitants of district Buner. Moreover, triglycerides levels in blood plasma of smokers (284.77 mg/dl) was higher in comparison to the non-smokers (168.72 mg/dl) inhabitants of district Buner. Further, it was observed that LDL levels in blood plasma of smokers (136.42 mg/dl) was higher in comparison to the non-smokers (97.40 mg/dl). Lastly, current investigation observed that VLDL levels in blood plasma of smokers (1436.35 mg/dl) was higher in comparison to the non-smokers (949.60 mg/dl). It is concluded that smoking elevate blood cholesterol, triglycerides, LDL, and VLDL levels in blood plasma. Higher levels of lipids in blood can lead to buildup of plaque in arteries, causing them to become narrower which ultimately increases the risk of heart disease, heart attack and stroke.

Keywords: Smoking, LDL, HDL, Cholesterol, CBC

Analysis of Heavy Metals in Catla catla collected from Sharki Dam Karak, Pakistan

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This study calculated the specific accumulation ratio of heavy metals (Copper, Cobalt, Chromium, Zinc, Iron, and Manganese) in *Catla catla* fish collected from Sharki Dam Karak, Pakistan. Fish samples were collected, dissected, and underwent organ-specific digestion using nitric acid and perchloric acid. The concentrations of heavy metals were determined using Atomic Absorption Spectrophotometry. SPSS software was used for further analysis to assess heavy metal contamination in fish. The study found that across fish tissue samples, the order of metals accumulation varied in different tissues. Chromium and Cobalt had the lowest accumulation, while Iron and Zinc had the highest accumulation in all samples. Manganese showed uniform accumulation in all tissues. The results emphasize the need for regular monitoring and targeted investigations to understand the build-up of heavy metals in aquatic organisms and to evaluate the ecological and health consequences. The study concludes that effective pollution control, habitat restoration, and conservation policies are necessary, and increasing public knowledge is critical to reduce the risks associated with heavy

metal contamination and protect aquatic environments and *Catla catla* populations for future generations.

Keywords: Metal, Accumulation, Fish, *Catla Catla*, SPSS software, Atomic Absorption

Prevalence of albinisms disease in district Buner Pakistan

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The present study was conduct on prevalence of Albinism Disease in district Buner Pakistan during August 2022 to August 2023. During the study a total of 168 Patents were reported in which 61 are Female and 107 are Male. We have collected the data of 168 patients from 6 tehsil of district Buner. The percentage of Tehsil Chagharzai are 44.88%, the percentage of tehsil Daggar are 25.59%, the percentage of Tehsil Gagra are 17.85%, the percentage of tehsil Khodu Khail are 10.71%, the percentage of tehsil Gadezai are 17.85% and the percentage of tehsil Mandanr are 13.9%. The most effected tehsil are Daggar and second top tehsil are Gadezai. We have collected data age wise of Albinism disease. The number of patients from 1 to 5 years patients are 42, from 5 to 10 patients are 43, from 10 to 15 patients are 30, from 15 to 30 patients are 28 and 20 to 25 patients are 25. The most patients age wise are present from 5 to 10 in district Buner.

Molecular detection of Toxoplasma gondii in mules of district Dir lower and Dir upper Khyber Pakhtunkhwa Pakistan

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Equines play vital roles in various sectors, including transportation, agricultural work, and rural livelihoods, especially in mountainous regions. In addition to their economic value, equines also hold cultural and historical importance, contributing to traditional practices and local heritage. However, their health and productivity are increasingly threatened by parasitic infections. Pakistan has 0.29 million of mules' population, while the study area possesses 3504 individuals of mules' population (Dir lower 551, Dir Upper 2953) (Pakistan Bureau of statistics 2024). Mules that serve as a primary mean of transportation in hilly terrain and are a vital component of local economies. As parasitic infections like toxoplasmosis can significantly impact the health and performance of these animals, therefore conduction of epidemiological studies in the region was necessary to identify the prevalence of *Toxoplasma gondii* in the mules of district Dir lower and Upper and investigate the association of various risk factors (age, sex, locality, altitude etc.) with *T. gondii.* Total 149 samples of mules' blood were collected from both districts and were stored in EDTA tubes. Genomic DNA was extracted using inorganic DNA extraction method. Through conventional PCR along with oligonucleotide primers DNA was amplified in all samples. A 300 base-pair portion of Internal transcribed spacer (ITS-1) gene was targeted of the ribosomal DNA of *T. gondii*. Out of total 16 % blood samples were detected positive with 300 base-pair gene (ITS-1). Two breeds were enrolled Kabali and Watani both were parasite infected 11% and 18 % respectively. Highest prevalence was observed in grey coat color 22% followed by brown 20% and bay 16%. Data analysis revealed that 23% of Dir Upper and 4% of Dir lower mules were *Toxoplasma gondii* infected. 93% of mules with treated history were *T. gondii* -ve. In the current data analysis, the infection rate is higher where the altitude is higher. The current study is reporting the detection of *T. gondii* in the mules of two rural districts of Pakistan, Dir Lower and Dir Upper. By looking at the wide range of host of *T. gondii,* we recommend wide and advanced molecular studies in different geoclimatic regions to explore epidemiology and diversity of pathogens among equines.

Keywords: Mules, Detection, PCR, Blood, infection, District, Pakistan

Association of malaria infection with the ABO blood group system

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Preventing malaria needs an understanding of the relationship between the ABO blood group and malaria infection. Numerous studies have demonstrated that a patient's blood type affects their susceptibility to several infectious diseases. Many researchers have investigated the connection between blood type and susceptibility to malaria, but the outcomes are conflicting. The glycophorins are found on erythrocytes and carry blood group antigenic determinants, as well as serve as receptors for a variety of intracellular pathogens. Human glycophorins and their impact on malaria resistance have drawn a lot of attention in recent years. Human Glycophorin Receptor Polymorphisms and blood groups are involved in severe malaria protection. Duffy antigen is located on the surface of RBC and is also referred to as Fy glycoprotein. It functions as a receptor for the human malarial parasite. In laboratories, several diagnostic techniques are used. The blood was taken in EDTA tubes from malaria-positive individuals, and thick and thin films were prepared, as well as conducting blood group detection by a commercially available kit. The most recurrent ABO blood group in the control group was A (n=34) followed by B (n=25), O (n=22), and AB (n=19), and most of them were Rh positive while in the infected group, the most frequent blood group was O (n=43) followed by A (n=31), B (n=14) and AB (n=12). Blood group O individuals had a higher rate of parasitic infection. The current study showed the occurrence of the O allele in an area. Blood group studies with malaria infection are important for controlling malaria infection among O blood group individuals.

Keywords: Malaria, ABO Blood Group, Glycophorin Receptor, Duffy antigen

Accumulation of Heavy Metals in Glyptothorax punjabensis and Schizothorax plagiostomus Collected from River Panjkora, Pakistan

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The study explore the accumulation of heavy metals in two fresh water fishes, *Glyptothorax punjabensis* and *schizothorax plagiostomus*, collected from the river Punjkora Pakistan. The primary objective was to assess the concentration level of selected metals (leads, chromium, cadmium and zinc)in the tissue of these fish and evaluate potential environmental and health issue. Fish sample were collected from different side of river and then analyzed by absorption spectrophotometer. The results revealed varying level of metal accumulation in different tissue, with the liver and gills showing higher concentrations compared to muscle. The presence of heavy metals in both species indicate contamination of the river ecosystem. The finding highlight the regular checkup is the way of to control water pollution and protect the aquatic life and human health.

Keywords: Heavy metals, Pollution, contamination, accumulation, ecosystem.

Comparative Effects of Multi-Species Probiotics on Growth Performance and Survival of Cyprinus carpio and Ctenopharyngodon idella under Different Rearing Environments

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This study investigated the effects of dietary multi-species probiotic supplementation on growth performance and survival of two commercially important freshwater fish species, *Cyprinus carpio* (common carp) and *Ctenopharyngodon idella* (grass carp), reared in glass and cemented tanks. A total of 480 fish (240 per species) were assigned to control and probiotic-treated groups and subjected to a 90-day controlled feeding trial. Fish in the experimental groups received a diet supplemented with 5 g kg^{-1} of probiotics containing *Bacillus subtilis*, *Bacillus licheniformis*, and *Enterococcus faecalis*. Probiotic supplementation significantly improved both survival and growth performance in both species compared to controls ($p < 0.05$). Survival rates of common carp increased from 83–85% in controls to 95–97% in probiotic-fed fish, while grass carp survival rose from 80–85% to 92–95%. Weight gain, percent weight gain, and specific growth rate (SGR) were consistently higher in probiotic-fed fish across both tank types. Notably, common carp in cemented tanks showed the highest final weight (23.0 ± 0.8 g) and SGR (2.10% day^{-1}), whereas grass carp in cemented tanks also exhibited enhanced growth (final weight 9.0 ± 0.5 g; SGR 1.40% day^{-1}). These results indicate that dietary inclusion of multi-species probiotics effectively enhances growth and survival of *C. carpio* and *C. idella* under different rearing conditions, offering a sustainable and eco-friendly strategy to improve productivity in freshwater aquaculture.

Keywords: Multi-species probiotics, *Cyprinus carpio, Ctenopharyngodon Idella,* Growth performance, Survival rate, Freshwater aquaculture, Glass and cemented tanks, *Bacillus subtilis, Bacillus licheniformis, Enterococcus faecalis*

Studies on analysis of minerals, phytochemicals, proximate composition, and in vitro biological activities of methanolic extracts from the fruits of Cordia dichotoma Forst and Cordia myxa L.

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Plants have been the primary source of sustenance, medicinal remedies, and refuge for humans throughout history. Wild edible fruits, in particular, have played a significant role in shaping people's diets due to their nutritional value. This research aimed to investigate the mineral content, antioxidant properties, chemical composition, and basic characteristics of selected wild edible fruits from Dera Ismail Khan, specifically *Cordia myxa* and *Cordia dichotoma*. The chosen wild edible fruits were utilized to determine their inorganic content through spectroscopy, assess their antioxidant potential using the DPPH assay, and analyze their proximate components such as crude fats via the soxhlet extraction method, proteins using the Kjeldahl technique, and ash content through muffle burning. Standard AOAC techniques were employed to ensure accurate measurements. The results of the proximate analysis revealed that *C. dichotoma* had a carbohydrate content of 88.35%, while *C. myxa* had 53.79%. Furthermore, the study found that the copper concentrations in *C. myxa* and *C. dichotoma* were 525.00 mg/kg and 477.70 mg/kg of dry weight, respectively. Notably, the highest levels of total phenols and their derivatives were detected in *C. myxa* (33.41 mg GAE/g) and *C. dichotoma* (19.59 mg GAE/g), with *C. myxa* exhibiting significant antioxidant activity (IC_{50} 46.75 g/mL). Additionally, both fruits displayed a total flavonoid content of 0.463 mg QE/g. Antibacterial activity was performed by microdilution method of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) whereas, *Cordia myxa* (L.) showed greatest activity of inhibition against *Pseudomonas aeroginosa* and *Cordia dichotma* inhibited *Klebsiella pneumonia* effectively. Cordia dichotma proved to be good anti-diabetic while Cordia myxa proved itself for better anti-inflammatory activity in defined laboratory environment, both activities were confirmed by performing enzyme inhibition assays. With high quantities of macronutrients, trace elements, antioxidants, and possible health advantages, these neglected food sources become more important in both conventional and contemporary diets.

Keywords Medicinal Plants Proximate Analysis Wild Edible Food Plants (WEFPS) Cordia family

Hidden Antimicrobial Peptides in Ichthyophthirius multifiliis: An In Silico Exploration

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The rapid escalation of antimicrobial resistance has created an urgent need for the discovery of novel therapeutic agents. Cryptic antimicrobial peptides (AMPs), concealed within larger protein sequences, constitute a largely untapped reservoir of bioactive molecules with significant potential for next-generation antimicrobial development. Among potential sources, protozoan parasites particularly those inhabiting microbe-rich host environments remain markedly underexplored despite their biological relevance. In this study, we conducted a comprehensive in silico investigation of the proteome of *Ichthyophthirius multifiliis* to identify cryptic AMP candidates. A total of 16,761 protein sequences were systematically analyzed using a Python/Biopython-based workflow that integrated sequence preprocessing, physicochemical characterization, and deep learning driven AMP prediction. Computational fragmentation of the proteome generated 197,916 peptide fragments, of which 10,949 were initially classified as high-confidence AMPs via AI (deep learning model). Subsequent sequential filtering based on net positive charge, hydrophobicity, and predicted structural stability refined this dataset to 1,574 biologically plausible candidates. From this refined pool, seven unique top candidates were selected for detailed structural characterization. Secondary structure prediction and helical wheel analysis revealed a consistent amphipathic α-helical architecture among these peptides, characterized by a clear spatial segregation of hydrophobic and positively charged residues hallmarks of classical membrane-active AMP mechanisms. Collectively, this study uncovers a previously hidden repertoire of cryptic AMPs within *I. multifiliis*, highlighting a novel and underexplored biological source. Furthermore, it establishes a robust computational framework for the systematic discovery and rational design of next-generation synthetic AMPs, with promising implications for both aquaculture and broader antimicrobial therapeutics.

