

PREFACE

The PARACON'24, 3rd International Parasitology Conference, organized by the Pakistan Society of Parasitology and Department of Parasitology, UVAS Lahore, is scheduled for October 9th – 10th, 2024. This event will feature a dynamic agenda including plenary and keynote lectures, oral and poster presentations, panel discussions, and digital proceedings, all aimed at addressing critical issues in parasitology and related disciplines. PARACON'24 serves as an excellent platform for participants to connect with peers, mentors, and potential collaborators, promoting knowledge exchange and fostering innovation to tackle parasitic diseases and their global health impact. With dedicated sessions and networking opportunities, the conference encourages interdisciplinary collaborations that can drive progress in the fight against parasitic diseases.

Conference Tracks

The conference will explore diverse topics through its various sub-themes, including:

1. Parasite Biology and Ecology
2. Phytopathology and Sustainable Agriculture
3. Host-Parasite Interactions
4. Diagnostic Methods and Tools
5. Chemical Treatments and Prevention Strategies
6. One Health Approaches
7. Parasite Vaccine Development
8. Vectors and Vector-Borne Diseases
9. Effects of Environment and Climate Change

This comprehensive approach ensures that PARACON-2024 will provide a valuable opportunity for presenting the latest advancements and research in parasitology, fostering discussions that address both scientific and practical challenges, with a focus on food security and public health.

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VICE CHANCELLOR'S MESSAGE

Livestock sector is an important segment of agriculture economy and contributed 60.84 % of the agricultural value added and 14.63 % of the national GDP during 2023-24. The gross value added of the livestock sector at constant factor cost was Rs. 5,804 billion during the same period. Historically livestock has been the subsistence sector dominated by the small holder to meet their needs of milk, food security and daily cash income. During the last decade there has been massive investment in poultry, fisheries, dairy and meat sectors to fulfill the demand for food of high biological value in the country and export in the regional markets.



Being flagship University in these disciplines in the country, UVAS has fully realized the needs of the sectors and initiated degree programs, research projects, technology centers and services to meet the challenges of growing industries, farming communities and stakeholders. The commercialization of research and expertise from this University generates significant economic growth and business opportunities within country. Our research-focused teaching ensures our students receive the highest quality education, which introduces them to the frontiers of knowledge and provides them with the skills and discipline to contribute fully to whatever career they follow. We have a strong professional orientation with a focus on academic excellence and graduate employability. For these reasons, UVAS graduates are highly sought after both locally and internationally.

By working closely with our communities, we are proud to play a leadership role in enhancing economic prosperity, contributing to improvements in the health and wellbeing of our nation.

In this connection PARACON-2024 will be organized to have a platform for the region so that scientists can collaborate and present their findings. It will be a great opportunity to learn latest cutting edge research designed to offer comprehensive global discussions that address current issues in the field of Parasitology.

**Meritorious Prof. Dr. Muhammad Younas (DLA.I, T.I)
Vice Chancellor/ Patron in Chief**

DEAN'S MESSAGE

On behalf of the Dean of the Faculty of Veterinary Science (FVS), it is both an honor and a privilege to host an international event like PARACON'24, under the auspices of the Pakistan Society of Parasitology and the Department of Parasitology, FVS, University of Veterinary & Animal Sciences, Lahore. The Department of Parasitology has been spearheading groundbreaking research in the domain of parasitology, addressing critical challenges in these fields.



PARACON'24 serves as a vital platform for exchanging ideas, developing innovative solutions, and fostering collaborations that have the potential to shape the future of parasitology and its applications. This conference is not merely a place to listen and learn; it is an opportunity to actively contribute towards driving progress for the community and the broader society.

Built on the foundation of multidisciplinary research and collaboration, PARACON'24 provides participants with a unique experience that enriches their understanding and contributes to the collective advancement of veterinary and parasitological sciences. I am confident that the interactions and discussions during this event will inspire new perspectives and pave the way for future achievements.

I welcome all participants and look forward to a fruitful and enriching conference.

Prof. Dr. Aneela Zameer Durrani
Dean, Faculty of Veterinary Science
University of Veterinary and Animal Sciences, Lahore, Pakistan

PRESIDENT'S MESSAGE

Greetings and welcome from the Pakistan Society of Parasitology (PSP)! PSP is a society which is made up of a diverse group of Parasitologists, researchers from government, industry and academia who are interested in the study, teaching and research related to Parasitology.

It was founded with the aim to have contribution not only in the development of parasitology as a discipline, but also to provide a solution oriented forum for parasitic challenges in the field. Since its inception, the society has grown by leaps and bounds, through annual conference, seminars, training programmes with various governmental and commercial organisations, supporting students and teaching of parasitology.



We welcome new members to PSP, and hope you will find the excitement and camaraderie to spur you in the search for knowledge in this field. To all our existing members from academia, governmental and research organisations and industry we wish you continued success in your work and do keep attending our events, giving suggestions to further improve the society. The society also serves as a networking opportunity for professionals and students involved with Parasitology including research, education, control and management of parasitic diseases.

PARACON-2024 is the annual highlight where you can update on the latest scientific information via oral, poster and plenary sessions as well as strong linkage with industry and corporate sector through booths showcasing the latest products and technology.

On behalf of the council of PSP, we hope you will be interested in joining our society or collaborate with our members. Once again welcome to our world of Pakistan Society of Parasitology.

Prof. Dr. Kamran Ashraf
President/Chairman Department of Parasitology

CONFERENCE SECRETARY MESSAGE

*Greetings and welcome from the
Pakistan Society of
Parasitology
(PSP)!*



As the Organizing Secretary of PARACON-2024, I am pleased to share with you important details to ensure a smooth and enriching experience at this International Parasitology Conference, organized by the Department of Parasitology, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan.

The conference will feature a comprehensive agenda covering oral and poster presentations, workshops, networking opportunities, and virtual and hybrid sessions. Detailed information on registration and check-in procedures will be provided to all participants closer to the event date.

Our dedicated committees, along with the UVAS administration, have been working diligently to create a highly successful and impactful event. We are confident that your participation will contribute significantly to the conference's success and provide a valuable platform for knowledge exchange and professional networking.

We look forward to welcoming you and hope that PARACON'24 will be both an informative and rewarding experience for you.

Warm regards,

Prof. Dr. Muhammad Imran Rashid

Organizing Secretary, PARACON-2024
Department of Parasitology,
University of Veterinary and Animal Sciences, Lahore, Pakistan

OUR PARTNERS



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ABSTRACTS
FOR
ORAL
PRESENTATIONS

Development of a therapeutic protocol against lumpy skin disease in fattening bulls

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Abstract: Lumpy skin disease is a transboundary disease and an economically significant problem that affects mainly cattle and water buffaloes. The disease affected Pakistan in the form of an outbreak in 2022. The current study was designed to develop a treatment protocol against lumpy skin disease. The total of 14% animals at the fattening farms of district Kasur were affected from disease. The experimental animals were selected on the basis of the characteristic's nodular lesions of the disease on the skin of the animals. The study included 36 animals in the three groups, and different treatment regimens were applied to cure the disease. Different combinations of Acyclovir, Flunixin meglumine, Ivermectin, Ketoprofen, and Sulphadimidine were injected at the recommended doses to treat lumpy skin disease. PCR was performed before and after the treatment to evaluate the efficacy of treatment. Group A, contained Acyclovir, ivermectin, and flunixin Meglumine, was more effective in the treatment of lumpy skin disease. The study suggested the administration of specific antiviral agents and the symptomatic treatment of the disease.

Keywords: Lumpy skin disease, Treatment, Antivirals, Antibiotics, Fattening Bulls, Pakistan

Assessing the percentage and impact of feline panleukopenia (FP) in stray cats of Lahore: implications for animal health management

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Abstract: Feline Panleukopenia Virus (FPV) is a major risk for stray cats, especially in kittens. The mortality rate is high, above 90% in kittens. This study assesses the positive percentage of FPV in stray cats of Lahore and its implications for animal health management.

A comprehensive survey was conducted on stray cats in district Lahore. The sample population was tested by using rapid antigen testing kits for FPV. This study involved the systematic collection and testing of fecal samples from stray cats showing the clinical signs of FP. The results reflect a higher percentage of FPV, thus indicating more susceptibility of stray cats towards infections. Moreover, it draws attention to a potential threat, the transmission of FPV to domestic cats and wild members of the Felidae family, which could pose a broader health crisis.

The findings suggest a strategic reconsideration of the health interventions and support systems that are currently working for the welfare of stray cats, with a special focus on veterinary care and community engagement for animal health. This study contributes to the basic understanding of the dynamics regarding FPV within the stray cat population and forms a basis for future initiatives to reduce the effects of this deadly virus.

Challenges of anthelmintic resistance: a study on fasciolosis prevalence and farmer practices in Kasur and Lahore

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Abstract: *Fasciola hepatica*, a common parasite of grazing livestock is prevalent globally and has economic and zoonotic implications. This study aimed to assess the burden of fasciolosis in small ruminants and associated anthelmintic practices in the districts of Kasur and Lahore. A comprehensive investigation was conducted at Central Veterinary Hospitals (CVHs) in both districts. The study revealed a significant burden of fasciolosis in the region, with a prevalence rate of 42.5%. Notably, the increased burden of fasciolosis was associated with a lack of awareness among farmers, leading to the irrational use of anthelmintic treatments. A majority of the livestock holder relied on over-the-counter anthelmintics without veterinary consultation, leading to improper dosage and irregular treatment intervals. The study revealed that the reduced efficacy of anthelmintics was directly linked to these malpractices. These findings underscore the urgent need for structured educational programs to promote the correct use of anthelmintics and proper treatment protocols in order to combat the rising resistance and the associated economic losses in these districts.

Keywords: Fasciolosis, Small Ruminants, Anthelmintic Practices, Farmer Awareness, Drug Resistance, Pakistan.

Schistosomiasis: understanding basics to accelerate disease elimination

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Abstract: Schistosomes, blood flukes, are an important global public health concern. Adult schistosomes can survive in their final hosts for several decades, and paired adult female schistosomes produce large numbers of eggs that are primarily responsible for the disease pathology and critical for dissemination. Consequently, understanding the mechanisms of parasitic survival in final hosts and sexual maturation and egg production may open avenues for developing novel strategies against schistosomiasis. In addition, rapid and accurate diagnosis of *Schistosoma* infection, particularly in the early stage is crucial for identifying infected hosts and taking effective control strategies to lessen disease dissemination. In the past few years, we explored the regulatory roles of small non-coding RNAs, extracellular vesicles carrying miRNAs and other molecules in *S. japonicum* sexual maturation and egg production as well as host-parasite interactions. Our findings uncover key roles for specific miRNAs and proteins in these processes. In addition, we also identified several catalogues of pathogen specific circulating DNAs, miRNAs and proteins, which could be ideal liver biomarkers for detecting *Schistosoma* infection. The development of novel diagnostic methods combined with the identified biomarkers showed a good promising for detecting murine or human schistosomiasis particularly for early stages. Overall, our studies could help to improve the elimination of Schistosomiasis.

The role of veterinary medicine in food security in light of the one health concept and the WEFE nexus (water-energy-food-environment nexus): an analysis focused on animal and zoonotic parasite

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Abstract: Food security is a globally significant concept, crucial for the stability of nations and populations around the world. Given the strategic importance of animal-based food products in ensuring food security, veterinary medicine is closely involved in this field. However, food security remains a relatively unknown concept among many veterinarians. In this presentation, we will explore the various dimensions of food security, emphasizing the role of veterinary medicine in its practical application across these areas, using examples of parasitic diseases with veterinary and zoonotic significance. We will also examine the key implications for veterinary education and research. Considering the systemic nature of food security and its interactions with other domains, it is essential that veterinary initiatives and solutions aimed at improving food security align with the principles of One Health and WEFE nexus (Water-Food-Energy-Environment nexus). This alignment is critical for minimizing trade-offs and fostering synergies, particularly with respect to environmental sustainability and human health.

BCG as adjuvant can increase the immunogenicity of DNA vaccine containing *Toxoplasma gondii* ROP2 gene in BALB/c mice

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Abstract: *Toxoplasma gondii* infects almost all mammals and birds worldwide. However, despite global distribution and considerable high prevalence, no vaccine is available for toxoplasmosis. The *Toxoplasma gondii* rhoptry protein ROP2 is one of the most important antigens that is expressed in three stages of the parasite life cycle and hence a proper target. On the other hand, Bacillus Calmette-Guérin (BCG) is an immune stimulator that can act as an important adjuvant in protection against infectious diseases. In this study, a recombinant plasmid containing the ROP2 gene was prepared, combined with BCG as an adjuvant, and injected into female BALB/c mice. The mice were then challenged with *Toxoplasma gondii* tachyzoites and immunological factors i.e. total IgG, IgG2a, and IgG1, cytokines (IFN- γ and IL-4), and the survival rates were compared in vaccinated and control groups. The experimental group receiving pcROP2 + BCG produced statistically higher IgG2a and total IgG. Furthermore, the amount of spleen cytokine IFN- γ was also significantly higher in mice receiving pcROP2 + BCG, but the level of IL-4 was lower in this group indicating the stimulation of Th1 immune response. We showed that pcROP2 + BCG is effective in inducing humoral and cellular immune responses and suitable for increasing the lifespan of mice against acute toxoplasmosis.

Keywords: DNA Vaccine; *Toxoplasma gondii*; BCG; Immune responses; pcROP2; BALB/c mice

Zoonotic parasites associated with horses and donkeys

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Abstract: Equids play critical roles in recreation, food production, transportation, and as working animals in different regions of the world. Because of their roles as working animals, pets, and livestock, close interactions of horses with humans support the potential transfer of equine pathogens to humans. Furthermore, the frequent international movement of show and race horses increases the risk of the transmission of novel equine pathogens to distant herds and to new persons who may be occupationally or recreationally exposed to horses.

Zoonotic diseases account for more than 60% of known diseases and 75% of emerging diseases. Previous reports identified 56 zoonotic pathogens that have been found in horses and donkeys. Pathogen transmission from equines to humans might occur directly via ingestion, inhalation, wound, bite, skin exposure, or indirectly by arthropod vectors. Zoonoses associated with horses and donkeys some of which are parasitic affect different organ systems in humans including gastrointestinal, respiratory, cutaneous, neurologic, reproductive, hepatic, circulatory, renal, ocular, and musculoskeletal systems. While information about the direct transmission of parasites from equines to humans remains low, there is a high potential for underreporting due to a lack of knowledge among health professionals. Awareness of these zoonotic pathogens, their disease presentation in equines and humans, and their associated risk factors for cross-species infection are important to public health officials, clinicians, and people with recreational or occupational equine exposure.

In this presentation, parasites of horses and donkeys with zoonotic importance including protozoa (*Cryptosporidium* spp., *Giardia duodenalis*, *Encephalitozoon* spp., *Enterocytozoon bieneusi*, *Toxoplasma gondii*, *Trypanosoma evansi*), and helminths (*Trichostrongylus axei*, *Echinococcus* spp., *Fasciola hepatica*, *Halicephalobus gingivalis*, *Trichinella*, *Onchocerca cervicalis*) are reviewed and diagnostic approaches in both equine hosts and humans are discussed.

Keywords: Diagnosis, Donkeys, Horses, Parasite, One Health, Zoonosis, Zoonotic Diseases

Protective potentials of mandarins lyophilized juice against acetaminophen induced toxicity in Wister rats

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Abstract: This study scrutinized the phytochemical composition, curative, hepato-and nephroprotective effect of different doses of the lyophilized juice of mandarins fruit against acetaminophen-induced toxicity. Phytochemical screening and RP-HPLC analysis were conducted to quantify total polyphenols and flavonoids respectively. For evaluation of *in vivo* curative and protective effects, thirty-six rats were randomly divided into six groups. In first four groups I, II, III and IV acetaminophen 75mg/kg, i.p, 150mg/kg, 250mg/kg and 500mg/kg p.o doses of lyophilized juice were administered to rats respectively. Blood samples were withdrawn at 0, 24, 48 and 72 hours in acetaminophen treated rats. For screening of hepato- and nephro-protective effect Group V and VI were fed on lyophilized juice (250mg/kg and 500mg/kg p.o) for seven days and on 8th day blood samples were collected at 0, 24, 48 and 72 hours. Hepatic and renal biomarkers were monitored. Phytochemical analysis revealed the presence of total polyphenols (20.7 ± 0.3 GAE mg/g) and flavonoid contents (21.2 ± 0.4 QE mg/g). Furthermore, RP-HPLC analysis also confirmed the presence of Myricetin, Quercetin, and Kaempferol in fruit juice. The lyophilized juice at 500 mg/kg dose have shown profound decrease in acetaminophen -induced elevated serum levels of liver and kidney functions under investigation, which suggests a possible therapeutic role of its constituents in hepatic injury and altered kidney functions.

Keywords: RP-HPLC, Hepatoprotective, Polyphenols, Myricetin, Paracetamol, *Citrus reticulata*

Weed infecting monopartite begomovirus *Ageratum enation virus* and associated DNA-satellites complex first time reported in woody plant (*Morus alba*) from Pakistan

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Abstract: Begomoviruses infect dicotyledonous host in the family *Geminiviridae* are transmitted by vector whitefly, these dicotyledonous infecting viruses usually infect diverse species of weeds, herbaceous plants, economically important crops, recent research work investigating that these are also infecting woody plants too. Begomoviruses infecting foliage parts of *Morus alba*; mulberry exhibiting leaf curling and yellowing viral symptoms were collected from Lahore- Pakistan. Molecular characterization of begomovirus showing old world begomovirus associated with DNA-satellites complex. These were amplified by using the RCA; rolling circle amplification product as PCR, cloned, sequenced and analyzed. Two full length begomovirus clones AEV; *Ageratum enation virus* associated with satellites complex, alphasatellites; *Ageratum enation alphasatellite* and Papaya leaf curl betasatellite have been first time reported from woody plant mulberry. Begomovirus and associated alphasatellite were showing recombination, usually infecting weeds and crops. This begomovirus associated complex was first time reported in woody plants and also from Pakistan.

