Can we combat worm infestation in developing countries: A broken link

Khattak, F

Print publication: 22/10/2014

Document Version
Publisher's PDF, also known as Version of record

INTERNATIONAL CONFERENCE ON ADVANCES IN PARASITOLOGY AND PUBLIC HEALTH

(ICAPPH-2014)

October 22-24, 2014

Organized by:

Department of Pathobiology, College of Veterinary and Animal Sciences, Jhang-Pakistan
In collaboration with

Pakistan Science Foundation

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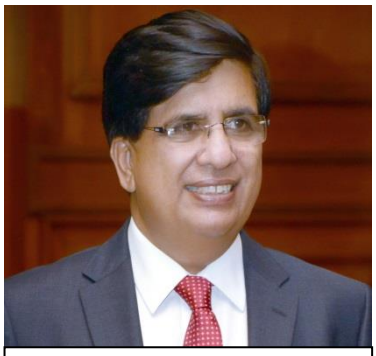
Vice Chancellor's Message



Prof. Dr. Talat Naseer Pasha
Vice Chancellor

Livestock sector is an important segment of agriculture economy and contribute over 55% to the agricultural value added and 11.6% to the national GDP. Research and training focused activities like conferences ensure to impart the highest quality education, skills and sharing of experiences.

The College of Veterinary & Animal Sciences (CVAS), Jhang, Pakistan is organizing an **"International Conference on Advances in Parasitology and Public Health (ICAPPH-2014)"** on October conference will provide a forum to the academicians, researchers, students, public as well as private entrepreneurs and stakeholders to present and discuss their research findings and experiences in areas of parasitology and public health. This conference will be helpful in creating awareness among the public about the risks and losses associated with parasites. This will remind them to take preventive measures about parasitic burden leading to enhanced productivity of their livestock. The conference will provide a platform to learn about latest trends of raising livestock by preventing parasitic problems and public health issues. This will help increasing the growth and productivity of livestock in the country. By working closely with our partners and stakeholders, we are trying our best in enhancing economic prosperity, contributing to improvements in the animal health, food production and ultimately well-being of our nation.

Prof. Dr. Talat Naseer Pasha

Vice Chancellor

Principal's Message

Prof. Dr. Muhammad Younus Rana
Principal

It is a great pride and delight for me to welcome all national and international delegates at an **"International Conference on Advances in Parasitology and Public Health (ICAPPH-2014)"** at College of Veterinary and Animal Sciences, Jhang, Pakistan. District Jhang has the largest livestock population in the Punjab province, Pakistan. The major theme of the ICAPPH-2014 is "Parasitology and Public Health" and would be consisted of scientific programs addressing Epidemiology of parasitic infestation, Diagnostics, Zoonoses and Public health sessions.

This conference will provide the opportunity to get insights from world-renowned experts in parasitology, public health and the other relevant area through the plenary sessions, key note lectures, and individual presentations. I hope this conference will be helpful to update the learning of galaxy of scientists, young researchers, students and farmers by sharing mutual knowledge. Beside the conference main scientific program we are also arranging satellite programs including elite animal show, calligraphic exhibition, stalls and animal products displays. Moreover, participants can make good networks for the future research and business with organizations and enterprises from in and around the country. We look forward to welcoming you in ICAPPH 2014 at Jhang, Pakistan on October 22-24, 2014. We will promise that your experience at the conference will be a rewarding and memorable.

(Prof. Dr. M. Younus Rana)
Principal/ Conference Chairman

Conference Organizer's Message

Dr. Muhammad Fiaz Qamar
Conference Organizer

We cordially invite you to join us in INTERNATIONAL CONFERENCE ON ADVANCES IN PARASITOLOGY AND PUBLIC HEALTH (ICAPPH-2014) on October 22-24, 2014 Organized by Department of Pathobiology, College of Veterinary & Animal Sciences, Jhang, Sub Campus University of Veterinary & Animal Sciences, Lahore-Pakistan.

Many zoonotic diseases are endemic in Pakistan. Some of them are trans-boundary diseases which are permanent threats to our neighboring countries. Some of these diseases are notifiable to OIE (World Organization for Animal Health) which cause huge economic losses in livestock sector and may impose sanctions regarding the trade of animals, their products and by-products. There is dire need to have a platform to address these issues and share the updates so that future policies may be chalked down to combat against these deadly maladies. Perhaps this conference would be a best platform to link the scientific community at national and international level to share their ideas as one health and devise new ways and means for the prevention and control of diseases having zoonotic implications (Healthy animals guarantee healthy human population).

