3rd International One Health Conference 2025

One Target: Long-Term Survival of Human Beings Through National

Eco-Security System

November 6–7, 2025

College of Veterinary Sciences & Animal Husbandry

Abdul Wali Khan University Mardan, Pakistan

PREFACE

It gives us immense pleasure to present the Abstract Book of the 3rd International One Health Conference, organized by the College of Veterinary Sciences and Animal Husbandry, Abdul Wali Khan University Mardan (AWKUM), on November 06–07, 2025.

The conference is being held under the overarching theme of *"One Health: Integrating Human, Animal, and Environmental Well-being for a Sustainable Future."* This timely and significant event brings together leading researchers, academicians, policymakers, professionals, and students from around the world to deliberate on emerging issues at the interface of animal, human, and environmental health.

The abstracts included in this volume reflect a diverse range of contemporary research, innovations, and ideas that collectively contribute to the realization of the One Health vision. Topics span across zoonotic diseases, food safety, antimicrobial resistance, wildlife health, public health policy, environmental management, and sustainable livestock production systems.

This compilation not only showcases the scientific contributions of our national and international participants but also underscores the growing importance of multidisciplinary collaboration in addressing global health challenges. We believe that the ideas shared through this conference and documented in this book will inspire future research and policy initiatives, paving the way for a healthier and more resilient world.

We extend our sincere gratitude to the Vice Chancellor, Abdul Wali Khan University Mardan, for his continued support, to our keynote speakers and contributors for their valuable insights, and to the organizing committee, reviewers, and volunteers whose dedication made this event a success.

We hope this abstract book serves as a useful reference for researchers, students, and practitioners committed to advancing the One Health agenda.

VICE CHANCELOR MESSAGE

Dear Esteemed Participants,

It gives me immense pleasure to extend my heartfelt congratulations to the organizers, participants, and distinguished guests of the 3rd International One Health Conference organized by the College of Veterinary Sciences and Animal Husbandry, Abdul Wali Khan University Mardan.

The theme of *"One Health"*—integrating human, animal, and environmental health—is of profound importance in addressing today's complex global challenges, including emerging zoonotic diseases, food security, antimicrobial resistance, and climate change. This conference serves as a vital platform for researchers, academicians, policymakers, and practitioners to share knowledge, exchange innovative ideas, and strengthen collaborative efforts for the betterment of global health.

I am confident that the deliberations and scientific discussions held during this event will contribute significantly to the advancement of multidisciplinary research and evidence-based policy development. Such initiatives are fully aligned with AWKUM's vision to promote cutting-edge research and its commitment to addressing societal challenges through scientific excellence.

I commend the College of Veterinary Sciences and Animal Husbandry for its continued efforts to foster academic excellence and international collaboration. I also extend a warm welcome to our international and national delegates whose participation enriches this academic endeavor.

I wish the organizers great success and hope that this conference will inspire new partnerships and research directions that will contribute meaningfully to the realization of the One Health vision.

Prof. Dr. Jamil Ahmed
Vice Chancellor
Abdul Wali Khan University Mardan

CHIEF ORGANIZER AND FOCAL PERSON MESSAGE

Dear Participants,

It is indeed a great honor and privilege to welcome all distinguished guests, eminent scientists, academicians, students, and participants to the 3rd International One Health Conference organized by the College of Veterinary Sciences and Animal Husbandry, Abdul Wali Khan University Mardan.

The concept of *One Health*—recognizing the interconnectedness of human, animal, and environmental health—has never been more relevant. The increasing emergence of zoonotic diseases, antimicrobial resistance, and climate-related health challenges demand collaborative, multidisciplinary approaches. Through this conference, we aim to bring together leading minds from diverse fields to share recent advances, innovative research, and practical solutions that contribute to achieving sustainable health for all.

This event reflects our college's continued commitment to promoting excellence in veterinary education, research, and outreach. I am deeply grateful to our respected Vice Chancellor, Abdul Wali Khan University Mardan, for his unwavering support and encouragement, and to our national and international collaborators for their participation and valuable contributions.

I extend my heartfelt appreciation to the organizing committee, faculty, and students whose dedication and hard work made this conference possible. I am confident that the outcomes of this event will pave the way for stronger scientific collaborations and contribute significantly to the implementation of the One Health approach in Pakistan and beyond.

I wish all participants a productive and enriching experience and hope that this conference serves as a milestone in our shared journey toward a healthier and safer world.

Dr. Sher Bahadar Khan
Principal & Chief Organizer
College of Veterinary Sciences and Animal Husbandry
Abdul Wali Khan University Mardan (AWKUM)

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CONFERENCE THEMES & SUB-THEMES

- One Health

- Health Security & Biosecurity

- Sustainable Resource Management

- Renewable Energy Applications

- Livestock Improvement and Species Competition

- Climate Change, Mitigation and Adaptation

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THEME 1: AGRICULTURE AND ECO SECURITY

WATER AND SANITATION SERVICES SUSTAINABILITY CHECK FOR IMPROVING HEALTH LADDER IN MARDAN, NORTHERN PAKISTAN

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ABSTRACT

Provision of continued water and sanitation services are vital for maintaining better health ladder and achieving sustainable development goal 6. The developing countries including Pakistan face the challenges of the non-functionality of water supply services and reverting of the open defecation free (ODF) villages into open defecation again. Sustainable water and sanitation program provide condition for the continuous provision of water supply and sanitation services without any interruption and depletion of environment. For these services, sustainability refers to the continuous supply of water and continuous operation of sanitation systems without harming the environment and water source depletion. This study was focused on assessing the overall sustainability of WASH program and its effectiveness, exploring the potential implementation gaps in the targeted areas in district Mardan, northern Pakistan. The overall sustainability of WASH program was assessed through sustainability assessment framework developed through a consultative process with sector expert and indicators and sub indicators were framed and scored in such a way which assessed the overall sustainability. A questionnaire surveys, key informant interviews (KIIs), and focus group discussions (FGDs) were also carried out for assessing the sustainability and effectiveness of the program and results obtained were displayed in tables and graphs through statistics, like frequency and percentage. The sustainability assessment framework (SAF) was scored in the field during KIIs and observation. The established methodological framework framed to assess the overall sustainability of water and sanitation system on the 5 key element of sustainability: namely technical, environmental, economic, social, and institutional. Results revealed that the overall sustainability of WASH program in study area was not sustainable. The overall sustainability of the water supply component was 28.85 percent while it was 24.77 percent for sanitation up scaling. The percentage of institutional sustainability was 2.72 percent for water supply and 2.74 percent for sanitation which was the lowest among all component. The implemented program showed some good result in social sustainability and effectiveness due to awareness, social mobilization, capacity building and provision of WASH facilities. Some of the key achievements observed through the study were achieving the status of open defecation free environment in more than a hundred villages, women empowerment, and inclusion of people with disabilities but on the other hand the ODF villages could not sustained its status and sustainability. The study suggests the use of SAF for assessing the likelihood of sustainability and

community capacity of community managed WASH program in pre implementation and design phase. The framework and scoring model would suggest the gap in sustainability which would be catered in program designing and pre implementation phase.

Keyword: Water and Sanitation; Health and Hygiene, Sustainability; Indicators; Assessment framework

CHARACTERIZATIONS OF SOOT PARTICLES AND ASSOCIATED PUBLIC HEALTH ISSUES IN NORTHERN PAKISTAN

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ABSTRACT

Air pollution and the public health related risks associated with poor air quality (both indoor and outdoor) in developing countries are the major environmental issues in recent years, which attract researchers, practitioners and policy makers' attention. This paper presents the findings of the two integrated studies conducted on the characterizations of soot particles released from indoor combustion and the associated health risks of brick kiln industries in Sawt and Mardan Districts of northern Pakistan. The combustion of firewood, cow dung and bagasse at households greatly release air pollutants into the atmosphere and the soot particles are the main precipitated compositions. Soot particles characterizations were performed to investigate their morphological, chemical, physical and elemental compositions released from the combustion of firewood, cow dung and bagasse. Characterization analyses including (XRD), (), (FTIR), energy dispersive spectroscopy (, Brunauer–Emmett–Teller (BET), and thermo-gravimetric analysis (TGA) were performed. Results demonstrated diverse compositions of soot particles from each household's fuel source. TGA results showed different thermal decomposition patterns at different temperatures. The presence of $C=C$ of aromatic and -C-H alkane as the major functional groups at the absorbance rate of about 1599 and 1431 cm-1, 1000-2000 cm-1 and 1431 and 1599 cm-1, respectively were more obvious in firewood soot particles. However, silicon band vibration was more prominent in bagasse soot particles. BET surface areas of firewood, cow dung, and bagasse were found to be 0.3, 0.4, 8.64 m2/g, respectively and the significantly high surface area of bagasse was recorded as compared to other soot particles. The emission of soot particles with high surface area in the atmosphere could lead to providing an elevated adsorption site for atmospheric pollution and trap more energy resulting in an increase in the atmospheric temperature. In the other hand, field-based investigation of the outdoor air pollutants from brick kiln industries in Jalala Mardan showed highest concentrations of PM1, PM2.5 and PM10. In addition, social surveys revealed the adverse impacts of air pollution on community health and directly associated with respiratory diseases, such as asthma and other general effects to all age groups in the study area. Findings from the present study suggested that

implementing strong penalty measures on brick kiln industries to install some sorts of PM and other air pollution reduction devices to protect community health. Similarly, the soot particles characterizations showed of vary compositions of the soot particles from different fuel sources exhibited different characteristics for its linkages with global warming and possible climate change phenomenon in northern Pakistan.

EXPLORING THE BIOLOGICAL APPLICATIONS OF EXOPOLYSACCHARIDES DERIVED FROM ENDOPHYTIC

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ABSTRACT

Endophytic fungi inhabiting plant tissues are gaining recognition as rich sources of bioactive secondary metabolites with diverse biomedical potential. Among these, exopolysaccharides (EPS) have drawn significant attention due to their antioxidant, anti-inflammatory, antidiabetic, and antibacterial properties. In this study, fungal endophytes were isolated from different parts of the wheat plant (Triticum aestivum) and identified as Wh1, Wh2, Wh3, and Wh4. Strains producing high EPS content were cultivated in yeast extract–peptone–dextrose broth at 25 °C for 7 days under shaking conditions. EPS were precipitated with ice-chilled ethanol and biochemically characterized, revealing considerable carbohydrate content in all isolates, with Wh4 showing the highest (10.8 µg/mL). Monosaccharide analysis showed varying concentrations of uronic acids, unsaturated uronic acids, pentoses, and hexoses. Biological assays revealed remarkable bioactivities. Wh2 and Wh3 demonstrated strong antioxidant potential, scavenging 73.00% ± 3.22 and 77.81% ± 1.42 of DPPH radicals, respectively, at 1000 µg/mL. Wh4 exhibited the highest anti-inflammatory activity (88.34% ± 1.14; IC_{50} = 163.60 µg/mL). Wh3 showed the most potent α-amylase inhibition (87.17% ± 4.21; IC_{50} = 301.39 µg/mL), signifying promising antidiabetic potential. In antibacterial testing, EPS from all strains exhibited reasonable to strong inhibition against Escherichia coli and Staphylococcus aureus, with Wh2 achieving maximum zones of inhibition of 6.75 mm (E. coli) and 10.75 mm (S. aureus) at the highest concentration. Overall, these findings highlight wheat-derived endophytic EPS as multifunctional bioactive compounds with significant therapeutic promise. Their natural origin, sustainability, and broad-spectrum biological activities position them as a valuable resource for developing eco-friendly and innovative biomedical solutions.

Keywords: Endophytes, Exopolysaccharides, Wheat plant, Bioactive compounds

DIVERSITY AND DISTRIBUTION OF TICK SPECIES IN THE HINDU KUSH MOUNTAIN RANGE OF PAKISTAN

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ABSTRACT

Ticks are important ectoparasites impacting livestock and human health globally, serving as vectors for numerous diseases. This study investigates the diversity and ecological distribution of tick species within the Hindu Kush ranges of Pakistan. This region is characterized by diverse altitudinal and climatic conditions. Tick specimens were collected from domestic animals, wildlife, and the environment, followed by morpho-molecular identification. Results revealed the presence of multiple tick genera including Rhipicephalus, Haemaphysalis, and Hyalomma, with species distribution strongly influenced by altitude, ranging from 1500 to 3200 meters, temperature ranges from 10 to 20°C, and humidity from 40 to 60%. In the study the Rhipicephalus mi croplus and Haemaphysalis montgomeryi were reported as principal species posing health risks. These findings will contribute in the epidemiological study which is necessary for understanding tick population dynamics and in developing the control measurement to mitigate the burden of tick-borne diseases in this ecologically sensitive mountain region.

Keywords: Tick diversity, ecological distribution, Hindu Kush, Pakistan, altitude, livesto ck health, vector ecology

FLEAS COLLECTED FROM DIFFERENT ANIMAL HOSTS IN DISTRICT CHARSADDA AND MARDAN, PAKISTAN

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ABSTRACT

Fleas are ectoparasitic hematophagous insects infesting a large number of mammalian hosts and playing key roles as vectors of zoonotic diseases. In the current research, an effort has been made to identify the flea species infesting domestic and commensal animals in District Charsadda and Mardan, Khyber Pakhtunkhwa, Pakistan. A total of 200 flea specimens were manually removed from goats, sheep, dogs, cats, and rats from field surveys. The collected specimens were stored in 70% ethanol and identified morphologically on a stereo zoom microscope employing standard morphological keys. Three flea species were identified, namely, Ctenocephalides felis, Ctenocephalides canis, and Xenopsylla cheopis. Among them, C. felis was the most common species found infesting cats and dogs, and X. cheopis was most commonly found in rats. The prevalence of X. cheopis, a flea species of plague (Yersinia pestis), murine typhus (Rickettsia typhi), and Rickettsia felis, highlights the likely risk of flea-borne zoonoses in

the area studied. Moreover, C. felis and C. canis are identified carriers of Rickettsia felis and Rickettsia canis, indicating their potential role in the propagation and transmission of rickettsial pathogens within domestic animals and humans. These findings highlight the regular monitoring and molecular testing of fleas to understand the types, their distribution, and diseases that fleas carry among domestic animals and their neighbors in Pakistan.

Keywords: Fleas; Ctenocephalides felis; Ctenocephalides canis; Xenopsylla cheopis; Rickettsia felis; Rickettsia canis; Flea-borne Zoonoses; Pakistan

LONG-TERM HUMAN SURVIVAL THROUGH NATIONAL ECO-SECURITY SYSTEMS: BUILDING RESILIENT FOOD SYSTEMS THROUGH FAO PAKISTAN

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ABSTRACT

The long-term survival of humanity depends on robust eco-security systems that safeguard natural resources, ensure environmental stability, and build socio-economic resilience. Central to this is food security, linking ecological sustainability with human well-being. In Pakistan, the Food and Agriculture Organization applies integrated, sustainable solutions to address climate change, resource degradation, and food insecurity. Its interventions enhance productivity, promote climate-resilient agriculture, and improve access to safe and nutritious food. FAO's initiatives such as sustainable irrigation systems, Farmer Field Schools, and community-based disaster risk management—strengthen national food systems while conserving ecosystems. These efforts illustrate the interdependence between eco-security and food security as foundations of sustainable development. Working with government, academia, and local partners, FAO fosters inclusive, science-based approaches to sustainability. By addressing systemic vulnerabilities and promoting resilient food systems, FAO Pakistan advances both national eco-security and the global agenda to end hunger, protect the planet, and secure humanity's future.

A STUDY ON THE CUMULATIVE EFFECT OF CHRONIC EXPOSURE TO THERMAL STRESS AND GLYPHOSATE-BASED HERBICIDE ON THE HEMATOLOGICAL PARAMETERS AND CORTISOL LEVEL OF COMMON CARP (*CYPRINUS CARPIO*)

ABSTRACT

Climate change and environmental pollution can alter the composition and productivity of biological communities in aquatic ecosystems. Sensitive species are at more risk due to these stressors, which can affect their physiology with adverse effects on their health and reproduction. Co-exposure to multiple environmental stressors may have synergistic effects on the health of aquatic animals. Therefore, present research was designed to explore the cumulative effect of chronic exposure to thermal stress and glyphosate-based herbicide on the hematological parameters and cortisol level of common carp (Cyprinus carpio). Twenty-eight common carp were exposed to thermal stress (30 and 32°C temperature) and a sub-lethal concentration of glyphosate for 28 days.A significant increase in white blood cells (WBCs), platelets and cortisol was observed in fish exposed to the sub-lethal concentration of glyphosate, while red blood cells (RBCs) and hemoglobin (Hb) decreased.Cortisol levels of fish exposed to thermal stress (32°C) increased significantly. RBCs, WBCs, platelets, hemoglobin concentration and cortisol levels increased significantly in fish co-exposed to thermal stress and a sub-lethal concentration of glyphosate. It was concluded that co-exposure to glyphosate and thermal stress causes physiological impairments in common carp.

ABSTRACT 008
IDENTIFICATION OF NOVEL BIOACTIVE COMPOUNDS FROM AMARANTHUS VIRIDIS EXTRACTS WITH POTENT ANTIMICROBIAL ACTIVITY AGAINST METHICILLIN -RESISTANT STAPHYLOCOCCUS EPIDERMIDIS

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ABSTRACT

The growing threat of drug -resistant pathogens, such as Methicillin -Resistant Staphylococcus epidermidis (MRSE), has intensified the demand for novel therapeutic agents, prompting exploration into medicinal plants as viable alternatives. Amaranthus viridis L ., recognized for its diverse pharmacological properties, was investigated for its potential to yield new antimicrobial compounds. This study aimed to identify bioactive cons tituents in A. viridis with antibacterial activity against MRSE, assess the antioxidant and hemolytic properties of its crude extracts, evaluate the drug -like characteristics of identified compounds through in silico ADMET analysis, and explore their molecular interactions with the MecA PBP2 protein via docking studies. MRSE was isolated and identified using advanced molecular and biochemical techniques. Methanolic extracts of A. viridis demonstrated significant antibacterial activity against MRSE, as confirmed by the well diffusion method, which produced a notable inhibition zone. The extracts also exhibited strong antioxidant activity at the same concentration, indicating their potential to mitigate oxidative stress. Furthermore, the extracts showed minima l hemolytic activity at a lower concentration, suggesting low cytotoxicity and high biocompatibility, essential for therapeutic applications. Gas chromatography -mass spectrometry (GC -MS) analysis

revealed a diverse array of compounds in the crude extract, including three novel compounds identified for the first time in A. viridis , expanding its phytochemical repertoire. In silico ADMET analysis indicated that 2,3 - dihydroxypropyl elaidate possesses excellent properties. Molecular docking studies revealed tha t cyclopropaneoctanal, 2 -octyl - displayed a strong binding affinity to the MecA PBP2 protein. These qualitative findings highlight the potent antibacterial and antioxidant capabilities of A. viridis extracts, coupled with promising drug -like characteristic s, positioning this plant as a highly promising candidate for the development of innovative antimicrobial therapeutics to address the global challenge of drug -resistant infections.

ADVANCING SUGARCANE BIOTECHNOLOGY: EFFICIENT REGENERATION VIA THIN CELL LAYER (TCL) CULTURE AND NANOTUBE-INDUCED RESPONSE INSIGHTS

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ABSTRACT

The present research work reports the impact of multi-walled carbon nanotubes (MWCNTs) on sugarcane morphological parameters, the production of secondary metabolite (SMs), and the antimicrobial activity of sugarcane callus culture in vitro. The induced calli from thin cell layers (TCLs) of leaf sheets were subjected to multiplication and proliferation with the application of various concentrations of MWCNTs (1-5mg/l). The highest multiplication was recorded on media containing 5mg/l of CNTs with fresh weight 2.8567g. Different concentrations (5, 10, 15 and 20 mg/l) of MWCNTs were applied to regenerate the callus. The highest number of shoots and roots were developed on 15mg/l concentration while highest shoot length and root length (in cm) were observed on 20 mg/l CNTs. Similarly, the sub-cultured callus was subjected to phytochemical analysis and the effect of MWCNTs on amount of biochemical contents like i.e total phenolic contents, flavonoids, saponins and terpenoids was determined. Highest content was produced in culture containing highest concentration of CNTs, in all cases. Moreover, the antioxidant activities of the MWCNTs induced in vitro sugarcane were also evaluated by the 1, 1 diphenyl 2, picryl hydrazyl (DPPH) methods. The antimicrobial activity was evaluated against two bacterial pathogens, Clavibactor michiganensis (CMM) and Ralstonia solanacearum (RS) with inhibition zone of 13 mm in case of CMM while 17 mm was calculated against RS Spp. Our results reveal that using MWCNTs in specific concentrations could act as a elicitors of growth and the in vitro biosynthesis of useful SMs with antimicrobial activity against broad spectrum microbial pathogens.