Keywords: *Ichthyophthirius Multifiliis*, Cryptic Antimicrobial Peptides, Deep Learning, Proteome-Wide, Ectoparasite

Agricultural Waste Transformation: Industrial Xylanase Production And Characterization From Bacillus Strains

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Lignocellulosic agricultural waste has become a useful and economic source for production of many commercially important products due to the presence of a wide variety of components. Xylan is a complex carbohydrate polymer which is composed of D-xylose units linked through β-1,4 linkage. Current study is an effort to produce xylanase from newly isolated microbial strain *Bacillus*. 30 strains were isolated from different areas (soil, water and air) of the Upper Dir. Out of 30, 13 were found to be Bacillus upon morphological, biochemical and microscopic characteristics. The production of xylanase was optimized using various physical and chemical parameters. 5 different reported media were used, and new medium was designed. The components of new media were also varied by incorporating agro-waste and wheat bran was found to be best induced at 3% concentration. Other salts were optimized as well. A high yield of xylanase was obtained at 40ºC, pH 7.0, and after 18 hours of fermentation, after optimization of culture conditions for maximal xylanase synthesis. Gradient precipitation was also used to partially purify crude xylanase, and the highest fold purification was obtained at 40.0% ammonium sulfate saturation. Enzyme kinetics showed that xylanase has Vmax of 660 U/mg and 1.25 mg/ml Km. The optimum reaction time, pH and temperature were found to be 30 minutes, 7 and 50 °C. Stability data showed that xylanase was quite stable for longer time periods at various temperature conditions. Thus, xylanase produced in current study can be further employed for biodegradation of lignocellulosic biomass which can be useful in circular bioeconomy.

Keywords: Xylanase, Fold Purification, Biomass, Biodegradation, Bioeconomy.

Species diversity and distribution of ladybird beetles (Coleoptera: Coccinellidae) in south Waziristan, Pakistan

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This study presents the first detailed record of ladybird beetle diversity from South Waziristan, a region previously unexplored due to prolonged geopolitical instability. The present study was designed to identify species richness, distribution patterns across eight selected localities in South Waziristan, Pakistan. An extensive survey was conducted from January to December 2022 in these areas. A total of 530 ladybird beetle specimens were collected from all types of agro-ecosystems using sweep netting and hand-picking methods. Samples were then taken to the laboratory, assigned a unique identification label and the ladybird beetle species were identified using published literature and identification keys. Among the collected specimens, fourteen species were identified, belonging to thirteen genera, three tribes and one subfamily. Ten of these species, viz., *Coccinella septempunctata*, *Hippodamia variegata*, *Cheilomenes sexmaculatus*, *Micraspis allardi*, *Propylea dissecta*, *Adalia bipunctata*, *Psyllobora bisoctonotata*, *Oenopia conglobata* and *Llleis confusa* belonged to the tribe Coccinellini, while three species were *Parexochomus nigripennis, Parexochomus pubescens* and *Chilocorus nigrita* classified under the tribe Chilocorini. One species, *Chnootriba elaterii*, represented the tribe Epilachnini within the subfamily Coccinellinae. Among all the species, *Coccinella septempunctata* was found to be widely distributed across all the studied localities, whereas *Illeis confusa* was identified as a rare species, present in only a few locations. Sholam Kot exhibited the highest species richness (14), the highest Shannon-Wiener's Diversity Index (H = 2.38), and the highest Margalef's Index (2.88), indicating the greatest diversity among the sites. Kaniguram showed moderate diversity, while Sarwekai had the lowest diversity across multiple indices. Additionally, similar indices between the localities revealed the highest similarity between Zarmilan and Sholam Kot (0.83) and the lowest similarity between Wana and Tiarza (0.33). This study revealed significantly different species diversity among all sites, with Sholam Kot exhibiting the highest species richness and diversity, characterized by orchard trees, wild vegetation, and agricultural crops. This finding provides baseline data for ladybird beetle diversity in understudied areas and is valuable for ecological conservation and agricultural sustainability planning worldwide.

Keywords: Coccinellid beetles; Species Richness; Diversity indices; Agro-ecosystem; Khyber Pakhtunkhwa

Impacts of Climate Change on Avian Ecology and Population Dynamics in Pakistan

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The origin of Climate change and greenhouse gas production, primarily caused by human and is quickly altering overall ecosystems. These changes pose serious threats to bird species which act as important indicators of environmental change. This study synthesizes current research on the impacts of climate change on birds, with particular emphasis on endemic species of the Pakistan. Evidence shows that rising temperatures are causing shifts in bird distribution toward higher latitudes and elevations, along with substantial contractions in suitable habitats projected under future climate scenarios such as 2050 and 2070. In addition to spatial changes, climate change is affecting avian phenology, leading to earlier migration and breeding periods, which may result in phenological mismatches and ecological disjunctions that disrupt food availability and reproductive success. These changes have complex effects on population dynamics, influencing survival, reproduction, and overall abundance through direct, indirect, nonlinear, and time-lagged processes. Despite growing documentation of these impacts, there remains a need for integrative models and long-term data to better understand and predict species responses, including frameworks such as Measuring, Understanding, and Predicting (MUP) and the concept of population robustness. The findings highlight significant extinction risks, especially for endemic species, and emphasize the importance of mitigation and adaptation strategies, including reducing fossil fuel use, adopting renewable energy, strengthening environmental policies, and implementing long-term monitoring systems. Coordinated global efforts, scientific innovation, and increased environmental awareness are essential to minimize the adverse effects of climate change on bird populations and maintain ecological balance.

Keywords: Climate Change, Avian Biodiversity, Species Distribution, Phenological Shifts, Population Dynamics

Effect of Irrigation Intervals on the Yield and Fiber Characteristics of Cotton Genotypes

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Cotton is a lifeline of Pakistan's economy. Drought stress is the major ecological factor that negatively impacts cotton yield and quality. A field experiment was conducted in 2016 at Cotton Research Station, Dera Ismail Khan, Pakistan to evaluate the effects of four moisture regimes on yield and fiber characteristics of two cotton genotypes. The experiment was laid out in split-plot within a randomized complete block design with three replications. Four irrigation intervals namely I1 (10 days interval), I2 (15 days interval), I3 (20 days interval) and I4 (25 days interval) were allotted to main plots while cotton genotypes (CIM-602 and CIM-616) were assigned to subplots having 10 m×3 m. Results revealed that cotton crop irrigated at 20 days interval showed greater bolls per plant; weight per boll; seed cotton yield and ginning out-turn (GOT %). The results further showed that the genotype CIM-602 gave optimum yield owing bolls per plant, weight per boll, GOT % plus higher staple length compared to CIM-616 at 20 days irrigation interval. Thus, it was concluded that the genotype-CIM-602 irrigated at 20 days interval suited well to the study area and had the potential to optimize cotton yield and quality in Dera Ismail Khan, Pakistan.

Keywords: Cotton, Irrigation intervals, Genotypes, Yield, Quality

Prevalence and diversity of gastrointestinal parasites of pet dogs and risk factors associated with infection in district Dir upper, KP, Pakistan

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Domestic dogs are among the most common companion animals worldwide and represent the most widely distributed carnivorous species. It is estimated that approximately one billion dogs exist globally, with more than half maintained as household pets. Pet dogs are frequently affected by a variety of gastrointestinal parasites that can compromise animal health and may also pose zoonotic risks. Therefore, the present study was conducted to determine the prevalence of gastrointestinal parasites in pet dogs. A total of 50 fecal samples were collected from pet dogs and examined for the presence of gastrointestinal parasites. Parasitological analysis revealed four types of gastrointestinal parasites: Toxocara canis**,** Strongyloides**,** Giardia**,** and Coccidia**.** The overall prevalence of gastrointestinal parasitic infection was 32% (16/50). Among the examined dogs, 18 were males and 32 were females. The prevalence of infection was higher in females, with 24% (12/50), compared to males, which showed 8% (4/50). Age-wise analysis indicated that puppies had the highest prevalence at 16% (8/50), whereas adult dogs showed the lowest prevalence at 4% (2/50). The findings of this study indicate that gastrointestinal parasitic infections are common in pet dogs, particularly in females and younger animals, highlighting the importance of routine screening and preventive parasite control measures in companion animals.

Keywords: Pet dogs, Gastrointestinal parasite, *Toxocara canis*, Strongyloides, Giardia, Coccidia, Prevalence

A first report on the Genetic Diversity of Both Local and Exotic Hens At District Dir Upper and Lower

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In the present study 40 different hen breeds were collected from different localities of District Dir Khyber Pakhtunkhwa, Pakistan. Total egg weight, egg shell weight, egg white and egg yolk weight were observed revealing variability for egg shell weight comparatively high followed by egg yolk weight which was low for egg weight and egg white weight. Pearson correlation coefficient revealed that egg shell weight is high positively significantly correlated with egg yolk weight while the remaining characters do not show any significant association. Dendrogram constructed for association/diversity between four different chicken varieties based on egg morphological traits showed intermingling depicting low diversity. Protein profiling of egg white protein showed significant differences in term of presence and absence. A total of 12 alleles were observed among which 2, 4, 5, 6, 7 8 and 9 were most frequent and were divided into two main clusters (C1 and C2). C2 gathered 3 allels into 3 subgroups individually. C1 was subdivided into 2 sub clusters between which allele-3 was seperated while the rest were subgrouped. Eggs of desi hens collected from Munda, Khal, Dir and Karo were genetically similar as they were grouped together. Similarly desi eggs from Darora and Nehag were similar. Genetic similarity in desi eggs from Wari, Sahib Abad and Shahi was also seen. Interestingly a great deal of difference was seen in desi eggs collected from the remaining locations. In conclusion we demonstrate high level of genetic polymorphism in egg white protein. This directes to the fact that variation within protein profile exist among different hen breeds which is important for trait preservation and can be achieved through advanced molecular techniques.

Keywords: Hen Breeds, Egg White protein, Genetic polymorphism, Protein Profiling

Prevalence of gastrointestinal and haemoparasites and its impact on hematological parameters in backyard poultry in district Dir (upper)

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Poultry is an essential component in agricultural industry providing a significant source of food and income worldwide. However, its efficiency has been challenged by certain factors like disease outbreaks, nutrient costs and environmental factors, particularly in backyard poultry. To address the issue, a specific study determining the effect of gastrointestinal and haemoparastic infections on hematological profile of different age and sex groups of various local breeds of backyard poultry was conducted in Dir Upper, Pakistan. For this purpose, 400 fecal and 400 blood samples were collected and examined. Fecal samples were either directly collected from the colon or freshly passed and were stored in plastic containers containing 10 % formalin while blood samples were collected from the wing vein by using sterile syringe with (22–27-gauge needles), forming a thin blood smear and stained with Giemsa and screened out for heamoparasites. Different gastro intestinal parasites like Nematodes (*Ascaridia galli, Capillaria, Heterakis gallinarum, Trichuris)* and Cestodes (*Hymenolopsis)* were identified revealing 52.5% overall prevalence. *Ascaridia galli* was the most prevalent parasite (53.75%). The prevalence of GIP was higher in female chickens (51.35%) compared to males (32.78%). By age, the prevalence was higher in growers (66.66%) compared to adult chickens (51.35%). Among different breeds, Aseel chickens showed the highest prevalence of GIP (54.76%), followed by undescriptive breeds (52.32%) and Naked Neck (50%). The study found that single gastrointestinal parasitic infections were more common, occuring in 89.04% cases, compared to mixed infections, which were found in 10.95% of cases. The overall prevalence of haemoparasites were 46.75%. The identified haemoparasites were *Aegyptienella, Haemoproteus, Leucocytozoon and Plasmodium*. Among these *Leucocytozoon* was the most prevalent found in (43.87%) of cases, followed by *Haemoproteus* (36.13%) and *Plasmodium* (18.07%). On the basis of gender and age haemoparasites were (57.14%) in male compared to female chickens (44.68%) and adult chickens exhibited a higher prevalence (47.29%) than growers (40%). Aseel chickens had the highest overall prevalence of haemoparasites at 66.66%, followed by undescriptive breeds at 44.77% and Naked Neck breeds at 35.71%. In the present study it was found that 82.88% of the infected chickens had single haemoparasitic parasitic infections, while 17.11% had mixed infections. In the current study we concluded that backyard poultry may harbor a number of gastrointestinal and haemoparasites in District Dir Upper (Tehsil Wari). The high prevalence rates, especially in specific breeds and localities, call for targeted interventions and improved management practices to control and reduce the burden of gastrointestinal and haemoparasitic infections.