Key words: Begomoviruses, Recombination, Old Word, DNA-Satellites Complex

Exploring potential of green synthesized nanoparticles to manage plant diseases with reduced application of synthetic pesticides

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Abstract: In recent years, various types of metal and metal-oxides nanoparticles attained copious interest as an alternate method for the management of plant diseases. Tomato as a perishable crop has become a successful model plant to investigate the initiation of defense pathways after exposure to disease agents which act as a trigger for resistance mechanisms. Indulgence to these mechanisms is a key focus of plant-pathology to enhance crop protection. The current investigation was aimed at the green synthesis of nanoparticles to induce resistance in tomato plants against fusarium wilt. Green synthesized nanoparticles via MA-AgNPs, CF-CuONPs and BC-IONPs were characterized and used to investigate the *in vitro* and *in vivo* antifungal activity at various concentrations. *In-vitro* antifungal potential of all three types of nanoparticles expressively inhibited mycelial growth and spore germination of *Fusarium oxysporum* f.sp. *lycopersici* in a dose-dependent manner. The highest percentage inhibition in mycelial radial growth ($96.8 \pm 0.23\%$) and decline in spore germination rate (4.67%) was observed at 140 $\mu\text{g/mL}$ of MA-AgNPs in contrast to the control and fungicide treatment. Monographs of the scanning electron microscope revealed the ultrastructural changes in fungal hyphae in response to higher concentrations of nanoparticles signifying the detrimental effect of these NPs on the fungal mycelial surface. DCFH-DA fluorescence revealed ROS accumulation in fungal mycelium by showing strong green color after treatment with studied NPs however, insignificant to very weak fluorescence was observed in control samples. Each type of nanoparticle was further characterized to get information about wavelength range, functional nature, crystallographic structure, size, shape and stability. The microwave-assisted MA-AgNPs showed a peak at 434 nm by using 5 mL of *M. azedarach* leaf extract and 2.5 mM of AgNO₃ solution at pH 8, exposed to 30 s of microwave irradiations. MA-AgNPs indicated stability even after six months. Spherical shaped nanoparticles ranged from 12–46 nm was confirmed by X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) analysis. MA-AgNPs indicates the negative zeta potential of -22.3 mV. In addition, uptake of these nanoparticles did not show any visible sign of toxicity on plant yield and productivity. The metal concentration checked in edible parts was found in permissible limits.

Evaluating the impact of temporal change and seasonal variability with urban trees on urban environment

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Abstract: Urban expansion, infrastructure development and increasing emissions have deteriorated the air quality, undermining both the environment and public health. Although many initiatives like Pakistan's Clean Air Program and the installation of monitoring stations have been implemented by the government but their ineffective management has led to failure to meet air quality standards ultimately posing a serious risk to environmental health and overall quality of life. This study was planned to investigate the effect of vegetation cover and seasonal variations on air quality. Primarily, the research examines the relationship between urban vegetation and atmospheric variables such as relative humidity, temperature, sound intensity, CO, CO₂, and particulate matter (PM_{0.5}, PM_{1.0}, and PM_{2.5}), as well as the impact of seasonal changes on the concentration and magnitude of these variables. The distribution of urban atmospheric variables in Faisalabad, Pakistan, was mapped using Pearson correlation analysis and principal component analysis to establish connections between urban air pollutants, urban vegetation, and seasonal changes. The findings indicate that urban vegetation and meteorological factors are significantly positively correlated with most atmospheric pollutants. Additionally, the concentration of PM is strongly associated with temperature and urban vegetation coverage. GIS distribution maps for PM_{2.5} and CO₂ reveal that pollutant concentrations are highest in areas with limited vegetation. Consequently, it can be concluded that urban vegetation requires careful design, planning, and cost-benefit analysis to maximize its positive impact on the environment.

Keywords: Air Quality Index, Ecosystem Services, Pollution, Urban Trees, Sound Intensity

Degradation of ochratoxin producing fungi using *Ocimum basilicum* l.

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Abstract: One of the primary challenges for global agricultural food production is ochratoxin exposure. Most essential crops, cereals, and dry fruits become impacted by ochratoxigenic fungi. This research's objective is to examine the antifungal effects of *Ocimum basilicum* L. against *Aspergillus niger* van Tieghem. To do this, methanolic extract of *O. basilicum* was made and tested *in vitro* against *A. niger*. Several conc. like, 1%, 2%, 3%, 4%, 5%, 6% and 7% of the test plant were prepared. The methanolic extract's 7% concentrations showed the strongest antifungal activity up to 93%. *O. basilicum* phytochemical analysis showed the presence of tannins, terpenoids, flavonoids, saponins, alkaloids and glycosides. The *O. basilicum* antioxidant activity was evaluated using spectrophotometric 1, 1 diphenyl-2-picrylhydrazyl (DPPH) radical test. The results showed that when concentrations increased, the antioxidant potential increased as well. Methanolic extract of *O. basilicum* were separated into fractions of n-butanol, n-hexane, ethyl acetate and chloroform. Among them, the ethyl acetate fraction revealed strongest antifungal activity against *O. basilicum*. The potential antifungal compounds were identified by using Gas chromatography mass spectroscopy (GC-MS) analysis of the ethyl acetate fraction. GC-MS analysis showed the presence of twenty five compounds including linalool, estragole, trans-alpha-bergamotene, bicyclodec-1-ene, 2-isopropyl-5-methyl-9-methylene, neophytadiene, n-hexadecanoic acid, phytol, oleic acid, 9,12 octadecadienoic acid, 9,12,15 octadecatrienoic acid, (z,z,z), octacosane, 1,19-eicosadiene, phytanic acid, heneicosane, 3-methyl, phenol, 2,2 methylenebis 1,1-dimethylethyl-4-methyl, tetracosane, glycerol palmitate, bis(2-ethylhexyl) phthalate, hexacosane, octadecane, squalene, pentadecanal, heptacosane, 2-dodecen-1-yl-succinic anhydride, 1-chloroeicosane. The plant extract was applied *in vivo* against ochratoxin producing fungus and the degradation of ochratoxin was assessed using HPLC (High Performance Liquid Chromatography). The ochratoxin degradation of *O. basilicum* by *in vitro* detoxification assay against pathogenic fungi (*A. niger*) was also performed. The TLC detection of ochratoxins from test fungi and plant sample result that *O. basilicum* effectively reduced the levels of ochratoxin in *A. niger*. Quantitative detection of ochratoxins by HPLC shows a great amount of reduction of OTA in experimental sample. The present study revealed that plant-based extracts are efficient in detoxification of ochratoxins.

Keywords: Ochratoxins, Methanolic Extract, Bioassay Guided Fractions, Antioxidant, GC-MS

Investigating charcoal rot infection caused by *Macrophomina phaseolina tassi* (goid) in sesame (*sesamum indicum* l.)

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Abstract: Dry and humid weather condition serves as favoring elements of Charcoal rot infection in sesame. Generally, the disease is identified by the symptoms appeared in adult plants which includes blackening of stem and root tissue disintegration because of presence of numerous microsclerotia. The population of viable sclerotia in the soil determines the severity of the infection. The present study investigated charcoal rot infection in sesame. Experimental assay was arranged in Earthen pots filled with sterilize soil and fungal inoculum. Surface sterilized sesame seeds were sown roots and stem pieces from both healthy and disease plants were subjected to histopathological studies after 45 days. Infected tissue showed invasive hyphal structure through cortex region of plant tissue that results in tissue browning, disintegration and formation of large spaces. Large no of swelled and lightly melanized structure were also observed intercellularly, which blocks the nutrients transport and caused wilting and death of the plant

Key words: Histopathology, Seclortia, Infection, Sesame, *Macrophomina phaseolina*

Efficacy of fresh plant extracts to control seed borne fungal contamination and therapeutic effect of tannins extracted from the callus culture of *Achyranthes aspera* as antifungal agent

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Abstract: Seed-borne fungi is detectable in various forms, including when studying them through in vitro cultures. The use of fungicides to combat these contaminations can have negative effects on the environment. The objective of this study was to explore the bioactive properties of callus cultures of *Achyranthes aspera* in order to overcome seed-borne fungal contamination during seed germination in a sterile environment. Seed discoloration caused by fungi was assessed using the Blotter and Towel methods. *Aspergillus niger* led to the highest black spot discoloration (16%), followed by dark brown discoloration (10%), while *Pseudozyma aphidis* induced creamy discoloration (5%). *A. niger* dominated the seed coat, while *Mucor hiemalis f. corticola* dominated the embryo. To control seed-borne fungal contamination, fresh extracts of ginger and neem were tested along with a fungicide, in the following order: fungicide, ginger, and neem. The antifungal potential of extracts from leaf, stem, root seedling, and callus cultures of *A. aspera* was evaluated against *A. niger* and *F. saloni*. The tannin content in methanol and petroleum ether extracts of leaf and root callus cultures from MSDN and MSDI culture media exhibited varying levels of inhibition against *A. niger* and *F. saloni* i.e., 34mm and 2mm, respectively. The inhibitory concentration (MIC) showed a positive correlation with tannin content, as the antifungal assay showed that the methanol extracts from leaf and root callus cultures grown in medium MSDN had the highest tannin content (7500 mg/g), while those from medium MSDI had the lowest tannin content (41.2 ml/g) of total antifungal activity. The petroleum ether extract of leaf callus culture from medium MSDN demonstrated the highest relative percentage inhibition against *A. niger*, whereas the chloroform extract of root callus culture from medium MSDI exhibited the lowest relative percentage inhibition against *F. saloni*. Overall, conducting in vitro research on this selected plant could lead to the development of callus cultures as a preliminary study for tannin extraction at the preclinical stage.

Keywords: Fresh Plant Extracts, Seed-Born Fungi, Tannins, Minimum Inhibitory Concentration, Total Antifungal Activity

Insights into the involvement of male *Hyalomma anatolicum* ticks in transmitting *Anaplasma marginale*, lumpy skin disease virus and *Theileria annulata*

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Abstract: Ticks can transmit viruses, bacteria, and parasites to humans, livestock, and pet animals causing tick-borne diseases (TBDs) mechanically or biologically in the world. Lumpy skin disease virus, *Anaplasma marginale*, and *Theileria annulata* inflict severe infections in cattle, resulting in significant economic losses worldwide. The study investigated the potential transmissions of LSDV, *A. marginale*, and *T. annulata* through male *Hyalomma anatolicum* ticks in cattle calves. Two 6-month-old Holstein crossbred calves designated as A and B were used. On day 1, 15 uninfected female ticks (IIa) and infected batch of 40 male ticks (I) were attached on calf A for 11 days. Filial transmission of the infections was observed in female ticks (IIb) collected from calf A, where 8 female ticks had been co-fed with infected male ticks. The blood sample of calf B was found positive through PCR for the infections. The larvae and egg pools obtained from the infected ticks were also tested positive in PCR. The study confirmed the presence of these mixed pathogens and potential intrastadial and transovarial transmissions of *A. marginale*, *T. annulata*, and LSDV in male and female ticks of *H. anatolicum* and experimental calves to establish the feasibility of infections through an in vivo approach.

Keywords: Lumpy Skin Disease Virus, *Anaplasma Marginale*, *Theileria annulata*, *Hyalomma anatolicum*, Cofeeding, Intrastadial Transmission

Challenges in controlling cattle tick-borne diseases in Sistan-va-Baluchestan province in South-Eastern Iran bordering Pakistan and Afghanistan

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Abstract: Tick-borne diseases (TBDs) are a substantial threat to the livestock industry globally, posing huge impacts on production, health, and welfare indices. Sistan-va-Baluchestan province, located in the south-eastern corner of Iran bordering Pakistan and Afghanistan, is home to approximately 100,000 indigenous and imported cattle breeds. The ruminant livestock industry in this region faces economically significant TBDs. The most prevalent tick species in the area are *Hyalomma anatolicum*, *Rhipicephalus annulatus*, and *R. sanguineus sensu lato* and our previous molecular investigation revealed the considerable prevalence of *Anaplasma marginale* (72.2%), *Theileria annulata* (68.3%), *A. phagocytophilum*/*A. platys*-like complex (66.1%), *A. centrale* (16.7%), *Babesia bigemina* (10.0%), and *A. bovis* (6.1%) in cattle populations. Currently, control and management of these TBDs are faced with significant challenges in the diagnosis and treatment of symptomatic cases as well as asymptomatic carriers since mixed infections involving two, three, and four pathogens are common. Considering TBDs' economic losses in this region and livestock movements between Iran, Pakistan, and Afghanistan, in this study management challenges and the available solutions are discussed.

Keywords: Cattle, Tick, Pathogen, Management, Challenge

Role of reservoir hosts in the ecology of arthropods and arthropod-borne parasitic diseases

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Abstract: Reservoir hosts are the asymptomatic carriers of parasites and can spread the infections to other susceptible hosts, including humans. They play an important role in the ecology of arthropods and/or arthropod-borne parasitic infections. Ecology has a profound link between the natural world and parasitic diseases as has been modeled through various causation models i.e., S.I.R, Monte Carlo simulation, and ARIMA model. The interaction between the arthropod vectors and reservoir hosts is complex, containing the ecological, physiological, and behavioral factors that impact the disease distribution. These wildlife host species mainly act as a source of infection for vectors including mosquitoes, ticks, and flies, and spillover mechanisms, maintenance, and propagation of arbo infections. Reservoir hosts for arthropod-borne encephalitis virus are woodland animals, bank voles, yellow-necked mice, and small mammals. *Rhombomys opimus* (gerbils) and various other animals, including wolves, bats, primates, rock hyraxes, *Tatera indica* rodents, dogs, cats, foxes, jackals, armadillos, and humans, are reservoir hosts for *Leishmania spp.* Rodentia, Chiroptera, and Euliptohyla are reservoir hosts of Hantaviruses for humans. More than 80 different viral species and other parasites are found in bats (*Chiroptera*). Small mammals and ground-dwelling birds are reservoir hosts of some tick species. Black rat (*Rattus rattus*) reservoir host for *Yersinia pestis*, *Leptospira spp.*, Hantavirus, and *Mycobacterium avium*. The rodent species of white-footed mouse (*Peromyscus leucopus*) are reservoir hosts of *Borrelia burgdorferi*. Sand lizards (*Lacerta agilis*) and woodland alligator lizards (*Elgaria multicarinata webbia*) are reservoir hosts of ticks and tick-borne diseases (*B. burgdorferi*, *Rickettsia helvetica*, *Anaplasma*, and *Ehrlichia spp.*). The diversity and abundance of reservoir hosts can influence the prevalence and distribution of arthropod-borne diseases, as different species may vary in their susceptibility to infection and their roles in transmission dynamics. Environmental factors such as habitat changes, climate variability, and land use can affect reservoir hosts' populations and, consequently, the patterns of disease spread. Understanding the ecology of reservoir hosts in the causation model systems and their surveillance to determine their competency as reservoir hosts are essential tools for effective disease surveillance and control strategies. Interventions, such as interventions targeting these hosts or their interactions with arthropods, can help mitigate the risk of outbreaks and reduce the burden of arthropod-borne diseases on human and animal populations.

Prevalence and morphological identification of Ixodid tick species collected from bovine population of district Poonch Azad Jammu and Kashmir, Pakistan

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Abstract: Ectoparasites, mainly ticks, pose serious health concerns in all domestic species of animals causing huge economic losses. Considering the economic impact of diverse ticks species on livestock, the present study was designed to investigate prevalence and identification of ticks (Ixodid), infesting bovine of District Poonch, Azad Kashmir, Pakistan. A total of 768 animals (Cattle: 384 and Buffalo: 384), were randomly selected from the proposed study area and examined for ticks existence. Overall (42.31%) animal (325/768) in District Poonch, were found tick infested. However, among bovines highest prevalence was recorded in cattle (53%), followed by buffalo (31%). Similarly, Tehsil wise highest prevalence of ticks (51.5%) was found in Tehsil Hajeera followed by Abbaspur (43.3%) and Rawalakot (32.03%). Bovine's breeds wise results have shown that Non-descriptive breeds were affected significantly higher than Australian cross (i.e. Friesian) and Sahiwal cross breeds in cattle. Similarly in buffalo, non-descriptive breed was also found the most affected than (Nili Ravi cross breeds and Nili Ravi). Gender wise tick prevalence was found more in female (cattle: 66.03%, buffalo 37.7%) than in male (cattle: 38.06%, buffalo 20.1%). Calves \leq 1 year of age were most affected age group as compared to adults (2-5 years) and older (5-8 years) animals. Inner thighs were the highest tick infested body part in both cattle and buffalo. Comparison of physiological state of animals revealed that in lactating cattle and buffalo prevalence percentage of ticks was more than non-lactating. There was significant difference in prevalence of ticks in different seasons. Highest tick infestation was recorded in summer season in the study area. Significant association was found between season and tick infestation in cattle while it was non-significant in buffalo. A total of 865 adult ticks were collected from animals. For the identification of ticks standard morphological keys have been used. Species identified belongs to three ticks genera i.e. *Rhipicephalus*, *Hyalomma* and *Haemaphysalis*. *Rhipicephalus microplus* was found to be the most significant (71.6%) followed in order by *Hyalomma anatolicum anatolicum* (21.8%) and *Haemaphysalis punctata* (6.4%). Logistic regression analysis showed that location, species, gender, and breeds were potential risk factors for ticks infestation. The present study will be helpful for researchers who wish to work in future on the subject matter. In addition, on the basis of present study findings proper control measures against ticks are advised in the study area.