Key words: Saccharum officinarumin, Multi-walled carbon nanotubes, morphological characteristics, secondary metabolite, antimicrobial activity.

EFFECTS OF MELATONIN ON BIOMASS AND SECONDARY METABOLITES PRODUCTION IN MORINGA OLEIFERA

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ABSTRACT

Melatonin is a pleotropic molecule, regulating key biological pathways in plant including growth, rooting, seed germination, photosynthesis and defense against biotic and abiotic stresses. Moringa oleifera, commonly known as drumstick tree, is a nutritional plant found across the globe. The current study aims to evaluate the effect of melatonin on the callogenesis and regeneration in Moringa and to analyze the biochemical and metabolomics profile of the calli and regenerated plants. Moringa seeds were cultured on MS basal medium containing 3% sucrose, 0.9% agar supplemented with different concentrations of melatonin (0, 5, 10, 15, 25, 35 and 50 mg/L) and incubated at $25 \pm 1°C$ for germination. Next, Moringa seeds were cut down into 2 to 4 pieces and then cultured on the callus medium with addition of BAP (1.0 mg/l), 2,4-D (1.0 mg/l) and various concentration of melatonin (0, 0.5, 1, 1.5, 3, 7 and 10 mg/l). Further, color, texture, fresh and dry biomass of the calli was evaluated. In addition, the seedlings were explored for their morphological and biochemical attributes. The results revealed that melatonin supplemented with BAP and 2,4-D, significantly improved the quality and biomass production. Biochemical characterization shows that enhanced concentration of phenolics, alkaloids and terpenoids were produced in the in vitro Moringa plant and callus. The current study concludes that melatonin as an effective elicitor in improving the quality and quantity of bioactive compounds in M. oleifera cultures. However, further studies are needed to explore the molecular pathways, involved in melatonin-induced metabolites production, contributing to the production of plant-derived pharmaceuticals.

Key words: Melatonin, Moringa oleifera, in vitro culture, bioactive metabolites

TO DRINK OR NOT TO DRINK, THAT IS THE QUESTION: CONTAMINATION OF SPRING WATERS WITH THE PROTOZOAN PARASITES

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ABSTRACT

Transmission of protozoan parasites through water is of great public and veterinary health importance. Cryptosporidium oocysts, in particular, are widespread in water sources and

can survive in this environment for months. Many people believe that drinking spring water is healthier than tap water. Therefore, using molecular tools, we analyzed spring water in three Iranian provinces for Cryptosporidium, Giardia, Acanthamoeba, and Microsporidia contamination. Twenty-nine springs from which locals, hikers, mountaineers, and livestock drink were selected in Hamedan (n=13), Kordestan (n=9), and Esfahan (n=7) provinces. From March to July 2023, the water samples were collected aseptically, and exactly 250 mL of each water sample was centrifuged to obtain sediment. Genomic DNA was extracted from approximately 200 µL of the obtained sediment and subjected to conventional PCR with genus-specific primers. The results showed that two sources in Kordestan province were contaminated with Cryptosporidium, and one source in Esfahan was contaminated with Acanthamoeba. Other parasites were not detected. The data presented herein is the first information on the contamination of spring water in Iran with Cryptosporidium and Acanthamoeba organisms. Considering that several species within these genera are capable of making people sick, especially children, the elderly, and immunocompromised individuals, people should avoid drinking water from sources from which parasites have not been removed or inactivated.

Keywords: acanthamebiasis, cryptosporidiosis, PCR, one-health, water, zoonosis

ABSTRACT 012
PROMOTING FOOD AND HEALTH SECURITY: ASSESSING THE TOXICITY OF CONSUMER PRODUCTS AND ENVIRONMENTAL TOXICANTS USING ZEBRAFISH MODEL
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ABSTRACT

Regulation of food and other human-use products is critical for ensuring food security, public health, and overall well-being. However, in many developing countries, the approval and regulation of locally produced human-use products are often inadequate, raising serious concerns about their safety and quality. To address these challenges, ee have established a zebrafish breeding and testing facility at The University of Agriculture Peshawar, where zebrafish is used as a model organism for toxicity screening of various locally available products. Zebrafish, a small teleost vertebrate, offers several advantages as an alternative to traditional mammalian models. It has a short life cycle, high fecundity, conserved biological pathways, and ethical acceptability. Using this model, we have evaluated the toxicological effects of a range of food additives (e.g., food colors, monosodium glutamate [MSG], tea whiteners) and cosmetics available in the local market, comparing the effects of local and multinational brands through in vivo assays. Additionally, we have examined the impact of environmental pollutants, including pesticides and microplastics, on zebrafish development and physiology. Following acute and chronic exposures of zebrafish embryos and adults, we analyzed multiple parameters

such as developmental anomalies, hatching rate, mortality (LC_{50}), growth performance, feed conversion efficiency, biochemical markers, histopathological changes, gene expression profiles, and gut microbiota composition. Results revealed significant dose-dependent effects of test substances on zebrafish growth, development, and survival. For instance, MSG exposure induced early hatching, deformities in up to 90% of embryos, and upregulated IL-1β (2-fold) and P53 expression, indicating inflammation and oxidative stress. Synthetic food dyes caused high embryonic mortality at concentrations as low as 1.5%, while glyphosate showed an LC_{50} of 8.1 mg/L, comparable to levels detected in contaminated water sources. Moreover, local cosmetics and tea whiteners exhibited greater toxicity and cholesterol accumulation, as well as vascular constriction in zebrafish, compared to their multinational counterparts. Exposure to glyphosate and microplastics also led to gut microbiota dysbiosis, characterized by a decline in beneficial bacteria and an increase in pathogenic taxa. Collectively, these findings demonstrate that zebrafish is a reliable, sensitive, and ethical model for assessing the toxicity of consumer and environmental chemicals. The outcomes emphasize the urgent need for strengthened regulatory oversight and safety evaluation frameworks for food and human-use products to safeguard public health within a One Health context.

WHEAT EXOPOLYSACCHARIDE-MEDIATED GREEN SYNTHESIS OF NICKEL OXIDE NANOPARTICLES WITH ENHANCED ANTIOXIDANT ACTIVITY

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ABSTRACT

Nanoparticles, fundamental to nanotechnology, are known for their distinct chemical, optical, and mechanical properties, which differ significantly from their bulk material counterparts. Due to increasing environmental toxicity concerns, this study focused on the eco-friendly biosynthesis of metallic nanoparticles (MNPs) using exopolysaccharides (EPS) extracted from endophytes of Triticum aestivum (wheat). The process involved dissolving 10 mg of wheat seed polysaccharides in distilled water, followed by mixing with nickel nitrate to achieve a final concentration of 10 mM. The formation of nickel oxide nanoparticles (NiO-NPs) was indicated by a color change and confirmed through UV-visible spectroscopy. The green-synthesized MNPs were characterized using double-beam UV and FT-IR spectroscopy to identify the specific biomolecules involved in their formation, with X-ray diffraction (XRD) analysis determining their crystalline structure. The biological potential of these nanoparticles was assessed through various bioassays, including antioxidant activity evaluation using the DPPH radical scavenging method and hydrogen peroxide inhibition. Among all screened samples, NiO NPs showed the most significant antioxidant potential, with an EC50 value of 37.93 µg/mL, followed by EPS at 66.04 µg/mL. At 200 µg/mL, NiO NPs effectively scavenged the H_2O_2 free radical by 81.9%±0.24, followed by EPS at 76.54%±0.65. These results highlighted the effectiveness of NiO NPs, emphasizing their potential for sustainable applications in nanomedicine and nanobiotechnology.

EFFECT OF MORINGA OLEIFERA LEAF POWDER SUPPLEMENTATION ON SERUM PARAMETERS, GUT MICRO-FLORA, MUSCLE AND BONE HEALTH IN BROILERS UNDER DEXAMETHASONE-INDUCED STRESS

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ABSTRACT

This research investigates the impact of Moringa oleifera leaf powder (MOLP) supplementation on broilers subjected to dexamethasone-induced stress. 200-day-old chicks were distributed into five groups (A, B, C, D and E), each group was divided into four replicates (ten chicks/replica). Negative control group (A) received only basal diet, positive control group (B) exposed to dexamethasone (day 21st onward), group C, D, and E received 0.8% ,1.2%, and 1.6% MOLP respectively. Results indicate significant (P ≤ 0.05) improvements in liver health, evidenced by decreased levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphate (ALP) in MOLP-treated groups, urea and creatinine levels remained non-significant. Obvious alterations in gut microbiota composition, with positive (P ≤ 0.05) changes in Escherichia coli, Bifidobacteria, and Lactobacilli in MOLP-treated birds, indicating potential benefits for gut health. Mineral content analysis revealed higher (P ≤ 0.05) phosphorus % in MOLP-administered groups, ash% and Ca% were non-significant. Bone morphological parameter demonstrated significant (P ≤ 0.05) improvements in tibia length, weight, tibiotarsal index and weight/length index in 1.2% and 1.6% MOLP groups, diaphysis diameter, medullary canal diameter, medial wall diameter, lateral wall diameter and robusticity index of tibial bones were non-significant. MOLP supplements significantly (P ≤ 0.05) changed muscle fascicle diameter and muscle fascicle cross-sectional area, while muscle fiber diameter, muscle fiber cross-sectional area and muscle fiber density were non-significant. It was noticed that up to 1.6% MOLP in broiler feed improves broilers health.

ISOLATION, BIOCHEMICAL CHARACTERIZATION, AND ANTIMICROBIAL ACTIVITIES OF EPS PRODUCING BACTERIAL ENDOPHYTES FROM *MORINGA OLEIFERA*

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ABSTRACT

Bacterial endophytes are a key part of the plant microbiome that influence developmental processes via different mechanisms. One of the known mechanisms is the production of exopolysaccharides (EPS) that are responsible for the response towards stresses and plant-bacterial interactions. In the current experiment, we isolated forty-two strains of bacterial endophytes from the sterilized parts of *Moringa oleifera* plant and then screened them for the production of EPS. Out of the forty-two, only three isolates; KR-27, KL-4, and KS-7 produced a significant amount of EPS. All these isolates showed invitro antagonistic activities against phytopathogenic fungi and bacteria. These antagonistic endophytic isolates, KR-27, KL-4, and KS-7 were identified as *Serratia marcescens, Klebsiella aerogenes,* and *Lelliottia amnigena* respectively based on *16s RNA* gene ribotyping. The selected bacterial strains were screened out for phytohormones and secondary metabolites production and all the identified strains produced significant amount of various phytohormones such as indole acetic acid (IAA), gibberellic acid (GA3) and salicylic acid (SA). In addition, the identified strains produced the antioxidant compounds like siderophores, phenolics, and flavonoids. The phosphate solubilization ability of all the strains was ascertained and found highest for KR-27 (30.46 ± 0.82 µg/mL), followed by KS-7 (18.30 ± 0.54 µg/mL),), and KL-4 (17.36 ± 0.68 µg/mL). Unlike previous research, which primarily focused on the nutritional and medicinal properties of *M. oleifera*, this research uncovers the new aspect by exploring the biotechnologically important bacterial flora of this miracle tree. In conclusion we isolated three novel bacterial endophytic strains with the ability to produce bioactive compounds for plant and antimicrobial activity against phytopathogens for the first time which can replace the synthetic fertilizers and pesticides in future sustainable agriculture practices.
Key words: *Moringa oleifera,* endophytic bacteria, exopolysaccharides, bioactive compounds, antimicrobial activity.

ABSTRACT 016

GREEN SYNTHESIS AND CHARACTERIZATION OF TRITICUM AESTIVUM DERIVED BIOCOMPATIBLE SILVER NANOPARTICLES AND EVALUATION OF THEIR ANTIDIABETIC POTENTIAL

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ABSTRACT

Current therapeutic options for various human ailments have faced challenges such as resistance, low efficacy, high costs, and increasing market demands, driving the search for novel, cost-effective natural alternatives. Nanoparticles, particularly those synthesized through green methods, have emerged as pivotal in biotechnology and therapeutics due to their high biocompatibility and potent bioactivities, including antimicrobial, anti-

inflammatory, antioxidant, and anticancer properties. This study focused on the biosynthesis of silver nanoparticles from endophytic exopolysaccharides (EPS) of Triticum aestivum. The EPS was extracted and precipitated using ethanol, followed by lyophilization. The presence of polysaccharides was confirmed through the phenol-sulfuric acid (PSA) method. Silver nanoparticles (AgNPs) were synthesized by treating the polysaccharide extracts with the respective metallic salts. The biosynthesis process was optimized using UV-visible spectrophotometry and characterized using FT-IR techniques to confirm the formation and stability of the nanoparticles. The in vitro potential of the synthesized nanoparticles was assessed through α-amylase and α-glucosidase inhibition assays. The results revealed that AgNPs significantly inhibited α-amylase activity, with an IC50 of 265.75 µg/mL, followed by EPS (357.18 µg/mL). NiO NPs, on the other hand, were found to significantly inhibit α-glucosidase, with an IC50 of 441.87 µg/mL, followed by EPS (590.68 µg/mL). These findings underscore the potential of biosynthesized MNPs as effective natural therapeutics, offering a promising alternative to conventional treatments, with significant implications for the development of cost-effective and biocompatible therapeutic agents.

Keywords: Triticum aestivum, Endophytic exopolysaccharides, Green synthesis, Silver nanoparticles

ABSTRACT 017
EXPLORING THE PREBIOTIC POTENTIAL OF OLIGOSACCHARIDES FROM CRESS SEED MUCILAGE ON HUMAN PROBIOTICS
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ABSTRACT:

Probiotics are beneficial microorganisms known for their positive impact on human health and require specific nutrients to thrive and exert their health-promoting effects. Oligosaccharides, a class of dietary carbohydrates, have gained attention as potential prebiotics, selectively nourishing beneficial gut microbiota. The current study aimed to isolate oligosaccharides from L. sativum mucilage and evaluate their in vitro prebiotic effects on human probiotics. Mucilage was extracted from cress seeds in distilled water overnight, and polysaccharides were precipitated with 75% ethanol. The precipitated polysaccharides were fermented with Pichia pastoris to produce bioactive oligosaccharides, followed by fractionation through size-exclusion chromatography. The fermentation process showed substantial growth of P. pastoris, with cell counts increasing from 4.98 ± 0.13 log CFU/mL to 8.76 ± 0.17 log CFU/mL over 6 hours. Biochemical analysis revealed that DF73 had the highest carbohydrate content (3.14246 mg/mL), while EPP contained the most unsaturated uronic acid (5.54436 ± 0.35138 µg/g) and hexose (6.54849 ± 0.30852 µg/g). DF58 exhibited the highest uronic acid concentration (7.98201 ± 0.73763 µg/g), and DF72 had the highest pentose content (4.54738 ± 0.11939 µg/g). The prebiotic effects on Bacillus clausii and Bifidobacterium bifidum showed that DF73 had the strongest acidifying effect (pH drop to 4.74333 ± 0.27538) and significantly supported bacterial growth. DF72 and DF73 had the highest prebiotic. These

findings highlight the potential of cress seed-derived polysaccharides, especially DF72 and DF73, as effective prebiotics for enhancing gut microbiota, offering a promising strategy for developing functional foods aimed at improving gut health.

Key words: Cress seed mucilage, Oligosaccharides, Pichia pastoris fermentation, Probiotics.

ISOLATION AND CHARACTERIZATION OF SIDEROPHORE PRODUCING ENDOPHYTIC BACTERIA FROM MORINGA OLEIFERA AND THEIR ROLE IN CHROMIUM STRESS MITIGATION
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ABSTRACT
The siderophores are low-molecular-weight secondary metabolites that are produced by bacteria and other microorganisms in iron-deficient conditions The aim of the study was to isolate, characterize and evaluate the biotechnological potential of siderophore producing endophytic bacteria from Moringa oleifera. Qualitative screening on CAS agar plates showed yellow-orange halo-shaped zones that indicate siderophore production and quantitative screening using CAS liquid assay demonstrated the variable production of siderophore production among the isolates. All isolates tested positive result in siderophore production, isolate NR-21 showed the most production (50.96%), followed by NL-3 and NR-33. In addition to siderophore synthesis, these isolates produced a wide range of metabolites such as indole-3-acetic acid (IAA) and salicylic acid (SA), phenols, flavonoids, carbohydrates, proline, and phosphate, which are quantitatively determined through spectrophotometer. Production of exopolysaccharide was confirmed by the chilled ethanol precipitation technique while catalase activity was verified by the use of slide catalase test in which all the isolates show positive response. Chromium resistance assay show that bacterial growths were reduced with the increase in the chromium concentrations, while IAA levels were decreased and SA production increased with increase in chromium concentration. Pot experiments on maize (Zea mays) showed that inoculation of siderophore producing isolates enhance germination rate, shoot and roots growth, biomass and photosynthetic pigments accumulation of maize under normal and chromium stress conditions. In addition, inoculated plants displayed greater sugar and IAA concentrations and a lower amount of proline than controls show improvements in stress tolerance. The results highlight the metabolic diversity and functional potential of endophytic bacteria isolated from Moringa oleifera in promoting plant growth and mitigating chromium toxicity. Their combined production of siderophore, phytohormone, and metabolites demonstrate a high potential for application in bioremediation and sustainable agriculture under heavy metal stress.

Keywords: Moringa oleifera, endophytic bacteria, Siderophore, chromium stress, bioremediation, plant growth promotion

EFFECTS OF FISH WASTE-BASED ORGANIC LIQUID FERTILIZER (LOF) ON THE SOIL FERTILITY, GROWTH, AND YIELD OF ONION (ALLIUM CEPA)

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ABSTRACT

The agricultural industry is striving to find sustainable alternatives to chemical fertilizers while simultaneously boosting food production. Therefore, a rich source of nutrients was selected by most farmers i.e., Fermented Fish Waste based fertilizer (FFW) that has been now gain a substantial agronomic value. This study investigates the effects of a Fish Waste-Based Organic Liquid Fertilizer (LOF) on the soil fertility, growth, and yield of onion (Allium cepa).The experimental design included seven treatment groups: a control group with no fertilizer, a positive control using commercial NPK fertilizer, and five experimental groups with varying concentrations of FFW (12 ml, 24 ml, 36 ml, 48 ml, and 60 ml). The trace metals found in these LOFs to promote growth, and nutrients in soil were analyzed by Atomic Absorption spectroscopy (AAS). The overall results of these treatments demonstrated that FFW is a valuable (specially 48ml and 60ml LOFs) would add essential nutrients in soil for raising growth rates and viable product for enhancing both soil fertility and onion cultivation. It is anticipated that the FFW treatment will lead to improved plant biomass production, larger and more productive bulbs, and a higher nutritional profile in the onion crop. These findings are expected to show that FFW is a feasible organic fertilizer, capable of promoting sustainable agricultural practices and reducing reliance on synthetic chemicals.

Keywords: Fish wastes; sustainable used of organic fertilizer , waste management; Organic fertilizer; Allium cepa

LIPASE PRODUCING POTENTIAL OF HALOTOLERANT BACILLUS SP. ISOLATED FROM SOIL OF SWAT, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Introduction: Halotolerant bacteria produce salt-resistant lipases used in food processing and other industrial applications. Halotolerant bacterial lipases are functional in detergents and surfactants as well as in varying pH, temperature, and salt concentrations. Objectives: This study explored indigenous lipase-producing halotolerant bacteria in the soil. Further, lipase extraction and physiochemical characteristics were evaluated. Materials and methods: Soil samples from different sites in Swat, Khyber Pakhtunkhwa, Pakistan, were collected in sterile bags and were transported to the Department of Microbiology, Abdul Wali Khan University Mardan. Lipase-producing bacteria were

isolated by serially diluting soil samples and spreading them on Tween 20 agar. The physiochemical properties of lipase-producing bacteria were examined by cultivating them at several pH levels, temperatures, and salt concentrations. The lipase-producing halotolerant bacteria were identified using 16S rRNA gene sequencing. The lipase enzyme was extracted, partly purified with ammonium sulphate, and resolved on SDS-PAGE. The activity of the extracted lipase was assessed at various pH values, temperatures, salt concentrations, and detergent concentrations. Results: Lipase-producing halotolerant Bacillus sp. were identified from a soil sample of Swat, Khyber Pakhtunkhwa. A 30 kDa lipase band was resolved on SDS-PAGE. Bacillus sp. possessed alkali-, acid-tolerant, psychotolerant, thermotolerant and halotolerant lipase. Their lipase was active in SDS and Triton X-100. Conclusion: Halotolerant lipase-producing Bacillus sp. found in soil. Further characterization and purification of lipase from this bacterium will help in their use for industrial applications.