Key words: Prevalence, Backyard poultry, Gastrointestinal, Haemoparasite

Assessment of socio–demographic and climatic factors associated with Dengue fever in District Dir Upper Khyber Pakhtunkhwa Pakistan

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Dengue fever is a vector borne disease found in the tropical and sub-tropical regions of the world and became a public health menace. This study was designed to find the distribution of dengue fever and its association with climatic and risk factor in District Dir Upper. Data was collected from four tehsils of District Dir Upper during the year 2023 i.e. January 2023 to December 2023. Various statistical tools were used to elaborate the real situation of Dengue fever and its relation with the climatic and risk factors in the district. During area wise analysis, higher number of cases were observed in Tehsil dir (3.4%) while least number of cases was found in Tehsil Sheringal (1.5 %). The frequency of dengue positive patients were significantly higher in male suspected patients as compared to female counterparts. The frequency of DEN fever in different age group patients was observed in the study. The people of age group 21 to 30 years were highly affected while least number of incidence were found in the age group 51 to 60 years. Month wise incidence showed that higher number of people affected in the month of July followed by August and September while fewest number of cases were found December followed by January. During analysis of Dengue fever and its relation with climatic and risk factors, temperature, humidity and rainfall has a direct effect on the distribution of Dengue fever. Maximum number of cases were observed in areas with high temperature, humidity and rainfall were observed. This study provides a base for the public health intervention to prevent and mitigate Dengue fever in District Dir Upper.

Keywords: Dengue Fever, Climatic factors, Dir Upper

Integrating mineral profiles and bone density imaging in osteoporosis risk stratification

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Patients worldwide are at risk of developing one of the most overtly dynamic but insidiously dangerous ailments – osteoporosis; a condition that is especially prevalent for those in need of specialized health care services. This is in concern with the target set of this particular investigation which is intended to explore

the rate of osteoporosis prevalence in Dir Lower, Khyber Pakhtunkhwa, Pakistan via dual bone density microscopy and mineral profiling system for better osteoporosis risk assessment. The study utilized a cross-sectional approach, and to address the goal, 500 respondents were chosen through composite random sampling segmentation according to age, gender, and geography. The data collection involved administering structured questionnaires, performing anthropometric measurements, conducting blood tests, and performing DEXA scans of the subjects. For the biochemical tests to be performed, a focus on calcium, phosphorus, magnesium, vitamin D, and parathyroid hormone (PTH) levels in the serum was taken. From the data obtained, it was noted that the prevalence of osteoporosis was markedly higher among women, post-menopausal women in particular, while most of the men were inclined towards osteopenia. In general, inadequately low body weight and tall stature, most pronounced in women, emerged as strongly predictive of low bone mineral density. Moreover, the vitamin D, calcium, and magnesium deficiency were critical in terms of strongly reduced bone mass – skeletal metrology and clinical paleopathology becoming a prime subject concern in terms of clinical considerations. Despite the common presence of can comorbid conditions like diabetes, hypertension, and osteoporosis, tertiary impact on bone density was questionable independently. It advocates the proposition of advancing general screening measures, nutritional amelioration schemes, and public health planning disposition as a means to delay osteoporosis among vulnerable people at risk.

Keywords: Osteoporosis, Bone Mineral Density (BMD), Vitamin D Deficiency, Mineral Profiling, Dir Lower

Prevalence of tuberculosis in district Dir upper associated with different risk factors

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Tuberculosis is bacterial zoonotic infectious disease causing severe health issues and mortality in humans and has a significant contribution in global diseases burden. The purpose of this study was to investigate the prevalence of tuberculosis and its association with different risk factors in the study area. For this purposed, a detailed survey was conducted through a structured questionnaire to determine the prevalence of tuberculosis and its association with various risk factors. The confirmation of tuberculosis was conducted through imaging studies, microbiological test and clinical evaluation. Results showed that the overall prevalence of tuberculosis is 43.7% in the study area. Regarding different risk factors, it was revealed that significantly ($P=0.01$) higher TB prevalence was recorded in female population (24.7%), children of 11-20 year’s age (15.3%). Similarly, higher TB prevalence was observed in the month of December (14%), at temperature range 11-20C° (21.4%), during

rainfall of 10-100 and 201-305 (17.7% and 15.8%, respectively). Higher TB prevalence was observed in Tehsil Dir Town (21.9%) of the study area. The results also revealed a strong correlation of different risk factors i-e Gender (X^2 =10.88 p-value = 0.01), Age (X^2=5.720 p-value = 0.047), Months (X^2= 82.049, p-value = 0.00), Temperature (X^2=42.358, p-value = 0.00), rainfall (X^2=29.443, p-value = 0.00) and Locations (X^2=24.641, *P-Value = 0.026)* with the prevalence of TB in the study area. It is thus concluded that different risk factors have significant relationship with the prevalence and distribution of TB in the study area. Therefore, it is recommended on the basis of the results of this study, that these risk factors must be addressed properly for the control and eradication of TB.

Keywords: Tuberculosis, Risk factors, Correlation, Dir Upper

Impact of Petroleum Crude Oil Exposure on the Gut Microbiota of Pigeons in District Kohat

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Petroleum crude oil contamination represents a persistent environmental challenge, particularly in industrial regions where it adversely affects wildlife. Urban birds such as *Columba livia* (rock pigeon) are frequently exposed to environmental pollutants due to their close association with human habitats. Disruption of gut microbiota in these birds can compromise digestion, immune competence, and overall physiological balance. This study was designed to investigate the effects of petroleum crude oil exposure on the gut microbial composition and metabolic activity of pigeons in District Kohat, Pakistan. Fecal samples were collected from pigeons inhabiting areas with differing levels of petroleum contamination. Microbial profiling was conducted using 16S rRNA gene sequencing to determine the presence and relative abundance of selected bacterial taxa, including *Escherichia coli*, *Salmonella* spp., and *Enterobacter* spp. Enzymatic assays were performed to measure β-glucuronidase and urease activities as indicators of microbial metabolic function. Additionally, Gas Chromatography–Mass Spectrometry (GC-MS) was employed to analyze metabolomic changes, focusing on the quantification of short-chain fatty acids (SCFAs) and amino acids. The analysis revealed notable alterations in the gut microbiota of pigeons exposed to petroleum contaminated environments. An increased prevalence of opportunistic and potentially pathogenic bacteria, including *E. coli*, *Salmonella* spp., and *Enterobacter* spp., was observed in contaminated areas. Enzyme activity assays indicated significant changes in β-glucuronidase and urease levels, reflecting disrupted microbial metabolism. Metabolomic profiling further demonstrated variations in SCFA and amino acid concentrations, suggesting impaired gut functionality and metabolic imbalance associated with pollutant exposure. The findings indicate that chronic exposure to petroleum crude oil can induce microbial dysbiosis and metabolic disturbances in *C. livia*. Such alterations may negatively impact avian health and ecological fitness. This study highlights the importance of monitoring environmental pollution and its biological consequences, contributing to improved conservation strategies and pollution management in affected regions.

Keywords: *Columba livia*, petroleum pollution, gut microbiota, 16S rRNA, GC-MS, avian health

Gastrointestinal parasites in pet cats and risk factors associated with infection in upper Dir, Pakistan

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Gastrointestinal parasites (GIP) are widespread in domestic cats and may impact animal health as well as the health of people because of their zoonotic characteristic. There is limited information regarding the prevalence and distribution of these parasites in pet cats in District Dir Upper Khyber Pakhtunkhwa Pakistan. The purpose of this study was to establish the prevalence rates of major gastrointestinal parasites (GIP) and the rates of their distribution by sex and age. Fecal samples of 50 pet cats in various localities of District Dir Upper were collected. Standard microscopic parasitological methods were used to examine samples in order to identify species of helminth and protozoa. Data analysis was conducted to identify general prevalence and difference in terms of age and sex. The general gastrointestinal parasite prevalence was 36%. There was a slight prevalence difference of 20% between females 20 and males 16%. The age analysis showed the highest infection rate to be in young cats 16% and then the kittens were 12% and then the lowest infection rate was in adult cats 8%. Specifically, the species-based outcomes demonstrated that nematodes were prevalent compared to protozoa with Toxocara 14% and Ancylostoma 10% being the most common helminths. Giardia spp. and Isospora spp. were protozoa, 8 and 4% respectively. The results indicate that gastrointestinal parasites are of moderate prevalence in pet cats in District Dir Upper with younger pets being more vulnerable. Toxocara and Ancylostoma predominance points to the zoonotic threat to humans. Deworming through routine, hygiene, and education of the owner is suggested to lower the infection and safeguard the health of people. This research offers valuable baseline information to control measures in the region in the future.

Keywords: Gastrointestinal parasites, pet cats, prevalence, Toxocara, Ancylostoma, Giardia, Isospora, Upper Dir

Heavy Metal Analysis in Different Organs of Clupisoma naziri Collected from River Kabul at Sardaryab, District Charsadda, Pakistan

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This study investigates the presence and distribution of heavy metals, including Cobalt (Co), Chromium (Cr), Zinc (Zn), Copper (Cu), and Iron (Fe), within various organs of *Clupisoma naziri* collected from River Kabul at Sardaryab, District Charsadda, Pakistan. Through meticulous analysis, the concentrations of these metals were examined across the gills, liver, skin, intestines, muscles, and stomach of the fish samples. The findings elucidate distinctive organ-specific accumulation patterns for each metal. Notably, the highest concentrations were observed in the intestines for Chromium, Copper, and Iron, indicating a potential role of this organ as a primary site for metal accumulation. The liver exhibited substantial accumulation of Iron and Copper, underscoring its significance as a storage organ for these metals. Conversely, Zinc displayed considerable concentrations in the gills and skin, suggesting potential accumulation related to environmental exposure or specific physiological functions. These outcomes emphasize the intricate distribution of Cobalt, Chromium, Zinc, Copper, and Iron within *Clupisoma naziri*, accentuating the importance of continual monitoring to assess the ecological impact and potential risks associated with heavy metal accumulation in aquatic organisms.

Keywords: *Clupisoma naziri,* Heavy Metals, River Kabul, Toxicity, Fish

Association of single nucleotide polymorphism, rs1800472 in TGF beta 1 gene with the incidence of chronic obstructive pulmonary disease in subjects enrolled from Multan: a case-control study.

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Chronic obstructive pulmonary disease (COPD) is a complex and progressive respiratory disorder characterized by persistent respiratory symptoms and airflow limitations that result from airway and alveolar abnormalities. The development of COPD is driven by combined effects of genetic vulnerability and environmental exposures, especially long-term smoking. Transforming Growth Factor Beta 1 (*TGF-β1*) is a multifunctional cytokine that plays an important role in regulating cellular growth, differentiation and immune responses and has been implicated in airway remodeling and chronic inflammation. Several single nucleotide polymorphisms (SNPs) have been identified in the *TGF-β1* gene, and these genetic variations have been associated with various pathological conditions; however, the genotypes at rs1800472 have been rarely investigated in relation to COPD. This study aimed to investigate the genotype and allelic frequency at SNP rs1800472 (C > T) in *TGF-β1* and to correlate them with the incidence of COPD. Following their informed verbal consent, a total of 220 clinically confirmed COPD patients and age and sex-matched controls (n = 220) were enrolled from Multan District during September 2023 till December 2024. Genotyping of rs1800472 was performed by using the Tetra-primer Amplification Refractory Mutation System-Polymerase Chain Reaction (T- ARMS). Data analysis revealed that genotypic frequency varied highly significant (P = 0.004) when compared between COPD patients and controls and a significant association between the polymorphic TT genotype at rs1800472 and incidence of COPD was observed. .. Subjects having this polymorphic genotype were at 2.13 time higher risk of developing COPD than those having wild CC genotype. While allelic frequency did not varied significantly (P = 0.9) between control and cases. Risk factor analysis indicated that male (P = 0.005), subjects in the age range of 51-65 years (P = 0.004) and smokers (P < 0.001) were more prone to develop this disease. In conclusion, the "TT" genotype at rs1800472 in the *TGF-β1* gene may influence COPD progression in the Pakistani population. Further large-scale and functional studies are needed to confirm this association and clarify its clinical relevance.