Key Words: Bovines, Ixodid, Ticks, Prevalence, Identification, Poonch, Azad Kashmir.

RPA: an efficient diagnostic tool of vector borne diseases

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Abstract: Theileriosis is an important disease of animals in tropical and subtropical countries and causes significant reductions in livestock productivity of domesticated animals especially. The arid zone of Punjab in Pakistan is notorious for the abundant presence of the vector tick (Acari: Ixodidae) and tick-borne diseases, such as theileriosis and babesiosis. The prevalence of *Theileria annulata* and *T. orientalis* in the Chakwal district of Punjab province was determined by developing a multiplex recombinase polymerase amplification (RPA) assay as a scientific basis for formulating control strategies for bovine theileriosis. Specificity of the test was evaluated using DNA from related piroplasm species, while analytical sensitivity was calculated using a long fragment of the enolase gene. A total of 158 blood samples were collected on FTA cards (Whatman[®]) from tick-infested asymptomatic breeds of cattle (*Bos indicus*, *Bos taurus*, and *Bos indicus* × *Bos taurus*) in the study district. Finally, infections with of *T. annulata* and *T. orientalis* were detected using the multiplex RPA and compared with the conventional multiplex polymerase chain reaction (PCR). The multiplex RPA specifically amplified 282-bp and 229-bp fragments of the enolase gene from *T. annulata* and *T. orientalis* and had no cross-reaction with other piroplasm species. It was determined that 41 (25.9%) and 4 (2.5%) out of 158 blood samples were positive for *T. annulata* and *T. orientalis*, respectively, when examined using RPA. Multiplex PCR detection indicated that 30 (19.0%) and 3 (1.9%) blood samples were positive for *T. annulata* and *T. orientalis*, respectively. In the present study, a specific RPA method was developed for simultaneous differentiation and detection of *T. annulata* and *T. orientalis* infections and used for the first time for the detection of the two bovine *Theileria* infections.

Keywords: Theileriosis, PCR, RPA, Pakistan

Ticks in Pakistan

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Abstract: Despite an agricultural country and suitable climatic conditions for development, growth and dispersal of several ticks, little is known about its taxonomy in Pakistan. Traditional methods of tick identification are based on morphological traits and requires a specialist's expertise. This is particularly difficult in the case of closely related species, sub-species, complex species, immature stages, engorged stages and damaged tick species, and a deep understanding of morphology, ecology, genetics, and geography is inevitable for tick taxonomy. The scarcity of valid knowledge and expertise over tick taxonomy in the country have resulted in the misdiagnosis of unlimited tick species, which have been related as a major obstacle in controlling ticks and tick-borne diseases. Commercially available acaricides have been used against ticks however, these drugs face increasingly high chances of failure, due to the resistance selection in different ticks. Tick-derived protective antigens have been an alternative approach that have gained focus for implementation in anti-tick vaccine development. To rectify taxonomy-related knowledge, we developed a reference museum of accurately identified tick species where some species were assigned as type specimens or described for the first time. Various markers were used to genetically characterize morphologically identified ticks, and the obtained sequences were uploaded to GenBank for the first time. Different tick-associated pathogens of public and veterinary health concerns were detected and characterized for the first time in Pakistan. *In-vitro* based acaricides resistance was confirmed through molecular studies in *Rhipicephalus microplus* for the first in Pakistan. Since livestock-holders in Pakistan hold significant financial burdens, there have been an increasing economic and social demand for alternative tick control methods. We developed an anti-tick vaccine and the potential of tick-derived protective mono antigens and cocktail anti-tick vaccines significantly decreased tick infestation. Long-term observations are needed for accurate assessment associated with ticks and TBPs, which are essential for effective health policies.

Keywords: Ticks, Acaricides, Control, Anti-Tick Vaccine, Pakistan.

Prevalence and contributing risk factors of *Neospora caninum* and *Toxoplasma gondii* infection in breeding ewes from south region of Punjab, Pakistan

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Abstract: *Neospora caninum* and *Toxoplasma gondii* are the potential factors that may cause abortion and reproductive losses in ewes all over the world. The current study aimed to investigate the prevalence of infection to abortifacient agents *Neospora caninum* and *Toxoplasma gondii* in ewes of breeding age from south region of Punjab, Pakistan. Blood samples were obtained from 276 ewes at the reproductive stage and evaluated using Commercial ELISA (ID Screen[®] *Neospora caninum* Competition, Garbels, France) and PCR tests. Logistic regression analysis was employed to evaluate the relationship between seroprevalence and various epidemiological management factors. The overall seroprevalence to *N. caninum* was 6.87.37% (19 of 276) by ELISA while DNA was detected in blood of 3.98% (11/276) objects. Positive cases were identified in 80% of herds sampled (12 of 15). Epidemiological risk factors associated with *Neospora caninum* infection was abortion history (OR 5.7) and poor disposal of placental waste (OR 7.9). *Toxoplasma gondii* anti-antibodies was observed in 13.4% (37 of 276) object at 14 herds studied, while no sample was found to be positive for *T. gondii* DNA. The primary risk factors to *Toxoplasma gondii* infection was; exposure to cats (OR 11.3), exposure to other livestock population (OR 8.1), inadequate hygienic practices (6.5), access to public water sources (OR 3.2). The co-infection with both pathogens was recorded in 1.43% (4 of 276) in breeding ewes. Prevalence to *T. gondii* was higher than that of *N. caninum* and both could be linked with reproductive losses in ewes.

Keywords: abortifacient agent, ELISA, anti-antibodies, Co-infection, DNA, breeding ewes

Exploring key pathways: The impact of mosquito-borne malaria on cancer risk through chronic inflammation and immunosuppression

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Abstract: Mosquitoes, primarily through their role as vectors for the malaria parasite *Plasmodium*, have a complex, indirect relationship with cancer development. Malaria, transmitted via mosquito bites, is a significant global health issue characterized by recurrent fevers, anemia, and other systemic symptoms. Although malaria itself does not directly cause cancer, it can contribute to conditions that potentially influence cancer risk. One of the key factors linking malaria to cancer risk is chronic inflammation. Persistent malaria infections can lead to prolonged inflammatory responses in the body. Chronic inflammation is known to create an environment conducive to cellular mutations and the development of cancer over time. Additionally, severe malaria can lead to immunosuppression, impairing the body's ability to detect and eliminate cancerous cells. This weakened immune response might increase susceptibility to various cancers. Moreover, the malaria-endemic regions often experience high rates of other infectious diseases and environmental stressors that may further influence cancer risk. For instance, co-infections with other pathogens or chronic exposure to environmental toxins can compound the risk factors for cancer development. Research into the direct connections between malaria and cancer is still evolving. However, understanding the indirect effects of malaria-related chronic inflammation and immunosuppression provides valuable insights into how mosquito-borne diseases might impact long-term cancer risk. Addressing malaria through effective prevention and treatment could potentially mitigate some of these indirect cancer risks, highlighting the importance of comprehensive health strategies in malaria-endemic regions.

Can chromosomal genome assemblies help to resolve gastrointestinal nematode control challenges facing Pakistani smallholders?

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Abstract: Gastrointestinal nematodes (GINs) are the most important infectious disease limitation to the production of food from pastoral livestock around the world. Control is hindered by the emergence of genetic adaptations, not least resistance to the available anthelmintic drug classes. Inefficient food production fails to alleviate rural poverty, or to prevent of hunger; and contributes to environmental degradation. Sustainably achieving better control of GINs is, therefore, an important ‘One Health’ challenge. Strategies aimed at reducing resistance gene flow, ensuring exposure to full therapeutic drug doses and maintenance of susceptible parasite populations in a refuge from drug selection, have been developed to reduce the risk of further selection for anthelmintic resistance. Despite being based solely on theoretical principles; complex practices such as the use of targeted selective treatments have achieved local success. However, a major constraint to their widespread adoption has been a lack of empirical evidence, arising as an inevitable consequence of poor understanding of the genetic basis for parasite adaptations. There have been step changes in the development of genomic and genetic methods and platforms for their use. Whole genome sequencing, used in conjunction with conventional parasitology methods and appropriate study design has enabled the development of publicly available, annotated chromosomal genome assemblies for *Haemonchus contortus* and *Teladorsagia circumcincta*; creating a resource for international post genomic research. Mining these genomes has afforded insight to the known β -tubulin SNPs conferring benzimidazole resistance; and has identified loci and candidate genes conferring resistance to levamisole and ivermectin. High throughput platforms such as Illumina MiSeq in conjunction with metabarcoding, enable resequencing of ~500 bp genomic regions of interest. Resequencing of ITS2 rDNA allows the species composition of GIN populations to be described; resequencing of drug resistance loci is needed to inform mitigation strategies; resequencing of mitochondrial genes informs parasite population origins, gene flow and infection patterns; and resequencing of genome wide loci can be used to show genetic linkage to regions coding for traits of interest. There is now a need to apply genomic research advances to address the critical ‘One Health’ challenge of more effective control of GINs in Pakistani pastoral livestock.

First molecular identification and 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 nested polymerase chain reaction (PCR) targeting the *18S rRNA* gene. 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. This study provides an inaugural report on the molecular identification and characterization of *T. luwenshuni* within the sheep population of Azad Kashmir, Pakistan.

First report regarding *Hepatozoon* and *Lankesterella* spp. infection in wild rodents from Pakistan with a note on effect of parasite on blood and oxidative stress markers from the vital organs of the host

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Abstract: Despite of having rich rodent fauna in Pakistan, they are never investigated for the presence of *Hepatozoon* spp. and hence their epidemiology and genetic diversity remains unknown from this subtropical part of the world. During present investigation, blood samples of four wild rodent species [*Rattus rattus* (N = 122), *Mus musculus* (N = 64), *Rattus norvegicus* (N = 57) and *Dryomys nitedula* (N = 1)] were collected during May 2022 till July 2023 from six three districts Pakistan: three in Punjab (Jampur, Dera Ghazi Khan and Multan) and three in Khyber Pakhtunkhwa (Upper Dir, Mardan and Bunar). Results revealed that 7/244 (2.86%) rodents amplified 18S rDNA of *Hepatozoon* spp. through PCR. DNA sequencing and BLAST analysis confirmed the presence of *Hepatozoon* sp. and *Lankesterella* spp. in rodent blood samples. Phylogenetic analysis showed that Pakistani isolates were genetically diverse and clustered with the isolates detected in reptiles, amphibians, birds and mammals from worldwide countries. Parasite prevalence varied between the rodent species. Highest rate of infection was found in *Rattus rattus* followed by *Rattus norvegicus* and *Mus musculus*. Parasite prevalence was more common in female rats than in males. *Hepatozoon* spp. infection significantly disturbed the the white, red blood cells and platelet associated parameters of infected *Rattus rattus*. Markers of oxidative stress analysis revealed that infected rodent had elevated superoxide dismutase levels in kidney, catalase levels in heart and malondialdehyde levels in liver and lungs as compared to uninfected animals. In conclusion, we are reporting for the very first time that Pakistani rodents are infected by *Hepatozoon* sp. and *Lankesterella* spp. Infection leads to disturbed complete blood count and markers of oxidative stress in the vital organs. We recommend large scale studies in various geo-climatic regions of Pakistan to report the incidence and prevalence of this pathogen among the rodents in order to prevent their infections in local people as well as in livestock.

Key words: *Hepatozoon* sp.; *Lankesterella* spp.; Molecular Prevalence; Phylogeny; Pakistan; Wild Rodents.

Recent biodiversity status of fishes inhabiting the Sulaimanki headworks Punjab Pakistan with special reference to parasitic diseases, ecohydrology, threats and their conservation strategies

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Abstract: The ichthyofauna of the Sutlej River has been experiencing a significant decline due to factors such as habitat destruction, flooding, pollution, pesticide use, and the introduction of exotic species. This survey aimed to assess the current status of fish populations in the Head Sulaimanki area and identify the impacts of environmental changes. During the study period, we collected specimens representing 25 different fish species from various landing sites. The predominant species included *Cyprinus carpio*, *Labeo rohita*, *Walago attu*, *Cirrhinus mrigala*, and *Oreochromis mossambica*, with *Cyprinus carpio* being the most frequently caught and *Channa marulius* the least. Water quality parameters were monitored throughout the study to evaluate the conditions affecting fish health. Measurements of dissolved oxygen (DO), temperature, pH, salinity, electrical conductivity (EC), total dissolved solids (TDS), and turbidity were taken using a multimeter. The water temperature ranged from 15°C to 19°C, with DO levels between 7 and 8 ppt, pH values from 6.9 to 7.5, and salinity ranging from 0.11 to 0.15 ppt. The study also revealed the presence of various parasites affecting fish, including external parasites such as *Lernaea spp.*, *Aeromonas spp.*, *Ergasilus spp.*, and *Saprolegnia spp.*, as well as internal parasites like *Neascus spp.*, *Heterosporis*, and *Ligula spp.* The findings underscore the critical need for immediate conservation actions to address the declining fish populations and deteriorating water quality. Implementing effective management strategies will be essential for preserving the ichthyofauna and ensuring the ecological balance of the Sutlej River.

Key words: Exotic Species, External Parasites, Ichthyofauna, Population

Epidemiology and impact of water and foodborne toxoplasmosis

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Abstract: Foodborne toxoplasmosis was prioritized within the foodborne parasites by the WHO and FAO, as the fourth most dangerous parasite for humans, due to its impact on human health and large prevalence worldwide. Foodborne toxoplasmosis can be acquired through contaminated water, undercooked meat or unwashed raw vegetables. Inspection scores of sites of production or preparation of food, such as restaurants, are a vital instrument of public health authorities and have made proof to identify sites with high risk for food contamination with bacteria. However, no evaluation exists of the concordance between inspection scores for parasites such *Toxoplasma gondii* on food. For food industry policies and for health inspection authorities, it is important not only to identify the risk factors involved in foodborne toxoplasmosis but how hygienic measures can reduce such risks and if food safety evaluation tools, such as questionnaires about food safety compliance, can identify restaurants that have a risk for foodborne toxoplasmosis.

Poultry – *Campylobacter jejuni*: An emerging food safety issue

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Abstract: Foodborne diseases are an important worldwide public health issue mostly resulting from ingestion of food contaminated with pathogenic bacteria. *Campylobacter* is the most important foodborne pathogen to cause human gastroenteritis worldwide and is primarily associated with poultry. Host–microbe interactions have attracted considerable attention; however, the mechanisms of the actions of *C. jejuni* on host physiology are poorly understood. Although ceca are the main site of *Campylobacter* colonization, the infection also influences functions in the small intestine. In addition, *C. jejuni* does not only reside in the gut but can also disseminate to various internal organs, such as the liver and spleen. Colonization of internal organs might pose an additional risk to consumers. It is still unclear whether *Campylobacter* is commensal or pathogen in chickens. Some groups, including our own, have reported changes in the chicken gut in response to *Campylobacter* colonization, as *Campylobacter* can induce histomorphological changes in the intestine, which are good indicators of the influence on bird health, clinically characterized by an impaired body weight. Changes in intestinal barrier function, the downregulation of certain nutrient transporters and the impact on mucin production influence nutrient uptake. In addition, the diet itself is also able to influence the *Campylobacter* load at certain points post infection, an effect associated with an alteration of nutrient digestibility. Such effects compromise the health of animals and the sustainability of production. Feed composition, age and breed also influence the outcome of *C. jejuni* colonization, immunity development and the gut microbiota. The biology of a *Campylobacter* infection in commercial broilers is influenced by the bacterium itself, the diet and the host. Some *Campylobacter* strains can disseminate to extra-intestinal organs (e.g. NCTC 12744) and significantly reduce the bird’s performance, while others do not (e.g. M1). Although campylobacteriosis is a widespread bacterial foodborne disease, there are no effective, reliable and practical intervention measures to reduce the colonization of the broiler gut with *Campylobacter*. We need to identify key events in the farm-to-fork chain that are appropriate targets for prevention and control. A possible way to reduce *Campylobacter* contamination in poultry is to develop new actions at the primary production level.

Understanding the influence of *Campylobacter* colonization of broiler chickens will also provide insights into its impact on production parameters and has the potential to reveal strategies to reduce viable numbers on the finished product and thus to improve food safety. Unravelling the link between feeding strategies and gut physiology/immunology in the context of *Campylobacter* colonization will help to elucidate the cascade of events that hampers *Campylobacter* colonization. The increasing importance of wheat/barley/oat in poultry diets should be reflected in a more detailed understanding of the functional consequences of feeding such grains. The challenge is to define efficient strategies to introduce a suitable level of dietary fiber to replenish the gut microbiome with essential missing microbes that significantly impact enteric poultry pathogens associated with the gut microbiota. A better understanding of diet-microbiota interactions will help the development of a nutritional approach that would target and efficiently reduce the colonization of *Campylobacter*, which is of a significant public and animal health concern.