SCREENING AND CHARACTERIZATION OF ALPHA -AMYLASE PRODUCING EXTREMOPHILIC SOIL BACTERIA
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ABSTRACT

Extremophilic microorganisms are valuable sources of industrially important enzymes due to their ability to survive and function under extreme environmental conditions. The present study was carried out to isolate and characterize alpha -amylase producing extremophilic bacteria from soil samples , collected from different sites and serially diluted to obtain distinct bacterial colonies on nutrient agar plates. A total of twenty -five isolates were obtained and screened for amylase production through starch hydrolysis using the iodine assay. Out of these, four isolates exhibited clear hydrolysis zones, indicating alpha -amylase activity. The positive isolates were f urther subjected to secondary screening in order to evaluate the alpha amylase production efficiency under optimized cond itions. Among them, the F1 bacterial isolated from soil (Karak) showed the maximum alpha -amylase activity , therefore, the F1 strain selected for further biochemical and molecular characterization. The study indicates that F1 could be a promising source of thermostable amylase , which can be effectively utilized in industrial processes such as fo od processing, textile desizing and pharmaceu tical applications. Overall, the findings suggest that soil extremophiles are valuable sources of novel and thermostable enzymes for industrial and biotechnological applications.

Keywords: Alpha -amylase, Extremophilic bacteria, Serial dilution, Soil isolate s, Thermostable enzymes, Biotechnology

ANTIBACTERIAL POTENTIAL OF MEDICINAL PLANT EXTRACTS AGAINST MDR PATHOGENS: HPLC AND MS ANALYSIS
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ABSTRACT

Background: Plants are a potential source of bioactive compounds. These compounds include a range of phytochemicals that have the ability to function in oxidative stress, anti-cancer, and antimicrobial. Furthermore, the development of therapeutic plants will impact the field of medicine because of the introduction of novel pathogens and diseases that require complementary therapies. Objective: The objective of this research was to evaluate the antibacterial activity of selected medicinal plant against selected multidrug -resistant (MDR) pathogens and to identify their bioactive compounds . Methods: HPLC fractions and crude extract of medicinal plant were checked against selected MDR pathogens by the well diffusion method. Mass spectrometry was performed for compound identification. Results: Both c rude extract and HPLC fraction s exhibited significant inhibitory activity against multidrug -resistant (MDR) pathogens . The HPLC fraction was processed through MS analysis . A total of fourteen compounds were identifie d, four compou nds were reported for the first time in the selected medicinal plant. Conclusion : Medicinal plant crude extract and HPLC fractions exhibited the strong inhibitory activity against selected MDR pathogens. The identified bioactive compounds may serve as promising candidates for pharmaceutical development against MDR pathogens.

Key words: MS (Mass spectrometry) , HPLC (High performance liquid chromatography), MDR (Multidrug -resistant).

GREEN SYNTHESIS OF BIOACTIVE SILVER AND NICKEL OXIDE NANOPARTICLES USING CHIA SEED MUCILAGE POLYSACCHARIDES

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ABSTRACT

The eco-friendly synthesis of nanoparticles has gained attention due to their diverse biological applications. This study presents the sustainable production of silver (Ag) and nickel oxide (NiO) nanoparticles (NPs) using water-soluble polysaccharides extracted from chia (Salvia hispanica) seed mucilage. Polysaccharides were extracted via ethanol precipitation, and the biosynthesized NPs were characterized by UV-visible spectroscopy, revealing absorption peaks at 473 nm for Ag-NPs and 343 nm for NiO-NPs. The antioxidant potential of the NPs was assessed using the DPPH assay, with Ag-NPs and NiO-NPs showing EC50 values of 118.85 ± 2.52 µg/mL and 97.21 ± 1.39 µg/mL, respectively, compared to 80.78 ± 1.65 µg/mL for ascorbic acid. Additionally, both NPs demonstrated promising in vitro antidiabetic activity, with IC50 values of 87.70 ± 3.65 µg/mL (Ag-NPs) and 146.15 ± 1.94 µg/mL (NiO-NPs) against alpha amylase, compared to acarbose (71.75 ± 2.63 µg/mL). The antibacterial effectiveness of the nanoparticles against three distinct variants of human pathogens was assessed using the agar well diffusion technique. The findings indicated that Ag-NPs effectively suppressed

the growth of three pathogenic strains: Staphylococcus aureus (9.5mm ± 0.5mm), Escherichia coli (11.75mm ± 0.82mm), and Hemophilus influenza (8.5mm ± 0.82mm). These findings indicate that chia seed-derived polysaccharides are effective in synthesizing bioactive Ag-NPs and NiO-NPs with strong antimicrobial and antioxidant properties, making them valuable for biomedical applications. This study underscores the potential of mucilage polysaccharides as a sustainable resource for producing bioactive nanoparticles.

Keywords: Nanoparticles, polysaccharides, Chia, Antimicrobial, antioxidant.

IN SILICO ASSESSMENT OF CYTOKININ SIGNAL TRANSDUCTION IN PLANT-ASSOCIATED MICROBES

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ABSTRACT

Cytokinins (CKs) are classical phytohormones regulating diverse aspects of plant growth and development. Their perception in plants occurs via membrane-bound Arabidopsis histidine kinases (AHKs), which transmit signals through histidine phosphor-transfer proteins and response regulators. Increasing evidence suggests that plant-associated microbes can also perceive and respond to CKs, yet knowledge of their perception pathways remains limited. This study employed a bioinformatics-based approach to investigate putative orthologues of CK signalling components in Pseudomonas fluorescens, Pseudomonas syringae, and Botrytis cinerea. Orthologous proteins with kinase, histidine phosphatase, and hybrid histidine kinase domains were identified, all containing a conserved histidine kinase (HK) domain essential for signal transduction. Notably, P. syringae AHP1 exhibited HK, HPT, and RR domains, suggesting functional versatility. Homology modelling revealed strong structural similarity of microbial orthologues to Arabidopsis CK receptors, and docking analysis demonstrated significant CK binding, particularly by the receiver domain of the P. syringae AHK4 orthologue. Key interacting residues included leucine, glutamine, and serine. These findings provide evidence that plant-associated microbes possess CK perception machinery and may respond to plant-derived CKs in the rhizosphere. Such microbial signalling capacity could have important implications for plant–microbe interactions, potentially influencing microbial colonization and plant health.

Keywords: phytohormones, cytokinin signalling, Arabidopsis thaliana, plant–microbe interactions

GREEN SYNTHESIS AND ANTIBACTERIAL EVALUATION OF IRON OXIDE NANOPARTICLES USING CHIA SEED POLYSACCHARIDES"

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ABSTRACT

Chia seeds (Salvia hispanica L.), a natural source of mucilage polysaccharides, exhibit remarkable biological potential with demonstrated antioxidant, prebiotic, and antimicrobial activities. This study presents the green synthesis of iron nanoparticles using chia seed polysaccharides as both a reducing and stabilizing agent. The biosynthesized iron nanoparticles were characterized through UV-visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and X-ray diffraction analysis, confirming successful nanoparticle formation. The antibacterial potential of Fe-NPs was assessed against five human pathogenic strains i.e Staphylococcus aureus, Salmonella typhi, Shigella spp., Klebsiella pneumoniae, and Salmonella spp using agar well diffusion, microbial biofilm inhibition, and minimal inhibitory concentration (MIC) assays. Results demonstrated that Fe-NPs at 100 µg/mL produced inhibition zones of 8.78 mm against S. aureus and 8.7 mm against S. typhi, compared to 7.73 mm for chia seed polysaccharides alone against S. aureus. The microbial biofilm inhibition assay revealed an IC50 of 15.67 µg/mL for Fe-NPs, while chia seed polysaccharides exhibited an IC50 of 14.21 µg/mL against Klebsiella pneumoniae. MIC assays indicated that Fe-NPs effectively inhibited the growth of S. aureus at a concentration of 28.12 µg/mL, with higher concentrations required for Shigella spp. (57.59 µg/mL), Klebsiella pneumoniae (87.49 µg/mL), and Salmonella spp. (54.22 µg/mL). These findings underscore the potent antibacterial activity of chia polysaccharide-based Fe-NPs, highlighting their potential as a natural, eco-friendly alternative for biomedical applications in combating bacterial infections.

Keywords: Chia seeds, Mucilage, Polysaccharides, Iron Oxide Nanoparticles, Antibacterial

FRACTIONATION OF ANTIOXIDANT POLYSACCHARIDES IN CRESS SEED COAT MUCILAGE BY SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

Elevated levels of reactive oxygen species (ROS) pose severe threats to the intracellular environment in terms of oxidative stress, and their continuous overproduction causes various clinical disorders. Antioxidant compounds combat oxidative stress in cells and are therefore useful in the management of various clinical disorders. Plants are an important source of bioactive compounds, especially mucilage containing polysaccharides have been extracted from plants and studied for various biomedical applications. Lepidium sativum, commonly known as garden cress, whose seeds release mucilage upon soaking in water. The aim of the current study was to extract polysaccharides via size-exclusion chromatography (SEC) from the seed coat mucilage of garden cress and to evaluate the bioactive fraction(s). The mucilage was extracted by soaking cress seeds in distilled water. The collected mucilage was then precipitated with 75% ethanol. The ethanol precipitated fraction was further fractionated through Bio-Gel P-10 (90 µm-180 µm). A total of 60 (2 ml each) fractions were collected tested for the

presence of carbohydrates. The results revealed the presence of pentoses, hexoses, uronic acids and unsaturated uronic acids. Among all the screened samples for their antioxidant potential through DPPH assay, significant EC50 value was shown by fraction F23 as its EC50 was 37.93 µg/mL, followed by fraction F52 (58.04 µg/mL) and fraction F53 (66.04 µg/mL), respectively. Selected SEC fractions of ethanol precipitated polysaccharides extracted from cress seed coat mucilage were then screened for H2O2 radical scavenging activity. Each fraction was screened at three different concentrations, including 50, 100 and 200 µg/mL. The results obtained showed that fraction F53 was the most effective fraction at 200 µg/mL as it scavenged the H2O`2 free radical by 81.9%±0.24, followed by fraction F18 (76.54%±0.65) and fraction F23 (76.4%±1.96). In vitro lipid peroxidation assay was performed on mice tissue samples where F52 fraction was the most effective one having IC50 value of 34.17 µg/mL, followed by F20 having IC50 value of 57.18 µg/mL. Our results concluded that water-soluble polysaccharides extracted from cress seed coat mucilage can be used as potential and effective natural antioxidant compounds.

Keywords: Endophytes, Exopolysaccharides, Wheat plant, Bioactive compounds

THEME 2: ANIMAL AND VETERINARY SCIENCES

COMPARATIVE EFFECTS OF VARIOUS INFECTIOUS BRONCHITIS VACCINES (IBV) ON THE GROWTH PERFORMANCES AND IMMUNITY OF BROILERS

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ABSTRACT

In this study efforts were made to answer the concern of local farmers that imported vaccines of Infectious Bronchitis (IB), being having the different strains from the local ones, leads to mild IB symptoms in the form of fever, mild respiratory symptoms a nd depressed growth. Hence, the purpose of this study was to compare the immunological responses, tracheal, lung, and kidney histomorphology, and growth performance of broilers given IB vaccines having different strains. A total 120 broiler chicks, each o ne day old, were split into four groups of three replicates, each with 10 chicks. Three most commonly used IB vaccines namely IB4/91, IB Mas and IB H120 were randomly provided as prime and booster doses to the experimental groups keeping one group as contr ol. Data on feed intake, body weight, gain, FCR, Immunity and histomorphology of trachea, lungs and kidneys were collected and analyzed for the results. There was no effect on growth performance, including feed intake, body weight gain, and feed conversion ratio, when various IBV strains including vaccines were given. Results were found to be statistically similar (P<0.05) in the vaccinated and control groups. In terms of numerical data, the vaccinated birds in IB 4/91 showed a superior trend in body weight growth (1.83 kg) and FCR (1.35) than the control group (WG1.82kg, FCR 1.36) and vaccinated groups 3 and 4 (WG1.82kg, FCR 1.35). In comparison to the control group, the vaccinated group had a considerably greater antibody titer (P<0.05). In comparison to I B mas (1202.47) and IB H120 (1295.93), Group 2, which consisted of vaccinated birds with the IB 4/91 strain of the IBV, had the best titer (1431.23) among the vaccinated groups (P<0.05). A similar trend though non -significant was also observed in the morta lity. Birds vaccinated with IB 4/91 strain showed no mortality. Birds in the rest of the groups were found with 3.33 % mortality. Mild to moderate respiratory symptoms were noted in the control group and the post mortem finding of the dead birds from cont rol group were showing the congestion in trachea and lungs as seen in the IB infection. The histomorphology of trachea, lung and kidneys collected from the birds of the experimental birds showed almost normal features in the broilers vaccinated with IB 4/9 1 strain. Mild to moderate lesions were observed in the IB mas and IB H 120 vaccinated birds. In comparison to the vaccinated groups, the control group's birds had more severe lesions in all three organs. The current research found that the three imported vaccines tested had no adverse effect on the birds' ability to develop, and in fact increased their IBV antibody levels as compared to the control group. However, some mild signs of respiratory disease were observed in the control as well as birds vaccinat ed with IB mas and IBH120 vaccine strains. The IB 4/91 among the tested vaccines was found better in term of antibodies titer, mortality and histo

morphological studies.

Key Words: Infectious bronchitis virus, Broilers, Immunity, Growth Performance and histomorphology

SERO -INCIDENCE AND PATHOLOGY OF INFECTIOUS BRONCHITIS VIRUS (IBV) IN COMMERCIALLY REARED POULTRY BIRDS IN DIFFERENT REGIONS OF KHYBER PAKHTUNKHWA

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ABSTRACT

Infectious Bronchitis is a disease of significant economic importance worldwide. The existence of Infectious Bronchitis Virus (IBV) in commercial poultry in various parts of Pakistan has been reported; however, literature regarding the recent status of IB in commercially reared poultry in Khyber Pakhtunkhwa was not available. The present study was therefore conducted for the examination of the sero -incidence of the IBV in different divisions of the province, including Hazara, Malakand, Kohat, and Peshawar. A basic random sampling technique was used for the collection of samples. Samples from various age groups of commercial poultry were collected and an indirect enzyme -linked immunosorbent assay (ELISA) was used for serological detection. In 2022, out of the total 1240 collected samples, 133 (10.7%) showed a positive antibody titer test against the IB virus. Similarly, in 2023, out of the total 1094 samples collected, 131 (11.9%) showed positive antibody test against the IB virus. The highest positive inciden ce was in October, with 28.7% prevalence, followed by December with 23.6% prevalence, May, and June with 15.8% and 11.6% prevalence, and September with 11.7% prevalence. The lowest prevalence of 3.2 % was observed in April. The highest incidence, calculate d at 19.6%, was observed in region -2, followed by region -1 with 14.8% and region -4 with 10.5% prevalence. Region 3 was noted with the lowest incidence of 4% only. Among the bird categories, the layers had the highest incidence of 14.2%, followed by the bro ilers and breeders, each with an incidence of 12.5%. Based on the number of birds reared on the farm, category 3 had the highest incidence of 15.8%, while category 2 had an 11.8% incidence. Category 1 showed the lowest percentage of positive cases (6.6%). Among age categories, early age showed the highest incidence of 12.91%, category 2 had 10.8%, and category 3 showed the least positive results of 3.56%. Gross observations of enlarged kidneys and hemorrhagic trachea were similar in all seropositive cases. The histopathology results revealed that the tracheal epithelium had sloughed off and the cilia had been lost. Acute tubular necrosis and moderate to severe congestion were shown on the renal microscopy. The results revealed that, despite vaccination, there is a persistent emergence of IB outbreaks in commercial poultry. Therefore, to prevent infectious bronchitis in commercial poultry, farmers should follow the locally prescribed vaccination schedule using particular viral strains.
Keywords: ELISA , Histopathology, Incidence, Infectious Bronchitis Virus ,

MOLECULAR CHARACTERIZATION OF INFECTIOUS BRONCHITIS VIRUS (IBV) ISOLATED FROM POULTRY IN KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Infectious bronchitis, caused by the Infectious Bronchitis virus (IBV), is a highly contagious disease that can infect poultry of all age groups. IBV is known for its diverse genotypes and strains, which can affect the respiratory system, reproductive orga ns, and kidneys of infected birds. This study aimed to explore the molecular epidemiology of IBV in districts with high poultry populations in the Khyber Pakhtunkhwa province. A total of 425 samples, including swabs and infected organs, were collected from various districts across the province. Specifically, 100 samples were obtained from Abbottabad and Mansehra, 90 from Mardan and Peshawar, and 45 from Malakand. The overall prevalence of IBV was 29.6%, as determined by RT -PCR. Area –wise prevalence was sign ificantly higher in Mansehra district (39%), followed by Mardan (31.1%), Abbottabad (31%), Peshawar (22.2%), and Malakand (7.7%). The prevalence of IBV was also notably higher in young birds (35.2%) and layers (33.7%) compared to broilers (30.7%). Good ventilation and disinfection between batches can significantly impact poultry health, as demonstrated by this study. Implementing all -in-all-out management practices, meaning birds of the same age are grouped, is recommended over maintaining different age gro ups on the same farm. Proper management combined with timely vaccination can significantly improve bird health and productivity, potentially reducing the risk of IBV outbreaks. The results indicate that infectious bronchitis in poultry from Khyber Pakhtunk hwa is primarily caused by IBV genotypes GI -19 and GI-13, which are the major circulating strains with limited similarity to GI -1 strains. This points to local endemicity and diversification of sub -lineages, likely driven by regional viral evolution and in adequate vaccination efforts. These findings underscore the urgent need for continuous molecular surveillance and the development of updated vaccine strategies tailored to regional IBV variants.

Keywords: Broilers, Genotyping, Infectious bronchitis virus, Layers, Risk factors, RT -PCR

A STUDY ON THE INCIDENCE AND CAUSES OF LAMENESS IN FARM-KEPT HORSES

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ABSTRACT

Lameness is a common and important problem in horse health that affects their performance, well-being, and value. This study looks at the causes, ways to diagnose, and treatments for lameness in horses. The main causes are musculoskeletal issues like arthritis, tendonitis, and hoof problems, as well as neurological conditions. Diagnosing lameness involves clinical exams and imaging techniques such as X-rays, ultrasound, MRI, and CT scans. Our study results conclude cause of lameness, accounting for 60% of the cases included arthritis, tendonitis, and fractures. Arthritis, characterized by inflammation of the joints, was prevalent among older horses. Tendonitis, an inflammation of the tendons, often resulted from overuse or strain. Fractures were typically caused by trauma or accidents. 10% of the lameness cases were due to bacterial and viral infections which had affect joints and soft tissues, leading to conditions such as septic. While 5% of the cases associated with metabolic imbalances. The findings of this study highlight the importance of a comprehensive approach to managing and preventing lameness.