The joint ICAPPH-2014 conference will cover scientific topics on disease risks and challenges - both for animals and for humans - posed by parasitic pathogens. Shared sessions will focus on the topics including: Parasites and climate change, Innovative pathogen detection tools, Host-parasite-pathogen relationships, Immunity and vaccines and Innovative solutions for parasiticidal drug discovery. Parallel sessions will include topics: Parasitic Lifecycle, ecology and epidemiology, Drugs and Drug-resistance. Whether your interests lie in basic or applied research in the fields of Livestock management of vectors and vector-borne diseases in human and veterinary health, this conference is an ideal forum for you. Two additional workshops focusing on Trypnosoma / Brucella will be held at the occasion.

The other salient aspects to be discussed at the event will include; ticks and tick-borne pathogens in veterinary medicine, Ticks and domestic animals, Zoonoses and Public Health, Natural products and Bio-control, Parasites in Laboratory animals and Wildlife. The system currently being used to prevent parasitic disease is not working. There are several reasons behind this failure. Compliance is a significant problem.

This belief will be shared by my fellow members to promote animal and human health through recommendations for diagnosis, treatment, prevention and control of parasitic infections. In fact, parasite prevention should be built into a lifelong wellness and prevention program associated with annual or biannual hospital visits, not with seasonality of parasitic disease. Many of these parasites can be prevented easily since they can be targeted. Ectoparasiticide control also can be built into programs to provide protection of animals and their owners from fleas and ticks. The time is right, the time is right to make a difference. Along with teaching, research is a major focus of The College of veterinary & Animal Sciences Jhang. Research groups at the campus have been involved in a variety of projects leading to increased Livestock productivity, vaccines and diagnostic products.

The broad aim of this conference is to promote discussion between scientists, practitioners and decision makers. In addition, this forum will update researchers, academics, governmental and non-governmental organization representatives, technicians, veterinarians and other professionals from industry and the private sector, interested in emerging and re-emerging epidemics affecting global health.

We are looking forward to meet you in CVAS in October 2014!

Can we combat worm infestation in developing countries: A broken link

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Abstract

Intestinal nematode infections were first highlighted in 1947 and since then several programmes such as special programme for training and research in tropical disease (TDR), partners for parasitic control (PPC) and preventative chemotherapy and transmission in control (PCT) have been launched under the umbrella of world health organization (WHO) to overcome global impact of zoonotic diseases including helminth. Worm infestation is inextricably linked with poverty, poor sanitation and dirty water. Treat, treat and treat is the global research agenda for control and elimination of Helminth diseases in humans because helminth disease is a re-emerging disease that is amplified due to external factors such as natural disasters, climate change, migration, shortage of clean water, poor sanitation and malnutrition.

To make any campaign sustainable requires community involvement. To make Pakistan a worm free country, it's crucial that people recognize, accept and commit to eradicate the helminth disease. It is not very easy for people to recognise this disease as the symptoms of mild worm infestation are often unnoticeable therefore majority of the people cannot even comprehend that worm infestation can turn into chronic irreversible diseases such as liver fibrosis, blindness, cancer of bladder and kidney failure etc. if preventive measures are not taken at early stage. Thus an awareness campaign is needed to translate research into a language which enables the people to link the route of infection with recognisable symptoms and the understand consequences if tailored treatment is not opted. A multi-pronged approach will be needed to ensure that the disease control programmes are nationally owned, embedded into national curriculum and health plans and backed up by the political agenda. Despite the advancement in controlling helminth diseases, the actual burden of the worm infestation remains uncertain. This necessitates a need to develop a link bridging the gap between researchers, community and government. The paper will explore the strategies how education, research, government and media can play a role to combat helminth infestation through eco-health approach and community participation.

The Secrete behind the successful survival of *Aedes aegypti*, the vector mosquito of dengue, yellow fever and chikungunya fever

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Abstract

The vector mosquitoes, *Aedes aegypti* of dengue and Chikungunya fever are closely associated with human habitations and adapted to feed on human blood. They undergo larval and pupal development in natural and artificial freshwater collections in the urban and peri-urban environment. Although reports are available about the feeding behaviour of the thriving mosquito larvae, much information is still required to understand the successful survival of *Aedes* mosquitoes in small and temporary water collections. We have investigated the role of microbes in the successful survival and sustainability of Aedes larvae. The role Bdelloid rotifer, *Philodina roseola* was explored and will be discussed.