Prevalence of poultry coccidiosis and associated risk factors in intensive farm and individual small holder poultry farm in Benadir region, Somalia

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Abstract: A cross-sectional study was carried out from September 2021 to February 2022 in Mogadishu, Somalia, aimed at determining the prevalence of poultry coccidiosis and identifying associated risk factors in both intensive and smallholder poultry farms in the Benadir region. The primary goal of the research was to estimate the prevalence of the disease and examine risk factors linked to coccidiosis. The flotation technique was employed to isolate coccidian oocysts from 384 chicken fecal samples, revealing a prevalence of 19.8%. No statistically significant difference was found in the prevalence of poultry coccidiosis across different chicken age groups ($P>0.05$), though younger chickens (chicks) exhibited a slightly higher prevalence (20.4%) compared to adults (18.8%). The influence of sex on disease prevalence was also analyzed, with a marginally higher prevalence in males (20%) than in females (19.4%), but this difference was not statistically significant ($P>0.05$). According to the management systems, a significant variation was noted between extensive and intensive management systems, where the extensive system had a higher prevalence (25.5%) compared to the intensive system (14%). Significant differences were also observed in body condition scores, with chickens in poor condition showing a higher prevalence (26.6%) than those in moderate (16%) or good condition (8.8%). Additionally, the study revealed a significant association between infection rates and housing methods, with chickens housed on the floor having a higher prevalence (25.5%) than those in cages (14%). Appropriate control measures should focus on key risk factors such as age, management practices, and housing systems, emphasizing biosecurity to prevent and control coccidiosis. Further research is necessary to identify the specific *Eimeria* species present for targeted control strategies.

Key words: - Coccidiosis, Risk Factors, Somalia

Translating innovations to improve malaria theranostics and predicting the outcomes of malaria in pregnancy

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Abstract: Malaria remains a significant global health challenge, in tropical countries. The disease is caused by Plasmodium parasites, with *P. falciparum* being the most prevalent and deadly species. Malaria elimination efforts rely on rapid diagnostic tests (RDTs) detecting histidine-rich proteins 2/3 (HRP2/3) and treatment with artemisinin combination therapies (ACTs). However, recent data suggest increasing *hrp2/3* gene deletions and ACT partial resistance due to *kelch13* (*k13*) mutations, complicating control efforts. Malaria in pregnancy (MiP) poses a great public health risk, with over 33 million women exposed to the disease in sub-Saharan Africa, alone. MiP often leads to serious morbidity and mortality, with anemia accounting for about 20% of all maternal deaths. Current diagnostic methods, including microscopy and RDTs, often miss low-level infections. To address these challenges, novel approaches are being developed. To this end, the Pillai laboratory conducted a three-year long "LAMPREG" clinical trial in Ethiopia and evaluated the effectiveness of loop-mediated isothermal amplification (LAMP) technology for malaria detection in pregnancy. We also investigated *hrp2/k13* deletions/mutations by digital droplet PCR (ddPCR)/sanger sequencing and found a high level of prevalence. In parallel, we also analyzed the differential host/parasite responses in malaria-infected versus non-infected pregnant women using targeted metabolomics, revealing significant differences in analytes associated with MiP. Additionally, we are developing predictive algorithms using machine learning based on multi-omic and clinical data to improve MiP outcomes. These innovative strategies are a step forward towards precision medicine and aim to provide more accurate diagnostics as well as personalized interventions for managing malaria, especially in pregnant women. Finally, the Pillai laboratory is also developing real-time isothermal assays for deployment at the point-of-care to combat infectious diseases in low- and middle-income countries (LMICs).

Molecular evidence of buparvaquone resistance in tropical theileriosis

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Abstract: *Theileria annulata* (*T. annulata*) is a protozoan parasite, causes tropical theileriosis in cattle. The drug of choice for treatment is Buparvaquone. However, recent reports published on drug failure indicates resistance against it. This study was designed to determine the existence and mechanism of buparvaquone resistance in the Southern Punjab, Pakistan. To confirm it, partial mitochondrial cytochrome b gene (Cyt-b) of *T. annulata* was amplified by polymerase chain reaction to know the point mutations in drug binding sites (Q_{o1} (130-148) and Q_{o2} (244-266)) of this gene. The sequences of this study were aligned (Bioedit) with a non-mutated reference sequence (Ankara strain XM949625) in order to find out non-synonymous mutation at drug-binding sites. Notably, the A146T mutation in Q_{o1} site was consistently observed in all isolates of this study. Additionally, we detected mutations at position I203V. This study highlighted the emergence of buparvaquone resistance in Pakistan associated with point mutation in Q_{o1} region of Cyt-b gene of *T. annulata*.

Keywords: *Theileria annulata*, Cytochrome b gene, Buparvaquone, Mutation, Drug Resistance.

Cardio-respiratory nematodes in mustelids from Romania

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Abstract: The family Mustelidae is the largest of the order Carnivora, comprising small to middle-sized animals. Some species prefer aquatic environments, while others dwell in open or forest habitats, and others are commonly found close to or in human settlements. Considering the diversity of habitats and food habits of mustelid species, lungworm infections are very likely to occur. In the present study, the aim was to determine the presence, prevalence, and diversity of lungworms infecting mustelids from Romania.

Between 2015 and 2023, 266 carcasses of different mustelid species were collected from various regions of Romania. Animals were either legally hunted or found as roadkill. The respiratory tracts were isolated and examined by careful examination under a stereomicroscope, and all detected nematodes were collected and morphologically and molecularly identified.

Overall, 30.45% (n=81) of the hosts were infected by at least one species of respiratory parasite. *Crenosoma* spp. (5.26%, n=14), *Perostrongylus falciformis* (14.66%, n=39), *Filaroides* spp. (0.75%, n=2), *Angiostrongylus daskalovi* (14.28%, n=38), *Dirofilaria immitis* (0.37%, n=1) and *Eucoleus aerophilus* (22.55%, n=60) were identified. Remarkably, small mustelid species seem to be more rarely affected by lungworms as besides Eurasian badgers, only 32.72% (n=36) of the rest of mustelids were infected by lungworms.

In conclusion, Romanian mustelids are commonly infected by lungworms. Further research is needed to assess the pathological effects on these lungworms on hosts.

Innovative strategies to control tick-borne pathogens in food animals

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Abstract: Ticks are hematophagous ectoparasites, and these are characterized by a wide variety of pathogens they transmit. The direct losses of ticks include decrease in animal productivity, damage to skin and hides, and heavy infestation may lead to anemia. The indirect losses include the transmission of disease agents such as pathogenic bacteria, viruses, and protozoans and rickettsial organisms. Furthermore, these are second to mosquitoes in transmitting human diseases. Ticks are categorized into *Ixodidae* (hard ticks) and *Argasidae* (soft ticks). *Rhipicephalus microplus*, and *Hyalomma anatolicum* are important ticks of family *Ixodidae* in transmitting livestock diseases. *Theileria annulata* is one of the most important protozoan parasites that is transmitted by *H. anatolicum* ticks. It causes tropical theileriosis or mediterranean theileriosis in cattle in tropical and subtropical regions of the world. The disease is prevalent across southern Europe, north Africa, and Asia; and approximately 250 million cattle are at risk of this parasite. *T. annulata* completes its sexual stages in the tick vector and asexual developmental stages in the cattle. The sporozoites of *T. annulata* invade the lymphocytes of cattle, transform to schizont stage and merozoites are then released which invade the circulating erythrocytes. The parasite affects the host signaling pathways and induce clonal multiplication of lymphocytes, which leads to parasite's continued propagation and evasion from host's immune system, a mechanism that is still poorly understood. The invasion and multiplication cause loss of red blood cells that leads to anemia. A good understanding of immune evasion by *T. annulata* is required to limit its propagation in host cells. *Theileria* is diagnosed using conventional microscopy in thin films of infected blood. Molecular techniques such as PCR are also used for its detection and confirmation. There is no single strategy to control this parasite, therefore integrated control methods are used to prevent its spread. The chemotherapeutic use of buparvaquone helps in controlling the disease at its early stages of infection, but longer withdrawal timing and higher costs limit its usage. Secondly, the control of ticks is also vital to control theileriosis but the irrational use of acaricides poses a threat to environment safety. A live-attenuated vaccine is also available to supplement the control of this parasite against its schizont stage in cattle. Now the focus of vaccination is on molecular vaccines based on antigens that are proved to induce immune response during experimental conditions. In recent decade, a great knowledge has been generated in understanding the potential immunogens against a variety of pathogens; and the focus is shifted towards recombinant vaccine production based on these potential immunogens. This strategy is based on selecting potential immunogens, finding their epitopes for helper-T-lymphocytes, cytotoxic T-lymphocytes, and B-cells, collectively known as reverse vaccinology. This strategy has a promising potential to control ticks and tick-borne pathogens. The studies based on multi-epitope predictions also carry the potential for large-scale applications with limited or no side effects.

Keywords: Tropical theileriosis; Mediterranean theileriosis; *Theileria annulata*; *Hyalomma anatolicum*; Ticks; Multi-epitope; Molecular vaccines.

A review of foodborne helminth parasites: modeling control and prevention strategies for echinococcosis and hydatidosis

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Abstract: Echinococcosis and hydatidosis pose significant public health risks, especially in areas with high stray dog populations and limited veterinary access. Effective control strategies, informed by modeling data, can enhance food security by reducing disease incidence. This review aims to analyze foodborne helminth parasites, with a specific focus on echinococcosis and hydatidosis, and to model effective control and prevention strategies that can mitigate their impact on public health and food security.

A comprehensive literature review was conducted, focusing on studies published in journals regarding *Echinococcus granulosus* (n=180). Data were collected on prevalence rates, transmission dynamics, and existing control measures (n=30).

In Iran, these zoonotic diseases are influenced by factors such as climate, livestock practices, and stray dog management. The rural population is especially vulnerable due to inadequate healthcare access. To mitigate the impact of these diseases, efforts should focus on public awareness campaigns, improved veterinary practices, and a One Health approach to integrate public health and agricultural strategies effectively.

The complex life cycles of *Echinococcus* species necessitate a multifaceted approach to control. In conclusion, addressing the public health risks posed by foodborne helminth parasites like echinococcosis and hydatidosis requires a One Health approach that fosters collaboration among veterinary, medical, and environmental sectors.

Keywords: *Echinococcus granulosus*, Cystic echinococcosis (CE), Control strategies, Veterinary Practices, Rural populations

ABSTRACTS
FOR
POSTER
PRESENTATIONS

Designing of a multi-epitope construct using immuno-informatic tools to prepare an mRNA vaccine against *Rhipicephalus microplus* ticks

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Abstract: Ticks are blood-feeding ectoparasites that transmit pathogens to animals and humans. One of the most important hard ticks in animals is *Rhipicephalus microplus*, which transmits *Babesia* and *Anaplasma* spp. Although many potential tick vaccine candidates have been identified, no effective vaccine that can provide sterile immunity against *R. microplus* tick infestations has been developed. This study aimed to design a construct using different computational tools to identify and predict immunogenic epitopes within protein sequences and to prepare a messenger RNA (mRNA) vaccine against *R. microplus* based on lipid nanoparticles (LNPs). The *R. microplus* proteins (Bm86, Subolesin, and ATAQ) were selected and their consensus sequence was obtained from the National Center for Biotechnology Information in FASTA format. The Immune Epitope Database and Analysis Resource (IEDB) server was used for the prediction of helper T-cell epitopes, the NetCTL 1.2 server was used to predict cytotoxic T-cell epitopes, and the ABCpred server was used for B-cell epitope prediction. Antigenicity testing, allergenicity assessment, and toxicity screening were immuno-informatic techniques used to identify potent epitopes within protein sequences. The multi-epitope construct was prepared and cloned into the pVAX1 plasmid. Plasmids were transformed in compatible competent cells, and restriction analysis was performed. After restriction analysis of the transformed plasmid, *in vitro* transcription was performed to prepare mRNA. The mRNA was purified, quantified, and converted into complementary DNA, and gene-specific primers were used to confirm *the in vitro* transcription of mRNA. A mixture of four lipids containing 1,2-dioleoyl-3-dimethylammonium-propane (DODAP), Distearoylphosphatidylcholine (DSPC), cholesterol, and 1,2-Dimyristoyl-sn-glycero-3-methoxypolyethylene glycol-2000 (DMG PEG-2000) was used to prepare LNPs. LNPs were characterized using a scanning electron microscope, Zeta potential, and Zeta Sizer tests. More than 1000 epitopes were predicted, from which only nine helper T-lymphocytes, 18 cytotoxic T-lymphocytes, and nine B-cell epitopes of all three proteins were selected with high antigenic scores of 0.958 for Bm86, 0.752 for Subolesin, and 0.964 for ATAQ, respectively. An adjuvant was used to enhance immune responses, all of which were linked to one another using GPGPG, AAY, and KK linkers, respectively. The physicochemical properties predicted that the instability index of the construct would be <40%, indicating that the construct is stable. Plasmids were transformed in compatible competent cells, and white-transformed colonies were observed. Restriction analysis was performed, DNA was transcribed into mRNA, and LNPs were prepared and characterized. More than 1000 epitopes were predicted using immune informatic tools, and only high-scoring epitopes were selected. A multi-epitope construct was designed using bio-informatic tools, and its physicochemical properties were predicted. The design construct was inserted into the pVAX1 plasmid, and *in vitro* transcription was performed to prepare the mRNA. LNPs of mRNA were prepared and characterized to be used as vaccines. It was found that LNPs were stable and nanometer-sized.

Keywords: *Rhipicephalus microplus*, Immuno-informatic tools. multiepitope construct, Lipid nanoparticles

Sero occurrence and molecular detection of *Dirofilaria immitis* in pet dogs of Lahore, Pakistan

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Abstract: Dogs showing clinical signs of exercise intolerance but otherwise appearing normal, are usually suffering from cardiopulmonary complications of a parasitic infection caused by *Dirofilaria immitis* commonly called as heart worm. This study aimed to investigate heartworm infection in dogs in the Lahore district for the first time, utilizing a combination of diagnostic methods, including microscopy, PCR, and rapid diagnostic tools. Mosquitoes serve as the primary vectors for disease transmission, with infected mosquitoes introducing the parasite to susceptible hosts during blood meals. The life cycle of *D. immitis* involves microfilariae being ingested by mosquitoes and developing into infectious larvae, which then mature into adult worms within the definitive host. Early detection of *D. immitis* is crucial for managing the disease and preventing severe organ damage. Microscopic detection, including wet mount and modified Knott's test, was used to identify microfilariae, while rapid diagnostic kits detected adult worm proteins in blood samples. Additionally, PCR provided precise identification of the parasite at various life stages. This combination of diagnostic methods allowed for a comprehensive evaluation of heartworm infection. Our findings highlight the need for implementing preventive strategies and monitoring the movement of infected animals to control the spread of dirofilariasis. This study represents a critical step in addressing heartworm infection in a previously unexplored region.

Key Words: *Dirofilaria immitis*, Pets, Epidemiology, Seroprevalence, PCR, Knott's test.

Fruit vinegars as biological control strategy against fruit born fungal pathogens

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Abstract: According to surveys done by NRDC (Natural Resources Defense Council), FAO (Food and Agriculture Organization of the United Nations), USDA (US Department of Agriculture) and the OECD (Organization for Economic Co-operation and Development), among food losses, that are estimated to be more than 33%, post-harvest losses of fruits and vegetables are even higher and are estimated to be 40–50%. Fungal pathogens are the major cause of these losses after fruit ripening. The aim of the study is to use natural sources as antifungals against food born fungi to avoid chemical toxicity on fruits and vegetables. In current study, antifungal property of different vinegars has been evaluated against some fruit born fungi. Fungal isolates were identified by macroscopic and microscopic study. Antifungal activity of vinegars was evaluated by agar well diffusion method. Four types of fungal isolates were identified i.e. *Acremonium* species, *Aspergillus flavus* and *Fusarium solani* and *Aspergillus niger* isolated from strawberry, guava, orange and lemon respectively. Synthetic white vinegar and mixed fruit vinegar showed maximum activity against *Acremonium* species forming zone of inhibition of 15 ± 0.05 mm and 12 ± 0.05 mm. *Syzygium cumini* vinegar showed maximum antifungal activity against *A. flavus* forming zones of inhibition 10 ± 0.05 mm. For *A. niger*, three different vinegars were used i.e., apple cider, grape vinegar and white distilled vinegar. Among them, apple cider showed the best effect against *A. niger* forming zone of inhibition of 0.7 ± 0.05 mm followed by grapes vinegar and white distilled vinegars respectively. The results showed that vinegars coatings, sprays or fumes can be used as an alternative to chemical fungicides to keep fungal pathogens and chemical toxicity away from food because vinegars have active components i.e., phenolics and flavonoids etc. which have antifungal and anti-oxidant properties.

Monkeypox infection: the global concern of today

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Abstract: After the pandemic of COVID-19, many countries are now suffering from monkey pox virus (MPXV) infection, one of the largest and most complex viruses. The rising number of patients in non-endemic countries is becoming a global concern. Hence, proper and quick identification, treatment, and prevention are essential. Proper sample collection, handling, and storage are essential. A variety of approved identification techniques like real-time PCR, multiplex real-time PCR assay, pan-orthopoxvirus PCR/ESI-MS, loop-mediated isothermal amplification-based diagnostic assay, recombinase polymerase amplification assay, GeneXpert, immunohistochemistry, electron microscopy, cell culture, and ELISA are available. The goldstandard technique is PCR. After this, treatment is required. Many drugs have proven to be effective for the treatment of MPXV. These are Tecovirimat (which inhibits viral replication and wrapping), Cidofovir (which inhibits the activity of viral DNA polymerase), Brincidofovir (a conjugate of Cidofovir), Ribavirin (which disrupts viral translation and helicase activity), Tiazofurin, Adenosine N1 oxide, and HPMPA (which inhibits IMPDH, DNA polymerase, and viral mRNA translation), Nilotinib, Simeprevir, and Dihydroergotamine (effective for Mpox). Additionally, vaccinations against Mpox, such as JYNNEOS, ACAM2000, and VIGIV, have been used. By following the prevention techniques, we can minimize the risk of MPXV and make the world healthy. Control measures and prevention in both the healthcare and public sectors are scrutinized, including contact tracing, isolation, vaccine programs, surveillance, and reporting. Campaigns for public awareness and for educating the population are crucial components of the strategy. The review highlights the genomics of the monkey pox virus, prevention techniques, the collection and handling of samples, identification techniques, drugs, and vaccines.