Keywords: diagnosis,arthritis, lameness, horse

ISOLATION AND IDENTIFICATION OF ESCHERICHIA COLI FROM RAW MILK SOLD UNDER MARKET CONDITIONS AT RAWALPINDI AND ISLAMABAD

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ABSTRACT

Pakistan is now the third largest producer of milk in the world. Production of maximum quantities of high-quality milk is an important goal of every dairy operation. High-quality milk must contain a low number of somatic cells and a low bacteria count, and must be free of human pathogens and antibiotic residues. The present study was undertaken to estimate the incidence of opportunistic pathogen, Escherichia coli (E. coli), in raw cow's milk from different localities of Rawalpindi and Abbottabad. Fifteen raw milk samples were randomly collected and inoculated on the relevant bacteriological media. Confirmation was performed using a series of biochemical tests. The results revealed that out of 15 samples 60% were E. coli positive.
Keywords: diagnosis, mastitis, E.coli,

CROSS-SECTIONAL INVESTIGATION OF THE VETERINARY AND HUSBANDRY PRACTICES IN A RACE CLUB LAHORE

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ABSTRACT

Objectives: Racehorses are elite athletes that require specialized care to optimize their performance and welfare. However, the intensity of racing and training can lead to

injuries and health issues. This study aimed to investigate the current veterinary and husbandry practices in a race club to identify areas of strength and weakness. Material &Methods: A cross-sectional study was conducted using a mixed-methods approach. An online survey was distributed to 150 racehorse owners/trainers, 75 veterinarians, and 30 farriers affiliated with the race club. Telephone interviews were also conducted with 20 owners/trainers, 10 veterinarians, and 5 farriers to gather more in-depth information. The survey and interview questions covered various aspects of veterinary and husbandry practices, including vaccination protocols, deworming schedules, dental care, hoof care, nutrition, and exercise routines. Results: The study found that: 80% of horses received regular vaccinations, but only 60% were vaccinated against infectious diseases such as influenza and strangles. 70% of horses were dewormed regularly, but 20% had evidence of parasite infestation. 60% of horses received dental care, but 30% had dental abnormalities. Only 40% of horses had regular hoof trimming, and 20% had hoof cracks or abscesses. 70% of horses were fed a balanced diet, but 20% had evidence of malnutrition. 60% of horses received regular exercise, but 20% were stall-bound for extended periods.   The study also found varying levels of collaboration and communication among owners/trainers, veterinarians, and farriers, which may have contributed to the observed deficiencies in care. Conclusion: This study highlights areas of strength and weakness in veterinary and husbandry practices in the race club. Improved communication and collaboration among stakeholders, increased emphasis on hoof care and pasture turnout, and enhanced veterinary care may benefit horse welfare and performance. These findings have implications for the development of evidence-based guidelines for racehorse care and the improvement of horse health and well-being in the racing industry.

Keywords: horse, race club , diseases

ABSTRACT 033

PREVALENCE, CLINICOTHERAPEUTIC TRIALS OF PULPY KIDNEY DISEASE(ENTEROTOXEMIA), AND ANTIBIOTIC RESISTANCE PATTERN OF THE CAUSATIVE AGENT CLOSTRIDIUM PERFRINGENS TYPE D IN GOATS OF PESHAWAR AND CHARSADDA DISTRICTS

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ABSTRACT

Pulpy Kidney Disease (Enterotoxemia) caused by Clostridium perfringens Type D is a fatal disease of Sheep and Goats with a maximum 100% reported mortality. The disease affects the goats more severely in the age group of 4-10 weeks. The current study aimed to determine the prevalence and therapeutic management of Pulpy Kidney Disease (PKD), and in-vitro evaluation of antibiotic susceptibility of the causative agent i.e. Cl. perfringens type D. Five hundred fecal samples (each ≈ 5g) from goats suspected for PKD were collected from different areas of Peshawar and Charsadda districts. Samples were transported to Pathology laboratory in ice box and were anaerobically cultured on selective medium i.e. Tryptose Sulphite Cycloserine (TSC). After incubation of 24-48h at 37 C°, 70 samples were found positive for Cl. perfringens Type D on bacterial culture and PCR, thus overall prevalence of 14% was recorded. A higher prevalence percentage

of the disease was recorded in District Peshawar (16%) than Charsadda (12%). Season-wise data showed highest prevalence of the disease in spring (18.9%) while minimum in summer season (7.1%). The purified isolates of Cl. Perfringens type D from field cases were subjected to antibiotic susceptibility testing using Kirby Bauer method. Results showed that the bacterium was highly susceptible to sulphamethoxazole in combination with trimethoprim, and teicoplanin. Moreover, bacterium showed intermediate susceptiblilty to amoxicillin and norfloxacin. Similarly, the bacterium was found resistant to gentamicin and tetracycline. Therapeutic trials showed that parenteral administration of sulphadiazine + Trimethoprim resulted in significantly ($p<0.05$) higher recovery rate in diseased goats in comparison to oral administration of antibiotics and antidiarrheal mixture. It is concluded from the current study that PKD is still occurring with notable rate (14%) in goats' population of Peshawar and Charsadda districts. The bacterium was highly susceptible to sulphamethoxazole in combination with trimethoprim, and teicoplanin hence it should be used for the treatment of PKD. Similarly, the bacterium was resistant to gentamycin and tetracycline hence it should be avoided for the treatment of this deadly disease.

Key words: Antibiotics susceptibility, Clostridium perfringens, Enterotoxemia, Goats, PCR, Pulpy Kidney Disease, Therapeutic trials.

ABSTRACT 034
PREVALENCE AND RISK FACTORS OF GIARDIASIS IN CATS AND DOGS IN DISTRICT MARDAN

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ABSTRACT

Giardia is a pathogenic flagellated and unicellular protozoan that mainly targets the duodenum of host and cause intestinal diseases called Giardiasis in both humans and animals including dogs and cats. Giardiasis in dogs and cats can be spread through ingesting, smelling, or drinking contaminated water or ground cysts. Due to malabsorption, maldigestion, or increased motility, giardiasis in dogs and cats can be asymptomatic or cause diarrhea. 15.6% of dogs and 10.8% of cats in the United States had Giardia infection, respectively. According to an ELISA test, the prevalence of Giardia infection in Romania is 34.6%. In Japan, puppies had a substantially greater rate of giardia infection (54.5%) than adults (30.9%). Giardia infection prevalence in dogs and cats has been found to be 10.4% in Lahore. In contrast, there is no research on the frequency of Giardia in cats and dogs in KPK. The purpose of this study was to identify the prevalence and associated risk factors of Giardia lamblia among dogs and cats' population in all four tehsils of District Mardan. 300 Fecal samples were collected randomly from 150 dogs and 150 cats and was tested for Giardia lamblia using commercial ELISA kit. 90 fecal samples (41 dogs and 49 cats) shown positive results with over all prevalence of 30.00%. It shown that the presence of the parasite Giardia lamblia was substantially correlated with the age, habitat, and diet of the pets. Age led to

a decline in prevalence. While domestic dogs and cats are less likely to contract Giardia lamblia than stray animals and those who eat outdoor food.

Key words: Giardia lamblia; prevalence; risk factors; ELISA; Giardiasis

ASSESSMENT OF CERTAIN BIOCHEMICAL, HEMATOLOGICAL AND HORMONAL PROFILE OF NORMAL CYCLIC, ANOESTRUS AND REPEAT BREEDER CROSSBRED CATTLE

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ABSTRACT

Livestock play an essential role in the socioeconomic development of the country. However, reproductive disorders including repeat breeding and anest rous syndrome in crossbred animals are adversely affecting the productivity and profitability of the animals, thus causing huge economic losses to the dairy sector in the country. Theref ore, the current research was conducted with the objective of investigating changes in biochemical metabolites and hematological indices of cows diagnosed with repeat breeding and anestrus, which aim to replicate the impact on animal health, performance, and reproductive efficiency under existing management Center of excellence for Bovine Genetics O kara district, Pakis tan. Execution of the current trial was carried out on a total of thirty (30) crossbred animals out of which ten each were used from regular cyclic healthy crossbred animals, repeat breeding and anestrous animals. Standard protocol wa s observed during the execution of the research trials. The concentration of Glucose, cholesterol, total protein and Blood urea nitrogen (BUN) were examined in repeat breeder and anestrous animals which were compared with regular cyclic animals. Some blood spe cimens were used for hematological purposes whereas some were used for the appraisal of biochemical profiles. Blood collection was done from jugular vein aseptically. Serum was obtained through centrifugation for various tests. Biochemical indices including total protein, Glucose, total cholesterol and BUN, Calcium, Phosphorous and Progesterone were investigated through commercial available kits. Furthermore, standard hematological laboratory procedure was adopted for the appraisal of hematological indices i ncluding Hemoglobin, TRBC, TLC, TRBC, PVC, Monocytes, Eosinophil, Basophil, etc. During the current study the mean total protein, glucose concentration and cholesterol were recorded as 6.14±0.41g/dl, 59.76±0.89mg/dl and 159.67±0.67mg/dl in the repeat breeder cow as compared for normal cyclic cows, where it was recorded as 8.04±0.63 g/dl, 82.99±0.76mg/dl and 209.78±3.87mg/dl respectively. Furthermore, theses indices were 6.13± 0.59, 61.62c±1.05 and 149.73±5.16 in the anestrous cows. On the other hand, the elevated concentration of BUN of 30.99±2.83mg/dl and 28.41±0.15 was recorded in the repeat breeding and anestrous cows as compared for normal cyclic cows, where it was recorded as 22.25±1.27 mg/dl of normal cyclic cows. Furthermore, during current investigation the value recorded for calcium concentration was 8.02±0.11 in the repeat breeding cows and 7.34c±0.14 in the

anestrous cows whereas, it was 10.06±0.15 in the health repro ductive cows. Likewise, the phosphorous concentration was 6.94±0.18 in the healthy fertile cows whereas it was 4.23±0.13 in the repeat breeding cows and 4.19±0.13 in the anestrous cows. Thus, the current biochemical indices study demonstrated significant d ecreases (P<0.05) of total protein, glucose and cholesterol, Calcium and Phosphorous. On the other hand, significant increase in BUN (P<0.05), in repeat breeding dairy and anestrous cows was observed when compared with those of the normal cyclic cows. Also the current study elucidated the significant difference in the hematological indices in the reproductive stages such as normal cyclic, repeat breeding and anestrous syndrome in the crossbred cows.

Key words : Anestrous, Repeat breeding, Crossbred animals, Biochemical profile .

THE LETHAL SEQUEL: HEMORRHAGIC BOWEL SYNDROME AS A SECONDARY FATAL CONDITION IN DAIRY ANIMALS

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ABSTRACT

It is known in the scientific world that a number of Clostridium spp. inhabit the intestine of ruminants and some other animals as normal commensals. The body keeps the count of this bacteria in a limit which is safe for itself in various ways e.g., the gut propulsive movements, mucous secretions and certain immunoglobulins in the lining secretions etc. However, there are certain conditions which results in down regulation of the above-mentioned mechanisms. The net result is favorable medium for the proliferation and consequent secondary hemorrhagic bowel syndrome which is predominantly attributed to Cl. perfringens Type A. Thus, creating a very fatal condition with clots and fresh blood oozing out with defecation and profound systemic toxemia with rapid progression to death. Formerly, the potential predisposing causes for this condition were reported to be high fermentable carbohydrates feeding and mycotoxins in feeds especially those produced by Aspergillus fumigatus. It has recently been observed that animals suffering from tick borne hemoparasitic diseases also have hemorrhagic bowel syndrome as potential sequelae. Hence this can be concluded that the spectrum of diagnosis and clinical assessment should expand to tick borne diseases as well in prevention of this fatal calamity. The therapeutic regimens designed to control the primary disease must also have some remedies to avoid secondary hemorrhagic bowel syndrome.

Keywords: Hemorrhagic Bowel Syndrome, Clostridium perfringens type A, Mycotoxins, Hemoparasitic diseases.

EFFECT OF INORGANIC SELENIUM SUPPLEMENTATION ON THYROID HORMONE AND REDOX STATUS IN BROILER UNDER DEXAMETHASONE-INDUCED STRESS

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ABSTRACT

Poultry industries are confronted to many difficulties which have made it a big problem for humans and birds. One of the major problems is oxidative stress which causes free radical damage and imbalance of thyroid functions. This research experiment was performed to check the effect of orally inorganic selenium on redox status and thyroid hormones of broilers under dexamethasone-induced stress. Day-old 100 chicks was purchased from the trading hatchery and divided randomly in five different groups having four replicates (n=5) in each groups. Broiler chickens feed with starter and finishing commercial corn-based basal diet with different concentrations of selenium powder. Basal diet (BD) was fed to Group A, which kept as a negative control group and no dexamethasone (DE) was be offered. Group B was considered as (+ve control group) fed with (BD+15mg DE/kg). The group C fed by (BD+0.2mg Se + 15mg DE/kg). Similarly, group D was feed by (BD+0.3mg Se+15mg DE). Group E, fed with (BD+0.4 Se+15mg/kg feed). At the end of the trail, two birds was randomly selected for slaughtering and collect samples of liver, muscles, kidney, and whole blood. The whole blood was centrifuges for further analysis. The redox status and thyroid hormones were measured through commercial available kits. The statistical analysis was through IBM SPSS 20.0. This current study showed that selenium supplementation improved(P≤0.05) the catalase level in the liver and muscles which fight against the free radicals and reduce oxidative stress. In the blood serum, the catalase and MDA both show significance (P≤0.05). In conclusion, I assume that 0.3mg/kg of selenium supplementation in redox status and thyroid hormone shows better results in comparison to the control group.
Keywords: Avian, antioxidant, thyroid hormone, trace element, redox status, Se.

ABSTRACT 038
PREVALENCE AND MULTIDRUG RESISTANCE IN SALMONELLA FROM RETAIL CHICKEN MEAT IN MARDAN, PAKISTAN

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ABSTRACT

Salmonella, a Gram-negative bacterium belonging to the Enterobacteriaceae family, is a major foodborne pathogen that causes salmonellosis worldwide. This study emphasizes the need for continuous monitoring and better antimicrobial practices in food production by illustrating the presence of Salmonella in poultry, its possible zoonotic impacts, and issues related to multidrug resistance. The goal of this study is to isolate and characterize Salmonella spp. from samples of chicken meat in Mardan district, and to evaluate the patterns of antibiotic resistance. For the purpose of bacterial isolation, 40 samples of chicken meat will be collected from four distinct locations in Mardan and processed using Brain Heart Infusion (BHI) and Xylose Lysine

Deoxycholate Agar (XLD) media. Following sample blending in sterile Brain Heart Infusion, isolates of Salmonella will be identified biochemically and characterized molecularly using Polymerase Chain Reaction (PCR), which targets the genus specific invA gene. Using the Kirby-Bauer disk diffusion method, the antibiotic susceptibility of isolate to five antibiotics—gentamicin, meropenem, cefepime, amoxicillin/clavulanic acid, and ciprofloxacin—will be assessed. The detection of antibiotic resistant genes BlaTEM, BlaCTXM and catA will also be checked using specific primers. The presence of Salmonella in processed samples can indicate the risk of consumers to be infected with salmonellosis through chicken flocks and the genotypic resistance pattern to antibiotics can draw attention in terms of both human and animal health. Can also, promote other options for poultry farming, avoiding antibiotics supplementation.

Keywords: Salmonella, Antimicrobial Resistance (AMR), Poultry Meat, Zoonosis

ABSTRACT 039

COMPARATIVE ASSESSMENT OF PROBIOTICS WITH DIFFERENT COMMERCIALLY AVAILABLE PROBIOTICSON BROILER PRODUCTION PERFORMANCE AT STARTER AND FINISHER PHASE

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ABSTRACT

To investigate and make a comparison of the probiotics (PB) with the different commonly used commercial probiotics available in the Pakistan poultry feed market. Birds growth performance and nutrient digestibility were assessed. Results showed the effect of probiotics supplementation feed on growth performance measurements in broiler chickens. Results clarified that broiler chickens fed with probiotics have significantly heavier BW and better FCR at 3wk of age compared with other treatment groups. Body weight gain and FCR was significantly improved by the supplementation of probiotics compared to other treatment groups supplement with different commercial probiotics. Feeding a diet with a probiotics at rate of 0.02% significantly improved the ileal digestibility of dry matter and CP efficiently on day 35 compared to those fed without or other commercially available probiotics. The digestibility of amino acid were significantly improved by the supplementation of probiotics in broiler diet deficient with crude protein and amino acid. The interaction of crude protein, amino acid and probiotics for ileal amino acid digestibility of methionine, lysine, alanine, cysteine, leucine, serine, threonine and tyrosine was significant. Maximum improvement was observed in-group supplemented with probiotics for methionine, lysine, threonine, cysteine and serine. There was no significant difference observed among the all treated groups in litter quality during the starter and finisher phase of the production. Litter quality decreased along the days of experiment, independently of litter material, as expected due to the increase in the humidity produced by the birds and their manure.

EPIDEMOLOGICAL INVESTIGATION OF PARASITIC INFESTATION IN SMALL RUMINANTS IN RULAR SETTINGS OF QUETTA , PAKISTAN

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ABSTRACT

Parasites poses serious threat to animal husbandry in term of direct and indirect losses. This study was designed to estimate the parasitic infestation in small ruminants in Quetta Pakistan. Retros pective data was collected from Livestock & Dairy Development Department Quetta regarding animal treatments during field visi ts, mobile camps, inside hospital s and dispensaries in and around Quetta during summer and winter in Jan - Dec 2023 . Total of sheep 138264(48%) and goats 146139(51%) were treated for parasit ic infestation. Among the internal parasites 65208(22%) cases of lungworm, 54249(19%) liver fluke and 56013(19%) wireworm were recorded . Similarly , 78719(27%) cases of ticks and 30214(10%) mange was seen and treated . On season basis during January - June, 2023 , 30900 (10%) sheep were affected by external parasites and 50097 (17%) by internal parasites. Whereas, goats 44796 (15%) internal parasites and external parasites 32714(11%) were recorded , respectively . Similarly July -December 2023, 22909(8%) sheep wer e affected by external parasites and 33203 (11%) by internal parasites . However, 22380(7%) goats were affected by e xternal parasites and 38376(13%) by internal parasites during the same period . This study highlights the prevalence of parasitic diseases in small ruminants in the area and draws the attention of policy makers to construct comprehensive strategy to control parasitic infestation in small ruminants in the area.

BIOMASS PRODUCTION OF PASTEURELLA MULTOCIDA FOR VACCINE PREPARATION USING BIOFERMENTER

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ABSTRACT

The organism Pasteurella multocida Robart B:6 strain was cultured on two different growth medium Brain Heart Infusion (BHI), and Casein Sucrose Yeast broth (CSY). After the seed activation in mice the organism was isolated from the heart blood of mice and was confirmed through presumptive methods and molecular based PCR test. A bsence of growth on the MacConkey agar was also recorded . The different growth factors and biomass production was compared with conventional and biofermenter method in Center for Advanced Studies in vaccinology & Biotechnology (CASVAB), University of Balochistan, Quetta. Results revealed the parameters established for manually harvested seed and biofermenter at pH 7.2 @ 37°C and 500 rpm agitation , operated ideally. The Biomass production by Casein Sucrose Yeast (C SY) broth relies heavily on its controlled parameter values higher yield of biomass production in biof ermenter than conventional technique. After 16 hours of growth, the biomass recorded was 2.205 mg/ml and 2.737 mg/ml concentration in comparison with conventional flask method and biofermenter , respectively u sing spectrophotometer (SHIMAZDU, JAPAN) @450 nm. These results suggest use of bioferementer for higher biomass yield especially in the production of cost effective vaccines.

ABSTRACT 042
EXPLORING THE IN VITRO COMPARATIVE EFFICACY OF ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF NIGELLA SATIVA (BLACK SEED) AND CROCUS SATIVUS (SAFFRON)
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ABSTRACT

Traditional medicinal plants have been known as possible sources of bioactive substances with antimicrobial potential. Of these, Nigella sativa (black seed) and Crocus sativus (saffron) are being Spotlighted for their potential therapeutic uses as alternatives to antibio tics. This study is designed to evaluate antimicrobial potential and antioxidant potential of extracts from black seed and saffron against some common pathogens, such as Staphylococcus aureus, Escherichi a coli, Pseudomonas aeruginosa, and Candida albicans. Bacterial cultures were grown on different selective and differential media su ch as Nutrient Agar (NA), Eosin -Methylene Blue (EMB) Agar, Brain Heart Infusion (BHI) Agar, and Muelle r-Hinton Agar. Whereas, C. albicans was grown on Sabouraud Dextrose Ag ar. The antimicrobial potential of the extracts was tested using disc diffusion method. The results reve aled that ethanolic extracts of black seed showed the inhibitory zone against S. aureus (20 mm), E.coli (18 mm) and Pseudomonas aeruginosa (16 mm).But Candida albicans was found resistant against Black seed. Similarly, the ethanolic extract of saffron showed the inhibitory zone against S. aur eus (26 mm), E.coli (24 mm) and Pseudomonas a eruginosa (20 mm). While the zone of inhibition (28 m m) was recorded against candida albicans. The antioxidant ability of the extract was

analyzed by DPPH (2 , 2-diphenyl -1-picryl -hydrazyl) scavenging assay in terms of concentration having 50% inhibition (IC50). Results showed that IC50 value of Black seed extract for DPPH% inhibition was 10.2 ± 1.20 mg/mL at a concentration of 43.71 mg/ml. whereas IC 50 value of saffron extract for DPPH % inhibition was $10.8\pm1 .02$ mg/mL at a concentration of 0.5 mg/ml.