Effect of Heavy Metals on the Proximate Meat Quality in Cyprinus carpio Collected from Selected Tributaries of River Kabul, District Charsadda, Pakistan

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Heavy metals adversely impact on the biota and meat quality as well. The current study was design to examine the health hazards of H.M. in the gills, liver, digestive tract, skin, and muscles of *Cyprinus carpio*, as well as the impact these risks had on the quality of meat derived from tributaries of the River Kabul. The chosen tributaries were Shah Alam, Naguman, and Sardaryab. Lead, copper, nickel, zinc, and cadmium were the Heavy Metals that were selected, in that order. The data was examined using SPSS software for heavy metal and meat quality assessments. It was determined that different organs in the fish samples had different distributions of Heavy Metals Heavy Metals. The results demonstrate that the levels of H.M. in various *C. carpio* organs collected from tributaries of the River Kabul varied. The gills of *C. carpio* had the most Heavy Metals compared to other organs such as the liver, digestive tract, skin, and muscles. Current research indicates that the tributaries of the River Kabul are negatively impacted by heavy metal deposition via a number of routes. The study also emphasizes the detrimental impacts of heavy metal pollution on *C. carpio* and its consequences for meat quality. These findings demonstrate how urgently habitat restoration programs and effective pollution management strategies are needed to safeguard aquatic ecosystems and all of their inhabitants, including C. carpio.

Keywords: Heavy Metals, *Cyprinus carpio*, Water quality, River Kabul, Amount

Molecular Phylogenetic study of Subfamily Coccinellinae (Coleoptera: Coccinellidae: Coccinellinae) of Malakand Region Pakistan

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The family Coccinellidae, consists of 6 subfamilies, are mostly predatory and phytophagous in nature, considered as biological control, which are distributed worldwide, including Pakistan. In our research we have reported only 26 species of Ladybirdbeetle and 15genera of subfamily Coccinellinae have been molecularly identified. The current study was from April 2022 to April 2023, a total of 725 samples were collected from Malakand region Pakistan, that were morphologically grouped. DNA extraction, PCR amplification through LCO1490 and HCO2198 were employed, Sanger Sequencing was carried out, and phylogenetic analysis was performed by use of different software. Based on morphological characteristics, collected samples of are *Coccinella septempunctata* (Linnaeus, 1758), *Coccinella transversalis* (Fabricius, 1781), *Adalia bipunctata* (Linnaeus, 1758), *Adalia tetraspilota* (Hope, 1831), *Coccinella novemnotata* (Herbst, 1793), *Menochilus sexmaculatus* (Fabricius, 1781), *Propylea dissecta* (Mulsant, 1850),*Propylea japonica* (Thunberg, 1781), *Coelophora bissellata* (Mulsant, 1850), *Angelies cardoni* (Weise, 1900), *Illies confusa* (Mulsant, 1850), *Illeis cincta* (Fabricius, 1798), *Coccinella undecimpunctata* (Linnaeus, 1758), *Oenopia conglobate* (Linnaeus, 1758), *Oenopia sauzuti* (Mulsant, 1866), *Oenopia mimica* (Weise, 1902), *Aiolocaria hexaspilota*(Hope, 1831),*Hippodamia (Adonia) variegata* (Goeze, 1777), *Calvia punctata*(Mulsant, 1850), *Psyllobora bisoctonotata* (Muslant, 1850),*Psyllobora vigintduopunctata* (Linnaeus, 1758), *Lemnia bisselata* (Mulsant, 1850), *Harmonia dimidiata*(Fabricius, 1781),*Harmonia axyridis* (Pallas, 1773), *Cryptogonus nepalensis* (Bielawski, 1972) and *Propylea quattuordecimpunctata* (Linnaeus, 1758)were identified as, and molecular phylogenetic analysis endorsed the morphological results. In the study, we have molecularly identified 26 species and 15 genera of Subfamily Coccinellinae. In which three new species of Subfamily Coccinellinae namely*Illeis confuse* (Mulsant, 1850), species wasadded to NCBI database for the first time.

Keywords: Coccinellidae, Subfamily Coccinellinae, DNA barcoding, Phylogenetic analysis, Malakand, Pakistan

In vitro evaluation of the individual and synergistic antibacterial effects of Withania coagulans and Withania somnifera against multidrug-resistant fish-borne Escherichia coli

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Rapid population growth, climate change, and the depletion of wild fish stocks are increasingly threatening global food security, highlighting aquaculture as an essential source of animal protein. The rise of multidrug-resistant (MDR) pathogens like *Escherichia coli* presents a significant hazard to fish health and public safety, requiring the creation of sustainable, plant-derived antimicrobial alternatives. This research assessed the antibacterial activity of *Withania coagulans* fruit extract and *Withania somnifera* leaf extract, both separately and in combination, against MD*R E. coli* obtained from *Labeo rohita*. Plant extracts were obtained using methanol, ethanol, and acetone, and their antibacterial properties were assessed via agar well diffusion tests. These extracts showed considerable inhibitory effects against MDR *E. coli*, with methanolic extracts displaying relatively greater activity. Notably, the simultaneous use of *W. coagulans* and *W. somnifera* demonstrated improved antibacterial effectiveness, resulting in larger inhibition zones compared to separate treatments and suggesting a synergistic effect. The analysis of plant extracts using gas chromatography–mass spectrometry (GC–MS) identified a variety of bioactive compounds, such as alkaloids, flavonoids, phenolic compounds, terpenoids, and withanolides, recognized for their antimicrobial effects. These compounds might influence their effects by disturbing bacterial cell membranes, blocking vital enzymes, and disrupting microbial metabolic pathways. These results indicate that *Withania* species, especially when used together, offer a hopeful, environment friendly option for managing bacterial infections in aquaculture and may help alleviate the rising challenge of antimicrobial resistance.

Keywords: Aquaculture; Multidrug-resistant *E. coli*; *Withania coagulans*; *Withania somnifera*; Antibacterial activity; Synergistic effect; GC–MS analysis

Association of the ABO Blood Group System in adults of the Tehsil Matta, District Swat

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Hypertension is one of the serious health issues all over the world. According to the World Health Organization (WHO) that global hypertension mortality rate is 13%. Blood pressure is higher than normal, known as hypertension. High blood pressure has been considered as “silent killer” due to no sign and symptoms in early stages, and due to this, an individual may develop prehypertension, and then the individual becomes hypertensive. The main objective of the current study was to identify and highlight the relationship between hypertension and blood groups in adults. The complete study has been carried out in the tehsil Matta, district Swat, Khyber Pakhtunkhwa, Pakistan. In six months, the data collection was carried out from January 2020 to June 2020 through a well-designed multifactorial questionnaire; a total of n=157 individuals, including both males and females, were identified during the study duration. The blood pressure of each person was measured by a digital sphygmomanometer (wrist monitor), and a three-times check-up of the blood pressure of a single individual was carried out with an interval of time, and the average of those three check-ups was considered the actual result of an individual's blood pressure. The blood groups were identified by anti-sera: anti-A, anti-B, and anti-D on an aseptic transparent glass slide. According to the current study, in both Rh-positive and Rh-negative individuals, blood group B (11.46 %) individuals have had prehypertension in both sexes. While hypertension was also found in both sexes in blood group B (5.0 %) followed by A (4.45 %), AB (3.82 %), and O (3.18 %).

This study concluded that blood group B showed a greater tendency to develop hypertension, followed by blood group A, AB, and O. Among blood group B individuals, prehypertension was observed in the 20-30-year age group, classified as lower middle. The identified individuals were found to be more vulnerable to heart diseases such as stroke, heart attack, heart failure, and kidney failure.

Keywords: Hypertension, Blood Group, Matta, WHO

Impact of Cypermethrin on Hematology and Mortality of Ctenopharyngodon idella Under Control System

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Toxic insecticides like cypermethrin are used to manage pests. The current study set out to carefully examine the effects of varying Cypermethrin Amounts at various time points on the mortality ratio and haematology of *Ctenopharyngodon idella* fish. After being kept in a glass aquarium under a control system, the fish were exposed to varying Amounts of Cypermethrin at various intervals. A hematology analyzer was used to examine the fish blood samples. The resulting results showed that exposing the fish to the acute and chronic toxicity of cypermethrin had an effect on the haematological markers. The resulting data showed that only the tested group (group 3) had a mortality ratio for 20 days (n=1) following the fish's exposure to chronic poisoning. This demonstrates that the mortality is directly related to the lengthening of the time interval. White blood cell counts, Red Blood Cell, platelets, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin Amount are the haematological changes, accordingly. To determine the toxicity range at different Amounts, these haematological parameters were investigated. The data shows Cypermethrin is one of the most harmful pesticides used in agriculture. The toxicity of cypermethrin will cause the fish population to diminish daily in the near future if agricultural operations exceed their allowable limitations. Cypermethrin essentially changes the biochemistry of fish blood, which affects the fish's growth and overall body composition.

Keywords: Cypermethrin,, pesticides, toxicity, mortality, hematology, Acute, Chronic

Prevalence of Beta Thalassemia and other Hemoglobinopathies in suspected Individuals visiting Fatmid Foundation Peshawar, Pakistan

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β-thalassemia is a common genetic hemoglobin state which is considered to be major health problem in Pakistan due to its high occurrence rate, long lasting consequences and transfusion reliance. In order to compare β-thalassemia major and β-thalassemia minor, this study sought to determine the prevalence of β-thalassemia subtypes and examine hematological parameters among diagnosed individuals. 349 patients with confirmed β-thalassemia participated in a cross-sectional analytical investigation. Descriptive statistics and inferential tests like one-way ANOVA and non-parametric techniques were used to analyze hematological parameters such as hemoglobin (Hb), hemoglobin A (HbA), fetal hemoglobin (HbF), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The results indicated that β-thalassemia minor accounted for 7.45% of the study population, whereas β-thalassemia major accounted for 92.26% of cases. There were no statistically significant differences between β-thalassemia major and minor for Hb, HbA, HbF, MCV, and MCHC, according to comparative analysis. Only the MCH values showed a marginally significant difference. The two subgroups showed significant overlap in regular hematological indices, especially in transfusion-dependent patients, suggesting that standard blood measures have limited discriminating power. Furthermore, hemoglobin levels did not significantly differ according to gender or transfusion status, indicating great inter-individual variability influenced by treatment history and illness severity. The study concludes by highlighting the significant prevalence of β-thalassemia major in the community under study and stressing that routine hematological indices are not enough for precise subtype classification. The integration of molecular diagnostics, comprehensive screening procedures, and stronger preventive interventions is crucial to reduce disease burden and improve clinical treatment in high-risk populations.

Keywords: Blood Transfusion, β-thalassemia, Hemoglobin, Pathophysiology, Polymorphism

Molecular diagnosis of Cutaneous Leishmaniasis in District Shangla, Khyber Pakhtunkhwa, Pakistan

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Leishmaniasis is a vector-borne parasitic disease that is caused by the intracellular parasite of the family trypanosomae and the genus leishmania that spreads through the bite of infected female sand flies of the genra phlebotomus in the old word and in the new world. CL is the most common infection in the world. This study is designed to investigate the prevalence and molecular diagnosis in local population of district Shangla Khyber Pakhtunkhwah Pakistan. During this study five localities including Alpuri, Puran, Martung, Chakisar, and Besham were surveyed for sampling. A total of (n=108) samples were collected from (n=373) people randomly comprised of (218) males and (155) females by using slide smears, exudate in tubes and filter paper impressions from lesions. Different age groups were also studied and the results show that the age group from 11-20 years was the highest effected group of the 101(27.1%). Among the gender males were mostly effected. 34.3% individuals having lesions on their hand and legs. Out of 108 collected samples from patients, 82(75.92%) found positive by microscopic examination, while 12 samples were examined by ITS1-PCR assay and 10 (83.33%) confirmed by kDNA-PCR. Hence, it's concluded that CL is prevalent in Shangla district's population. In addition, kDNA-PCR is more sensitive and specific method for parasite identification followed by microscopy.

Keywords: Cutaneous leishmaniasis, DNA, Khyber Pakhtunkhwa, leishmania parasite, PCR, Shangla

Prevalence of anaplasmosis babesiosis and theileriosis among cattle reared in harichand dairy farm and adjacent areas of tehsil Tangi Pakistan

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Tick-borne diseases (TBDs) remain a major constraint to cattle productivity in Pakistan, particularly in regions where climatic and management conditions favor tick proliferation. This study assessed the prevalence and risk factors associated with the transmission of anaplasmosis, babesiosis, and theileriosis among cattle reared at Harichand Dairy Farm and adjoining rural areas of Tehsil Tangi, District Charsadda. A cross-sectional survey was conducted in 2025, involving 735 cattle selected through stratified random sampling. Blood samples were examined using Giemsa-stained smears, while epidemiological and tick related determinants were evaluated using multivariate logistic regression, GLMM, Random Forest models, and Bayesian network analyses. The overall prevalence of TBDs was 59.1%, with theileriosis (28%) being the most dominant hemoparasitic infection. Mixed infections—particularly Anaplasmosis + Theileriosis—were frequently observed. Host-associated risk factors significantly influenced disease occurrence: females had higher odds of infection (OR = 4.67, $p < 0.001$), Achai cattle exhibited 8.4 times greater risk compared to Friesian breeds ($p < 0.001$), and adults and young cattle were more susceptible than calves ($p < 0.05$). Tick-related risk factors played a central role in disease transmission. High tick infestation levels— particularly of *Hyalomma* and *Rhipicephalus* species—were more common among cattle managed under free-grazing and low-hygiene systems. Climatic suitability (high summer temperatures and humidity) further enhanced tick survival and pathogen transmission. Machine-learning models identified gender, breed, tick load, and management system as the strongest predictors of infection, while network analysis highlighted *Anaplasma spp.* as the core pathogen within co-infection clusters. The study concludes that the high burden of TBDs in the study area is driven by a combination of host susceptibility, inadequate tick-control practices, and ecological conditions favorable to vector expansion. Strengthening integrated tick management, improving farm hygiene, enhancing veterinary diagnostic capacity, and promoting breeding for tick-resistant genotypes are recommended to reduce disease transmission and improve cattle health and productivity.