Implementation of poly(lactic-co-glycolic acid)(plga) nanoparticle-based vaccine to vaccinate gastrointestinal parasite (*Ostertagia*) to reduce cattle infection

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Abstract: Gastrointestinal parasitism, particularly by *Ostertagia* species, poses a significant threat to cattle health and productivity. Traditional vaccination methods have shown limitations such as antigen degradation and limited mucosal immunity. This study investigates the possibilities of Poly(lactic-co-glycolic acid) (PLGA) polymer-based nanoparticles as an innovative vaccine delivery method to fight *Ostertagia* infections, aiming to improve vaccine efficiency and reduce cattle infection. The polymer matrix of PLGA contains antigens from *Ostertagia* parasites and these nanoparticles aim to target the mucosal surfaces of the gastrointestinal tract in order induce a strong immune response. PLGA nanoparticles protect antigens from degradation and formulate to release antigens in order to provide a sustained immune response over time. PLGA nanoparticles are generally well-tolerated by the body, minimizing adverse reactions. Because of various capabilities of nanoparticles like biocompatibility, biodegradability and encapsulation, it can be used for the purpose of vaccine delivery. Polymer-based nanoparticles offer several advantages over traditional techniques including improved antigen delivery, enhanced immune responses, controlled release and reduced side effects. PLGA based-vaccines are time-consuming and expensive due to its manufacturing complexity and regulatory requirements but the long-term benefits in terms of improved health outcomes and reduced disease burden can justify the cost. The findings of this study suggest that PLGA nanoparticles holds a promising approach for the development of effective vaccines against *Ostertagia* infection in cattle.

The implementation of an automated dosing system (ads) within the framework of precision livestock farming (plf)

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Abstract: Parasites are organisms that live on or within a host, deriving nutrients at the host's expense. Coccidia are protozoan parasites that belong to the phylum Apicomplexa. Coccidiosis, the disease caused by Coccidia, is one of the most significant parasitic diseases in poultry, affecting chickens, turkeys, and other birds. The implementation of an Automated Dosing System (ADS) within the framework of Precision Livestock Farming (PLF) offers a cutting-edge approach to controlling coccidiosis caused by *Eimeria necatrix* in chickens. This parasite, notorious for causing severe intestinal damage, poses a significant threat to poultry health and productivity. Traditional methods of managing coccidiosis often depend on blanket administration of anticoccidial drugs, leading to challenges such as drug resistance and uneven dosage distribution. An ADS integrated with PLF technologies addresses these challenges by ensuring precise, targeted administration of anticoccidial treatments based on real-time data collected from environmental sensors, health monitoring systems, and predictive analytics. By continuously assessing the flock's health status and environmental conditions, the ADS can adjust dosage levels in real-time, delivering the exact amount of medication required for each bird or specific flock segments. This approach not only optimizes drug use but also reduces the risk of resistance development also it is effective as it reduces cost by reducing drugs waste and labour cost on manual dosing . The adoption of this technology enhances the overall health management of poultry, minimizing the impact of *E. necatrix* and improving growth rates, feed efficiency, and ultimately, food security. The abstract concludes that the integration of ADS within PLF represents a significant advancement in poultry farming, offering a more sustainable, efficient, and effective method for controlling coccidiosis, with potential applications in broader livestock management contexts.

The application of CRISPR/Cas9 in targeting the genes of interest in *Eimeria maxima* to control coccidiosis in poultry animals

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Abstract: Parasites are organisms that live on or within a host, deriving nutrients at the host's expense. Coccidia are protozoan parasites that belong to the phylum *Apicomplexa*. Coccidiosis, the disease caused by coccidia, is one of the most significant parasitic diseases in poultry, affecting chickens, turkeys, and other birds. CRISPR/Cas9 technology offers a revolutionary approach to controlling *Eimeria maxima*, a major pathogen causing coccidiosis in poultry. By enabling precise genetic modifications, CRISPR/Cas9 facilitates the development of targeted interventions such as attenuated *Eimeria* strains and enhanced vaccines. This methodology includes designing specific guide RNAs (gRNAs) to target key genes, constructing and validating plasmids for gene editing, and performing rigorous in vitro and in vivo testing. In Pakistan, where poultry farming is a significant industry, this model can be implemented by collaborating with local research institutions to adapt the technology to regional strains and conditions. The step-by-step process involves objective setting, gene identification, vector construction, transfection, validation, and scaling up production. Cost-effective method as it may reduce long term cost on disease management by providing more durable solution. Compared to traditional methods, CRISPR/Cas9 provides enhanced precision and efficacy, reducing the risk of drug resistance and offering long-term solutions for disease control. Additionally, the technology's ability to produce specific and effective vaccines could lead to significant economic benefits by improving flock health and productivity, thus addressing food security challenges in Pakistan. The only complexity this method has that it requires genetic experts to implement as implementing CRISPR/Cas9 requires careful planning, adherence to regulatory standards, and ongoing evaluation to ensure its effectiveness and safety.

Targeted drug delivery systems to treat infections caused by parasites.

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Abstract: Targeted drug delivery systems (TDDS) represent an effective way to improve treatment of parasites by delivering drugs directly to the site of infection or function. This approach can improve delivery, minimize side effects, and help control drug resistance, all of which are critical to solving food safety issues caused by parasites. Drugs target cells or tissues. For example, liposomes are spherical vesicles made of lipid bilayers that encapsulate drugs and target them to specific tissues. The Nanoparticles containing materials such as gold or silicon dioxide can be designed to deliver drugs with high efficiency. Microspheres and nanospheres are small particles that encapsulate drugs and provide controlled or time released. Hydrogels are three-dimensional polymer networks that swell and release chemicals in response to environmental stimuli, providing control over delivery. Focusing on this will lead to effective treatments for parasites that can cause significant losses to crops and animals. For livestock that contribute a major role as food, TDDS can contribute in treatment of parasites, increasing animal health which increase the efficiency of food production. TDDS can be adapted for field use in limited areas, making it a versatile tool for controlling parasite in different areas which help us to overcome the food shortage. Overall, the financial outlay of this system is very high running up to millions of dollars for clinics trials, preclinical studies and regulatory approvals, but it can prove valuable in tackling diseases caused by parasites.

Genetic characterization of *Dermacentor* sp. and associated *Anaplasma marginale*, infesting small ruminants in Pakistan

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Abstract: Ticks are hematophagous ectoparasites and vectors of a wide range of infectious diseases after mosquitoes. *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 important 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 at the morphological and molecular identification of *Dermacentor* sp. and its associated pathogen *Anaplasma marginale*. A total of 30 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 *cox* 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. marginale*. The phylogenetic analysis based on 16S DNA and *cox* sequences revealed a close evolutionary relationship with *Dermacentor pavlovskyi* from China. All the 16S rDNA sequences of *Anaplasma* in the phylogenetic tree clustered with *A. marginale*. This study emphasizes the need for further investigation to explore tick diversity and associated pathogens across the country.

Keywords: *Dermacentor* sp.; *Anaplasma marginale*; Tick; Pakistan.

Status of the genus *Haemaphysalis* in KP, Pakistan

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Abstract: *Haemaphysalis* is the second most diverse genus after ixodes having 176 species throughout the world. Excluding other genera, totally 11 species of *Haemaphysalis* exist in Pakistan. For the species-specific control measures, it is important to study *Haemaphysalis* tick fauna. The selected districts for this study were Charsadda, Peshawar, Mardan, Swabi, Nowshera, Bajaur, Upper Dir, Lower Dir, Shangla, and Swat. By observing different hosts for tick's collection, various tick's species were found that belonged to three genera of hard ticks; *Haemaphysalis*, *Rhipicephalus*, and *Hylomma*. Moreover, the *Haemaphysalis* ticks were collected mostly in northern region of Khyber Pakhtunkhwa while infesting small ruminants. The diversity of *Haemaphysalis* in Khyber Pakhtunkhwa was (*Haemaphysalis danieli*, *Haemaphysalis indica*, *Haemaphysalis montgomeryi*, *Haemaphysalis sulcata*, *Haemaphysalis kashmirensis*, *Haemaphysalis cornupunctata*, and *Haemaphysalis bispinosa*). These ticks are known for the transmission of *Rickettsia* (*Rickettsia hoogstraalii*, *Rickettsia aeschlimannii*), *Coxiella* spp (*C. burnetii*, *C. endosymbionts*). This study covers the diversity and its associated pathogens in Khyber Pakhtunkhwa. Further study is needed to investigate its diversity and pathogenicity throughout the country.

Keywords: Ticks, *Haemaphysalis sulcata*, Pakistan.

Ticks collected from different animal hosts and environment in selected areas of Kashmir

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Abstract: Ticks are obligate blood sucking ectoparasites that acts as a vector for several pathogens includes bacteria, protozoans and viruses. Kashmir, which is in north of Pakistan, have suitable temperature, humidity and hosts, making this region favorable for ticks. Despite potential threat to animals, including humans, studies on ticks have been largely neglected in Kashmir. Therefore, this study aims to focus on tick diversity in Kashmir. Ticks were collected manually from domestic animals and open environment. A total of 236 ticks were collected from different open habitats: meadows and agriculture area, and from domestic animals: hens, cows, goats and buffaloes. Ticks were identified representing three genera of hard ticks (*Ixodidae*) and one genus of soft ticks (*Argasidae*). Among the hard ticks, the identified species were: *Rhipicephalus microplus*, *Hyalomma anatolicum*, *Haemaphysalis sulcata*, *Haemaphysalis montgomeryi*. Among soft ticks, the identified species were: *Argas persicus* and *A. reflexus*. Further molecular study should be conducted to monitor ticks and tick-borne pathogens in the region, which can help to control the emerging ticks-borne disease in Kashmir.

Keywords: Ticks, Domestic Animals, Open Environment.

***Amblyomma* ticks and associated *Borrelia*: Insight from wild reptiles**

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Abstract: As hematophagous ectoparasites, ticks may transmit microorganisms to their vertebrate hosts, ranging from semi-terrestrial to terrestrial. With highest proportion of reptile-infesting ticks, the genus *Amblyomma* comprises approximately nine subgenera. Two of these subgenera are *Aponomma* and *Adenopleura*, which also contains *Amblyomma gervaisi* and *Amblyomma javanense*. *Borrelia* are common spirochetes in ticks, while a new group has been identified in association with reptiles and their associated ticks. To overcome the knowledge deficit in Pakistan, this study aimed to morphologically and molecularly identify ticks collected on wild reptiles, as well as to molecularly assess *Borrelia* spp. associated with these ticks. For this purpose, free-ranging wild reptiles belonging to different genera from Khyber Pakhtunkhwa province, Pakistan, were examined for ticks. Only the monitor lizards (*Varanus bengalensis*) were found infested, and ticks were morphologically described as *A. gervaisi* and a new *Amblyomma* species, *Amblyomma pakhtunensis* sp. nov. Of the total 776 *A. gervaisi* ticks, there males (316, 40.7%), females (184, 23.7%), nymphs (267, 34.4%) and larvae (9, 1.2%). Of the total 57 *A. pakhtunensis* ticks, there were males (23, 40%), females (14, 25%) and nymphs (20, 35%), while no larvae were recorded. The morphological identification of ticks was molecularly confirmed through sequencing of complete mitochondrial genome or its partial fragment. The extracted DNA from both tick species was subjected to two PCR assays (16S rRNA and flaB genes) for detecting associated *Borrelia*. Only *A. gervaisi* ticks were found to be *Borrelia* positive, with an infection rate of 18% (19/108). Considering the closest identities from BLAST analysis, *A. gervaisi* showed 94.6% identity to conspecifics (16S rRNA). In contrast, *A. pakhtunensis* showed the following maximum BLAST identities: 98.25% with an *Amblyomma* species (12S rRNA), 96.07% with *A. javanense* (16S rRNA). Moreover, the mitogenome of *A. pakhtunensis* revealed maximum BLAST identities of 80.75%, 80.48% and 79.42% with *Amblyomma testudinarium*, *A. javanense*, and *Amblyomma* sp., respectively. In the Phylogenetic trees for *A. gervaisi*, this tick species from the current study clustered with same species from India (16S rRNA) and clustered alone (ITS2). In the phylogenetic trees for *A. pakhtunensis*, this tick species clustered with *Amblyomma* sp. from Sri Lanka (12S rRNA), clustered with *A. javanense* from Sri Lanka (16S rRNA), and clustered with *A. javanense* from China and *Amblyomma* sp. from China (mitogenome). In the phylogenetic trees for *Borrelia* sp., this microorganism clustered with reptile-associated *Borrelia* group (REP) from various countries across the Asia, Australia and Africa. This research could assist to know about the systematics and phylogeny, as well as molecular epidemiology, of the genus *Amblyomma* and the genus *Borrelia*.

Keywords: Ticks, *Amblyomma gervaisi*, *Amblyomma javanense*, *Amblyomma pakhtunensis* sp. nov., *Borrelia*

Teratological abnormalities in ixodid ticks of Pakistan

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Abstract: Teratological abnormalities are malformations in the normal morphology of a tick and are important to be considered in any tick related study, as it causes complications in morphological identification. Globally, this phenomenon is reported in different states of the world, but its studies are missing in the ticks' fauna of Pakistan. This study was conducted to find out teratological anomalies in ticks of Pakistan infesting different livestock (buffalos, cattle, camels, goats and sheep). Visits of animal markets across 17 different districts (mainly in Khyber Pakhtunkhwa) were made in the summer season (May-October) of 2023 for tick samples collection. After studying these ticks under stereo zoom microscope, 89 out of 3271 (2.17%) were found to have teratological anomalies. The anomalous tick comprises of eight *Ixodidae* species (*Rhipicephalus microplus*, *R. turanicus*, *R. haemaphysaloides*, *Haemaphysalis bispinosa*, *H. montgomeryi*, *Hyalomma anatolicum*, *H. scupense*, *H. dromedarii*). The teratological anomalies were classified into, local anomalies present in 67 specimens, general anomalies in 12 specimens and nine specimens have a combination of both general and local anomalies. This is the first report of teratological anomalies in the ticks' fauna of Pakistan whereas abnormalities in *Hy. anatolicum*, *Ha. bispinosa* and *Ha. montgomeryi* were reported for the first time throughout the world.

Keywords: Ticks, Pakistan, Anomalies, *Hyalomma*, *Haemaphysalis*.

Comparison of prokaryotic and eukaryotic expression systems and evaluation of immunogenicity for theilerial proteins

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Abstract: Parasitic diseases, especially tick-borne diseases (TBDs), are great reasons that have restricted the potential for growth of the livestock sector. Ticks act as a vector of various diseases, including babesiosis, anaplasmosis, and theileriosis, causing huge losses among cattle. *Theileria annulata* infection is considered one of the biggest threats to livestock in Pakistan, causing immense morbidity and mortality. For its control, several methods have been tried; these involve acaricides and vaccines. However, some limitations exist to this approach, such as resistance against acaricides and several drawbacks regarding the current vaccine types available. So current study was designed to use an expression system for higher recombinant protein production to elicit a higher immune response. This study has successfully expressed two recombinant proteins: *Theileria annulata* (TA) and rTasp and purified them against *Theileria annulata* through the expression systems of *Escherichia coli* (*E. coli*) and *Pichia pastoris* respectively. Quantification of both proteins was done by using a BCA kit (G-Biosciences, Cat# 786-570). The calves were administered with a vaccine comprising of 100 microgram of our protein contained in two ml mixed with Montanide ISA 50 V as an adjuvant, for evaluation of immune potential of our construct. ELISA was used to determine antigen specific antibody titer. The cellular response was monitored through flow cytometric analyses. Our study finding revealed that the expressed recombinant Tasp through the *Pichia pastoris* expression system is more immunogenic than the recombinant protein TA expressed through the *E. coli* expression system. This study results showed that recombinant protein expressed by the eukaryotic expression system *Pichia pastoris* is produced in larger quantities and generates higher immunogenic responses in calves against theileriosis

Key Words: *Theileria annulata*, rTasp, *E. coli*, Purification, *Pichia pastoris*

Research on alternative medicine against avian coccidiosis: a huge challenge being faced by the poultry industry

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Abstract: Avian coccidiosis, an intestinal tract disease, caused by a protozoan parasite *Eimeria* is an increasing economic issue in the world because of its severe damaging effects on the poultry industry. Chemical drugs play an important part in conventional treatment approaches that include frequent use of anticoccidials. Challenges due to drug resistance and residues in poultry products threaten food safety, highlighting the necessity for alternate medicinal approaches.

The objective of our study is to focus on exploring alternate therapies that might have the potential to replace the conventional treatment approach. By reviewing the literature and experimental studies, the most effective alternates that have shown the coccidiostatic effect are herbal extracts, probiotics, prebiotics and essential oils. The alternative approaches can improve the poultry health and thus minimize the threats in the context of the food safety. Hence, there is a dire need to investigate the efficacy of these alternate medicines.