Key Words: Medicinal Plants, Antimicrobial Effects, Anti -oxidant, Extracts, Pathogenic

ABSTRACT 043
EVALUATION OF PHYSICAL PROPERTIES OF SALIVA AS NON-INVASIVE AND POINT-OF-CARE DIAGNOSTIC TOOLS FOR EARLY PREGNANCY DETECTION IN COW

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ABSTRACT

The study aims to design a cost-effective pregnancy detection model by combining early pregnancy predicting parameters, altering saliva's physical properties in response to early pregnancy in cattle, and developing a point-of-care testing system. The study analyzed the physical properties of saliva in 100 pregnant and 100 non-pregnant cows at Government Cattle Breeding and Dairy Farm, Harichand, Sucha Dairy Farm and Jaan dairy farm at district Charsadda, Khyber Pakhtunkhwa Pakistan. The samples were collected through pre-weighed sponges that were attached to a thin, flexible metal rod, into the cows mouth for 30 seconds and processed at the respective farms. The results showed that mean pH in pregnant cows was significantly higher ($P\leq0.05$) (9.325 ± 0.13) than non-pregnant animals (8.133 ± 0.13). The mean Specific gravity (0.000173 ± 0.00), and Conductivity (0.666 ± 0.029) in non-pregnant were higher ($P\leq0.05$) than the pregnant cows (0.000146 ± 0.00) and (0.538 ± 0.028) respectively. The mean buffer capacity (7.40 ± 0.10) and flow rate (91.92 ± 1.13) of non-pregnant were higher ($P\leq0.049$) than in the pregnant animals (2.42 ± 0.151) and (91.92 ± 1.13). The mean density (0.172 ± 0.03) non-pregnant cow were higher ($P\leq0.05$) than the pregnant cow (0.172 ± 0.03).The study examined cows' salivary crystallization patterns during the pregnancy. Six different patterns were identified, including branches, ferns, fir dot none and a combination of these patterns. In pregnant cows, Fern-like patterns are 26.19, fir-like 4.76, and Branch-like 11.90, Dot-like 9.52, Branche-fir 7.14, Branche-fir-fern 4.76, branch-fern 19.04, fern-fir 7.14, none 9.52. In non-pregnant cows, fern-like 0.00,fir-like 6.45, bran-like 0.00, Dot-like 19.35,branch-fir 29.03,branch-fern-fir 19.35,branch-fern 22.58,fern-fir 0.00, none 3.22. The significant and numerical variations in the physical aspects of pregnancy seem to be influenced by hormonal fluctuation pregnancy. Further investigations are required to validate these variations for early pregnancy diagnosis in cattle and other animal species.

Keywords; Biomarker, Early pregnancy, non-invasive, physical properties, saliva

ANTIOXIDATIVE PROTECTION BY STRAWBERRY AND GREEN TEA EXTRACTS DURING CRYOPRESERVATION OF SAHIWAL BULL SEMEN.

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ABSTRACT

The reactive oxygen species (ROS), produced during semen cryopreservation, have detrimental effects on sperm survivability. The negative impact of ROS may be countered by adding antioxidants. This study was designed to evaluate the effects of natural antioxidants (green tea and strawberry extracts) on post-thaw semen quality. Semen samples from three Sahiwal bulls were collected, pooled, extended in Triscitric acid based extender and mixed with various dilutions of strawberry and green tea extracts. The post thaw parameters of semen motility, viability and plasma membrane integrity for 0.25% green tea supplementation (42.66 ± 3.08, 54.13 ± 3.36, 43.33 ± 2.40) were significantly improved than control (29.66 ± 1.85, 43.40 ± 2.43, 33.33 ± 2.06) ($P<0.05$). The strawberry supplements also improved the quality of semen but to alesser extent. So, it was concluded that addition of green tea extract in semen preservative can improve post-thaw quality of Sahiwal bull semen.

EFFECTS OF MORINGA OLEIFERA LEAF POWDER SUPPLEMENTS ON HISTOMORPHOMETRY OF LYMPHOID ORGANS AND IMMUNE RESPONSES OF BROILERS UNDER DEXAMETHASONE INDUCED STRESS

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ABSTRACT

This study aimed to explore histomorphometry and immune responses of broilers to Moringa oleifera leaf powder (MOLP) supplements under dexamethasone (De) induced stress. Hundred-day-old chicks were divided into five groups having four replicates, (5 birds/replica). Group A (negative control) was fed basal diet (BD), B (positive control) with De (15mg/kg of diet after day 21st) + BD, C with De + 0.8% MOLP, D with De + 1.2% MOLP, and E with De + 1.6% MOLP. On 35th day, (2 birds/replica) were slaughtered for sampling (bursa of Fabricius (BF), cecal tonsils, intestine). Lymphatic follicular width and area of BF was higher ($P \leq 0.05$) in 1.6% MOLP group, lymphatic follicular number and length was non-significant in all groups. Lymphatic nodules number, length, width and area of cecal tonsils were non-significant in all groups.

Antibody titers against Newcastle disease virus were unaffected and antibody titers against sheep red blood cells were significant (P ≥ 0.05) in 1.6% MOLP treated birds on 21st and 35th day, cell-mediated immunity was non-significant. Also, after 72 hours, cell-mediated immunity was significant (P ≤ 0.05) in 1.2% and 1.6% MOLP groups and acidic, mixed, and total goblet cells count were significant (P ≥ 0.05) in all intestinal portions in MOLP groups except acidic goblet cells in duodenum. Number of Intra-epithelial lymphocytes in MOLP groups were not affected except ileum (P ≤ 0.05). It was determined that 1.6% MOLP in broiler feed had a moderate effect on broiler's immunity.
Key words: Broiler, dexamethasone, immunity, phytobiotics, stress.

MOLECULOR EPIDEMOLOGY AND PHYLOGENETIC ANALYSIS OF PIROPLASM INFECTIONS IN VARIOUS CATTLE BREEDS OF KHYBER PAKHTUNKHWA PAKISTAN.

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ABSTRACT

Piroplasmids are tick-borne protozoans, which cause substantial damage to the livestock industry by causing piroplasmosis in cattle. In the study area, there is no information is present regarding the molecular characterization of Babesia bigemina and piroplasmosis in cattle hosts. The aim of this study was to conduct a molecular survey and phylogenetic analysis of Babesia spp. and Theileria spp. infecting different cattle breeds population in Khyber Pakhtunkhwa. Based on signs and symptoms, 219 blood samples were collected from various cattle breeds including; Holstein Friesian (143/219; 65.3%), Jersey (47/219; 21.5%) and Sahiwal (29/219; 13.2%). Genomic DNA was extracted and after quantification, PCR were performed for the amplification of 18S rRNA fragment of protozoan parasites. Obtained 18S rDNA sequences from cattle hosts showed 99.5% identity with B. bigemina, and 100% with T. annulata. Having an overall infection rate of 61.6% (135/219), the highest infection rate was recorded for, T. annulata (43.8%; 95/219), followed by B. bigemina (18.3%; 40/219). Phylogenetic analysis of 18S rDNA sequences revealed that B. bigemina grouped with corresponding species reported from Bolivia, and South Africa, while T. annulata clustered with same species reported from India, Turkey, and Italy. The study provides the first molecular surveillance and phylogenetic position of B. bigemina and confirms the previous molecular detection of T. annulata in the study region. To reduce the losses and boost up the livestock sector, such investigations based on molecular and phylogenetic analysis are crucial for efficient control measure of piroplasmosis in cattle populations.
Key words. Babesia bigemina, Theileria annulata, Cattle, Piroplasmosis.

EFFICACY OF ZINGIBAIN PHYTO-PROTEASE ON GROWTH PERFORMANCE, LITTER QUALITY AND GUT MICROBIOTA IN BROILERS FED HIGH ANIMAL PROTEIN CONCENTRATES

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ABSTRACT

This study examined the effects of dietary supplementation with animal protein concentrate (APC) and ginger phyto-protease (GPZ) on growth performance, carcase yield, litter quality and gut microbiota in broiler chicks over a 35–day period. A total of 700-day-old chicks were divided into give groups with five replicates as follow: control (standard diet), diet with 4% APC, diet with 6% APC, diet with 4% APC + GPZ at 0.02% and diet with 6% APC + GPZ at 0.02%. The results showed that GPZ supplementation improved body weight gain (BWG) and feed conversion ratio (FCR) in diets containing APC. Carcase yield analysis revealed that diet with 4% APC + GPZ, and diet with 6% APC + GPZ had the highest yields (p<0.01). Immune organ weights of spleen and bursa of fabricius were significantly increased in groups having 4 and 6% APC with GPZ (p¼0.02). Additionally, gut microbiota analysis indicated that treatments with GPZ effectively reduced harmful bacteria (Clostridium perfringens, Escherichia coli, and Salmonella) while promoting the growth of beneficial Lactobacillus. Overall, GPZ supplementation at the level of 0.02% in 4% APC added diet proved more effective in improving feed conversion, reducing harmful bacteria, and promoting the growth of beneficial gut microbiota.

HIGHLIGHTS

GPZ improved body weight gain and feed conversion ratio with APC diets.

Diets supplemented with GPZ achieved highest carcase yields.

GPZ reduced harmful bacteria and promoted beneficial Lactobacillus in gut microbiota.

ASSESSMENT OF VITAMIN-E AND SELENIUM SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE, INTESTINAL MORPHOLOGY AND EGG SHELL QUALITY IN JAPANESE QUAIL (COTURNIX JAPONICA) CHALLENGED WITH As AND Cu TOXICITY.

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ABSTRACT

This study was conducted at avian research and training center UVAS to evaluate the dietary effects of Vitamin E and Selenium supplementation on reproductive performance, intestinal morphology, and eggshell quality in Japanese quail (Coturnix japonica) exposed to arsenic (As) and copper (Cu) toxicity. A total of 300 adult quails were randomly assigned to six dietary treatment groups for 9 weeks from 7th day of age to 67th day of age. Three hundred seven-day old quails were divided into six groups (n=10/replicate, 5 replicates/group): T1 basal diet (BD; negative control), T2 BD + 20 mg/kg As (positive control), T3 BD + 0.3 mg/kg Se + 250 mg/kg Vit E, T4 BD + 20 mg/kg As + 250 mg/kg Vit. E, T5 BD + 20 mg/kg As + 0.3 mg/kg Se, and T6 BD + 20 mg/kg As + 0.3 mg/kg Se + 250 mg/kg Vit. E). At 63th day, six birds per group were slaughtered for different histological and reproductive performance analysis. Parameters measured included egg production, fertility, hatchability, intestinal villi height and crypt depth, and eggshell thickness. Results revealed that exposure to As and Cu significantly

(p < 0.05) impaired reproductive performance, reducing egg production, fertility rate, and hatchability, and caused marked degeneration of ovarian follicles. Histological analysis showed shortened villi, increased crypt depth, and disrupted epithelial integrity in the intestine of quails exposed to the toxic metals, indicating impaired nutrient absorption. Eggshell quality was also compromised, with reduced thickness, shell weight, and calcium deposition. Supplementation with Vitamin E and Selenium, either individually or in combination, significantly ameliorated these toxic effects. The combination of both antioxidants provided the greatest protective response, restoring reproductive parameters close to control levels, improving intestinal villus height-to-crypt depth ratio, and enhancing eggshell quality traits. It is concluded that Vitamin E and Selenium play synergistic roles in mitigating oxidative damage and restoring physiological functions in Coturnix japonica under heavy metal stress. Their inclusion in diets can serve as an effective nutritional strategy to counter As and Cu- induced reproductive and intestinal toxicity, ultimately improving egg production and shell quality in quails reared under such conditions.
Keywords: Reproductive performance, intestinal morphology, eggshell quality.

PREVALENCE AND RISK FACTORS ASSOCIATED THEILERIOSIS AND ITS IMPACT ON BLOOD PARAMETERS IN CATTLE OF DISTRICT SHANGLA, KPK, PAKISTAN

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Theileriosis is a disease, caused by a blood-borne protozoan parasite belonging to the genus theileria and infect cattle around the globe. Theileriosis causes a huge economical loss including reduction in the production of meat and milk. The prevalence of theileriosis is associated with many risk factors such as age, sex, altitude level and warm and humid environmental conditions. The current study was conducted to examine prevalence of theileriosis, its associated risk factors and impact of theileriosis on blood parameters in cattle of district Shangla. Blood samples of 270 cattle were randomly collected in six tehsils along with limited data including age, sex, altitude and latitude of the area with the permission of the owner from March 2023 to August 2023. After microscopic examination 14.07% cases were confirmed positive. Among the six tehsils the maximum number of cases were found in Chakisar (16.67%), which was followed by Alpuri(16.36%), Martung(15%), Besham(14.28%), Puran(12.5%) and then Shahpur Kuzkana(11.12%) respectively. Age-wise distribution showed that older cattle (> 5 years) were highly infected as compared to other age groups of below five years. Females' cattle were confirmed more infected than males and the rate of infection was in peak in the month of August due to monsoon rain in the study area thus produce a favorable site for ticks. Similarly, the minimum number of cases were in the month of April. Impact on blood parameters were assessed and high significance difference was found. Further more sensitive methods like PCR is recommended to ensure the exact and more authentic prevalence of Theileriosis in the area.

PREVALENCE AND MOLECULAR CHARACTERIZATION OF THEILERIA LUWENSHUNI IN SHEEP FROM AZAD KASHMIR, PAKISTAN

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ABSTRACT

Ovine theileriosis, a protozoal disease prevalent among small ruminants in tropical and subtropical regions, is a significant concern worldwide. To discern the distribution patterns and prevalence of this disease within the sheep population, a molecular survey was undertaken in Azad Kashmir, Pakistan. A total of 300 blood samples were collected from six tehsils spanning the districts of Kotli and Bagh. DNA samples were subjected to PCR targeting the 18S rRNA gene of ovine Theileria (T) species. Subsequent nucleotide sequencing of PCR products demonstrated complete alignment with T. luwenshuni sequences documented in GenBank originating from sheep in China. The overall prevalence of Theileria within the sheep population was 29.66% (n=89). District wise analysis indicated a greater infection rate among sheep in District Kotli (42%) than among those in District Bagh (17.33%). Phylogenetic analysis revealed shared homology between the isolated T. luwenshuni strain and other strains from Asia.

MOLECULAR CHARACTERIZATION, ANTIBIOTIC RESISTANCE, AND BIOFILM FORMATION OF ESCHERICHIA COLI ISOLATED FROM COMMERCIAL BROILERS FROM FOUR CHINESE PROVINCES

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ABSTRACT

Escherichia coli (E. coli) represents a significant etiological agent of colibacillosis in poultry, resulting in considerable economic losses for the global poultry sector. The present study aimed to determine molecular characterization, antibiotic resistance, and biofilm formation of E. coli strains isolated from diseased broilers from four provinces of China. A total of 200 tissue samples were collected from the intestine, liver, crop, heart, and spleen and processed for microbiological examination. Molecular detection of E. coli strains, virulence genes, and serotypes was performed using polymerase chain reaction (PCR). Antibiotic susceptibility testing and biofilm formation were assessed using disk diffusion and 96-well microtiter plate assays. The study retrieved 68% (136/200) of E. coli strains from collected samples. Most of the E. coli strains were resistant to enrofloxacin (56%), followed by cefepime (54%), amoxicillin/clavulanate (52%), streptomycin (50%), ampicillin (48%), clindamycin (47%), kanamycin (41%), polymyxin B (37%), tetracycline (35%), sulfamethoxazole/trimethoprim (33%), ceftazidime (31%), meropenem (4.7%), and florfenicol (2.9%). Similarly, the E. coli strains tested positive for at least one virulence gene and specific serotypes. Among these, O145 was the most prevalent serotype, identified in 22 isolates (16.2%), followed by O8 (12.5%), O102

(11.8%), and O9 (11.0%). The tsh gene (10.2%) was the most prevalent virulence gene. This study found that 47.1% of E. coli strains were biofilm-producing, with 62.5% exhibiting weak biofilm production, 29.7% mild biofilm production, and 7.8% strong biofilm production. Similarly, 24.2% of the E. coli strains were avian pathogenic E. coli strains due to the presence of five or more virulence genes, specifically tsh, ompA, fimC, iss, fyuA, and astA, in a single strain by multiplex PCR. The present study recommends continuous surveillance and effective control measures to reduce the burden of avian pathogenic E. coli-related infections in poultry.

Keywords: Escherichia coli; APEC; virulence genes; serotypes; biofilm; antibiotic resistance.

ABSTRACT 052
ROLE OF FDHD IN THE PATHOGENICITY AND ITS REGULATORY CHARACTERISTICS IN AVIAN PATHOGENIC ESCHERICHIA COLI
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ABSTRACT

Avian Pathogenic Escherichia coli (APEC) is responsible for significant economic losses in the poultry industry and may pose a risk to human health. The pathogenic mechanisms of APEC are still not fully understood. The fdhD in E. coli is essential for the function of formate dehydrogenases (FDHs), which play a crucial role in bacterial metabolic pathways. However, little is known about its role in APEC pathogenicity. In this study, the LsrR quorum-sensing regulator expresses the fdhD and binds to the region between ☐ 96 to ☐ 66 of the fdhD promoter. The two motifs, 6 bp and 9 bp of the fdhD promoter, are crucial for the LsrR binding. The results showed that the inactivation of fdhD in APEC94 (a strain isolated from diseased poultry) did not affect the growth and motility of APEC94 but led to decreased biofilm formation ($P < 0.01$), reduced serum resistance ($P < 0.05$), and altered antibiotic susceptibility. Similarly, the deletion of fdhD declined the adhesion ($P < 0.05$) and invasion ($P < 0.01$) of APEC94 towards host cells, reducing APEC94 colonization in blood, lungs, liver, spleen, and intestine ($P < 0.05$) in mice, and the mortality rate of mutant APEC94ΔfdhD and APEC94 was 12.5 % and 87.5 % in a mouse infection model respectively. Furthermore, the fdhD positively affects the expression of fimbrial, flagellar, and virulence genes in APEC. Moreover, the transcription level of the inflammatory cytokines IL-2, IL-4, IL-6, IL-10, TNF-α, and INF-ɣ ($P < 0.05$) was significantly decreased in mutant APEC94ΔfdhD compared with the wild-type strain. Thus, fdhD is a global regulator that activates the transcription of several genes. The present results suggest that fdhD plays an important role in pathogenicity, making it a good target for managing infection with APEC.

Keywords: Avian pathogenic E. coli, FdhD, Quorum sensing regulator LsrR, Virulence

AVIAN PATHOGENIC ESCHERICHIA COLI (APEC): CURRENT INSIGHTS AND FUTURE CHALLENGES

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ABSTRACT

Avian pathogenic Escherichia coli (APEC) causes colibacillosis in avian species, and new investigations have implicated APEC as a possible foodborne zoonotic pathogen. This review analyses APEC's pathogenic and virulence features, assesses the zoonotic potential, provides an update on antibiotic resistance and vaccine research efforts, and outlines alternate management approaches. Aside from established virulence factors, various additional components, including 2-component systems (TCS), adhesins, secretion systems (SS), invasions, iron acquisition systems, quorum sensing systems (QS), transcriptional regulators (TR), toxins, and genes linked with metabolism, contribute to APEC pathogenesis. APEC may spread to diverse species of birds in all business sectors and can infect birds of varying ages. However, younger birds experience more severe sickness than mature ones, probably due to their developing immune systems, and stress factors such as vaccination, Mycoplasma Infections, poor housing circumstances, respiratory viruses, and other risk factors for secondary infections can all make APEC both primary and secondary pathogens. Understanding these factors will help in generating new and effective treatments. Moreover, APEC O145 was the most prevalent serotype recently reported in all of China. Thus, the APEC's zoonotic potential should not be underrated. Furthermore, it has already been noted that APEC is resistant to almost all antibiotic classes, including carbapenems. A robust vaccine capable of protecting against multiple APEC serotypes is urgently needed. Alternative medications, particularly virulence inhibitors, can provide a special method with a decreased likelihood of acquiring resistance.