Keywords: Tick-borne diseases; Anaplasmosis; Babesiosis; Theileriosis; Cattle; Prevalence; Risk factors; Harich and Dairy Farm; Tehsil Tangi; Epidemiology; Hemoparasites; Pakistan;

Evaluation of Heavy Metals in Water, Sediments and Schizothorax plagiostomous Collected from Streams of Dargai Malakand, Pakistan

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This study investigated heavy metals level in aquatic environment, sediments deposition in specific physique organs, of *Schizothorax plagiostomus* in Dargai tehsil of Malakand. Atomic absorption spectrometry was employed for sample analysis spectroscopy to determine the occurrence of metallic elements, including cadmium, chromium, copper and zinc. High concentration of contaminants were detected both within the aquatic environment and in the biological tissues of *schizothorax plagiostomus*. Maximum levels of heavy metals remained found in skin, gills besides muscles indicating bioaccumulation. The results showed that the concentration of heavy metals charted the following pattern within water samples: zinc > copper > chromium > cadmium, while in sediments zinc > copper > chromium > cadmium. Zinc > Copper > Chromium > Cadmium The concentration of heavy metals in different tissues of fish samples followed the following concentration order: Zinc > Copper > Chromium > Cadmium. Analysis of water and sediments revealed high concentrations of heavy metals, suggesting that industrial and human activities ,including mining ,smelting ,combustion, wastewater generation, and metal processing, are key contributors. These findings indicate the severe risks associated with heavy metal pollution, underscoring the urgent need for continuous environmental surveillance and the implementation of effective pollution mitigation measures to protect aquatic resources and public health in the freshwater reservoirs of Malakand District.

Keywords: *Schizothorax*, Heavy Metals, Water, Sediments, pollution.

Prevalence and Seasonal Diversity of Haemoparasites in Rodents of Dir Upper, Pakistan: Implications for Climate-Responsive Zoonotic Disease Surveillance

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Rodents are principal reservoirs for haemoparasites of zoonotic concern, and climate change is predicted to intensify disease transmission by altering vector population dynamics and extending seasonal activity. This study reports the first systematic survey of haemoparasite prevalence and diversity in rodents inhabiting Tehsil Sheringal, District Dir Upper, Khyber Pakhtunkhwa, Pakistan. A total of 56 rodents were live-captured across a one-year period, comprising four species: *Rattus rattus* (n=33), *Rattus norvegicus* (n=10), *Mus musculus* (n=12), and *Dryomys nitedula* (n=1). Blood samples were collected and thin smears were stained with Giemsa for microscopic examination. An overall haemoparasite prevalence of 64.29% (36/56) was documented. Five distinct parasite species were identified: *Trypanosoma lewisi* (42.85%) was most prevalent, followed by *Anaplasma marginale* (19.64%), *Babesia microti* (14.28%), *Plasmodium berghei* (7.14%), and *Anaplasma centrale* (5.35%). Mixed infections accounted for 25% of positive cases. A pronounced seasonal effect was observed, with prevalence peaking significantly in summer (84.61%) compared to winter (46.66%), underscoring the critical role of temperature and vector abundance in transmission dynamics. *Rattus rattus* exhibited the highest infection burden (50.00% overall). These findings establish essential baseline data for the region and emphasize the potential for climate-driven shifts to amplify haemoparasite circulation in rodent populations. Continued surveillance integrating molecular diagnostics is recommended to inform public health preparedness in the context of environmental change.

Keywords: Rodentia, Haemoparasite Prevalence, *Trypanosoma lewisi*, Seasonal Variation, Climate Change, Dir Upper

Phenotypic Plasticity and Meristic Stability in Labeo rohita Across a Hydrological Gradient in Kohat, Pakistan: Baselines for Climate Adaptation

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Environmental heterogeneity drives morphological variation in freshwater fish, yet region-specific data on phenotypic plasticity in response to hydrological gradients remains scarce in semi-arid Pakistan. This study assesses how lentic, lotic, and controlled hatchery conditions influence the morphology of the commercially vital Rohu (*Labeo rohita*) to inform climate-resilient aquaculture strategies. A total of 45 adult specimens (n=15 per habitat) were collected from Tanda Dam, Toi River, and a Government Hatchery in District Kohat. Nineteen morphometric characters (standardized to total length) and six meristic counts were analyzed using digital calipers and standard statistical descriptors. Significant morphometric divergence was observed across habitats. Hatchery specimens achieved the greatest mean total length (45.10 cm) and fin development, indicating optimized growth in controlled environments. Riverine specimens exhibited the highest mean body weight (290.00 g) and streamlined profiles, reflecting lotic adaptation. Dam populations consistently displayed the most compact morphology (Mean TL: 36.70 cm). Conversely, meristic characters—including lateral line scales (40–44) and fin rays—remained highly conserved across all groups, confirming the taxonomic integrity of local stocks. *L. rohita* exhibits high morphometric plasticity in response to environmental conditions while maintaining genetically fixed meristic structures. The distinct morphological signature of the riverine stock underscores the need to conserve wild gene pools as reservoirs of adaptive traits for climate change resilience. These findings establish critical baseline data for habitat-specific management and selective breeding in an era of increasing hydrological variability.

Keywords: *Labeo rohita*, Phenotypic Plasticity, Morphometrics, Climate Adaptation, Stock Structure, Aquaculture Management, Kohat

Seasonal Effects on Icthyofaunal Diversity in River Swat and its Tributaries

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The study's main objective was to ascertain how the climate affected the ichthyofunal diversity in the River Swat and its tributaries. 1835 fish samples were chosen at random from the river and its tributaries during the spring and summer months of March through July 2024, and they were identified through established identification keys. Out of 1835 specimen, 977 belonged to the Cyprinidae family, 627 were from the Nemacheilidae, 45 were from the Sisoridae, 57 were from the Channidae, 53 were from the Mastacembelidae, and 76 were from the Salmonidae. The current study found 29 species from the River Swat and its tributaries, representing 15 genera, 6 families, and 5 orders With 15 species, the Cyprinidae family was found to be the most dominant.. The most common species were *Triplophysa naziri*, *Schistura naseeri*, *Garra Gotyla*, *Crossochelius diplocheilus*, and *Shizothorax plagiostomus*, while the least common and endangered species were *Tor putitora*, *Glyptosternon reticulatum*, *G. maculatum*, and *Glyptothorax naziri*, In particular, *Tor putitora* faces extinction. Some once common fish species are now extinct due to habitat degradation, pollution, illegal fishing, and the floods in 2010 and 2022. The mean temperature was (11.35°C), the pH was (7.15), the electrical conductivity was (96µS/cm), the TDS was (61.28 mg/l), and the dissolved oxygen was (7.63 mg/l) when the water quality was measured in the spring. These levels slightly increased in the summer but remained within acceptable limits, demonstrating that the reduction of species was not primarily caused by water quality. Species richness and abundance were positively correlated with water temperature, which increased in the summer and decreased in the winter. The Shannon-Wiener diversity index (H'), which ranges from 4.39 in the spring to 4.421 in the summer, reflects this seasonal change. This demonstrates how fish diversity is dynamic and adapts to changes in the environment. Overfishing and habitat degradation were the most important factors affecting species diversity. We can maintain ichthyofunal diversity by protecting endangered species, managing fisheries, restoring ecosystems, and establishing community-based water quality monitoring.

Keywords: Icthyofauna, diversity, Seasonal variation, River swat, tributaries, cyprinidae

Avian Biodiversity Assessment in Dara Adam Khel, District Kohat: Implications for Conservation in a Transitional Ecotone

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Avian biodiversity serves as a key indicator of ecosystem health, particularly in ecologically sensitive transitional zones. Dara Adam Khel, located in District Kohat, represents a unique ecotone between semi-arid plains and the north-western Himalayan foothills. Despite its ecological significance, the region has remained largely unexplored in terms of avifaunal diversity. This study aimed to document bird species composition, ecological roles, and habitat associations in the area, while identifying potential conservation concerns. The study was conducted over four months (November 2024 to February 2025) across six selected sites representing mountainous/forested and residential habitats. Standard ornithological survey techniques, including point counts, transect walks, and photographic documentation, were employed. Species identification was verified using regional field guides and taxonomic references. Data were collected on species richness, residency status, habitat preference, and ecological guild classification. A total of eleven bird species were recorded, representing six orders, eleven families, and twelve genera. Four species—*Garrulus lanceolatus* (Black-headed Jay), *Dendrocitta vagabunda* (Rufous Treepie), *Anthus similis* (Long-billed Pipit), and *Pycnonotus leucogenys* (Himalayan Bulbul)—were documented for the first time in the region, extending their known distribution range. The majority of species belonged to the order Passeriformes. Observed birds were categorized into diverse ecological guilds, including omnivores, insectivores, granivores, and scavengers, indicating functional ecological diversity. Certain species, such as *Bubulcus ibis* (Cattle Egret), exhibited sensitivity to environmental conditions and were observed only during rainfall events. Overall species diversity was comparatively low relative to nearby regions. The study highlights Dara Adam Khel as an ecologically valuable yet underexplored region with limited avian diversity potentially impacted by anthropogenic pressures such as habitat degradation, overgrazing, climate change, and noise pollution. The findings emphasize the need for long-term biodiversity monitoring, habitat conservation, and strategic policy interventions to protect and sustain avian populations in this transitional ecosystem.

Keywords: Avian biodiversity, Dara Adam Khel, ecotone, bird diversity, habitat degradation, conservation biology

Harnessing Stem Cell Derived Extracellular Vesicles for Vision Restoration

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Oxidative stress (OS) plays a pivotal role in the pathogenesis of age-related macular degeneration (AMD) and other ocular disorders. Mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) offer a promising, cell-free therapeutic approach due to their antioxidant and regenerative properties. To generate clinical-grade MSCs from human umbilical cord (hUC) tissue and evaluate the regenerative potential of their EVs in an in vitro AMD model. Ethical approval was obtained for processing human umbilical cord (hUC) tissue. MSCs were isolated and characterized by Immunophenotyping, immunocytochemical staining, trilineage differentiation, population doubling time and number, gene expression profiling for proliferation and cell cycle progression, a senescence-associated β-galactosidase assay, human telomerase reverse transcriptase (hTERT) expression, and detection of mycoplasma, cytomegalovirus, and endotoxin. EVs were isolated from hUC-MSCs by differential centrifugation and characterized using fluorescence microscopy, scanning electron microscopy (SEM), atomic force microscopy (AFM), and dynamic light scattering (DLS). Oxidative stress was induced in RPE cells, as confirmed by the H_2DCFDA assay, and the therapeutic effects of EVs were subsequently evaluated. Analysis of pluripotent gene markers *Oct4*, *Sox2*, and *Nanog* in hUC-MSCs revealed no significant differences in culture conditions. The immunophenotypic markers CD90, CD73, CD105, CD44, vimentin, CD29, Stro-1, and Lin28 were positively expressed by MSCs. Proliferative gene expressions of *Pax6*, *BMP2*, and *TGFb1* showed no significant variation between hUC-MSC groups. Nevertheless, a significant increase ($P < 0.001$) in the mitotic phase of the cell cycle was observed in hUC-MSCs. Cellular senescence markers (hTERT expression and β-galactosidase activity) did not show any negative effect on hUC-MSCs. Additionally, quality control assessments consistently confirmed the absence of mycoplasma, cytomegalovirus, and endotoxin. DLS, SEM, and AFM topography revealed vesicles with an average size of 181.65 ± 11.49 nm and a zeta potential of -10.31 ± 1.692 mV. Treatment with EVs significantly reduced intracellular ROS in RPE, restored cell viability, and promoted migration and proliferation. Uptake experiments confirmed efficient internalization of EVs by RPE. The scratch assay demonstrated accelerated wound closure in EV-treated groups. Gene expression analysis further showed that EVs upregulate oxidative stress markers, indicating a protective effect against oxidative damage. MSC-derived EVs demonstrate potent antioxidant and regenerative effects on RPE cells, supporting their potential as a safe, cell-free therapy for AMD. This approach may overcome limitations of stem cell transplantation and accelerate clinical translation in ophthalmology.