Our current study underlines the urgent demand for innovative methods to manage the avian coccidiosis through alternative approach to ensure the food safety and food security along with coping up the challenges of drug resistance, that will ultimately uplift the economy of our country. Pomegranate (*Punica granatum*) peel extract is rich in phenolic compounds, having higher antioxidant contents (punicalagin, anthocyanins, and ellagic acid), with potential anti-parasitic properties, especially against coccidiosis. This context provides a strong basis for conducting research to evaluate the anti-coccidial effects of pomegranate in the avian hosts.

Key words: Coccidiosis Control, *Eimeria*, Herbal Medicine, Pomegranate Peel Powder.

Exploring parasites in house sparrow (*Passer domesticus*)

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Abstract: This study aimed to detect parasites in house sparrows (*Passer domesticus*) from the Lahore region of Pakistan using optimized microscopy and molecular diagnostics. The primary objective was to enhance the early detection of parasitic infections to mitigate health risks and maintain ecological balance. Blood samples from 10 house sparrows were collected and examined microscopically at 1000X magnification for Haemosporidia and other blood parasites. Necropsies were performed, and PCR was used for molecular confirmation. Microscopic examination revealed Haemoproteus like structures in sample S5 and structures resembling Trypanosomes, Leucocytozoon, and Microfilaria in sample S2. To confirm the presence of *Trypanosoma evansi* and Haemoproteus, PCR was performed and analyzed using agarose gel electrophoresis. Distinct bands in the PCR products from samples S2 and S5 confirmed the presence of parasitic DNA. For *T. evansi*, both positive (C.P) and negative controls (C.N) were included, while for Haemoproteus, test samples L5H2, S5H1, L5H1D and S5H2 were analyzed without a negative control. Previous studies suggest that *P. domesticus* is of particular interest in ecological and evolutionary research due to its widespread distribution and sedentary behavior. Parasite prevalence is generally higher in native populations than in introduced ones. Environmental factors such as climate change, insect vectors, and habitat loss significantly affect parasite distribution. Further molecular-level studies in Pakistan are required to better understand parasitic infections in *P. domesticus* and their broader ecological implications.

Keywords: Parasites, *Passer domesticus*, PCR

Molecular characterization of *Anaplasma* in goat ticks from Southern Punjab, Pakistan

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Abstract: Ticks are prominent vectors of various pathogens impacting livestock, including goats. Molecular characterization of tick-borne pathogens, specifically *Anaplasma* species, is essential for developing effective control strategies. This study focuses on the molecular characterization of *Anaplasma* in goats from Southern Punjab, Pakistan. We collected approximately 200 tick samples from six distinct regions: Sadiqabad, Rahim Yar Khan, Khanpur, Liaquatpur, Ahmadpur, and Bahawalpur. Following DNA extraction from the collected ticks, we employed polymerase chain reaction (PCR) to amplify *Anaplasma* DNA. Subsequent gel electrophoresis and gene sequencing allowed for the precise identification and characterization of *Anaplasma* species. Phylogenetic analysis was performed to assess the genetic diversity and relationships of the detected *Anaplasma* strains. Preliminary results indicate variable prevalence and diversity of *Anaplasma* across the regions studied, with some areas exhibiting higher infection rates. These findings enhance our understanding of *Anaplasma* distribution in Southern Punjab and underscore the need for targeted control measures to address the impact of *Anaplasma* on goat health. This research provides a valuable foundation for developing region-specific strategies to mitigate tick-borne diseases affecting goats.

Keywords: *Anaplasma*, Molecular Characterization, PCR, Gene Sequencing, Phylogenetic Analysis.

Molecular detection and evolutionary insights of emerging tick-borne bacterial fauna in ticks from Pakistan

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Abstract: Ticks pose serious threats to the health of humans and animals, as they are significant transmitters of numerous infections. The function of newly developing bacterial infections in tick populations is still poorly understood, despite a great deal of study on well-known tick-borne infections. Environmental factors significantly contribute to the spread of tick populations in Pakistan, which underscores the need for further study. This study explored the genetic diversity and presence of emergent bacterial species that are within both hard and soft tick populations in various locations all over Pakistan. *Argasidae* and *Ixodidae* are the most suitable tick families for due to their ecological distribution and pathogens transmission. The collected hard and soft tick species from different ecological zones, including woodland, farmland, and cattle grazing land. Upon tick collection, the samples were cleaned and kept in 100% ethanol to retain the quality of their DNA. Since morphological identification requires the use of taxonomic keys, only the recognized ones were utilized to accurately describe tick's species. To facilitate DNA extraction and produce accurate samples for further analysis, the phenol-chloroform method was used. The molecular identification of tick species and bacteria species was based on PCR using the 16S rDNA and *cox1* genes and *groEl* gene respectively. Sanger sequencing was used to bidirectionally sequence the PCR products, and SeqMan software was used to trim and purify the sequences. After the sequences trimming, they were put for BLAST analysis to determine the closest matches. The sequences with the highest identity scores were picked for the phylogenetic analysis. By using MEGA-XI software, the phylogenetic relationships and genetic diversity among bacterial species within tick hosts evaluated using a maximum likelihood method. These investigations identify a spectrum of bacterial pathogens including, *Enterobacter bugadensis*, *E. cloaca*, *Acinobacter pseudolwoffii*, *Klebsiella pneumoniae*, *Neisseria* species, *Pantoea agglomerans*, *Providencia manganoxydans*, *Psychobacter* species in Pakistani hard and soft tick populations, which indicate new associations between ticks and emerging, zoonotic, or invasive bacteria species. The phylogenetic analysis shows their closed similarities with the same species reported from neighboring countries. The goal of the research was to illuminate, by showing, what these infections may do to public health if they emerge in nature, something that has probably been under-examined so far as their distribution and heterogeneity are concerned. The current study provides a major help in the diseases carried by ticks, particularly in Pakistan. Filling critical gaps in our knowledge, the study was of a high level of importance for future research and public health policies aimed at reducing tick-borne infection by focusing on both hard and soft ticks as well as investigating their neglected clades of bacterial species. The findings update strategies to mitigate the risks of emerging tick-borne pathogens.

Spatiotemporal distribution of tick species in the Hindu Kush Mountain range of Pakistan

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Abstract: Ticks are significant vectors of various pathogens affecting both humans and animals. Understanding their distribution patterns is crucial for disease management and prevention strategies. This study investigates the spatiotemporal distribution of ticks in the Hindu Kush Mountain range of Pakistan, a region characterized by diverse ecological zones and climatic conditions. Over a period of two years, tick samples were systematically collected from various altitudes and habitats, including forests, grasslands, and agricultural areas. The tick species identified in this study include *Haemaphysalis montgomeryi*, *H. danieli*, *H. sulcata*, *H. cornupunctata*, *H. kashmirensis*, *Rhipicephalus microplus*, *R. sanguineus*, *R. haemaphysaloides*, *R. turanicus*, *Hyalomma anatolicum*, and *Dermacentor marginatus*. Geographical Information System (GIS) tools and remote sensing data were employed to map the spatial distribution of tick populations. Temporal patterns were analyzed in relation to seasonal variations and climatic factors such as temperature, humidity, and precipitation. The findings reveal significant spatial heterogeneity in tick distribution, with higher densities observed in forested and humid regions. Seasonal peaks in tick abundance were noted during the spring and summer months, correlating with optimal environmental conditions for tick survival and reproduction. This study highlights the complex interplay between ecological variables and tick distribution in the Hindu Kush Mountain range. The results underscore the importance of integrating spatiotemporal data in vector surveillance programs to predict potential hotspots and implement timely control measures. These insights are crucial for public health authorities and veterinary services to mitigate the risk of tick-borne diseases in this geographically and climatically diverse region.

Keywords: Ticks, Hindu Kush Mountains, *Haemaphysalis*, *Rhipicephalus*, *Dermacentor*, *Hyalomma*, Vector Surveillance.

First report on detection and genetic characterization of tick-borne rickettsioses in livestock holders: identification of *Candidatus rickettsia shennongii* in Pakistan

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Abstract: Tick-borne *Rickettsia* species have long been recognized as causative agents of zoonotic diseases. We have previously characterized *Rickettsia* species in various ticks infesting a broad range of hosts in Pakistan however, knowledge regarding the detection and genetic characterization of tick-borne rickettsioses in humans is missing. This study aimed to uncover human rickettsiosis in the study region and to update our understanding of the disease's current global epidemiology. Blood samples were collected from symptomatic (n = 40) and asymptomatic (n = 65) livestock holders with tick-infested animals in various regions of district Peshawar and Mohmand. DNA was extracted from both symptomatic and asymptomatic livestock holders' blood and tested using PCR targeting various genes, including *gltA*, *ompA*, and *ompB*. All positive specimens were genetically characterized by sequencing of the *gltA*, *ompA*, and *ompB*. The obtained sequences of *gltA*, *ompA*, and *ompB* from symptomatic blood showed 99.71–100% identity with “*Candidatus Rickettsia shennongii*” respectively and phylogenetically clustered with the corresponding species reported from different countries. Phylogenetic trees revealed that they grouped with the corresponding species of *Rickettsia*, and for the first time in human, *Candidatus Rickettsia shennongii* was characterized. None of the tested blood from asymptomatic livestock holders was found positive for rickettsial DNA. The presence of *Rickettsia* species in the study region suggests a potential risk of zoonotic disease transmission to humans, emphasizing the importance of regular surveillance and diagnosis of tick-borne rickettsioses.

Keywords: *Candidatus Rickettsia shennongii*, Livestock-holders, Tick-borne rickettsioses.

Molecular epidemiology 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 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.

The status of *Dermacentor* sp. (Ixodidae) and *Alectorobius coniceps* (Argasidae) in Pakistan

Quid Ali

Abstract: *Dermacentor* are mainly hard ticks which affects animals, such as small ruminants, while *Alectorobius* species are mainly *Argasidae* that affects birds. For the first time in Pakistan these two species, *A. coniceps* and *Dermacentor* sp. were characterized by morphological and molecular bases. *Dermacentor* sp. and *A. coniceps* were found in Khyber Pakhtunkhwa and Balochistan respectively. In the entire study, we collected 25 tick species, in which 11 *Dermacentor* sp. and 14 species of *A. coniceps* were identify morphologically. The molecular characterization of the collected ticks was done by targeting 16S rDNA and *cox1* gene. In case of *A. coniceps* the blast results of 16S rDNA and *cox1* showed greatest similarity of 97.46% and 96.49% with *A. coniceps* from Malta. The 16S rDNA as well as the *cox1* sequences of *Dermacentor* sp. showed maximum similarity of 98.42% and 97.45% in blast with *Dermacentor pavlovskyi* identify from China. With the help of 16S rDNA and *cox1* sequences, *Dermacentor* sp. and *Dermacentor pavlovskyi* from China show close phylogeny with each other.

Targeting *Bartonella henselae* for novel drug target identification and multi epitope vaccine construction using subtractive proteomics and reverse 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.

Molecular based confirmation of *Anaplasma bovis*, *Candidatus Anaplasma boleense* and *Rickettsia* spp. in ticks infesting cattle, goat and sheep

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Abstract: *Anaplasma* spp. and *Rickettsia* spp. have great public concern, and their surveillance provides key information about their epidemiology. In Pakistan, studies have been documented regarding tick-borne pathogens, but previously, unidentified diversity of *Anaplasma* spp. and *Rickettsia* spp. have not been investigated. Therefore, to address this knowledge gap, using suitable genetic markers, is important to report unidentified tick-borne pathogens. This study aimed to molecularly detect *Anaplasma* spp. and *Rickettsia* spp. in ticks collected from cattle, goats, and sheep in nine districts of Khyber Pakhtunkhwa (KP), Pakistan. Among 254 examined hosts (cattle, goats, and sheep), 112 (44.04%) were infested by 850 ticks. Highest ticks infestation was observed on cattle 55.17% (32/58), followed by goats 42.6% (38/89), and sheep 39.25% (42/107). Among ticks, *Rhipicephalus microplus* (317/850, 35.64%), was highly prevalent species, followed by *Haemaphysalis sulcata* (255/850, 28.35%), *Hyalomma anatolicum* (146/850, 15.52%), and *Haemaphysalis bispinosa* (132/850, 13.88%). A subset of 108 ticks were screened for *Anaplasma* spp. and *Rickettsia* spp. based on the amplification of partial 16S rDNA and outer-membrane protein A (*ompA*) fragments, respectively. In total screened ticks DNA samples, 16 (14.81%) were found positive for *Anaplasma* spp. and *Rickettsia* spp. The sequences obtained based on 16S rDNA for *Anaplasma* spp. detected in *Ha. sulcata* and *Ha. bispinosa* showed 99.98% identity with *Anaplasma bovis*, while detected in *Rh. microplus* showed 99.84% identity with *Candidatus Anaplasma boleense*. Similarly, sequence detected based on *ompA* in *Rh. microplus* showed 100% identity with *Candidatus Rickettsia shennongii*, and another sequence detected in *Ha. Sulcata*, showed 100% identity with *Rickettsia* sp. and 97.93% with *Rickettsia slovaca*. In phylogenetic trees, these sequences clustered with corresponding species from Pakistan, China, South Africa, South Korea, Turkey, and Herzegovina. To the best of our knowledge, this study first time reporting detection of *A. bovis* in *Ha. sulcata* and *Ha. bispinosa*, *Ca. A. boleense* in *Rh. microplus* collected from goats, and *R. slovaca*-like in *Ha. sulcata*. Our results will update the knowledge and enforce the need regarding comprehensive surveillance of Rickettsiales in the country.

Keywords: Hard Ticks, *Anaplasma bovis*, *Candidatus Anaplasma boleense*, Domestic Animals.

Food safety challenges: Understanding the potential risk associated with echinococcosis, and fascioliasis in Pakistan

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Abstract: Foodborne trematodes and cestodes are considered serious global health issues declared as one of the 20 neglected tropical diseases (NTDs) by the World Health Organization (WHO). More than 1 billion people are affected by NTDs in low and middle-income countries due to poor surveillance systems. Human Fascioliasis is an emerging zoonotic disease in southern Asia, particularly rampant in densely populated areas of Pakistan. The prevalence of *Fasciola* spp. is the highest in Sindh and Baluchistan as compared to other provinces. Globally distributed cystic echinococcosis causes severe challenges for animal and human health. A study highlighted clinical CE human records; the condition has been endemic in Khyber Pakhtunkhwa province over the past 15 years. Proper Food safety practices and regulations, such as appropriate washing and cooking of food by households and food handlers are advised. Moreover, the safe disposal of offal by farmers and livestock keepers during slaughtering (to prevent echinococcosis), plays a key role in maintaining food quality and reducing the risk of zoonotic disease transmission. With One Health approach, develop national operational plans to implement interventions in neglected tropical diseases (NTDs) operating at the human-animal-environment interface. These plans should clearly define the roles and responsibilities of stakeholders, ensuring accountability across human, animal, food, and ecosystem-related actions, which is in line with the One Health framework. There is a need for sound surveillance systems to provide detailed data about the disease epidemiology in different areas of Pakistan. While developing an effective and sustainable strategic control program, it is essential to have a comprehensive understanding of disease distribution, associated risk factors, and economic impacts.

Keywords: Fascioliasis, Echinococcosis, NTDs, Food Safety, One Health

Implementation of machine learning-based image analysis technology to develop decision support system in precision agriculture

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Abstract: In the world we live in, there are numerous food security challenges around the globe, starving people to death. To tackle an agricultural-based food security challenge, especially in countries where economic development is based mostly on the agricultural firm, e.g., Pakistan, a research model is prepared with the aim to detect the pests and diseases in crops to optimize the application of fertilizers and pesticides with the highest efficiency as possible. This model proposes to implement the Machine Learning Image Analysis (MLIA) technology to develop a decision-support software-based system (DSS) for pest and disease detection considering the precision agriculture principles. By capturing and analyzing crop images, the MLIA model can provide real-time interventions to farmers, enabling them to take early interventions. Two of the modern diagnostic methods like early warning systems and precision agriculture are implemented in this model. The first step in this model is data preparation and collection of images of crops which can be done through smartphones, drones, and UAVs (depending on the budget). The second step is to use the Convolutional Neural Networks (CNN) model to employ deep learning architectures for image feature extraction. The third step is to build an app or website for a decision support system. Farmers can capture crop images using smartphones or drones and upload them to the DSS for real-time analysis. The system provides tailored recommendations on optimal fertilizer and pesticide applications, considering factors such as crop type, growth stage, and disease severity. This technology can effectively contribute to food security.

Economic significance of vector borne parasitic infections in livestock industry: a critical food security concern

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Abstract: Vector-borne parasitic infections pose a significant economic threat to the livestock industry, with far-reaching implications for food security. The multifaceted economic impacts of these infections, focusing on their direct and indirect effects on livestock productivity and the broader agricultural economy should be addressed. Tick-borne, mosquito-borne and fly-borne parasites cause diseases that lead to drastic reductions in livestock health such as decreased productivity rates as well as reproductive inefficiencies. The economic consequences are reflected in higher veterinary costs associated with treating diseased animals, decline in meat and milk production and loss of breeding potential. Furthermore, trade restrictions imposed during disease outbreaks increase management cost from biosecurity measures put in place to control disease spreading while limiting access to markets. Climate change coupled with changing vector dynamics presents further challenges for controlling vector-borne parasitic infections. These can change patterns of infection or worsen outbreak intensity levels. This abstract emphasizes the urgent need of integrated control strategies i.e., improved vector management, vaccination programs and sustainable agricultural practices that would reduce these economies' adverse effects while enhancing food security. Addressing this problem is critical for stability and resilience of livestock industry against expected shocks from climate change consequences. Moreover, these responses also promote resilience within pastoral communities which depend largely on livestock rearing as their only source of livelihood.