Keywords: avian pathogenic Escherichia coli, foodborne, zoonotic, virulence

MOLECULAR CHARACTERIZATION OF CLOSTRIDIUM PERFRINGENS AND DEVELOPMENT OF TOXOID VACCINE

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ABSTRACT

An extensive research on the presence of Clostridium perfringens infections was undertaken in the Khyber Pakhtunkh Province (KPK) of Pakistan. For the purposes of this study, the province was stratified into four zones; Southern, Plain, Northern, and Mountainous for the collection of samples. From all zones, 4,000 fecal and rectal swabs were collected from the livestock of selected districts. For each of the epidemiological

occurrences, history, symptoms, mortality data were systematically collected as per the structured questionnaires. For the microbiological analyses, the samples were placed in thioglycollate broth and incubated under CO2 conditions. Out of the 4,000 samples, 1,116 samples were found to be positive for C. perfringens as per the growth detection criteria. Using TSC agar and PCR for the alpha, beta, and epsilon toxin genes, the colonies were confirmed. Out of the isolates, 22 were toxin positive through PCR producing 247bp, 317bp, and 206bp fragments. Whole genome sequencing was performed on 2 confirmed isolates, Type B and Type D, using Illumina NGS and analyzed on the PATRIC platform thereafter. For C. perfringens type B, 7.9 million reads were generated yielding a genome size of approximately 12.2 Mb, GC content of 34.72%, and 225 contigs. From the functional annotation, 12,277 protein coding genes, 206 tRNA, 26 rRNA genes, and 4033 hypothetical proteins were identified. For C. perfringens type D, we retrieved 7.95 million reads, resulting in a genome approximately 18.7 Mb in size with 907 contigs and a GC content of 44.02%. Annotation revealed 19,715 CDSs, 230 tRNAs, and 19 rRNAs with 13,010 assigned as functional proteins. A toxoid vaccine was constructed with Type B and D cultures grown in glucose broth, inactivated with formalin, and adjuvanted with alum sulfate. Safety was confirmed with rabbit inoculation, sterility was validated with multiple culture media, and safety was confirmed. Vaccinated sheep showed higher antibody response via HAI compared to those injected with the commercial vaccine. This research advanced the molecular and genomic characterization of local C. perfringens isolates for better diagnostic and vaccine planning for enterotoxemia in livestock.

Keywords: Clostridium perfringens, PCR, Toxoid Vaccine, Enterotoxamia, Khyber Pakhtunkhwa, Molecular typing, Alpha-beta-epsilon toxins

MOLECULAR DETECTION, PHYLOGENETIC IDENTIFICATION, AND RISK FACTOR ANALYSIS OF ANAPLASMA SPP. IN CATTLE OF BHIMBER, AZAD KASHMIR, PAKISTAN

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ABSTRACT

Background: Anaplasma spp. are emerging tick-borne pathogens of veterinary and zoonotic concern, yet their molecular epidemiology in many regions of Pakistan remains poorly characterized. This study aimed to detect, identify, and analyze the risk factors associated with Anaplasma spp—infections in cattle from Bhimber, Azad Kashmir. Methodology: A total of 210 blood samples were collected and screened using PCR targeting the 16S rRNA genes, followed by sequencing and phylogenetic analysis. Results: Overall, 5.71% (12/210) of samples were positive for Anaplasma spp., including A. capra (2.38%), A. ovis (0.95%), A. marginale (0.47%), A. phagocytophilum (0.47%), A. bovis (0.47%), and uncultured Anaplasma spp. (0.95%). Sequences showed 99–100% identity with global isolates in GenBank. Chi-square analysis revealed a significant association between infection and age group ($\chi^2 = 12.91$, $p = 0.0003$), while sex and breed were not significant ($p > 0.05$). Multivariate logistic regression confirmed age ≥ 2

years as a significant independent predictor ($\beta = 1.31$, $p < 0.001$). Phylogenetic analysis positioned local isolates within clades that included known zoonotic and livestock-origin strains from Asia, Europe, and America. Conclusion: These findings provide the first molecular evidence of multiple Anaplasma species in cattle from this region, underscoring the need for targeted control strategies and zoonotic risk assessment in endemic areas.

THEME 3: ONE HEALTH

ABSTRACT 056

UNVEILING THE MOLECULAR MECHANISM OF BACOPA MONNIERI AGAINST HEPATOCELLULAR CARCINOMA VIA THE P13/EGFR AXIS

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ABSTRACT

Background: Bacopa monnieri L., commonly known as Brahmi, has long been recognized in traditional medicine for its neuroprotective and anti-epileptic effects. Recent studies have revealed its anti-cancer potential against several cancer cell lines, including MDA-MB-231 and MCF-7; however, the precise molecular mechanisms underlying its activity remain unclear. Hepatocellular carcinoma (HCC) is a multifactorial malignancy with rising global incidence, necessitating the discovery of novel natural therapeutics. Objective: This study aimed to elucidate the molecular mechanisms and key therapeutic targets of B. monnieri against HCC using an integrative network pharmacology, molecular docking, and experimental validation approach. Methods: Active phytoconstituents of B. monnieri were retrieved from pharmacological databases, and drug-likeness and oral bioavailability indices were used to identify pharmacokinetically favorable compounds. The intersection of compound-associated and HCC-related targets was analyzed through Gene Ontology (GO) and KEGG pathway enrichment analyses. Compound–target–pathway networks were constructed to visualize the molecular interactions. Molecular docking was then employed to validate the binding affinity of key compounds with core HCC-related proteins, including AKT1, PPARG, EGFR, and ESR1. Results: Eight bioactive compounds—ascorbic acid, Bacogenin A, nicotinic acid, apigenin, luteolin, 3,4-dimethoxycinnamic acid, asiatic acid, and quercetin—were identified as major contributors to the anti-HCC activity of B. monnieri. Among them, quercetin demonstrated the strongest binding affinity with AKT1, EGFR, and ESR1. In vitro validation using HepG2 cells revealed significant downregulation of these targets, consistent with in silico predictions. Transcriptomic profiling of quercetin-treated HepG2 cells revealed 23 upregulated and 44 downregulated genes, including TNFSF10, CSF2RA, EGFR, STAT3, and ERK1/MAPK1-3, all of which are crucial components of oncogenic signaling pathways. Functional assays, including CCK8, colony formation, wound-healing, and cell cycle analysis, demonstrated that quercetin markedly suppressed cell proliferation, migration, and colony formation in a dose-dependent manner, with maximal inhibition at 50 nM. Knockdown of the P13 gene—identified from transcriptomic and literature data as an upstream regulator of the EGFR pathway—further potentiated the inhibitory effects of quercetin, suggesting a synergistic mechanism. Conclusion: Our integrative study demonstrates that B. monnieri exerts potent anti-HCC effects primarily through its bioactive compound quercetin, which targets the P13/EGFR axis and downstream signaling pathways including AKT and

ERK/MAPK. These findings provide novel mechanistic insights and support the potential development of B. monnieri-derived compounds as complementary therapeutics for hepatocellular carcinoma.

Keywords: Bacopa monnieri, Quercetin, Hepatocellular carcinoma, EGFR, Network pharmacology, Transcriptomics

COMPLETE GENOME SEQUENCE OF A NOVEL FMH32 VARIANT ESCHERICHIA COLI LD91-1 ST1737, RECOVERED FROM A HEPATITIS B PATIENT IN YANGON, MYANMAR

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ABSTRACT

Objectives: In this study, we report the complete genome sequence of a multidrug-resistant Escherichia coli   LD91-1 recovered from a 57-year-old hepatitis B patient in Myanmar. Methods: E. coli LD91-1 was subjected to antimicrobial susceptibility testing. Extracted genomic DNA from   the E. coli LD91-1 clone was sequenced using a Nanopore PromethION sequencer (ONT, UK) and   the highthroughput Illumina HiSeq 2500 platform. The clean reads were de novo assembled using   Flye   (v.2.9).   The complete genome was annotated using MLST, phylogeny, serotyping, plasmid replicons, fmH typing, chromosomal point mutations, antibiotic resistance, and virulence genes using   web   tools   available   at   the   Center   for   Genomic   Epidemiology. Results: E. coli LD91-1 had a circular genome of 4,838,891 bp. In silico analysis revealed that E. coli LD91-   1comprised plasmid pLD91-1 and belonged to the ST1737-O22:H16 serotype carrying a fmH32 variant that had not been reported in such strains. Core genome phylogeny demonstrated clear divergence among major E. coli clades. Mutations in the quinolone resistance-determining regions gyrA, gyrB, parC, and parE conferred fluoroquinolone resistance; mutations in pmrA and pmrB   conferred colistin resistance. Cephalosporin, fluoroquinolone, and tetracycline resistance genes were located on plasmid pLD91-1. Notably, several virulence genes, including 12 encoding   yersiniabactin and yersiniabactin siderophores were also revealed in the E. coli LD91-1 genome. Conclusions: To our knowledge, this is the frst complete genome report of a new E. coli LD91-1 carrying   the fmH32 variant in Myanmar. This MDR E. coli isolate may pose potential risks to human and   animal   health,   highlighting the urgent need for control and preventive strategies.

CRESS POLYSACCHARIDES MEDIATED SYNTHESIS OF NICKEL OXIDE AND SILVER NANOPARTICLES FOR BIOLOGICAL APPLICATIONS

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ABSTRACT

Phytochemical-assisted biosynthesis of nanomaterials offers an eco-friendly alternative to traditional methods. This study presents the synthesis of silver (Ag-NPs) and nickel oxide nanoparticles (NiO-NPs) using cress seed mucilage polysaccharides (CSMP) as a reducing, stabilizing, and capping agent. AgNO3 and NiNO3 served as precursors in the hot plate combustion method. Characterization of the NPs was conducted using UV-visible and Fourier-transform infrared (FT-IR) spectroscopy. The biocompatibility of Ag-NPs and NiO-NPs was confirmed through hemolysis assays, showing no toxicity at low concentrations. Cytotoxicity assessments revealed CC50 values greater than 10,000 µg/mL for CSMP, 2346 µg/mL for Ag-NPs, and 1987 µg/mL for NiO-NPs, indicating their safety at lower concentrations. The antioxidant activity of the NPs, evaluated using the DPPH assay, showed Ag-NPs stabilizing DPPH radicals by 85.67 ± 1.01% at 400 µg/mL, comparable to ascorbic acid (96.91 ± 2.05%). Ag-NPs also exhibited significant inhibition of α-amylase (89.03 ± 3.71%) and α-glucosidase (90.83 ± 3.54%) at 400 µg/mL, surpassing NiO-NPs and CSMP, and approaching the effectiveness of the standard drug acarbose. Antibacterial assays demonstrated that both Ag-NPs and NiO-NPs effectively inhibited the growth of Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus influenzae, and Escherichia coli. These findings suggest that CSMP offers a sustainable and effective method for the green synthesis of bioactive Ag-NPs and NiO-NPs with potential applications in biomedicine and agriculture.
Keywords: Silver nanoparticles, nickel oxide nanoparticles, antioxidant, antidiabetic, antibacterial, green synthesis.

ABSTRACT 059

EVALUATION OF IN VITRO AND IN VIVO ANTI-INFLAMMATORY POTENTIAL OF BIOACTIVE POLYSACCHARIDES FROM SALVIA HISPANICA L. (CHIA) SEED MUCILAGE

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ABSTRACT

Inflammation is a crucial immune response to pathogens, cell damage, and harmful substances. Although various drugs are available to treat inflammation, they often have side effects. Plants, especially those with bioactive compounds, offer alternative therapeutic options. Salvia hispanica L, commonly known as chia, is a significant medicinal plant rich in bioactive ingredients and therapeutic polysaccharides. This study aimed to extract and fractionate the bioactive polysaccharides from chia seed mucilage and determine their anti-inflammatory effects in vivo and in vitro. Chia seeds were soaked in distilled water, and mucilage was precipitated using 75% ethanol. The resulting fraction underwent size-based fractionation using Bio-Gel-P-10 gel filtration chromatography, yielding 60 fractions screened for carbohydrate content. Biochemical analysis revealed the presence of uronic acids, pentoses, and hexoses. In vitro anti-inflammatory activity was assessed using the HRBC membrane stabilization assay, while in vivo effects were evaluated with the carrageenan-induced paw edema model in mice. Results showed that the Ch19 fraction significantly stabilized HRBC membranes

(EC50=8.75 μg/mL), and the Ch39 fraction at 1000 μg/mL resisted heat-induced membrane destabilization by 97.20%. Furthermore, the in vivo activity showed that Ch31 fraction significantly reduced the volume of the mice's paw edema by 91.37%. These findings suggest that the ethanol-precipitated fraction of chia seed mucilage contains medium-sized polysaccharides with substantial anti-inflammatory potential, indicating their promise for further preclinical and clinical trials as treatments for inflammation and related diseases.

Keywords: Anti-inflammatory, Bioactive polysaccharides, Salvia hispanica L., Mucilage.

ABSTRACT 060

INFLUENCE OF AGE ON IMMUNOGLOBULIN M (IGM) SEROPOSITIVITY FOR TOXOPLASMA GONDII AMONG PREGNANT WOMEN PRESENTING AT MARDAN MEDICAL COMPLEX

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ABSTRACT

Toxoplasmosis is a globally distributed zoonotic infection caused by the protozoan parasite Toxoplasma gondii (T. gondii). Pregnant women are particularly at risk, as infection may result in miscarriage or congenital abnormalities, especially involving the fetal central nervous system. This study aimed to assess the prevalence of T. gondii among women seeking treatment at Mardan Medical Complex (MMC) and to evaluate ELISA-based IgM detection across different age groups. Serum samples from pregnant women of various age groups were tested for anti- T. gondiiimmunoglobulin M (IgM) antibodies using enzyme-linked immunosorbent assay (ELISA). All the cases were evaluated according to the age, cats' ownership, consumption of raw or uncooked meat or vegetables, occupation, source of drinking water, gestational age and abortion history. A total of 182 serum samples were tested. The quantitative ELISA revealed IgM positivity in 82 cases (45.1%), while the qualitative ELISA showed 120 positive cases (65.9%). In conclusion the results showed a considerable prevalence of T. gondii, indicating the implementation of control and serological screening programs in Mardan for the accurate and prompt detection of Toxoplasma infections.

Keywords: Age; immunosorbent assay; immunoglobulin M; Toxoplasma; Pregnant women

ABSTRACT 061

GENETIC CHARACTERIZATION OF DERMACENTOR SP. AND ASSOCIATED ANAPLASMA BOVIS, INFESTING SMALL RUMINANTS IN PAKISTAN

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ABSTRACT

Ticks are hematophagus ectoparaiste and vector of a wide range of infectious diseases after mosquito. Dermacentor are hard ticks infesting mammals including small ruminants. They have a wide geographic range across the Palearctic, Nearctic, and Oriental regions. Dermacentor species are of significant importance and pose a serious threat to veterinary and public health, transmitting different pathogens. To date, little information is available on the occurrence of Dermacentor species in Pakistan. The current study aimed the morphological and molecular identification of Dermacentor sp. and its associated pathogen Anaplasma bovis. A total of 35 ticks were collected from small ruminants in Balochistan province. Collected ticks were morphologically identified as Dermacentor sp. Extracted DNA was subjected to PCR to amplify 16S rDNA and cox1 for ticks and to screen for Anaplasma species by amplifying 16S rDNA sequences. The obtained BLAST results of 16S DNA and cox1 sequences showed 98.42% and 97.45% identity with Dermacentor pavlovskyi reported from China. Furthermore, the detected 16S rDNA sequences of Anaplasma species in Dermacentor sp. showed 100% identity with A. bovis. The phylogenetic analysis based on 16S DNA and cox1 sequences revealed close evolutionary relationship with Dermacentor pavlovskyi from china. All the 16S rDNA sequences of Anaplasma in phylogenetic tree clustered with A. bovis. This study emphasizes the need for further investigation to explore tick diversity and associated pathogens across the country.

Key words: Dermacentor sp.; Anaplasma bovis; tick; Pakistan

ABSTRACT 062

DIAGNOSTIC VALUE OF ADENOSINE DEAMINASE (ADA) IN PLEURAL FLUID FOR PULMONARY TUBERCULOSIS IN HIGH-BURDEN SETTINGS

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ABSTRACT

Tuberculosis (TB) continues to be an important worldwide health concern, and it is especially problematic in Pakistan. Accessible and accurate method of diagnostic is needed for effective early diagnosis and management. The current study evaluated the diagnostic value of adenosine deaminase (ADA) levels in pleural fluids as a means of diagnosing pulmonary tuberculosis (PTB) and made a comparison with data of the GeneXpert(R) MTB/RIF Ultra PCR assay, which served as the molecular reference standard. A total of 70 pleural fluids were tested, and their ADA concentration and MBT status were established using a colorimetric enzymatic technique and using GeneXpert, respectively. ADA yields were classified into four variants of diagnoses, including Normal (<30U/L), Suspected (30-40U/L), Strongly Suspected (40-60U/L) and Positive (>60U/L). One-way ANOVA, along with LSD post-hoc analysis, was used in statistical analysis. In 19 samples, the presence of MTB was detected with GeneXpert. Severe ADA levels (more than 60 U/L) were decisively linked with registered PTB. One way ANOVA

showed highly significant differences (p < 0.001) across the four ADA categories and that the Positive group surpassed others with the highest mean ADA levels. The post-hoc analysis was used to confirm that each successive category was significantly higher than the value of the ADA of the preceding category. ADA is a quick, economically efficient biomarker of PTB particularly with the molecular confirmation.

Keywords: Tuberculosis, Adenosine Deaminase (ADA), Biomarker, Pulmonary Tuberculosis, Diagnosis

PHYTOCHEMICAL PROFILING AND ANTIBACTERIAL EVALUATION OF ESSENTIAL OILS FROM SELECTED MEDICINAL PLANTS AGAINST CLINICAL ISOLATES OF SALMONELLA PARATYPHI IN LAHORE

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ABSTRACT

The rising resistance of Salmonella species to conventional antimicrobial agents has intensified the need to identify alternative therapeutic options, particularly those derived from natural sources. Medicinal plants are a promising source of bioactive compounds with potent antimicrobial activity. The present study investigated the in vitro antibacterial potential and phytochemical composition of essential oils extracted from Syzygium aromaticum, Ocimum sanctum, Allium sativum, and Triphala churna against clinical isolates of Salmonella Paratyphi collected from Lahore. Antibacterial efficacy was assessed using the agar well diffusion assay and minimum inhibitory concentration (MIC) determination via broth microdilution method. Gas Chromatography–Mass Spectrometry (GC–MS) was employed to characterize the chemical constituents of the most active essential oil. Among the tested oils, S. aromaticum exhibited the highest antibacterial activity, with an average inhibition zone of 30 mm and an MIC value of 50 mg/mL. GC–MS analysis revealed eugenol as the predominant bioactive compound responsible for the observed activity. The findings of the study demonstrate that the essential oil of Syzygium aromaticum possesses strong antibacterial potential and may serve as a natural therapeutic candidate for managing enteric infections caused by Salmonella Paratyphi.