Immunogenomics and Extracellular Vesicle–Driven Vaccine Target Discovery Linked with One Health Surveillance of Zoonotic Poultry Pathogens and Environmental Transmission Dynamics in Pakistan

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Poultry farming incurs huge economic costs due to increased mortality, reduced productivity, and higher treatment costs, which are further exacerbated by the growing burden of antimicrobial resistance (AMR). In Pakistan, major bacterial pathogens that includes, *Escherichia coli*, *Salmonella spp.*, *Mycoplasma spp,*, *Pasteurella spp*, and *Citrobacter spp.* poses the significant threat to both animal and public health, particularly in regions affected by climate stress and limited biosecurity. Bacterial extracellular vesicles (BEVs) are physiologically active, cell-free lipid bilayer nanostructures that carry genetic material including, immunogenic components, virulence factors, and antimicrobial resistance genes. These vesicles play a crucial role in inter-bacterial communication, horizontal gene transfer, and environmental persistence. This study aims to isolate, characterize, and perform genomic analysis of BEVs derived from locally circulating poultry pathogens and to investigate their role in environmental persistence, zoonotic transmission, and disease dynamics within a One Health framework. Poultry pathogen isolates will be collected from diverse geographic regions of Pakistan and identified through biochemical assays and 16S rRNA sequencing. BEVs will be extracted and characterized using different analytical techniques. Subsequently, immunogenomics and BEV genomics techniques will be used for the downstream analysis. This study is expected to elucidate the role of BEVs in the maintenance and dissemination of antimicrobial resistance genes across microbial populations and environmental reservoirs. It will provide insights into BEV biogenesis, cargo selectivity, and their contribution to pathogen survival and transmission. Additionally, the identification of conserved immunogenic components within BEVs may support the development of novel cell-free vaccine candidates and immunotherapeutic strategies targeting poultry pathogens. By integrating BEVs in immunogenomics, this study establishes a novel One Health oriented framework linking microbial resistance, zoonotic risk, and environmental dissemination. The findings will contribute to sustainable disease control strategies, reduced antibiotic dependence, and the development of next-generation vaccines, ultimately supporting poultry industry resilience and public health protection in Pakistan.

Keywords: Bacterial Extracellular Vesicles (BEVs), Antimicrobial Resistance (AMR), Poultry Pathogens, One Health, Immunogenomics, Vaccine Development

In Silico Mapping of the Nipah Virus G-Protein Mutational Landscape Reveals Changes in Receptor Affinity and Neutralization Resistance

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The Nipah virus remains a highly pathogenic zoonotic agent with pandemic potential, largely mediated by its surface attachment glycoprotein (G), which governs host receptor recognition and viral entry. In this study, we performed a comprehensive in silico mapping of the mutational landscape of the Nipah virus G glycoprotein to investigate the structural and functional consequences of amino acid substitutions on receptor binding and immune escape. A panel of reported and computationally predicted mutations was modeled, followed by structural refinement and stability assessment. Protein–protein docking analyses were conducted to evaluate the binding affinity of mutant G proteins with host receptors Ephrin-B2 and Ephrin-B3. Several mutations were found to significantly enhance or reduce receptor-binding affinity, indicating their potential role in viral transmissibility and host adaptation. Molecular dynamics simulations further revealed mutation-induced conformational fluctuations affecting key binding interfaces. In parallel, epitope mapping and antibody docking analyses suggested that specific mutations may contribute to reduced neutralizing antibody recognition, highlighting mechanisms of immune evasion. Collectively, these findings provide critical insights into the evolutionary adaptability of Nipah virus and identify key residues that may influence receptor specificity and neutralization resistance. This study offers a structural framework for surveillance of emerging variants and supports the rational design of vaccines and therapeutic interventions targeting the G glycoprotein.

Keywords: Nipah virus, G glycoprotein, Neutralization resistance

Assessment of Antibacterial Activity of Nigella sativa Extract Versus Erythromycin on Staphylococcus aureus Isolated from Cyprinus carpio

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Staphylococcus aureus is a significant pathogenic bacterium responsible for a wide range of infections in both humans and animals. The growing resistance of this organism to commonly used antibiotics, including erythromycin, has become a major public health concern. Plant-based alternatives, such as *Nigella sativa*, are increasingly being explored due to their bioactive compounds with antimicrobial properties. This study aimed to evaluate the antibacterial efficacy of aqueous extract of *N. sativa* in comparison with erythromycin against *S. aureus* isolated from naturally infected *Cyprinus carpio*. Bacterial isolates were obtained from infected *C. carpio* and identified using standard microbiological

and biochemical techniques, including Gram staining. The antibacterial activity of *N. sativa* extract was assessed using the agar well diffusion method at concentrations ranging from 0.75 to 1.5 mg/ml. Erythromycin was used as a control antibiotic to compare susceptibility patterns. Zones of inhibition were measured to determine antibacterial effectiveness. The aqueous extract of *N. sativa* demonstrated a clear dose-dependent antibacterial effect against *S. aureus*. The highest concentration (1.5 mg/ml) produced the largest inhibition zone of 30.2 mm, indicating strong antibacterial activity. Lower concentrations showed comparatively smaller zones of inhibition. In contrast, erythromycin exhibited no inhibitory effect, confirming resistance of the bacterial isolate. These findings suggest that *N. sativa* extract is effective even against antibiotic-resistant strains. The study highlights the potent antibacterial activity of *Nigella sativa* against erythromycin-resistant *Staphylococcus aureus*. The presence of bioactive compounds, such as thymoquinone, may contribute to its efficacy. These results support the potential use of *N. sativa* as a natural and effective alternative to conventional antibiotics in both aquaculture and clinical applications. Further in vivo investigations and detailed phytochemical analyses are necessary to confirm its safety and therapeutic potential.

Keywords: *Nigella sativa, Staphylococcus aureus*, antibacterial activity, erythromycin resistance, phytotherapy, aquaculture

Comparative In Vitro Antibacterial Activity of Nigella sativa Extract and Ciprofloxacin

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The emergence of antibiotic resistance has intensified the search for effective plant-based antimicrobial agents. *Nigella sativa*, known for its rich phytochemical composition, has been widely studied for its therapeutic properties. This study aimed to compare the antibacterial activity of *N. sativa* extract with the standard antibiotic ciprofloxacin using an in vitro approach. The antibacterial activity was evaluated using the agar diffusion method. *N. sativa* extract was tested at three concentrations (0.75, 1.25, and 1.5 mg/ml), while ciprofloxacin was used as a control. Zones of inhibition were measured in millimeters across three independent trials, and mean values were calculated. Statistical analysis was performed to determine the significance of differences between treatments. Ciprofloxacin demonstrated the highest antibacterial activity, with a mean inhibition zone of 34.1 mm across all trials. The *N. sativa* extract also exhibited substantial antibacterial effects in a concentration-dependent manner. At 0.75 mg/ml, the mean inhibition zone was 27.3 mm, which increased to 28.1 mm at 1.25 mg/ml and 28.6 mm at 1.5 mg/ml. Although the activity of *N. sativa* was slightly lower than ciprofloxacin, the results showed a consistent increase with higher concentrations. Statistical analysis indicated that the observed differences were highly significant ($p = 0.000 < 0.05$). The findings confirm that *Nigella sativa* possesses strong antibacterial activity, though slightly less potent than ciprofloxacin. The significant, dose-dependent response highlights its potential as a natural antimicrobial agent. These results support further investigation into *N. sativa* as an alternative or complementary treatment to conventional antibiotics.

Keywords: Nigella sativa, ciprofloxacin, antibacterial activity, zone of inhibition, phytochemicals, antimicrobial resistance

In Vitro Evaluation of Antibacterial Activity of Methanolic Mucus Extract from African Catfish (Clarias gariepinus) Against Aeromonas hydrophila

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The increasing resistance of pathogenic bacteria to conventional antibiotics has prompted the search for alternative antimicrobial sources. Fish mucus is known to contain a variety of bioactive compounds that play a protective role against microbial infections. This study aimed to assess the antibacterial potential of methanolic mucus extract obtained from healthy African catfish (*Clarias gariepinus*) against *Aeromonas hydrophila*, a common fish pathogen. Mucus samples were collected from healthy specimens of *C. gariepinus* and extracted using methanol. The antibacterial activity of the extract was evaluated in vitro using the agar diffusion method. Three different concentrations of the extract were tested against *A. hydrophila*. Zones of inhibition were measured in millimeters across three independent trials, and mean values were calculated. A standard antibacterial agent served as the positive control, while the solvent was used as the negative control. The methanolic mucus extract exhibited antibacterial activity at all tested concentrations. The lowest concentration produced a mean inhibition zone of 2.9 mm, while intermediate and highest concentrations showed mean values of 3.0 mm and 3.1 mm, respectively. The positive control demonstrated a mean inhibition zone of 2.3 mm, whereas no inhibition was observed in the negative control. A clear concentration-dependent increase in antibacterial activity was observed, with consistent results across all trials. The findings indicate that methanolic mucus extract of *C. gariepinus* possesses effective antibacterial properties against *A. hydrophila*. The dose-dependent response suggests the presence of potent bioactive compounds in fish mucus, highlighting its potential as a natural and sustainable antimicrobial agent in aquaculture and related fields.

Keywords: African catfish, fish mucus, methanolic extract, antibacterial activity, *Aeromonas hydrophila*, natural antimicrobials

Influence of Probiotic Supplementation on Growth Performance and Hematological Parameters in Broiler Chickens

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The poultry industry plays a crucial role in meeting the global demand for high-quality protein. With increasing concerns over the use of antibiotics, probiotics have emerged as a natural and safer alternative for enhancing poultry productivity and health. This study aimed to evaluate the effects of dietary probiotics on growth performance, organ development, and hematological parameters in broiler chickens. A total of four groups were established for this experimental study. The control group received no probiotic supplementation, while three treatment groups were administered probiotics at concentrations of 100 mg/L, 300 mg/L, and 500 mg/L, respectively. The trial was conducted over a period of 22 days. Parameters assessed included body weight, morphometric measurements, organ weights (liver, heart, lungs, and gizzard), and hematological indices such as red blood cells (RBC), hemoglobin (Hb), white blood cells (WBC), and platelets (PLT). Probiotic supplementation resulted in a dose-dependent improvement in growth performance and organ development. The highest body weight and organ growth were observed in the 500 mg/L group. Morphometric parameters also showed consistent enhancement with increasing probiotic levels. Hematological findings indicated that RBC and Hb levels remained stable in the 300 mg/L group, suggesting optimal physiological balance at this dosage. Variations were observed at higher concentrations, indicating the importance of controlled dosing. Overall, probiotic-treated groups demonstrated improved health and growth compared to the control group. The study concludes that dietary probiotics positively influence growth and physiological health in broiler chickens. Supplementation levels between 300 and 500 mg/L were found to be most effective, with 300 mg/L providing a balance between growth enhancement and hematological stability. These findings support the use of probiotics as a natural growth promoter in poultry production, offering a viable alternative to conventional additives.

Keywords: Broiler chicken, probiotics, growth performance, hematology, organ development, poultry nutrition

Assessment of Heavy Metals in Schizothorax labiatus, Water and Sediments Collected from River Siran Mansehra, Pakistan

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The present Research has been carried out to assess the comparative evaluation of the health risk Heavy Metals in water, sediments and various organs of *Schizothorax labiatus* in River Siran Mansehra, Pakistan. Comparison of the health hazards of heavy metal in *Schizothorax labiatus* gills, liver, intestinal, skin and muscle. This was through the use of lead, copper, nickel, zinc and cadmium as the selected heavy metals. To analyze the data, SPSS software was applied. The conclusion made was that the amount of heavy metals was different in water, sediments and fish samples. Gills of *Schizothorax labiatus* showed the greatest level of heavy metals in like skin muscles, liver and intestinal tract, The present resulted information shown that the heavy metal deposition by various

ways of path has adverse effect on the River Siran. These findings convey the seriousness of habitat restoration programs and effective control of pollution to preserve aquatic life.