Acaricidal efficacy of encapsulated *Eucalyptol* particles against *Rhipicephalus microplus*

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Abstract: Agriculture is the most important pillar of Pakistan's economy. According to the Pakistan Economic Survey 21-22 Livestock has a major share of 61.9% of this sector and more than 8 million families are directly or indirectly associated with the livestock sector for their income. Parasitic diseases are of major concern in this matter as they cause loss of more than 50 USD per animal per year. About 80% of the animal population is infested with ticks and tick-borne diseases with *Hyalomma* and *Rhipicephalus* being predominant genera. They cause financial losses directly by production losses in milk, meat, and hide and indirectly by disease transmission through ticks. *Rhipicephalus microplus* is a vector for many hemoparasitic diseases such as *Babesia*, *Theileria*, *Anaplasma*, and *Ehrlichia*. The most commonly adopted approach to control parasitic diseases is synthetic acaricides but acaricidal resistance, residues in human diet, and non-target populations are certain demerits associated with their use. To overcome these demerits and economic losses there should be an eco-friendly approach for acaricidal control. Plant essential oils and their active ingredients are one of the alternative approaches for tick control. Nano-encapsulated *Eucalyptol* through Ionic gelation into chitosan polymer provided a prolonged acaricidal efficacy (highest 94% and average 71% mortality) against *R.microplus* for 35 days. The potent mechanism involved is through inhibition of AChE (Acetylcholinesterase Enzyme). *Eucalyptol* proves to be an effective acaricidal agent and its efficacy should be evaluated through *in-vivo* studies.

Keywords: Hemoparasitic diseases, *Rhipicephalus microplus*, Nano-encapsulation, *Eucalyptol*, Acetylcholinesterase.

Sustainable livestock protection: acaricidal efficacy of *Trichoderma longibrachiatum* secondary metabolites against *Rhipicephalus (Boophilus) microplus*

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Abstract: Livestock contributes significantly to the economy, especially as a growing industry in Pakistan. It is a source of meat, milk, and leather. More than half of the global population is susceptible to vector-borne diseases (VBDs), particularly tick-borne diseases (TBDs), which cause more than a million deaths yearly. These diseases are on the rise worldwide. Pakistan's economy relies extensively on livestock, particularly in rural areas. Conventional pesticides, although effective, have led to environmental concerns, human health risks, and acaricidal resistance due to their widespread misuse. Entomopathogenic fungus (EPF) is a promising tool in integrated pest management (IPM) due to its environmental safety, non-target specificity, and low resistance risk. Among the other EPFs *Trichoderma longibrachiatum* has been reported to produce a wide range of secondary metabolites that suppress the immune system of the insects. The current study focused on the use of secondary metabolites (SMs) produced by the *Trichoderma longibrachiatum* directly against engorged female *Rhipicephalus (Boophilus) microplus* instead of its conidia in an adult immersion assay as SMs show enhanced and rapid acaricidal activity. The fungal culture was retrieved from the Entomology Lab, Department of Parasitology, UVAS, Lahore. The fungus plates were scrapped for the conidial suspension formation. The concentration of the suspension was adjusted to 10⁸ conidia/ml. On the other hand, fungal biomass was harvested and extracted with the ethyl acetate solvent with the help of the solvent extraction method. The dried crude extract was subjected to GC-MS and FTIR. The crude extract and the prepared conidial suspension were checked for their acaricidal activity against *R. microplus*. The adult immersion assay involved five concentrations of conidial suspension and secondary metabolites, with trials conducted triplicate with negative and positive controls. Crude extract of SMs has shown fast and enhanced larvicidal effect according to the LC₅₀s and LC₉₀s. nhexadecanoic acid was the most abundant SM present in the crude extract which is also the cause of the death of the adult faster than the conidial suspension. According to the paired t-test the results of conidial and SMs suspension were compared and there was a significant difference noted between the two treatments. The SMs of *T. longibrachiatum* showed a rapid acaricidal effect against *R. microplus*. Hence, the present study has accentuated the importance of biological control by using *T. longibrachiatum* derived SMs. Furthermore, it is necessary to isolate individual compounds from the crude extract to make an environmentally sustainable acaricide against *R. microplus*.

Keywords: *Trichoderma longibrachiatum*, Secondary Metabolites, Acaricidal Resistance, *Rhipicephalus (Boophilus) microplus*, Livestock Protection

Screening of different genotypes/ lines of onion against *Iris Yellow Spot Virus (IYSV)*

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Abstract: Onion (*Allium cepa* L.) is a major vegetable crop and an essential part of our diet. According to the Food and Agricultural Organization (FAO), Pakistan ranked in 5th position in onion production. Among other pathogens, *Iris yellow spot virus (IYSV)* is an economically important virus and a significant threat to onion seed and bulb crops in many parts of the world. It can decrease bulb size and seed yields and cause crop losses up to 100%. It is a member of the genus *Tospovirus*, family *Bunyaviridae*, transmitted by onion thrips (*Thrips tabaci* L.) in a persistent and propagative manner. The disease Symptoms were characterized by spindle-shaped, straw-colored, irregular chlorotic and necrotic eye spots with a green island at the center, known as diamond eyes. In the present study, 17 varieties/lines were screened against IYSV. Data was collected at an interval of 15 days after one month of sowing till the crop's maturity. The level of susceptibility/ resistance in varieties was graded according to the disease rating scale. Results indicated that SUPER WHITE F1 has the highest disease incidence (DI) rate while HON-305 F1 has the lowest DI rate. All other varieties have a DI rate between these two varieties. PINKAR has the DI rate less than SUPER WHITE and then GGAS-AC-63, VX-314, SCARLET F1, ICS-ON-2051, HSU-552, TCP-ON-2001, SULTAN, ICS-ON-2052, GGAS-AC-65, GGAS-AC-64, GGAS-AC-63, PHULKARA, TCP-ON-2002 and GGAS-AC-66 have likewise less DI rate.

Keywords: Susceptibility, Resistance, *Tospovirus*, Onion Thrip

One Health One Fight: Prevent Vectors Protect All Life

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Abstract: A vector is a living organism that transmits an infectious agent from an infected animal to a human or another animal. Diseases transmitted by vectors are called vector-borne diseases (VBDs). 80% of the world's population is at risk of one or more VBDs. 17% of the global burden of communicable diseases is due to VBDs. And over 700,000 deaths are caused by VBDs annually. Climate change or global warming modifies the environment that favors vectors' abundance, growth, and reproduction, and consequently, the rate of development of pathogens within the vectors. There are several VBDs transmitted by mosquitoes, sandflies, and other insects. Major VBDs in humans include Malaria, Dengue, Lymphatic filariasis, Chagas disease, Onchocerciasis, Leishmaniasis, Chikungunya, Zika virus disease, Yellow fever, Japanese encephalitis and Schistosomiasis. The spread of VBDs is on the rise globally, and the globalization of infectious diseases is becoming a palpable threat to global health. VBDs are also considered one of the most important one health issue. For most VBDs, prevention by targeting vectors is the first and best approach. There are four pillars of action for effective locally adapted sustainable vector control 1) Strengthen inter and intra-sectoral action and collaboration 2) Engage and mobilize communities 3) Enhance vector surveillance, and monitoring and evaluation of interventions 4) Scale up and integrate tools and approaches with a Foundation of enhance vector control capacity and capability and increase basic and applied research, and innovation. Millions of people have already benefitted from vector control, with major reductions in malaria, and Chagas disease. However, vector control has not been used to its full potential or sustained for maximum impact. A comprehensive approach is required that enables: increased capacity, better coordination, improved surveillance, and integrated action (effective proven vector control approaches and promising new approaches on the horizon).

Key words: Vector borne diseases, One health, Control strategy

Dengue virus: a comprehensive exploration of molecular mechanisms and public health implications

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Abstract: The Flavivirus known as dengue virus (DENV) propagates by *Aedes aegypti* mosquitoes. An alarming global increase has been observed in dengue fever (DF), notably in tropical and subtropical countries, along with its more severe variants, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). With 390 million infections and roughly 3.6 billion individuals at risk, the virus causes significant economic and social challenges, especially in developing nations with scant healthcare resources. Climate change, urbanization, and enhanced vector activity brought on by rising temperatures, factors that promote viral transmission and vector survival are credited for the geographical spread of dengue.

Dengue fever is endemic in Pakistan and has seasonal peaks. Unprecedented floods in 2022 contributed to an increase in dengue infections in the nation. Over 25,000 confirmed cases and 62 deaths were reported in the National Institute of Health report, with the majority of cases occurring in the Sindh and Punjab areas. This outbreak highlights Pakistan's health system's fragility, which is made worse by natural disasters driven on by climate change.

DENV is composed of a single-stranded RNA genome that codes for both structural and non-structural proteins. These proteins are essential for pathogenesis, immune suppression, and viral replication. Early diagnosis and treatment are vital since dengue may appear with a wide range of clinical symptoms, from mild febrile illness to severe outcomes like shock, plasma leakage, and multi-organ failure. Effective medicines are still scarce despite continuous research, underscoring the critical need for sustained international efforts in dengue prevention, surveillance, and control.

Keywords: *Aedes aegypti*; Variants; Viral Transmission; Single-Stranded RNA Genome; Viral Replication; Prevention.

Anthelmintic efficacy of aloe vera against gastrointestinal nematodes in sheep in district Muzaffargarh

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ABSTRACT: Traditional farmers in southern Punjab have identified plant materials selected in the current study from various sources to serve as anthelmintic agents. The *Aloe Vera* plants were selected because of the reported anthelmintic activity in their different species and their parts or the different concentrations of the plant drugs used in the present study based on the values available. There was a reduction in FEC in sheep by using the different dose rate of *Aloe Vera* leaves in dry powdered form against mixed GI parasite infections. Results showed that these plants possess anthelmintic properties against various gastrointestinal parasites in sheep. It is good to note that the powder extracts of these plants were effective against *H. contortus*, the most commonly occurring nematodes in sheep. This plant is also effective in all age of animals and both in male and female. This plant is easily available and cheap so I will suggest utilize this plant as anthelmintic in sheep and other species.

Keywords: Sheep, Nematodes, *Aloe Vera*

Economic significance of vector borne parasitic infections in livestock industry: a critical food security concern

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Abstract: Vector-borne parasitic infections pose a significant economic threat to the livestock industry, with far-reaching implications for food security. The multifaceted economic impacts of these infections, focusing on their direct and indirect effects on livestock productivity and the broader agricultural economy should be addressed. Tick-borne, mosquito-borne and fly-borne parasites cause diseases that lead to drastic reductions in livestock health such as decreased productivity rates as well as reproductive inefficiencies. The economic consequences are reflected in higher veterinary costs associated with treating diseased animals, decline in meat and milk production and loss of breeding potential. Furthermore, trade restrictions imposed during disease outbreaks increase management cost from biosecurity measures put in place to control disease spreading while limiting access to markets. Climate change coupled with changing vector dynamics presents further challenges for controlling vector-borne parasitic infections. These can change patterns of infection or worsen outbreak intensity levels. This abstract emphasizes the urgent need of integrated control strategies i.e., improved vector management, vaccination programs and sustainable agricultural practices that would reduce these economies' adverse effects while enhancing food security. Addressing this problem is critical for stability and resilience of livestock industry against expected shocks from climate change consequences. Moreover, these responses also promote resilience within pastoral communities which depend largely on livestock rearing as their only source of livelihood.

Parasitic plants in agriculture: chemical ecology of germination and host-plant location as targets for sustainable control: a review

Justin B. Runyon, John F. Tooker, Mark C. Mescher and Consuelo M. De Moraes

Presenter: Sahib Hussain

Abstract: Parasitic plants are among the most problematic pests of agricultural crops worldwide. Effective means of control are generally lacking, in part because of the close physiological connection between the established parasite and host plant hindering efficient control using traditional methods. Seed germination and host location are critical early-growth stages that occur prior to host attachment, and provide promising targets for ecologically sound management of parasitic weeds. Knowledge of parasite-host interactions, particularly chemical cues that induce parasite seed germination and mediate host location, should facilitate the development of novel management approaches. In parasitic plants that attach to host roots—e.g., *Striga* and *Orobanche* spp.—seed germination is known to occur only in the presence of chemical stimulants released from plant roots. The recent finding that these same chemicals promote the colonization of beneficial fungi has potentially important implications for the control of parasitic plants. Far less is known about the early stages of parasitic plants that attach above-ground to host shoots—e.g., *Cuscuta* spp. Seeds of these parasites lack germination stimulants, and it was only recently shown that foraging *C. pentagona* seedlings use airborne cues to locate and select among hosts. We review research on seed germination and host location by the major parasitic weeds that attack agricultural crops, and discuss the implications of recent findings for the development of sustainable and effective management strategies.

Keywords: *Striga*–*Orobanche*–*Cuscuta*–Strigolactones–Volatiles–Plant-plant communication

Comparative efficacy of silymarin and *Lycopodium clavatum* against liver diseases in domestic cats: a study on herbal interventions

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Abstract:

This study examines the effects of Silymarin and *Lycopodium clavatum* on domestic cats with liver disorders, exploring the benefits of their combined use.

Thirty domestic cats were divided into three groups: Group A received Silymarin, Group B received *L. clavatum*, and Group C received both treatments. Clinical evaluations and liver function tests (ALT, AST, ALP, GGT, and bilirubin) were performed before and after 6 weeks of treatment. Ultrasonographic assessments evaluated liver changes.

Improvements in liver enzyme levels were noted in all groups, with the combination therapy showing the most significant reduction in enzyme levels and bilirubin. Ultrasound results indicated better liver texture and reduced echogenicity in the combination group.

Both Silymarin and *L. clavatum* are effective in treating liver diseases in cats, with their combination providing superior results.

Keywords: Silymarin, *Lycopodium clavatum*, Liver Diseases, Domestic Cats, Herbal Interventions

Evaluation of anthelmintic efficacy of drugs against hookworm infections in dogs

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Abstract: Dogs are regarded as loyal companions of mankind. About 75% of dogs around the world are stray and end up in shelter houses due to injuries and infections. Shelter houses provide favorable environment for the development of infections of Gastrointestinal Tract (GIT) in dogs. Hookworm infections are most common GIT infections in dogs. These infections can be transmitted to humans, thus prompt action for immediate eradication is required. Various anthelmintic drugs are used for this purpose, but development of resistance has been noted against drugs recently.

This study aimed to identify the prevalence of hookworm infections and to compare three anthelmintic drugs, namely, Levamisole (1 mg/kg orally), Mebendazole (22 mg/kg orally), fenbendazole (50 mg/kg orally), to determine the one being most effective. Fecal samples from 100 dogs were collected and screened for hookworm eggs. The dogs were divided into groups with each group receiving comparable anthelmintic drugs at day 0 and day 14. Fecal sample of affected dogs were again evaluated to identify the efficacy of drugs.

The results demonstrated a prevalence of 50% dogs infected with hookworms. Among the anthelmintic drugs, Fenbendazole proved to be the most effective in eradicating hookworm eggs.

Keywords: Hookworms, Dogs, Anthelmintic Drugs

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9.	Dr. Mian Muhammad Awais Associate Professor, Department of Pathobiology, FVS, BZU	Member
10.	Dr. Muhammad Oneeb Assistant Professor, Department of Parasitology	Member
11.	Dr. Hafiz Muhammad Rizwan Assistant Professor, Department of Parasitology, Narowal campus	Member
12.	Dr. Muhammad Suleman Assistant Professor, Department of Pathology	Member
13.	Dr. Abdullah Saghir Ahmad Assistant Professor, Department of Parasitology, CUVAS	Member
14.	Dr. Fasiha Qurashi Visiting Lecturer, Department of Biological Sciences	Member
15.	Dr. Sikndar Ali Research Associate, Department of Parasitology	Volunteer
16.	Mr. Yousaf Gul Kakar Ph.D Schiolar , Department of Parasitology	Volunteer
17.	Prof. Dr. Muhammad Imran Rashid Department of Parasitology	Organizing Secretary

TORs: This committee will be responsible for planning, arrangement, management, and organization of two-days International Conference on PARACON: Farm to Fork Manageme

2. RESOURCE MOBILIZATION AND LIAISON COMMITTEE

1	Dr. Nisar Ahmed Associate Professor, Department of Parasitology	Convener
2.	Dr. Abdullah Saghir Ahmad Assistant Professor, Department of Parasitology, CUVAS	Member
3.	Dr. Qasim Assistant Professor, The University of Agriculture, D. I. Khan	Member
4.	Mr. Ubaid Ullah Research Associate, Department of Parasitology, UVAS Lahore	Volunteer
5.	Mr. Yousaf Gul Kakar Ph.D Scholar , Department of Parasitology	Volunteer
6.	Dr. Muhammad Oneeb Assistant Professor, Department of Parasitology	Secretary

TORs:

1. This committee will be responsible for generation of resources for conference, gala dinner and advertisement of materials.
2. Members will contact industry to gather resources for conference.