Keywords: Salmonella Paratyphi, Essential oils, Antibacterial activity, Minimum inhibitory concentration, GC–MS, Syzygium aromaticum, Eugenol

GENOMIC EPIDEMIOLOGY, EVOLUTIONARY HISTORY, AND POPULATION DYNAMICS OF THE OMICRON VARIANT OF SARS-COV-2 IN PAKISTAN

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of COVID-19, has had a significant impact on global health and the economy. The virus is rapidly mutating, and several variants of concern (VOCs) have appeared recently, with Omicron being the latest. The Omicron wave has also affected Pakistan, but its genomic-based molecular epidemiology, evolutionary history, phylogeny, and population dynamics have not yet been studied in detail. Therefore, this study was conducted to analyze whole-genomic sequencing data from Omicron in Pakistan to determine its evolutionary history, genomic epidemiology, and phylogeny. The whole-genome sequence data were obtained from public databases and underwent quality filtering. The filtered data were then used for further analyses. First, the genetic diversity and population structure of the Omicron variant were evaluated, revealing a low level of genetic diversity within Pakistani samples (Pi=0.00177±0.00048). Population differentiation analysis showed that the Omicron variant shared similarities with the delta VOC but was most genetically diverse from the Wuhan variant. Next, amino acid sequence-based haplotyping identified 10 haplotypes, each with 53 amino acid changes in the spike protein. Among these, 13 mutations, including 2 insertions and 3 deletions, were unique to Pakistani samples and had not been previously reported for Omicron VOC. Then, a median-joining network was used to reconstruct the evolutionary history of SARS-CoV-2, which originated in Wuhan and diverged into various VOCs, including Omicron. Mutations along the S protein were mapped, and the unique set of mutations was visualized on 3D protein structure models for each haplotype. To understand the evolutionary history of Omicron in Pakistan, we first reconstructed a time-calibrated phylogeny of Pakistani Omicron sequences using a Bayesian framework. The results indicated that Omicron was introduced into Pakistan through two distinct lineages (clades A and B), which then spread across the country. Further phylogenetic analysis showed that clade A of Pakistani Omicron samples shared a most recent common ancestor (MRCA) in January 2022, originating from a South African lineage isolated in late December 2021. Meanwhile, clade B samples from Pakistan had their MRCA in December 2021 and were introduced via lineages from Thailand (December 2021), Spain (late December 2021), and Belgium (early January 2022). We also observed the spread of Omicron from Pakistan to other countries and back. To analyze the evolutionary history of Omicron, we performed phylogenetic reconstructions of all VOCs. The analysis revealed that Omicron is a distinct monophyletic group with an extremely long branch length (sharing an MRCA with other VOCs very early in 2020) and no intermediate evolutionary branches. To assess past population dynamics, we reconstructed a Bayesian skyline plot (BSP). The results indicated a steady population growth until 2021, followed by a decline in mid-2021, and a sudden expansion at the end of 2021, aligning with historical records and reflecting the Omicron outbreak. Overall, by using a population genomics approach, we successfully inferred the evolutionary history, genomic epidemiology, and population dynamics of the Omicron VOC.

Keywords: Covid-19, Omicron, genomic epidemiology, SARS-CoV-2, VOCs, Omicron outbreak in Pakistan

PUERARIN ATTENUATES THIRAM -INDUCED CYTOTOXICITY IN CULTURED CHICKEN GROWTH PLATE CHONDROCYTES : INSIGHTS INTO THE INVITRO THERAPEUTIC EFFICACY BY MODULATING HIF-1A, TIMP -3, AND BCL -2 EXPRESSIONS

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ABSTRACT

Endochondral ossification is a crucial process in longitudinal bone growth, relying on chondrocytes proliferation, hypertrophy and cartilage matrix secretion. Disruption in this process, particularly due to cytotoxic agents like tetramethyl thiuram disulfide (Thiram), can impair skeletal development and induce apoptosis. The present research aimed to explore the invitro protective role of Puerarin, a reputable bioactive isoflavone from Traditional Chinese Medicine on the growth plate (GP) chondrocyte's morp hology and survival, as well as mRNA and protein expressions of hypoxia -inducible factor -1α (HIF-1α), tissue inhibitor of metalloproteinase -3 (TIMP -3), and B - cell lymphoma -2 (BCL -2) against Thiram -induced cytotoxicity in chicken growth plate chondrocytes. The chondrocytes from chicken tibial growth plates were obtained, cultured, refined, and divided into Control, Thiram and Puerarin groups. The chondrocytes in Thiram and Puerarin groups were subsequently treated with a sub -lethal dose of Thiram at 2.5 μg/mL to cause cytotoxicity, followed by an optimal dose of Puerarin at 2.5 μg/mL to Puerarin group. Microscopy, RT-qPCR and Western blotting were used to investigate chondrocyte morphology and viability, and molecular expressions of key regulators i.e., HIF -1α, TIMP -3 and BCL -2. Thiram exposure resulted in diminished surv ival and drastic structural anomalies of chondrocytes, upregulated HIF - 1α, and downregulated TIMP -3 and BCL -2. Nonetheless, Puerarin treatment efficiently counteracted these Thiram induced str uctural and molecular alterations (P<0.05).
Key Words: Chickens, Chondrocytes, Genes, Proteins, Puerarin, Thiram

PREVALENCE AND ANTIMICROBIAL RESISTANCE OF GRAM-NEGATIVE BACTERIA IN DAIRY FEED AND WATER: A ONE HEALTH CONCERN

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ABSTRACT

Introduction: Dairy animals are constantly exposed to contaminated environments, which predisposes them to infections by pathogenic bacteria such as Salmonella spp. and Escherichia coli. These pathogens not only affect animal health and productivity but also pose significant zoonotic risks through the food chain. Understanding their prevalence and antimicrobial resistance (AMR) profiles is vital for protecting both animal and public health within the One Health framework. Objective: This study aimed to determine the prevalence of Salmonella spp. and E. coli in feed and water samples collected from dairy farms and to evaluate their antimicrobial resistance patterns, thereby assessing potential transmission risks within the One Health context. Methodology: A total of 160 samples, including 98 feed and 49 water samples, were collected from various dairy farms. Bacterial isolation and identification involved Gram staining, motility testing, and endospore staining, followed by standard biochemical characterization using IMViC tests. Antimicrobial susceptibility was assessed by disk diffusion testing against commonly used antibiotics. The Multiple Antibiotic Resistance Index (MARI) was calculated to evaluate antibiotic exposure risk. Results: Of the 144 samples analyzed, 76 (52. 8%) tested positive for E. coli and 68 (47. 2%) for Salmonella spp. E. coli isolates showed the highest susceptibility to ampicillin, cefotaxime, and ciprofloxacin (19%), while Salmonella spp. were most susceptible to cefpodoxime and ampicillin (17%). MARI values above the 0. 2 threshold were observed in 6 (7. 7. 8%) E. coli and 4 (5. 5. 8%) Salmonella spp. isolates, indicating significant antibiotic exposure. The average ± SD inhibition zones were 9. 87 ± 6. 16 mm for E. coli and 8. 8.5 ± 5. 34 mm for Salmonella spp., demonstrating comparable resistance profiles. Conclusion: The high prevalence of E. coli and Salmonella spp. in dairy farm feed and water emphasizes these as major reservoirs for potential pathogen spread. The observed multidrug resistance patterns highlight the urgent need for integrated monitoring, rational antibiotic use, and coordinated antimicrobial stewardship programs at the farm level.
These findings reinforce the importance of a One Health approach to mitigate the spread of resistant pathogens across animal, human, and environmental interfaces.
Keywords: antimicrobial resistance, antibiotic resistance index, One Health, farm-level surveillance, hygiene practices, regulatory measures

ABSTRACT 067
CHAPTER 45 THE FUTURE OF CHIROPRACTIC: AND THEN WHAT?

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ABSTRACT

Chiropractic care, an evolving branch of complementary medicine, focuses on diagnosing and managing musculoskeletal disorders, particularly spinal dysfunctions, through manual manipulation and holistic healing approaches. This chapter explores the foundations, principles, and clinical applications of chiropractic care in both humans and animals, emphasizing its non-invasive, drug-free nature and its integration into modern veterinary and human healthcare. Current challenges such as inconsistent regulations, limited public awareness, and variations in educational standards are discussed alongside the growing global trend of evidence-based chiropractic practice. The chapter highlights the "Six Pillars" framework as a strategic model for guiding the future of chiropractic care, aiming for improved regulation, interdisciplinary collaboration, and global professional recognition. Materials and Methods A comprehensive literature review was conducted using peer-reviewed sources, textbooks, and institutional guidelines to evaluate chiropractic principles, therapeutic techniques, case applications, and regulatory frameworks. The analysis included both human and veterinary chiropractic studies published between 2008 and 2024. Special focus was given to the integration of chiropractic care in animal health, emphasizing case-based data involving dogs, horses, and exotic species. The Six Pillars analytical framework was employed to assess future developmental trends, challenges, and opportunities for professional growth within the chiropractic field. Results Findings indicate that chiropractic manipulation significantly improves musculoskeletal mobility, neural function, and patient well-being in both humans and animals. Clinical case studies demonstrated positive outcomes in conditions such as vertebral subluxations, limb lameness, and neurological deficits. Despite its clinical potential, the chiropractic field continues to face regulatory inconsistency and variable public perception. The Six Pillars model identified key areas for advancement: regulatory uniformity, research-based practice, technological adaptation, public education, and professional identity enhancement. A global trend toward holistic, drug-free therapies supports chiropractic care's increasing integration into modern healthcare systems. Conclusion Chiropractic care is emerging as a vital component of integrative medicine due to its focus on natural, non-invasive treatment modalities and evidence-based outcomes. The future of chiropractic practice lies in enhancing its credibility through scientific validation, regulatory reform, and interdisciplinary cooperation. Adoption of innovative technologies, improved education, and adherence to the Six Pillars framework can strengthen chiropractic's global recognition and contribution to both human and animal health. Young practitioners and researchers play a crucial role in modernizing and legitimizing chiropractic care as a respected healthcare discipline.
Keywords: Chiropractic care, spinal manipulation, holistic medicine, veterinary chiropractic, Six Pillars, musculoskeletal health, complementary therapy

ABSTRACT 068
ISOLATION AND MOLECULAR CHARACTERIZATION OF STAPHYLOCOCCUS AUREUS FROM RAW MILK

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ABSTRACT

In the present study a total of 60 raw milk samples were collected from different commercial dairy farms, milk collection centers and Gawalas of District Kasur. Through culture technique 27(45%) of samples were found positive for S. aureus. The maximum isolates positive for Staphylococcus aureus were obtained from Gawalas raw milk 11(55%) as compared to milk collection center and commercial dairy farms which were 9 (45%) and 7 (35%), respectively. Further molecular characterizations of positive isolates were carried out using PCR for the detection of enterotoxin gene of Staphylococcus aureus. It was found that in commercial dairy farms the prevalence of gene encoded for enterotoxin C was significant i.e. 42.86% as compared to enterotoxin A and B which were 28.57% each, while milk from collection center showed high incidence of enterotoxin A of S. aureus 42.85% followed by B (28.57%) and C (28.57%). In Gawalas raw milk, enterotoxin encoded A gene was 50%, while enterotoxin B (30%) and C (20%) respectively. As a whole enterotoxin A, B and C gene was found (41.67%), (29.16%) and (29.16%) percent, respectively. Our study showed that incidence of enterotoxigenic S. aureus is high in Gawalas raw milk. It suggested that unhygienic conditions during milking should be avoided, otherwise the chances of milk born diseases will increase. It is recommended that pasteurized milk should be used to avoid milk born diseased.
Key words: Raw milk, Staphylococcus aureus, PCR

ABSTRACT 069

EVALUATING THE PREBIOTIC EFFECTS OF CHIA SEED-DERIVED OLIGOSACCHARIDES ON HUMAN PROBIOTICS

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ABSTRACT

Prebiotics, non-digestible compounds that enhance beneficial gut microbiota, have gained attention for their role in improving gut microbiome health. Mucilage from Salvia hispanica (chia), rich in polysaccharides, is a promising dietary component with multiple health benefits. The current research aimed to produce bioactive oligosaccharides from chia seed mucilage and evaluate in vitro prebiotic effects on human probiotics. Polysaccharides extracted from chia seed mucilage were precipitated with 75% ethanol, digested by Pichia pastoris, and purified via size exclusion chromatography. Biochemical analysis revealed that fraction ChF78 had the highest unsaturated uronic acid (7.79 ± 0.71 mg/mL), fraction EPP had the highest uronic acid (5.57 ± 0.32 mg/mL) and pentose (6.54 ± 0.30 mg/mL), while fraction ChF90 had the highest hexose content (4.60 ± 0.12 mg/mL). Prebiotic effects were notable in fractions ChF91 and ChF90, which significantly enhanced the proliferation of Bifidobacterium bifidum and Bifidobacterium clausi, achieving cell counts of 9.11 ± 0.17 log10 CFU/mL and 9.96 ± 0.30 log10 CFU/mL, respectively, at 48 hours. The acidifying effect was strongest in EPP and ChF91 fractions, with pH levels dropping to 4.14 ± 0.13 and 4.46 ± 0.28. Prebiotic index and score analyses further highlighted ChF91 and ChF90 for their potent prebiotic

potential. The prebiotic analysis highlighted ChF91 with the highest scores for both B. bifidum (5.67 ± 0.13) and B. clausi (5.47 ± 0.23), while ChF90 showed a strong prebiotic index (2.15 ± 0.07) for B. bifidum and (3.25 ± 0.12) for B. clausi. These findings support the potential for developing functional foods, paving the way for novel dietary interventions and functional food development to enhance gut health.

Keywords: Chia seed mucilage, Oligosaccharides, Pichia pastoris fermentation, Probiotics.

EFFECT OF OLIVE OIL AND COCONUT OIL MASSAGE ON PRODUCTION PERFORMANCE, INTESTINAL HEALTH, GROWTH HORMONE AND MUSCLE H ISTOMORPHOMETRY, USING MICE MODEL

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ABSTRACT

In the current scenario, massage therapy with oils are gaining significance day by day, reason for this being massage therapy has many beneficial effects, promoting weight gain, increasing secretion of oxytocin, endorphins, dopamine and serotonin hormones, enhancing blood flow in the skin and muscles, improving small intestine and reducing infection rates. Olive and coconut oil are commonly used as massage therapy due to its beneficial health effects. This study's objective was to investigate the effect of massage therapy with olive oil along with coconut oil on production performance, growth hormone and histomorphometric parameters of muscle and small intestine, using mice model. A total of 24 (n=24) albino mice (BALB/c mice) were used in this research. The se Albino mice were aged between 6 to 8 weeks. These albino mice were chosen at randomly selected animals and divided into four groups. These four groups of six mice each (one cage –six mice were kept). These groups were denoted by G1, G2, G3, and G4. In th e experiment, head and lumbar area of mice were massaged for six minutes once a day using $100\mu l$ of distilled water (G2), $100\mu l$ of olive oil (G3) and $100\mu l$ of coconut oil (G4). The distilled water, olive oil and coconut oil were evenly spread on the dorsal skin. Three steps were done in the experiment, first one is tissues sampling, second one is tissues treating and the third and last one is H and E staining technique were used. Results concluded that massaging of albino mice with olive oil and coconut oil boosted growth performance, increased growth hormones (T3 & T4), improved villus height, villus width, villus surface area, lamina propria thickness, muscularis mucosa and externa thickness, and ratio of a villus height: crypt depth of ileum, duodenum and jejunum of the small intestine but the crypt depth remained unchanged. Furthermore, numbers of fibers diameter, fascicles diameter, surface cross sectional areas and fibers density of muscles also increased. This study explained olive oil and coconut oil i s admirable alternative therapy for growth hormones, weight gain, muscles and intestinal health.

Key words: olive oil, coconut oil, massage, mice.

PREVALENCE AND MOLECULAR CHARACTIZATION OF CRYPTOSPORIDIUM INFECTION IN RURAL HUMAN POPULATION OF

DISTRICT MARDAN , PAKISTAN

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ABSTRACT

Cryptosporidium spp . is an important gastro -intestinal protozoan parasite which c ause diarrhea in humans, cattle and wild animals. Cryptosporidium spp . infection remai ns a main public health problem but the epidemics in human beings is still uncertain particularly in developing countries. This study aim s to investigate the prevalence of Cryptosporidium infection and its molecular characterization in the human population of district Mardan , Pakistan . For this study questionnaire was designed to obtain the de mographic and clinical data from the participants. Approximate ly 350 fecal samples were collected from those individual who attending different hospitals and villages of district Mardan , Pakistan . The sample were then analyzed for the presence of suspected parasite on two methods i.e., Mic roscopy and PCR. Modified Zheil Neelsen staining is used for daily diagnosis due to its low cost, easy availability and less time consumption. The PCR method was found to be more authentic and accurate in detecting the pos itive samples with a 100 percent sensitivity and specificity. The highest prevalence was found in individual having age $\leq$ 5 years was 24.75% while those at the age > 5 years have prevalence 20.13%. The prevalence found in males was 52.86% which was found h igher than that of female individual 47.14%. The highest prevalence on microcopy was found in Ghalla Dher area of dist rict Mardan (46.15%) while their prevalence on PCR was 34.46%. The lowest prevalence was detected in Chamtar region of district Mardan (11.62%) while on PCR their prevalence was 9.30%, the lowest prevalence on PCR was found in Dosehra Chowk area of district Mardan i.e.,6.66%. For evaluating the sensitivity of two diagnostic methods (PCR and microscopy), the data were statistically compared using the Pearson's chi -square test.

Key words: Cryptosporidium spp ., prevalence, Modified Ziehl Ne elsen, molecular charactization, humans.

ABSTRACT 072

BRIDGING ETHICS AND INNOVATION: THE ROLE OF ARTIFICIAL INTELLIGENCE IN ADVANCING ONE HEALTH BIOMEDICINE

ABSTRACT

This talk will explore the ethical dimensions of integrating artificial intelligence (AI) into biomedical research, focusing on key considerations for ensuring responsible and equitable use. We will begin by defining the scope of AI in biomedicine, followed by a discussion on its prospective applications, such as personalized medicine, drug discovery, and diagnostic tools. However, the promise of AI in these fields is accompanied by significant challenges. We will examine three critical ethical concerns: data privacy and security, the transparency and fairness of algorithmic decision-making, and the potential for AI to exacerbate inequities in healthcare access and outcomes. A case study will be presented to illustrate these challenges in practice, highlighting how ethical principles can guide the development and deployment of AI technologies. The talk will conclude by

offering recommendations for ethical frameworks to ensure that AI serves the public good while minimizing harm in biomedical contexts.

ISOLATION, IDENTIFICATION, MOLECULAR CONFIRMATION AND ANTIBIOTIC RESISTANCE PATTERNS OF SALMONELLA ISOLATED FROM BEEF IN DISTRICT MARDAN

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ABSTRACT

Salmonella is one of the important pathogens which cause different zoonotic diseases in humans around the world. Clinically salmonella infection is associated with food born disease which manifested as Vomiting, diarrhea, abdominal pain and gastroenteritis which is related to non typhoidal salmonella, and cause typhoid fever which involve septicaemia and can be life threatening. Salmonella can be ingested through the consumption of contaminated food from animal origin. Beef can be a source if it is contaminated by salmonella. Beef is contaminated during unhygienic practices in retail shops. The present study aimed to estimate the prevalence of salmonella, their molecular confirmation and to determine the resistance patterns of salmonella isolates to empirically used antibiotics, and to analyse bla-TEM, bla-CTXM and catA resistance genes of salmonella isolates in beef samples collected from three different tehsil of district Mardan. A total 40 samples were collected from different tehsil of district mardan. Beef samples were collected from tehsil Mardan (n=12), tehsil Katlang (n=12) and tehsil Takht Bhai (n=16). Out of total 40 samples 18 (45%) were positive for salmonella, and all salmonella isolates were confirmed through biochemical tests (catalase, gram staining, and IMViC tests). Molecular confirmation was done by targeting the invA gene. Culturing was done on Salmonella Shigella Agar which is a selective media for salmonella identification. Antibiotic sensitivity revealed that all 18/18 isolates were MDR (multi drug resistant). All of isolates were resistant to Amoxicillin (100%), cefotaxime (100%) and Meropenem (100%). Gentamycin, ciprofloxacin and chloramphenicol was (100%) sensitive to all isolates. ESBLs are enzymes that degrade beta lactam antibiotics and make them ineffective. catA gene develop resistance to chloramphenicol. ESBL genes are typically present on plasmids that can quickly spread among commensal and pathogenic bacteria. All off the 18 samples were screened for invA, bla-TEM, bla-CTXM and catA genes. And it was found that all isolates were positive for bla-TEM and bla-CTXM genes. While no isolate was found positive for catA gene.