Safety and efficacy of mosquito control from marine natural products

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Abstract

Mosquito control is facing a threat due to the emergence of resistance to synthetic insecticides. Insecticides of botanical origin could serve as potential alternatives in future. Large numbers of plant samples have been screened for their insecticidal and/or repellent activities and a few of them have been found promising and their products are commercially available. We have investigated for the mosquito larvicidal efficacies of different marine plants resources, some essential oil and nanoparticles from marine plants belonging to tested against *Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus* and *Armigeres subalbatus.* Some of them were effective in killing the larvae, repellency and adultdicidal female mosquitoes. Isolation and identification of active compounds from the effective samples would be useful in synthesising mosquito larvicides, repellents on a large scale.

Reducing the disease burden of Vector borne Neglected Tropical diseases in South-East Asia Region

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Abstract

The theme of the World Health Day 2014 focused the attention of the countries to augment the control efforts against vector transmitted diseases. Some of them are dengue, lymphatic filariasis (LF) and visceral leishmaniasis (VL) which are listed as neglected tropical diseases by WHO. The Road map to eliminate/eradicate by 2020 endorsed during "London Declaration" in 2012 and the World Health Assembly (WHA) resolution (WHA 66.12) in 2013 increased opportunities to overcome these diseases. The paper presents the review of the progress made by the South-East Asia Region of WHO to elimination of LF and control of dengue.

Lymphatic filariasis (LF) transmitted by mosquitoes; is one of the leading causes of disability worldwide. The disease is targeted for elimination as a public health problem by 2020. An estimated 63% of the 1.34 billion people globally are at risk of infection and 50% of the 120 million infected people are in nine countries in the South East Asia region. The Region bears nearly 60% of the total global burden estimates. All the three lymphatic filarial parasites, prevalent in the region. The LF endemic countries implemented annual single dose preventive chemotherapy to the entire at-risk population with DEC and albendazole, minimum for five years. MDA coverage increased from 19.4 million in 2001 to 332 million in 2012. As a result, the microfilarial (Mf) rate declined to less than 1% in 493 of the total 1104 implementation units (IU) endemic for LF in the region by 2011. Maldives, Sri Lanka and Thailand completed the process of verification of LF elimination in 2011 and soon will be out of the LF endemicity map. Morbidity management and disability prevention is being implemented.

Dengue: It is estimated that 2.5 billion people are at risk in more than 100 countries in the world; and of them, 1.3 billion people are at risk in South-East Asia (SEA) Region(WHO2013). An estimated 50 million dengue infections occur each year globally. *Ae.aegypti* and *Ae. albopictus* are responsible for transmission of dengue. All the 4 types (Denv1, 2, 3, 4) have been reported.

Maximum burden of the infection (dengue/dengue haemorrhagic) occur in the 10 out of 11 Member countries of the SEA Region. A total 2.4 million cases were reported from 2000 to 2011 in the region. In 2012, the region reported around 120 000 cases(WHO2012).There is a sudden

upsurge in dengue cases in Thailand and Sri Lanka. However, the reported case fatality rate is less than 1%.

A review of the available epidemiological data from the dengue programmes in South‐East Asia Region indicated a changing pattern in the human host, the dengue virus and the vectors biology. Age and sex-wise analysis of trend data showed a shift in the affected age groups and sex groups. More and more cases were reported among the adults than children <15 years old. Over a period of time, more and more cases are being reported by the rural areas than urban locations. In addition, the severity and time interval between the episodes of infection depended in the virulence and genotype of the virus. The vectors are very adaptive in nature and have been affected by climatic changes, socio‐cultural and economic factors, and rapid urbanization. The health policy makers and programme managers may consider looking at the changing patterns to implement control/prevention measures to reduce the disease burden and its impact.