3. INVITATION COMMITTEE

1.	Dr. Muhammad Oneeb Assistant Professor, Department of Parasitology, UVAS Lahore	Convener
2.	Dr. Muhammad Imran Rashid Professor, Department of Parasitology	Member
3.	Prof. Dr. Haroon Akbar Department of Parasitology	Member
4.	Dr. Sakandar Khan Visiting lecturer, University of Narowal	Member
5.	Toqir Anwar Mughal Accountant, Department of Parasitology	Member
6.	Dr. Sikandar Ali Research Associate, Department of Parasitology	Volunteer
7.	Mr. Asadullah Abid Visiting Lecturer, Department of Parasitology	Secretary

TORs:

1. Draft invitation letter for Chief Guests for inaugural, concluding and other sessions and proceed for confirmation
2. This committee will Draft invitation letter and communicate with all National and International Invited Speakers
3. Preparation of invitees, i.e., alumni members of syndicate, other statutory bodies, Members of Pakistan Society of Parasitology and Govt. officials in consultation with Organizing Secretary.
4. Provide list of special guests to hall management committees for the arrangement of seats
5. Provide list of guests to accommodation committee to make necessary arrangement for their accommodation

4. PROCTORIAL AND HOSPITALITY COMMITTEE

1.	Dr. Nisar Ahmed Associate Professor, Department of Parasitology	Convener
2.	Dr. Haroon Akbar Professor, Department of Parasitology	Member
3.	Mr. Ubaid Ullah Ph.D Scholar, Department of Parasitology	Volunteer
4.	Mr. Yousaf Gul Kakar Ph.D Scholar , Department of Parasitology	Volunteer
5.	Ms. Aneeqa Rashid M.Phil Scholar, Department of Parasitology	Volunteer
6.	Mr. Asadullah Abid Visiting Lecturer, Department of Parasitology	Secretary

TORs

1. Proctorial duties will be inside and outside conference hall and near registration desk
2. Proctors shall perform their duties in following categories:-
 - a. For participants
 - b. For delegates
3. Proctors will guide delegates and participants and will escort them to conference venue
4. Get the rosettes, tags and stickers etc. Prepared

5. REGISTRATION COMMITTEE

1.	Dr. Muhammad Lateef Associate Professor, Department of Parasitology	Convener
2.	Prof. Dr. Muhammad Imran Rashid Department of Parasitology	Member
3.	Dr. Muhammad Rashid Assistant Professor, Department of Parasitology, IUB	Member
4.	Dr. Muhammad Zahid Associate Professor, Islamia College Peshawar	Member
5.	Dr. Muhammad Shoaib Assistant Professor, College of Veterinary Sciences, The University of Agriculture Peshawar	Member
6.	Dr. Muhammad Shafee Professor, CASVAB, Quetta	Member
7.	Dr. Hafiz Muhammad Rizwan Assistant Professor, Department of Parasitology, Narowal	Member
8.	Dr. Fasiha Qurashi Visiting Lecturer, Department of Biological Sciences	Member
9.	Mr. Asadullah Abid Visiting Lecturer, Department of Parasitology	Member
10.	Toqir Anwar Mughal Accountant, Department of Parasitology	Member
11.	Mr. Abubakar PhD Scholar, Department of Parasitology	Volunteer
12.	Mr. Yousaf Gul Kakar Ph.D Scholar , Department of Parasitology	Volunteer

13	Mr. Shabab PhD Scholar, Department of Parasitology	Volunteer
14	Mr. Ahsan Elahi PhD Scholar, Department of Parasitology	Volunteer
15	Ms. Palwasha Gul M.Phil Scholar, Department of Parasitology	Volunteer
16	Ms. Aneeqa Rashid M.Phil Scholar, Department of Parasitology	Volunteer
17	Mr. Abdur Rehman Khan DVM student, UVAS Lahore	Volunteer
18	Dr. Muhammad Oneeb Assistant Professor, Department of Parasitology	Secretary

TORs:

1. This committee will keep record of registration
2. It will decide number of registrations
3. It will decide what type of written material to be distributed among summit participants
4. It will be responsible for timely distribution of registered entitlement to participants
5. It will prepare name tags for already registered members and on desk registration of members

6. SCIENTIFIC COMMITTEE

1.	Prof. Dr. Rao Zahid Abbas Chairman, Department of Parasitology, UAF	Convener
2.	Dr. Muhammad Suleman Assistant Professor, Department of Pathology	Member
3.	Dr. Abdullah Saghir Ahmad Assistant Professor, Department of Parasitology, CUVAS	Member
4.	Mr. Asadullah Abid Visiting Lecturer, Department of Parasitology	Member
5.	Dr. Fasiha Qurashi Visiting Lecturer, Department of Biological Science	Member
6.	Mr. Muhammad Abdullah Bin Saleem PhD Scholar	Volunteer
7.	Dr. Sikandar Ali Research Associate, Department of Parasitology	Volunteer
8.	Prof. Dr. Muhammad Imran Rashid Department of Parasitology	Secretary

TORs:

1. This committee will call and manage submitted abstracts
2. It will be responsible for acceptance and rejection of abstracts
3. It will plan technical session
4. It will formulate conference technical schedule
5. It will decide conference Chair, Co-Chair and Moderator
6. Committee will have powers to change conference theme and mode of presentation
7. It will collect all scientific presentations and poster before conference

7. POSTER AND STALL EXHIBITION COMMITTEE

1.	Dr. Muhammad Suleman Assistant Professor, Department of Pathology	Convener
2.	Dr. Abdullah Saghir Ahmad, Assistant Professor, Department of Parasitology, CUVAS	Member
3.	P.O Estate Management /His Representative	Member
4.	Mr. Ubaid Ullah Ph.D Scholar, Department of Parasitology	Volunteer
5.	Mr. Muhammad Abdullah Bin Saleem Ph.D Scholar, Department of Parasitology	Volunteer
6.	Mr. Ghulam Mustafa Ph.D Scholar, Department of Parasitology	Volunteer
7.	Mr. Ahsan Elahi Ph.D Scholar, Department of Parasitology	Volunteer
8.	Ms. Sana Ashiq M.Phil Scholar. Department of Parasitology	Volunteer
9.	Ms. Rabia Malik M.Phil Scholar	Volunteer
10.	Dr. Fasiha Qurashi Visiting Lecturer, Department of Biological Sciences	Secretary

TORs:

1. This committee will be responsible for collection of posters from scientists
2. This committee will be responsible for selection of poster format and dimensions.
3. It will decide venue of poster display
4. It will have powers of rejection of any poster which will not be according to the format.
5. This committee will also upload Poster format on conference website
6. The Committee will be responsible for allotment of stall

8. PRINT AND PUBLICATION COMMITTEE

1.	Prof. Dr. Muhammad Yasin Tipu Department of Pathology	Convener
2.	Dr. Ghulam Mustafa Lecturer, Department of Pathology	Member
3.	Mr. Muhammad Nouman Public Relation Officer, UVAS	Member
4.	Mr. Ubaid Ullah PhD Scholar, Department of Parasitology	Volunteer
5.	Mr. Yousaf Gul Kakar Ph.D Scholar, Department of Parasitology	Volunteer
6.	Ms. Rabia Malik M. Phil Scholar, Department of Parasitology	Volunteer
7.	Ms. Sana Ashiq M. Phil Scholar, Department of Parasitology	Volunteer
8.	Dr. Muhammad Oneeb Assistant Professor, Department of Parasitology	Secretary

TORs:

1. To collect soft copies of abstracts at least three weeks prior to conference from scientific committee and print books of abstract
2. To prepare and provide all materials for conference (brochure, flexes and back drop)
3. To design and print souvenirs/ abstract book for all registered participants.
4. To coordinate with registration and invitation committee to ensure number of participants, chief guest, other national and international participants.
5. To design and print registration cards along with meal tickets for participants and delegates.
6. To design news ad and other advertisement materials for conference
7. To prepare shields and certificates for presenters and participants

9. STAGE MANAGEMENT AND GALA DINNER COMMITTEE

1.	Prof. Dr. Muhammad Imran Rashid Department of Parasitology	Convener
2.	Prof. Dr. Mian Abdul Hafeez Department of Parasitology	Member
3.	Prof. Dr. Muti Ur Rehman Khan Department of Pathology	Member
4.	Mr. Muhammad Rizwan Saleem Director IT Centre, UVAS Lahore	Member
5.	Mr. Ubaid Ullah Ph.D Scholar, Department of Parasitology	Volunteer
6.	Mr. Ahsan Elahi Ph.D Scholar, Department of Parasitology	Volunteer
7.	Mr. Fasih ur Rehman Ph.D Scholar, Department of Parasitology	Volunteer
8.	Ms. Rabia Malik M.Phil Scholar, Department of Parasitology	Volunteer
9.	Ms. Palwasha Gul M.Phil Scholar, Department of Parasitology	Volunteer
10.	Ms. Aneeqa Rashid M.Phil, Department of Parasitology	Volunteer
11.	Mr. Rawal Khan DVM student, UVAS Lahore	Volunteer
12.	Ms. Javeria DVM student, UVAS Lahore	Volunteer
13.	Ms. Fatima DVM student, UVAS Lahore	Volunteer
14.	Dr. Muhammad Faiz Rasool Assistant Director ORIC	Secretary

TORs:

1. This committee will ensure stage management at inaugural and concluding sessions
2. Stage management at the time of technical sessions
3. Nominate person for Holy Quran and Naat for different sessions
4. This committee will make proper arrangement
 - a. Dice for stage secretary
 - b. Backdrop for inaugural and concluding session
 - c. Tables for guests
 - d. Tables for shields and awards

- e. Table for dignitaries and reserving seats
5. Proper stage and Hall decoration for technical session and gala dinner
6. To allocate ushers during the program
7. To check and ensure readiness of complete venue one hour prior to event
8. To make seat according to number of participants and chief guests
9. To arrange seats of Chair and Co-chair prior to technical sessions

10. ACCOMMODATION COMMITTEE

1.	Prof. Dr. Haroon Akbar Department of Parasitology	Convener
2.	Dr. Javeria Khan Associate Professor, Department of Veterinary Medicine	Member
3.	Dr. Muhammad Oneeb Assistant Professor, Department of Parasitology	Member
4.	Dr. Asif Ali Lecturer, Department of Food Science and Human Nutrition	Member
5.	Mr. Muhammad Awais PA to Vice Chancellor	Member
6.	Mr. Ubaid Ullah Ph. D Scholar, Department of Parasitology	Volunteer
7.	Mr. Muhammad Abdullah Bin Saleem Ph. D Scholar, Department of Parasitology	Volunteer
8.	Mr. Fasih ur Rehman Ph. D Scholar, Department of Parasitology	Volunteer
9.	Ms. Palwasha Gul M. Phil Scholar, Department of Parasitology	Volunteer
10.	Ms. Javeria DVM student, UVAS Lahore	Volunteer
11.	Ms. Fatima DVM student, UVAS Lahore	Volunteer
12.	Mr. Asadullah Abid Visiting Lecturer, Department of Parasitology	Secretary

TORs:

1. This committee will make arrangements for stay of national and international speakers, students and other guests in university rest house, foreign faculty, and other hotels
2. This committee will communicate with speakers and transportation committee for receiving of guests at airport till their accommodation place

11. REFRESHMENT COMMITTEE

1.	Prof. Dr. Kamran Ashraf Chairman, Department of Parasitology	Convener
2.	Prof. Dr. Muhammad Imran Rashid Department of Parasitology	Member
3.	Prof. Dr. Muhammad Avais Department of Veterinary Medicine	Member
4.	Toqir Anwar Mughal Accountant, Department of Parasitology	Member

5.	Mr. Ahsan Elahi PhD Scholar, Department of Parasitology	Volunteer
6.	Mr. Shabab Ahmad Ph.D Scholar, Department of Parasitology	Volunteer
7.	Mr. Yousaf Gul Kakar Ph.D Scholar, Department of Parasitology	Volunteer
8.	Ms. Aneeqa Rashid M.Phil Scholar, Department of Parasitology	Volunteer
9.	Ms. Palwasha Gul M.Phil Scholar, Department of Parasitology	Volunteer
10.	Mr. Hassan Ali M.Phil Scholar, Department of Parasitology	Volunteer
11.	Dr. Muhammad Oneeb Assistant Professor, Department of Parasitology	Secretary

TORs:

1. Proposal and approval of Venue and Menu
2. To estimate approximate number of guests who will attend meals in coordination with invitation and registration committees
3. Magnificent arrangements should be made and Caterers / Firms of outstanding performance should be indentured.
4. To ensure proper timings of meals (Tea, Lunch and Dinner)
5. To ensure mineral water bottles for guests
6. To prevent the entry of irrelevant persons in the venue

12. PRESS, MEDIA AND ADVERTISEMENT COMMITTEE

1	Prof. Dr. Aneela Zameer Durrani Dean, FVS	Convener
2	Dr. Javeria Ali Khan Associate Professor, Department of Veterinary Medicine	Member
3	Dr. Muhammad Oneeb Assistant Professor, Department of Parasitology	Member
4	Mr. Basharat Ali Khan Chief Photographer	Member
5	Mr. Yousaf Gul Kakar Ph.D Scholar, Department of Parasitology	Volunteer
6	Mr. Muhammad Nouman Public Relation Officer, UVAS	Secretary

TORs:

1. To communicate with press and media partners and ensure their availability at the time of conference
2. To provide them access to conference hall and expo
3. To communicate with press and provide them sufficient proper space
4. To provide them timely and adequate information regarding conference
5. To record video of all events of conference

13. TRANSPORT AND PARKING COMMITTEE

1	Prof. Dr. Muhammad Yasir Zahoor Institute of Biochemistry and Biotechnology	Convener
2	Mr. Zulfiqar Ali Dogar, EMO	Member
3	Ch. Muhammad Shafique (Transport Officer (City Campus Lahore))	Member
4	Mr. Shabab Ahmad Ph.D Scholar, Department of Parasitology	Volunteer
5	Mr. Yousaf Gul Kakar Ph.D Scholar, Department of Parasitology	Volunteer
6	Dr. Muhammad Oneeb Assistant Professor, Department of Parasitology	Secretary

TORs:

1. To make appropriate schedule of transportation.
2. To make appropriate arrangements for transport of special guests to and from hotel and summit venue.
3. To arrange and provide transport facilities to participants when requested.
4. To ensure transport facilities one prior and post event.
5. To care for parking facilities of participants.

14. IT MANAGEMENT COMMITTEE

1	Mr. Muhammad Rizwan Saleem, Director IT, UVAS, Lahore	Convener
2	Mr. Imran Younas Chohan Network Manager IT Center	Member
4	Mr. Yasir Ameen System Manager	Member
5	Mr. Muhammad Farooq Software Manager	Member
6	Mr. Muhammad Atif IT Lab. Assistant	Member
7	Mr. Shahid Mughal IT Administrator	Member
8	Toqir Anwar Mughal Accountant, Department of Parasitology	Member
9	Mr. Ahsan Elahi PhD Scholar, Department of Parasitology	Volunteer
10	Mr. Asadullah Abid Visiting Lecturer, Department of Parasitology	Secretary

TORs:

1. To upload information regarding PARACON on University Website.
2. To manage ALL IT related issues (sound system/multimedia etc.) during conference.
3. To take presentations from Scientific Committee in time.
4. To ensure spreading information through social media etc.

15. SECURITY COMMITTEE

1	Prof. Dr. Mati ur Rehman Khan Niazi Department of Pathology	Convener
2	Muhammad Nawaz Security Officer	Member
3	Mr. Shabab Ahmad Ph.D Scholar, Department of Parasitology	Volunteer
4	Mr. Yousaf Gul Kakar Ph.D Scholar, Department of Parasitology	Volunteer
5	Mr. Asadullah Abid Visiting Lecturer, Department of Parasitology	Secretary

TORs:

1. To ensure security of all national and international delegates attending conference
2. To ensure all event safely and security clearance of foreign delegates

16. FINANCE MANAGEMENT COMMITTEE

1	Prof. Dr. Kamran Ashraf Chairman, Department of Parasitology	Convener
2	Prof. Dr. Wasim Shehzad IBBT	Member
3	Ch. Muhammad Shafique (Deputy Treasurer)	Member
4	Dr. Zia ur Rehman Tara Group of Industries, Raiwind	Member
5	Mr. Asadullah Abid Visiting Lecturer, Department of Parasitology	Member
6	Toqir Anwar Mughal Accountant, Department of Parasitology	Member
7	Dr. Muhammad Oneeb Assistant Professor, Department of Parasitology	Secretary

TORs:

1. This committee will be responsible for all finance management
2. This committee will maintain record of all expenditures and fill the Performa of different funding agencies
3. This committee will meet different private companies and generate resources for conference
4. The committee will be solely responsible for release of funds to different committees

SOUVENIR COMMITTEE:-

1	Dr. Muhammad Oneeb, Assistant Professor, Department of Parasitology	Convener
2	Prof. Dr. Haroon Akbar, Department of Parasitology	Member
3	Prof. Dr. Raheela Akhtar, Department of Pathology	Member
4	Mr. Yousaf Gul Kakar Ph.D Scholar, Department of Parasitology	Volunteer

5	Ms. Sana Ashiq M.Phil Scholar, Department of Parasitology	Volunteer
6	Ms. Aneeqa Rashid M.Phil Scholar, Department of Parasitology	Volunteer
7	Ms. Palwasha Gul M.Phil Scholar, Department of Parasitology	Volunteer
8	Mr. Ahsan Elahi PhD scholar, Department of Parasitology	Volunteer
9	Mr. Asadullah Abid Visiting Lecturer, Department of Parasitology	Secretary

TORs

- 1- Arrangement of Souvenirs/shields for all related events.
- 2- To arrange/design appropriate souvenirs for all conference participants
- 3- To ensure souvenirs/shields are ready at least one week before conference
- 4- To coordinate with registration and invitation committee to ensure number of participants, chief guest, other national and international delegates
- 5- To arrange display of awards at the events and assisting in award distribution.
- 6- To decide which award to be given in which session.