Keywords: Salmonella, Antimicrobial Resistance (AMR), Poultry Meat, Zoonosis

IN SILICO ANALYSIS AND ASSOCIATION STUDY OF IL-17F GENE VARIANT(S) WITH OBESITY

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ABSTRACT

Obesity is one of the most prevalent global health issues and a major risk factor for non-communicable diseases such as diabetes, dyslipidemia, hypertension, and cardiovascular disorders, thereby increasing morbidity and mortality. According to the World Health Organization, obesity incidence has tripled since 1975. Obese individuals show elevated levels of acute phase proteins, pro-inflammatory mediators, and circulating cytokines, predisposing them to autoimmune and inflammatory diseases. This study investigated the correlation between the IL-17F rs23977084 variant and obesity using a case-control design, while also identifying the most damaging nsSNPs with bioinformatics tools. Blood samples from 200 participants (100 obese, 100 controls) were collected, and DNA was extracted using the phenol-chloroform method. Genotypic and allelic distributions of IL-17F SNP (rs2397084) were analyzed via ARMS-PCR. Results indicated no significant difference in genotype distribution (P=0.5698, $\chi 2$ =1.125) or allele frequency between cases and controls, suggesting that this SNP is not strongly associated with obesity risk. Advanced bioinformatics platforms, including SNPs&GO, SIFT, PolyPhen2, PHD SNP, and Panther, were used to identify deleterious nsSNPs in IL-17F. Additional validation of protein stability, structure, and functional impact was performed using I-Mutant, MutPred, and ConSurf. Three-dimensional protein models were generated with I-TASSER and visualized using Chimera v1.11, while RMSD and TM-scores were predicted using TM-align. Gene-gene interaction networks were also constructed. Several damaging nsSNPs were identified, including R77S, R77C, S78Y, P81S, D89H, R92G, R92W, R92Q, A100T, A100V, C107R, G112R, I123T, V129A, R131W, R132S, C152R, C152Y, C152F, T153A, T153I, V155G, and P157L. These variants may cause significant structural and functional defects in IL-17F, potentially contributing to obesity and autoimmune disorders.

ABSTRACT 075

MACHINE LEARNING MODEL BASED ON FTIR SPECTROSCOPY AND CHEMOMETRICS FOR THE IDENTIFICATION AND CLASSIFICATION OF THYROID MALIGNANCIES

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ABSTRACT

The integration of artificial intelligence (AI) and machine learning (ML) in healthcare has revolutionized diagnostic and therapeutic approaches, offering unprecedented precision in disease detection and management. This study focuses on the application of ML to analyze Fourier Transform Infrared (FTIR) spectroscopy data for identifying molecular compounds associated with neoplasia in thyroid tissues. Thyroid cancer, characterized by abnormal cell growth, presents diagnostic challenges due to its molecular complexity and subtle spectral signatures. We developed a novel ML model leveraging supervised and unsupervised learning techniques to classify and identify key biochemical compounds linked to neoplastic transformations in thyroid tissue samples. FTIR spectra were collected from both healthy and neoplastic thyroid tissues, preprocessed to remove noise and baseline distortions, and subjected to feature extraction to isolate relevant molecular

signatures. The ML pipeline, incorporating algorithms such as Random Forest, Support Vector Machines, and Convolutional Neural Networks, was trained on labeled spectral datasets to differentiate neoplastic from non-neoplastic samples with high accuracy. Dimensionality reduction techniques, including Principal Component Analysis (PCA), were employed to enhance model interpretability and computational efficiency. The model achieved a sensitivity of over 90% and specificity of 85% in identifying neoplasia-associated compounds, such as altered proteins, lipids, and nucleic acids, which are indicative of malignant transformations. These findings demonstrate the potential of AI-driven FTIR analysis as a non-invasive, high-throughput diagnostic tool for early detection of thyroid neoplasia, paving the way for personalized treatment strategies. Future work will focus on validating the model across larger, diverse patient cohorts and integrating multi-omics data to further enhance diagnostic precision.

ABSTRACT 076
ANTIMICROBIAL EFFICACY OF SEED EXTRACTS FROM TRIGONELLA, SALVIA, OCIMUM, AND CORIANDRUM AGAINST RESISTANT PATHOGENS

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ABSTRACT

The increasing risk of bacterial resistance to antibiotics and associated side effects has driven researchers to explore medicinal plants as natural alternatives to synthetic drugs. This study investigates the antimicrobial activities of four seeds (Trigonella foenum-graecum, Salvia hispanica, Ocimum basilicum, and Coriandrum sativum) commonly used in traditional medicine against five bacterial strains (E. coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella spp., and Staphylococcus aureus) and one fungal strain (Candida spp) isolated from fruit samples. The bacteria were identified based on cultural morphology, Gram staining, and Analytical Profile Index (API 20E) testing. The antimicrobial activity of the seed extracts was tested using ethanol, methanol, acetone and distilled water as solvents in a disc diffusion method. Ethanol extracts generally exhibited higher activity than methanol, acetone, and distilled water, except for Trigonella foenum-graecum, which showed better activity in methanol. Trigonella foenum-graecum demonstrated a significant zone of inhibition against Proteus mirabilis, while Salvia hispanica and Ocimum basilicum were particularly effective against E.coli. Coriandrum sativum exhibited broad-spectrum activity against all tested bacteria, with notable efficacy against Salmonella spp. None of the seed extracts in distilled water showed activity against the bacteria, and Coriandrum sativum did not exhibit antifungal activity against Candida spp. Antibiotic sensitivity tests with Azithromycin and Linezolid showed that the ethanol extracts were more effective than the reference drugs. The findings suggest that seed extracts could be a valuable source for developing new antimicrobial compounds

Keywords: Medicinal plants, Antimicrobial resistance, Antibacterial activity.

PREVALENCE AND RISK FACTORS ANALYSIS ASSOCIATED TO CRYPTOSPORIDIUM INFECTION IN GOAT KIDS IN PUNJAB, PAKISTAN.

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ABSTRACT

Cryptosporidium is an important zoonotic protozoan affecting wide range of hosts and a major constraint to the production of livestock globally. In south Asian countries very little are known about its prevalence and associated risk factors in working small ruminants where the disease is epidemic so a study was conducted in three communities, Punjab Pakistan. Fecal samples (n=1080) were collected randomly for presence of Cryptosporidium spp. by using floatation technique for concentration and stained by modified Ziehl-Neelsen acid fast staining technique. As a result highest prevalence was recorded in Sheikhupura (23.33%) followed by Qadir Abad (20%) while lowest in Sargoda (18.35%) and statistically significant difference (p=0.028) was recorded. The highest percent prevalence was recorded in the month of August (36.66%) followed by May (30%), June, July and October (26.66%), April (23%), September (20%), March (16.66%), November (13.3%), December (10%) while the lowest percent prevalence was recorded in the month of January and February (6.66%) and statistically significant difference (P<0.028) was recorded. The highest season wise percent prevalence was recorded in the summer season (30.83%), followed by autumn and spring season (21.66%) while lowest prevalence in the winter season (9.16%) and statistically significant difference (P<0.0045) was recorded. The highest percent prevalence was observed at the age of ≤1-15 days(33.92%), followed at the age of 16-30 days(17.69%) while the lowest percent prevalence at the age of 31-60 days and above(15.25%) and statistically significant difference (P<0.0045) was recorded. Similarly, the highest percent prevalence was recorded in female (20.98%) followed by male (19.19%) and statistically non-significant difference (P >0.229). As a result it was concluded that high prevalence and potential risk factors can cause an outbreak of cryptosporidiosis in small ruminants and humans because Cryptosporidium is highly prevalent and a significant pathogen in goat kids which act as a reservoirs and have the public health significance.

A STUDY ON PREVALENCE, INCIDENCE, ISOLATION AND ANTIBIOGRAM OF E. COLI FROM TABLE EGGS DISTRICT PESHAWAR, PAKISTAN.

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ABSTRACT

Escherichia coli is one of the common microbial flora of poultry gut. Most of E. coli isolates are nonpath-ogenic but are considered to be an indicator of fecal contamination in food industry. A study was carried-out on the prevalence, incidence, isolation and antibiogram of E. coli from table eggs. A total of 21 table eggs were collected from various locations of district Peshawar, Pakistan and divided into three parts viz., the egg-yolk, egg-white and eggshell. These were cultured on different media and identified

organism was subjected to antibiogram study using the disk diffusion method. The overall prevalence of E. coli was found as 29%. While, incidence was recorded as 15% in egg-shells, 12% in egg-whites and 10% in egg-yolks. It was concluded that the table eggs were contaminated with E. coli and higher incidence of E. coli was recorded in eggshells as compared to other components of the eggs. The antibiotics ciprofloxacin and enrofloxacin were recorded highly active against E. coli. The overall prevalence recorded as 29% during my study.

Keywords: Escherichia coli, table eggs, prevalence, incidence, antimicrobial resistance, food safety

FIRST REPORT ON ISOLATION AND CHARACTERIZATION OF BACILLUS SP. ASSOCIATED WITH HONEY BEE BROOD DISEASE

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ABSTRACT

Bacterial infections in honey bee brood pose a significant threat to bee populations, leading to decreased honey production and disrupting critical crop pollination networks. While Paenibacillus larvae and Melissococcus plutonius are well-established as major pathogens of honey bee eggs and larvae, the presence of other harmful bacteria in the Khyber Pakhtunkhwa (KPK) province of Pakistan remains largely unexplored. Molecular characterization of Bacillus species and B. cereus isolates revealed the presence of key virulence genes, including the cry gene, known for its insecticidal properties. The ability of B. cereus to produce cytotoxins, haemolysins, and enterotoxins raises concerns about its impact on larval immunity and survival. Similarly, B. mycoides, a close relative of B. cereus, was identified in diseased broods, highlighting its potential involvement in microbial dysbiosis within the hive. Reports from North America and Europe have also linked these Bacillus species to declining honey bee health, emphasizing the need for further investigation into their pathogenic mechanisms. This study investigated bacterial contamination in broods collected from infected beehives across various cities of KPK. Biochemical and molecular analyses revealed a widespread presence of bacteria the Bacillus species, as the emerging most dominant, followed by Bacillus cereus. Phylogenetic analysis indicated a close evolutionary relationship between Bacillus species, and Bacillus cereus, highlighting their shared spore-forming characteristics. This research is the first to report the involvement of Bacillus species in infecting honey bee eggs and larvae, shedding light on a previously unrecognized threat to apiculture and pollination in the region.

TARGETING BARTONELLA HENSELAE FOR NOVEL DRUG TARGET IDENTIFICATION AND MULTI EPITOPE VACCINE CONSTRUCTION USING SUBTRACTIVE PROTEOMICS AND REVERE VACCINOLOGY APPROACHES

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ABSTRACT

Bartonella henselae, a gram-negative bacterium, causes diverse health issues, and is spread through infected fleas. B henselae is a newly recognized zoonotic pathogen and can survive within host red blood cells and endothelial cells underscores its medical importance. Despite progress in deciphering the disease-causing mechanisms of B. henselae, there is still limited knowledge about the unique virulence factors and regulatory pathways specific to this bacterium. Thus, conducting research on these aspects is crucial for developing targeted therapeutic strategies against this adaptable pathogen. In accordance with reverse vaccinology and subtractive proteomics methodologies, this study aimed to find highly antigenic proteins to design a multi-epitope vaccine against B. henselae. One significant antigenic and virulent protein, sensor histidine kinase was determined. Later, B-cell, MHC-I and MHC-II epitopes for this protein were predicted and assessed for their antigenicity, allergenicity, solubility, as well as toxicity. These peptides were linked together using linkers then an adjuvant was added to form a vaccine model. Further refinement of the vaccine structure resulted into 92% amino acids residing within accepted regions. Docking simulations conducted with the TLR4 receptor showed a binding potential of −1047 Kcal/mol, indicating substantial bindings. MD simulations verified the stability of the bound complex, underscoring the structure and connections among the molecules. Additional in vitro/in vivo experimentation is necessary to analyze the vaccine's effectiveness against B. henselae.
Keywords: Bartonella henselae; subtractive proteomics; reverse-vaccinology; multi-epitope vaccine; molecular docking; molecular dynamic simulation.

ABSTRACT 081

EXPLORING ANTI-DIABETIC AND ANTI-INFLAMMATORY EFFECTS OF ENDOPHYTIC FUNGI-DERIVED EXOPOLYSACCHARIDES FROM WHEAT AND PARTHENIUM

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ABSTRACT

Endophytes are microbes that colonize living, internal tissues of plants without causing any negative effects. In recent years, endophytic fungi have been demonstrated to be excellent exopolysaccharide (EPS) producers. EPS are high-molecular-weight carbohydrates with a wide range of biological activities. This study was, therefore, designed to extract and purify EPS-producing endophytic fungi from wheat (Triticum aestivum) and Parthenium (Parthenium hysterophorus) and explore their biological applications using different bioassays. The purified EPS were labelled as EPSW1, EPSW2, EPSW3, EPSP1, EPSP2, and EPSP3. The purification of EPS involved inoculating isolated endophytic fungi into a broth medium at 25°C for 32 hours on a shaking incubator and EPS precipitation using 95% ice-chilled ethanol. The purified EPS were processed for quantification of total carbohydrate content and bioactivity through α-amylase, α-glucosidase, and HBRCs membrane stabilization assay. The results indicated

that EPSW1 inhibited α-amylase, showing an effective IC50 value of 265.75 μg/mL, while EPSP2 inhibited α-glucosidase with an IC50 value of 441.87 μg/mL. In terms of in vitro anti-inflammatory activity, EPSP1 significantly stabilized HRBCs membrane, with an EC50 value of 8.75 μg/mL, followed by EPSW1 (155.17 μg/mL). Furthermore, the study assessed in vivo anti-inflammatory activity through a carrageenan-induced paw edema model in mice. Simultaneously, the in vivo antidiabetic activity was investigated in Balb/C mice by inducing diabetes via a 1% alloxan injection. Three concentrations (400 mg/kg, 200 mg/kg, 100 mg/kg) were tested for each activity. At 400 mg/kg, EPSP1 showed the highest anti-inflammatory impact (91.37%) while EPSW2 at the same concentration displayed a significant 74.85% reduction in blood glucose levels in alloxan-induced diabetic mice. In conclusion, the study emphasizes the potent bioactivity of EPS from wheat and Parthenium, showcasing therapeutic benefits, including anti-diabetic and anti-inflammatory properties.

Keywords: Endophyte, Exopolysaccharides, Anti-diabetic, Anti-Inflammatory, Wheat, Parthenium

ABSTRACT 082
HAPLOTYPE DIVERSITY, GENE FLOW AND GENETIC DIFFERENTIATION, AND PHYLOGENY OF H. ANATOLICUM FROM DIFFERENT COUNTRIES.

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ABSTRACT

Among the Ixodid ticks, Hyalomma anatolicum is a well-known vector that transmits various pathogens to vertebrate hosts including humans. Expansion of this tick to new areas changes the genetic structure, and lead to affect the vector-pathogen interaction and disease outcomes. The present study was designed to infer the haplotype diversity, gene flow and genetic differentiation, and phylogeny of H. anatolicum from different countries based on the cytochrome oxidase I (COI) and 16S rDNA sequences. A total of 220 ticks were collected from cattle, buffaloes, and sheep in five districts of Khyber Pakhtunkhwa, Pakistan, morphologically identified as H. anatolicum, and subjected to genetic analysis. A total 55 and 130 sequences for COI and 16S rDNA, including 9 and 2 sequences generated in this study, respectively, were analyzed to assess haplotype network, population structure, demographic changes, and phylogenetic analysis. Analysis based on COI sequences yielded 29 haplotypes in which haplotype 1 and 15 were the predominant consisting of 35 and 20 sequences, respectively, from Pakistan, India, China, Bangladesh, Iraq, Saudi Arabia, Kazakhstan and Egypt. The 16S rRNA yielded 30 haplotypes in which haplotype 1 was predominant consisting of total 86 sequences from Pakistan, India, China, United Arab Emirates, Tajikistan, Kazakhstan, Turkey, Egypt, and Iraq. Complete haplotype network based on COI and 16S rRNA confirmed stellate structure, together with high haplotype diversity (COI 0.77899, 16S rRNA 0.60774) and low nucleotide diversity (COI 0.00445, 16S rRNA 0.00431), which support recent population expansion. In the phylogenetic tree based on the COI and 16S rDNA sequences, with exception of one sequence for a single haplotype, which appeared independently, there is a single main clade that includes the largest number of sequences for all other haplotype.

Based on COI and 16S rDNA sequences, the present study provided first detail information about the population genetics and haplotype networks of H. anatolicum

ABSTRACT 083
PREVALENCE AND MULTIDRUG RESISTANCE IN *SALMONELLA* FROM RETAIL CHICKEN EGGS IN MARDAN, PAKISTAN
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ABSTRACT

Salmonella is one of the important zoonotic pathogens (by disease spread from animals to people). Food-borne illnesses in people all over the world. Symptoms of such salmonella infection are: with food-borne disease, presenting with vomiting, diarrhea, and abdominal pain, diarrhea (gastroenteritis) related to non-typhi salmonella, cause: typhoid fever (a disease which is associated with septicemia and can be fatal). Salmonella can be contracted through the consumption of contaminated food of animal origin. Eggshell is a potential source, if contaminated with Salmonella. Eggshell is passed in unhygienic conditions in retail outlets. The present study: Objective: To estimate the prevalence and molecular confirmation of Salmonella. Our study identified resistance phenograms of Salmonella isolates to empirically applied, antibiotics and to drill bla-TEM, bla-CTXM, and catA resistance genes of S. campylobacter in eggshell samples from three tehsils of the district Mardan. A total of 40 samples were collected from various tehsils of the district of Mardan. Eggshell was sampled from tehsil Mardan (n=12), tehsil Katlang (n=12), and tehsil Takht Bhai (n=16). Of 40 total samples, 5 were positive for Salmonella bacteria, and all Salmonella isolates identified were confirmed by biochemical tests. (catalase, Gram stain, and IMVIC reactions). Molecular confirmation was carried out by targeting the invA gene. Culturing was performed on Salmonella Shigella Agar, which is a selective medium for the detection of Salmonella. Resistance to antibiotics was found in all (5) isolates were MDR (multi-drug resistant). All of the isolates were Resistant to Amoxicillin (100%), Cefotaxime (100%), & Meropenem (100%). Gentamycin, ciprofloxacin, and chloramphenicol were 100% susceptible to all strains. isolates. ESBLS are enzymes that metabolize beta-lactam antibiotics (break them down) and render them inactive. Mutations leading to resistance to chloramphenicol occur in the catA gene. ESBL genes are typically encoded on plasmids, which readily exchange among commensal and pathogenic bacteria. All 5 samples were surveyed for invA, bla-TEM, bla-CTXM, and catA genes. And all isolates tested positive for bla-TEM and bla-CTXM genes. None of the isolates were positive for catA. gene.

IN SILICO ASSESSMENT OF CYTOKININ SIGNAL TRANSDUCTION IN PLANT ASSOCIATED MICROBES

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Abstract

Cytokinins (CKs) are classical phytohormones regulating diverse aspects of plant growth and development. Their perception in plants occurs via membrane-bound Arabidopsis histidine kinases (AHKs), which transmit signals through histidine phosphor-transfer proteins and response regulators. Increasing evidence suggests that plant-associated microbes can also perceive and respond to CKs, yet knowledge of their perception pathways remains limited. This study employed a bioinformatics-based approach to investigate putative orthologues of CK signalling components in Pseudomonas fluorescens, Pseudomonas syringae, and Botrytis cinerea. Orthologous proteins with kinase, histidine phosphatase, and hybrid histidine kinase domains were identified, all containing a conserved histidine kinase (HK) domain essential for signal transduction. Notably, P. syringae AHP1 exhibited HK, HPT, and RR domains, suggesting functional versatility. Homology modelling revealed strong structural similarity of microbial orthologues to Arabidopsis CK receptors, and docking analysis demonstrated significant CK binding, particularly by the receiver domain of the P. syringae AHK4 orthologue. Key interacting residues included leucine, glutamine, and serine. These findings provide evidence that plant-associated microbes possess CK perception machinery and may respond to plant-derived CKs in the rhizosphere. Such microbial signalling capacity could have important implications for plant–microbe interactions, potentially influencing microbial colonization and plant health.

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