Keywords: Heavy Metals, *Schizothorax labiatus,* Sediments, concentration, Health hazards

Acute and Chronic Toxicity of Cypermethrin on Hematology and Mortality of Carassius auratus

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The main aim of this study was to meticulously analyze the various concentrations of Cypermethrin at different time intervals on hematology and mortality ratio of *Carassius auratus* fish. The fishes were placed in the glass aquarium under a control system and then subjected to various concentration of Cypermethrin at different interval of times. The blood samples of the fish were analyzed by using Hematology analyzer. The resulting data revealed that the hematological parameters are impacted by exposing the fish to the acute and chronic toxicity of Cypermethrin. The resulting data demonstrated that mortality ratio was found only in the tested (group 3) for 48 hrs (n=1) and 72 hrs and (groups 3) for 72 hrs (n=2) after exposing the fish into the chronic toxicity. This shows that increasing the time interval is directly proportional to the mortality. The hematological alterations are White Blood Cell, Red Blood Cells, Platelets, Hemoglobin, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin Concentration respectively. These hematological parameters were examined to find out the toxicity range in various concentrations. The overall resulting data concluded that Cypermethrin is one of the most toxic pesticides used in agriculture. If agricultural activities cross their permissible limits in near future, the fish population will decline day by day due to the toxicity of Cypermethrin. Cypermethrin basically alter the fish blood biochemistry as a result the fish growth and overall body function are badly affected.

Keywords: Pesticides, Cypermethrin, Acute, Chronic, Toxicity, Mortality, Hematology

Prevalence and diversity of hemoparasites of birds in tehsil Lal Qila and tehsil Sheringal district lower Dir and upper Dir, KP, Pakistan

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Birds belong to the phylum Chordata, sub-phylum vertebrata and class Aves. Worldwide over 20000 different species of birds can be found, while in Pakistan there are 790 species of birds. Birds can be infected by different types of parasites, in which the most prevalent blood parasites belong to the genus *Plasmodium* and *Haemoproteous*. The aim of this work was to determine the prevalence and diversity of avian blood parasites in district Lower Dir and Upper Dir, KP, Pakistan. Out of 149 examined birds collected from two distinct sampling points Lal Qila (39/149) and sheringal (110/149), comprising 06 orders, 23 families and 42 species, 16 individuals were detected positive. An overall prevalence of blood parasites approximately 11% (16/149) was determined in 12 bird's species. The prevalence between the two sampling sites was different with higher prevalence in Sheringal 12.72% (14/110) compared to Lal Qila 5.12% (02/39). During microscopic study we found three different genera of parasites, *Haemoproteous* (12/16), *plasmodium* (01/16), and *Microfilaria* (03/16). Among these parasites the most prevalent was *Haemoproteous* 75% (12/16) infecting higher number of species (08/42) followed by *Microfilaria* 18.75% (03/42) and *Plasmodium* 6.25% infecting only one specie. The prevalence of haemoparasites was different among the twelve infected species. The highest prevalence was detected in Luscinia svecica 12.8% (04/05).

Keywords: Birds, Haemoparasites, Prevalence, Diversity, Lal Qila, Sheringal

PCR based amplification of 16S rRNA gene of Anaplasma capra in the sheep blood samples collected from six districts in Punjab

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Sheep population in Pakistan was approximately 37.2 million during 2023-24 and despite this huge population, sheep remained unexplored for the presence of majority of vector borne pathogens. Present study was aimed to report the molecular prevalence and phylogenetic evaluation of Anaplasma capra in the sheep blood samples (N = 272) that were collected from six districts (Muzaffargarh, Rajanpur, Dera Ghazi Khan, Khanewal, Layyah and Taunsa) in Pakistan during during August till December 2024. Molecular analyses revealed that 35/329 (11%) of the screened sheep amplified a 347 base pair fragment from 16S rRNA gene of Anaplasma capra. Anaplasma capra prevalence varied significantly between the sampling district (P = 0.007). Highest bacterial prevalence was detected in sheep from Taunsa district (20%) followed by Rajanpur (18%), Dera Ghazi Khan (12%), Layyah (10%), Khanewal (9%) and Muzaffargarh (3%). Similarly, the bacterial prevalence varied significantly among the sheep breeds but was not associated with herd size, presence of ticks on sheep or presence of dogs with herds or sex of sheep. In conclusion, we are reporting a higher prevalence of Anaplasma capra in Pakistani sheep and animals from all six districts were infected indicating that the bacteria are endemic. We recommend similar large-scale studies in various geo-climatic regions of Pakistan to further explore the epidemiology, genetic diversity, host-parasite interactions that will lead to effective control of this bacterial infection among the local sheep population.

Malariometric assessment and plasmodium species distribution in district Khyber, Khyber Pakhtunkhwa, Pakistan: implications for vector control strategies

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Malaria persists as a formidable public health challenge across Pakistan, yet its transmission dynamics in the remote, topographically diverse District Khyber of Khyber Pakhtunkhwa have remained largely undocumented. In an era when climate-driven shifts in rainfall, temperature, and vector ecology are reshaping disease landscapes, this study presents the first detailed malariometric assessment of the district, offering critical baseline data to inform targeted vector control amid accelerating environmental change. Between January 2021 and December 2022, a cross-sectional survey screened 30,337 individuals presenting with suspected malaria at government health facilities in Landikotal, Ali Masjid, and Jamrud. Using both rapid diagnostic tests and Giemsa-stained microscopy, we documented an overall positivity rate of 11.96% (3,629 positive cases). Plasmodium vivax overwhelmingly predominated (89.20%), followed by P. falciparum (9.18%) and mixed infections (1.63%); no P. malariae or P. ovale were detected. Males were marginally more affected (52.36%) than females, while children aged 1–15 years bore the highest burden. Transmission exhibited pronounced seasonality, peaking sharply in October 2021 (25.40%) and November 2022 (23.14%), periods coinciding with post-monsoon conditions that favor Anopheles breeding. These findings reveal a persistent P. vivax-dominated transmission landscape disproportionately impacting children and driven by seasonal environmental cues that are likely to intensify under climate change. By highlighting focal hotspots and demographic vulnerabilities, this research provides an evidence-based foundation for precision vector control—integrating larval source management, insecticide-treated nets, and strengthened surveillance—tailored to the ecological realities of this underserved high-risk district. Ultimately, such localized zoological and epidemiological insights are essential for resilient malaria control in the face of a changing climate.

Keywords: Plasmodium vivax; malaria prevalence; seasonal transmission; vector control

Molecular Detection of Toxoplasma gondii in cats of two Districts of Khyber Pakhtunkhwa, Pakistan

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Cats are among the most common domestic animals worldwide and serve as companions to humans. Due to frequent environmental exposure, they may carry various pathogens, including zoonotic parasites. Among these, *Toxoplasma gondii* is a particular importance because of its global prevalence, public health impact, and heteroxenous life cycle, involving felids as definitive hosts and warm-blooded animals, including humans, as intermediate hosts. Rising concerns over zoonotic infections and the lack of local data motivated this study to investigate the molecular prevalence and genetic diversity of *T. gondii* in domestic cats of Pakistan. This study aimed to address knowledge gaps regarding *T. gondii* in cats from Khyber Pakhtunkhwa (KPK) and evaluate potential risk factors associated with infection. Specifically, it assessed whether cat species, sex, body weight, or hematological parameters influenced parasite prevalence. A total of 144 blood samples were collected from domestic cats in two districts of KPK, Lower Dir (n = 74) and Buner (n = 70), from December 2024 to January 2025. Molecular detection was performed using Polymerase Chain Reaction (PCR) targeting a 300 bp fragment of the ITS-1 gene specific for *T. gondii*. Positive samples were confirmed through Sanger sequencing and BLAST analysis for comparison with global isolates. Risk factors and hematological profiles were analyzed to determine associations with infection. PCR results revealed a 6.9% prevalence (10/144 samples), indicating moderate infection rates. Genetic analysis showed high similarity with sequences reported worldwide. Statistical analysis indicated that infection was not associated with cat species, sex, body weight, or sampling site, but was linked to reduced red cell distribution width–coefficient of variation (RDW-CV) and platelet counts, suggesting potential hematological alterations in infected cats. In conclusion, this study provides the first molecular evidence of moderate *Toxoplasma gondii* prevalence in domestic cats from Pakistan. These findings highlight the need to include cats in zoonotic disease surveillance programs to reduce human infection risk and underscore the importance of large-scale studies across diverse geo-climatic regions to explore the parasite's genetic diversity, host interactions, and public health implications.

Keywords: cat, *Toxoplasma gondii*, PCR detection, genetic diversity, phylogenetic tree, zoonotic disease

Depression and Post-Traumatic Stress Among Children During the COVID Pandemic: Evidence from Parental Reports

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The COVID-19 pandemic has created unprecedented disruptions in the lives of children, including school closures, social isolation, and increased exposure to health-related stressors. Such conditions have raised concerns regarding the psychological well-being of children, particularly in relation to depression and post-traumatic stress symptoms. This study aimed to evaluate the prevalence and severity of these mental health issues among children aged 7 to 14 years based on parental observations. A descriptive quantitative research design was adopted for this study. Data were collected from 60 parents of children enrolled in GGCMS Heera Banda using structured questionnaires. The Short Mood and Feelings Questionnaire–Parent Version (SMFQ-P) was used to assess depressive symptoms, while the Impact of Event Scale–Revised (IES-R) measured post-traumatic stress-related symptoms. Demographic information, including parental education and occupational status, was also recorded to examine potential associations with children's mental health outcomes. Findings revealed that a substantial proportion of children exhibited mild to moderate levels of depressive symptoms, including persistent sadness, fatigue, restlessness, and feelings of loneliness. Similarly, post-traumatic stress symptoms such as intrusive thoughts, avoidance behaviors, heightened arousal, and sleep disturbances were reported at mild to moderate levels. The analysis further indicated that children from households with lower parental education levels and higher occupational stress were more susceptible to psychological distress. The study demonstrates that the COVID-19 pandemic has had a measurable impact on the mental health of school-aged children. The presence of both depressive and trauma-related symptoms underscores the need for timely psychological support. Implementation of school-based mental health initiatives increased parental awareness, and community-level interventions are recommended to mitigate these effects. Early detection and preventive measures are essential to strengthen resilience and promote long-term psychological well-being in children.

Keywords: COVID-19, children, depression, post-traumatic stress, mental health, parental reports

Birth defects in different localities of district Buner, Khyber Pakhtunkhwa, Pakistan

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Any structural or functional abnormality that has happened during intrauterine life due to any cause is called congenital birth defect. Birth defects occur in approximately 2% to 3% of births with a variable frequency in different populations. This study was conducted with the objective to explore different birth defects and their frequency/pattern in district Buner, KP, Pakistan from October 2022 to September 2023. A total of 2310 neonates (n=2310) data were recorded from different hospitals (NICUs, Surgical wards, and OPDs) inhabiting in different localities of the district Buner through specifically designed proforma with the help of already available literature on the topic. These medical centers were frequently visited with proper consent from the physicians. Data was collected randomly on weekly basis from each and every medical center. Analysis of results revealed that 245 (10.60%) individuals born were belonged to fetal growth restriction (FGR)/intrauterine growth restriction (IUGR). Moreover, 1508 (65.28%) individuals were having birth complications/diseases, and the remaining 557 (24.12%) displayed at least one birth defects. Urogenital system defects represented 24.05% individuals, followed by body wall defects represented 21.55% individuals, and then oral cavity defects represented 15.97% individuals. Furthermore, musculo-skeletal system defects represented 10.05% individuals, gastrointestinal defects represented 7.55% individuals, CVS defects represented 5.20% individuals, CNS defects represented 3.25% individuals, and other defects represented 12.38% individuals. This study also reported that individuals born with birth defects represent 82.40% male whereas the remaining 17.60% were female. These higher birth defects were belonged to urogenital system of male. The ultimate reason for this frequency of defects may be due to malnutrition, folic acid and iron deficiency, lack of medical facilities and awareness, consanguinity/heredity, lower mother age, and even viral infection.

Keywords: Congenital, Birth defect, Fetal growth, CNS defects

Blood profile of patients attending cardiovascular clinics in district Buner, Pakistan

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Cardiovascular diseases (CVDs) are the leading cause of sudden death. CVDs are a major health problem in Pakistan, and the number of patients is increasing daily. The objective of the current study was to investigate the blood profile of patients attending cardiovascular clinics in district Buner in order to aware people about information on the high rates of mortality and morbidity due to CVDs in this region of Khyber Pakhtunkhwa, Pakistan. Data was collected from different clinics located in district Buner from March 2022 to September 2022. A total of ninety four (n= 94) patients in terms of age, height and weight, blood glucose, cholesterol and blood pressure were screened during this study. Data was analyzed as Mean±SE. Average age recorded for male was 52.6±1.41 (n =54) followed by female as 43.94±1.51 (n = 49). Average height for male was noticed as 5.79±0.08 (n = 26) followed by female 5.31±0.07 (n= 29), whereas average weight recorded for male was 66.29±2.63 (n = 24) followed by female as 56.70±1.95 (n = 30). BMI of Six patients was recorded as higher than 25 Kg/m2 and were classified as overweight. BMI of Eight patients was recorded as higher than 25 Kg/m2. Out of which 2 patients were classified as obese and six were categorized as overweight. It was reported that 6/24 (25 %) in case of male were overweight. Moreover, 6/ 29 (20.69 %) in case of female were overweight, whereas 2/ 29 (6.90 %) were belonged to obese category. The average glucose concentrations recorded for male was 143.17±8.44 and female was 139.63±6.63. Furthermore, the average cholesterol concentrations recorded for male were 167.63±5.12 and female were 166.04±7.54. In conclusion, CVDs are the main cardiac problem among people in Buner, KP, Pakistan. The survey also revealed other associated CVD diseases. Inactive lifestyle, inadequate activity and family history of disease could be risk factors of diseases. The prevalence of CVDs was more common in females than males in the studied area. The onset age of CVDs was lower in females in the studied area. The way forward from these findings is to investigate the reasons for the high susceptibility to CVD in an extended study using modern research techniques. This research establishes a baseline study for future survey projects in this area.