Developing in house reagents for the diagnosis of Chikungunia and Dengue

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Abstract

In recent years Chikungunya virus reemerged and reported numerous outbreaks all over the world. This infection leads to joint pains and arthraligia. Similarly, the dengue infection in human can result in two well defined syndromes: dengue fever and dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). Hence the early diagnosis is an important parameter for the control and management of both diseases. Even though the diagnostic kits are available which are procured from abroad, the present study aims in developing in house reagents for the diagnosis of both IgM and IgG antibodies in the patients' sera. In this connection we have made panel of peptide epitopes of dengue antigen and chikungunya antigens and screened with patients sera to develop early diagnostic marker. After screening Chikungunya positive/dengue sera we had already reported many dominant epitopes within the envelope protein of Chikungunya virus (CHIKV) and domain III and NS1 protein. Also multiple antigen peptide (MAP) approach was used with four different E2 and domain peptide combinations in order to increase its sensitivity over linear peptides. The MAPs reacted with all chikungunya positive sera as well as dengue sera. Our results indicate that MAP could be an alternate antigen for the immunodiagnostics of Chikungunya/dengue infections with high sensitivity and specificity.

Emergence of Dengue Problem in India – A Public Health Challenge

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Abstract

India contains approximately half of the 205 billion people worldwide who are at risk of dengue fever. The virus causing Dengue/DHF is believed to have established in almost all parts of India and has emerged as major public health concern. Dengue is found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas. It is the most common mosquito-borne viral disease of humans. Globally, 2.5 billion people live in areas where dengue viruses can be transmitted. *Aedes aegypti* is the main vector playing major role in the transmission of dengue/DHF. Dengue fever and its severe complication i.e. DHF are caused by one of four types of distinct, but closely related, viruses namely DEN1, DEN2, DEN3 and DEN4 of genus flavivirus.[1] By the last decade of the 20th century *Aedes aegypti* and the 4 dengue viruses had spread to nearly all countries of the tropical world. Some 2 billion persons live in dengue-endemic areas with tens of millions infected annually. Dengue pandemics were also documented in the 18th and 19th centuries; they were contained by organized anti-*Aedes aegypti* campaigns and urban improvements. The 20th century dengue pandemic has brought with it the simultaneous circulation of multiple serotypes and in its aftermath, endemic dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS)[2].

At the National level, dengue control is coordinated by the National Vector Borne Diseases Control Programme (NVBDCP). NVBDCP is the agency responsible for framing national dengue guidelines and policies for guiding the implementations of programme strategies at the state level.

In the absence of a vaccine, vector control is the main strategy to prevent dengue outbreaks. The country paradigm for dengue control is largely passive surveillance and early case detection coupled with rapid mobilization in the case of an outbreak. The first outbreak of DHF occurred in Calcutta in 1963. After that disease outbreaks reported from different states. The First major outbreak of dengue fever (DF) was reported in Calcutta during 1963. Since then more than 60 outbreaks have been reported in India. During recent years it has become a major public health problem in urban areas of India and gradually spreading to rural areas. The problem of dengue is increasingly becoming important in most tropical countries due to the expanding urban areas, limited piped water supply, constant influx of people from rural to urban

areas, creation of slums, high rise buildings with increased use of water coolers during summer season.

An epidemic of dengue was also reported in city, Rajasthan in 1985. A severe outbreak of dengue was reported in 1996 in Delhi with more than 400 deaths. Gurgaon town of Haryana state faced similar outbreak of dengue with 1137 cases and 9 deaths in 2008. During 2009, Pune Corporation in Maharashtra state reported outbreak of dengue. The state reported 2255 cases and 20 deaths. The maximum dengue deaths were reported from Haryana (20), Kerala (17) and Punjab state (15).

Aedes aegypti was only vector in these all outbreaks. The epidemic which occurred during 2005-06in certain islands of Indian Ocean and in Kerala strongly suggests that *Aedes albopictus* played an alternate role. *Aedes albopictus* has invaded the peridomestic and peri domestic settings, hitherto the exclusive domain of *Aedes aegypti*. The aggressive nature of *Aedes albopictus* when compared to *Aedes aegypti* may help them to out compete the later and could play a crucial role in disease transmission due aggressive bites in Kerala state. Ae. albopictus, a secondary vector for dengue is likely a significant factor in the persistence of dengue in the environment through vertical transmission and may be replacing *Ae. aegypti* in semi-urban areas

The diverse breeding habitats of the dengue vector, Aedes spp. Mosquitoes, demand community education and mobilization for effective control. However, community involvement for dengue control has mixed results in the country. Improper water management, lack of public awareness, inadequate solid waste disposal mechanisms, urbanization, lack of communication and integration between governing agencies, all contribute to increased number of dengue cases. This paper highlights the state of dengue control in India, explore vector control mechanisms that have worked elsewhere, to strengthen dengue control activities by policy and practice.