Keywords: Cardiovascular diseases, BMI, Blood pressure, Blood glucose

Demographic Determinants and Prevalence of Hypertension in a Rural Male Population

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Hypertension is a major public health concern and a leading risk factor for cardiovascular diseases worldwide. Its prevalence is influenced by various demographic factors, including age, education, and living conditions. This study aimed to examine the demographic characteristics and associated trends in hypertension prevalence within a rural male population. A cross-sectional study was conducted involving 144 male participants residing in rural areas. Data on demographic variables, including age and educational level, were collected. Hypertension prevalence was assessed across different subgroups. Frequencies, percentages, and prevalence rates were calculated to identify patterns and associations. All participants were male and from rural settings, with an overall hypertension prevalence of 29.2%. Educational status showed a notable inverse relationship with hypertension. Individuals with no formal education had the highest prevalence (41.2%), followed by those with school education (27.0%), while lower rates were observed among college (17.4%) and university-educated individuals (15.4%). Age-wise distribution revealed a strong positive association with hypertension. Minimal prevalence was observed in younger individuals aged 18–29 years (12.9%), which increased progressively in middle-aged groups (30–44 years: 29.0%; 45–59 years: 50.0%) and peaked in older age groups (60–74 years: 62.5%; ≥75 years: 66.7%). The youngest category (≤17 years) showed no cases of hypertension. The findings indicate that hypertension prevalence is significantly associated with increasing age and lower educational attainment in rural male populations. These results emphasize the need for targeted awareness programs, early screening, and preventive interventions, particularly among older and less-educated individuals, to reduce the burden of hypertension.

Keywords: Hypertension, rural population, demographic factors, age distribution, education level, prevalence

Prevalence of *Cutaneouse leishaniasis* from 2010 to 2020 in district Dir lower Khyber Pakhtunkhwa, Pakistan

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Cutaneous leishmaniasis is one of the most important infectious diseases in northern areas of Pakistan. The aim of this study was to determine the prevalence of cutaneous leishmaniasis across a 11-year period in the Dir Lower, KP. This study was considered necessary due to the lack of new information in recent years on the CL of the disease. This is a descriptive study. This study has been undertaken in Dir lower to check the prevalence and risk factors of cutaneous leishmaniasis. For this purpose, the patients of leishmaniasis were examined directly from 2010 to 2020 at leishmania center, DHQ Timergara. The study area was divided into 4 Tehsils. i.e., Tehsil Adenzai, Tehsil Lalqilla, Tehsil Samer Bagh, and Tehsil Timergara and were analyses using SPSS software. The 7729 registered patients the prevalence of CL was 92.37% in local population and 7.62% in Afghan refugees, 68.11% were male and 31.89% were female. The prevalence of CL was highest 11-20 age group 33.78%. The highest incidence rate was observed in the year 2018 with a rate of 61.82% and the lowest was in 2015 at 5.8% the lesion were more frequently on nose 23.35. the highest prevalence was recorded in kaccha housing system and lowest were recorded in banker roof. The largest size of lesion was recoded 5cm and lowest size of lesion was 1cm. the highest prevalence was recorded in those people which have no proper contact with animals and the lowest was recorded in those people which have the contact with the cattle. the highest prevalence in the time duration was recorded in those cases which have the duration of 51-100 days and the lowest was recorded in those cases which have the duration of 351-400 days. It can be concluded from this study that; in term of sex the prevalence of CL is more frequent in males as compared to females. It also showed the younger people have more cases of CL than the people of older age. The cases of cutaneous leishmaniasis are more common in local residents than the Afghan people. In order to control the spread of CL the people should be properly educated about harmfulness of cutaneous leishmaniasis, and mosquito nets are recommended to be regularly used

Mapping of Genes Underlying Inherited Retinal Disorders in Consanguineous Families of Dir Lower, Pakistan

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Inherited retinal diseases (IRDs) are a group of heterogeneous, rare genetic disease that range in prevalence from 1 in 2000 to 1 in 3000 individuals worldwide. This study was designed to explore the genetic landscape of inherited retinal diseases in consanguineous families of district Dir Lower. To evaluate clinical spectrum and understand the molecular basis of IRDs in population of district Dir Lower. A total of 12 IRDs families were collected from different Regions/tehsils of district Dir lower Families carrying at least two patients affected by IRDs were recruited and diagnosed at district headquarter hospital of district Dir lower. blood samples were collected from patients, their parents, and a minimum of one healthy sibling as a control. Following extraction of genomic DNA from blood samples, six families were selected for whole exome sequencing (WES). Whole exome sequencing (WES) was performed on one individual per family. WES data were filtered through an in-house Computational pipeline. A total of 12 IRDs families were collected from different Regions/tehsils of district Dir lower. Clinically, RP was the most predominant IRDs phenotype in our cohort followed by Bardet Biedl syndrome. Parental consanguinity was reported in 91% cases. Pathogenic variants were detected in 04 IRDs families resulting in a total diagnostic yield of 67%. A total of 8 pathogenic alleles were observed in 4 different genes. Mutations in PRPF31 gene were (25%), RPGRIP1 gene (25%), BBS7 (25%), and AIPL1(25%). Overall, there was equal presence of all kinds of mutations which was 25 % for all (Missense, Nonsense, Splicing, and Indel mutation). As much as 25% the mutations, which were found in homozygous and 8% in heterozygous state. Among the four solved families, three (n=3) families reported variants in potentially RP causing genes and only one family, reported variants in gene potentially causing BBS Syndrome. Overall, our findings indicate that population of Dir present a unique genetic architecture of IRDs and mostly dominated by homozygous pathogenic mutations apparently due to the high consanguinity rate in the region.

Keywords: Inherited retinal diseases, Retinitis pigmentosa, Consanguineous families, Genetic landscape, Whole exome sequencing, PRPF31 gene, RPGRIP1 gene, BBS7 gene, AIPL1 gene, Homozygous mutations.

Exploring fish fauna and flora of different streams at tehsil Adenzai, Dir lower Khyber Pakhtunkhwa Pakistan

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The present study was designed to demonstrate the “The exploring of Fish fauna and Flora of different Streams of Tehsil Adenzi at Dir Lower, KP, Pakistan” during mid of May 2021. The aims and objectives of the study were estimating the ecology of freshwater fauna and biodiversity of fishes in the selected streams. During the present study the collection was made once from each stream and collected 550 specimens during the study. In them Nineteen (19) fish spices were identified from all Streams of Tehsil Adenzai District Dir Lower. The identified fish species belonging to four (4) orders, five (5) families, four (4) subfamilies and eleven (11) genera. Twelve (12) species belonged to family Cyprinidae and two species family Channidae and one species of each family Balitoridae and Mustacembelidae, and *Cichlidiae* family have three species *Oreochromis Aureus* , *Oreochromis mossabicmus*.and *Oreochromis Niloticus* The family Cyprinidae was represented by twelve (12) species these are; *Schizothorax esocinus, Schizothorax plagiostomus, Barilius pakistanicus, Barilius vagra, Crosochilius diplochiliu, Caracius auratus, Puntius ticto, Puntius chola, Puntius sophore, Garra gotyla, Tor putitora* and *Puntius* conchonius. Family Channidae and Cichlidiae represented by two species each such as *Channa punctatus, Channa gachua* and *Oreochromis aureus* and *Oreochromis mossabicmus, Oreochromis Niloticus.* Furthermore, family Balitoridae represented by one species *Schistora alepidotas* and family Mustacembelidae represents *Mestacembielus armatus*. During the current study a total of 521 specimens of plants were collected. A total of forty (40) aquatic plants species were identified from selected streams the identified plants species belonged to (17) orders, (25) families, and thirty (39) genera. Family Potamogetonaceae and Asteraceae represented four plant species each while family Hydrocharitaceae, Araceae, Hydrocharitaceae, Scrophulariaceae, Pontederaceae, Ceratophyllaceae, Cyperaceae, Juncaceae, Poaceae, Brassicaceae, Dryopteridaceae, Pteridaceae, Polygonaceae, Apiaceae, Equisetaceae, Euphorbiaceae, Balsaminaceae and Polypodiaceae represented by one species each. In the current study a total of 247 specimens of insects were collected from selected streams. In them ten (13) insect spices were identified from all Streams of Tehsil Adenzai District Dir Lower. The identified insect species belonged to four (4) orders, Nine (9) families and Thirteen (13) genera.

Exploring Fish Fauna and Flora of different Streams at Tehsil Adenzai, Dir Lower Khyber Pakhtunkhwa, Pakistan

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Fishes of the stream, such as trout and loaches, are adapted to living in fast-flowing, oxygen-rich freshwater. The present study was designed to demonstrate the “The exploring of Fish fauna and Flora of different Streams of Tehsil Adenzi at Dir Lower, Khyber Pakhtunkhwa, Pakistan” during mid of May 2021. Objectives of the study were to estimate the ecology of fresh water fauna and biodiversity of fishes in the selected streams. During the present study the collection was made it once from each stream and collected 550 specimens during the study. In them Nineteen (19) fish spices were identified from all Streams of Tehsil Adenzai District Dir Lower. The identified fish species belonging to four (4) orders, five (5) families, four (4) subfamilies and eleven (11) genera. Twelve (12) species belonged to family Cyprinidae and two species family Channidae and one species of each family Balitoridae and Mustacembelidae, and *Cichlidiae* family have three species *Oreochromis Aureus* , *Oreochromis mossabicmus*.and *Oreochromis Niloticus*.The family Cyprinidae was represented by twelve (12) species. Identified insect species belonged to four (4) orders, Nine (9) families and Thirteen (13) genera.

Key Words: Biodiversity, Fresh water, Fish, Streams, Adenzai, Dir Lower

POSTER ABSTRACT

Extraction of podophyllotoxins from endophytic fungi of Podophyllum hexandrum and evaluation for anticancer potential

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The current studies were aimed at the discovery of novel and sustainable sources of podophyllotoxin (PTOX), a potent anticancer drug. In this pursuit, endophytic fungi, which resides within the plant bodies in a symbiotic relationship were isolated from the roots of *Podophyllum hexandrum*. The cultural conditions were accurate and suitable for the growth of individual fungi. Briefly, an infusion of the surface sterilized roots was inoculated on sterilized potato dextrose agar (PDA+ antibiotic) at 28 ± 2 °C for several days until the first growth appeared. The individual fungi were cultured through hyphae tip method on separate PDA to obtain 14 different fungi. The identification was made through macro and micro morphological features (colony growth, color, texture, zones, and diameters). The species identified belonged to *Aspergillus*, *Fusarium*, *Candida*, *Trichoma*, *Phomopsis*, *Neofusicoccum*, *Penicillium* and *Verticillium* genera. A methanolic extract of all the fungal cultures was screened for the presence of PTOX where *F. solani* and *A. niger* showed positive result in Thin Layer Chromatography (TLC). The extensive HPLC analysis quantified the PTOX contents in *F. solani* extract (40 %) and *A. niger* (14 %) at Retention time of 5.14 minutes at detecting wavelength of 287 nm. The *F. solani* ethyl acetate extract (500 mg) was subjected to isolation of PTOX via column chromatography to produce 25 mg PTOX in chloroform: methanol solvent system. The physical data obtained through UV/Visible and FT-IR analysis was sufficient to confirm the structure of PTOX. Furthermore, the anticancer assays against liver HepG2 cancer cell lines revealed that *F. solani* extract inhibited the liver cancer cell lines more effectively (40 %) in comparison to *A. niger* (10 %) due to more PTOX contents. In conclusion, the endophytic fungi of *P. hexandrum roots* are huge sources of PTOX for its sustainable and bulk production that could be used in commercial production of PTOX.

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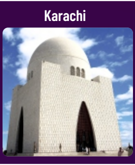

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