Mosquito malaria interaction and recent control strategies

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Abstract

Malaria remains the most important of the mosquito-borne disease resulting in more clinical cases and fatalities worldwide than any other parasitic disease. This disease is transmitted by the anopheline mosquito based on which mosquitoes are categorized into a) susceptible mosquito; in which the malaria parasite succeeds to complete its life cycle and infect a new vertebrate host and b) refractory mosquito; which has innate immune responses that able to block the parasite development inside its gut. The refractory mosquito has recently attracted the attention of scientists for controlling malaria. This is mainly because of the recently evolved problem of the pesticide resistance of the mosquito vector and the rapid spread of multiple anti-malarial drug-resistance of malaria parasite. Therefore, the nowadays alternative novel control strategies are to block the malaria life cycle in the susceptible mosquito midgut to reduce its competence of transmitting malaria. This prime objective includes the recent rapidly evolved area of research on malaria, “the transmission-blocking immunity strategy”. In this context, two recent strategies are implemented; the first is the genetic selection of malaria-refractory mosquito and/or genetically modifying mosquito vectors. These genetic strategies are aiming to produce mosquito vector that is incompetent to the development of malaria parasite in its gut. The second strategy is based on using transmission-blocking targets in mosquito midgut. These targets block the passage of malaria ookinetes across the midgut. Both of strategies consider the vector immune system as the prime target to be utilized for blocking the development of *Plasmodium* inside its mosquito vector. However, there is a reproductive cost, as a price of immune induction, that must be paid by the vector. Evidences for this have been shown by mosquito models that have been selected for refractoriness to malaria in terms of reduced reproductive fitness. This, in fact, may reduce the importance of immune-based control strategies in the battle against malaria disease. On the other hand, recent studies have refocused on mosquitocidal microorganisms as useful alternatives to conventional insecticides, suggesting these pathogens as bio-control candidates in the battle against mosquito-borne diseases. Thankfully, the mosquitocidal bacterium, *Bacellus thuringiensis* (Bt) yet faced no resistance from mosquito host. This talk will discuss the above mentioned strategies and my immunity-based studies as well as my current ongoing studies of isolating and identifying new native isolates of mosquitocidal Bt.

Integrated approach in malaria vector control

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Abstract

Each year, an estimated 300 million to 500million people become ill with malaria, and nearly 1 million die. Every 60 seconds, an African child dies of malaria. More than 80 percent of the world's malaria deaths occur in sub-Saharan Africa. Malaria is a leading cause of death of young children in Africa. Malaria is a preventable and treatable disease. Integrated vector management (IVM) is a decision-making process for malaria vector control. The aim of the IVM approach is an efficient vector control process for achieving the global targets for vector-borne disease control in a cost effective & sustainable manner. Key elements of an integrated vector management (IVM) are Advocacy, social mobilization and legislation, Inter sector Collaboration, Integrated approach & Capacity-building. Integrated approach element is one major element which addresses chemicals & non-chemical vector control tools. Vector control remains the most generally effective measure to prevent malaria transmission. The current malaria control strategy calls for the selection of those control measures which are most appropriate to local circumstances and capabilities and malaria risk. Vector control methods vary considerably depending on situation. Choice of vector control will depend on the magnitude of the malaria burden, the feasibility of timely and correct application of the required interventions and, most important of all, the possibility of sustaining the resulting modified epidemiological situation. Basic objective of integrated approach is reduction of human-mosquito contact by using Insecticide-treated nets, repellents, protective clothing, screening of houses; destruction of adult mosquitoes by Insecticide-treated nets, indoor residual spraying, space spraying, ultra low-volume sprays; destruction of mosquito larvae in peri-domestic sanitation by Larviciding of water surfaces, intermittent irrigation, sluicing, biological control; source reduction by environmental sanitation, water management, drainage etc. Any strategy should be responsive to changes in local ecological and epidemiological conditions. The issues to be taken into account in planning vector control are: the target vectors, the timing of implementation, the areas of implementation, the entities involved in implementation and the entities responsible for implementation and external monitoring and evaluation.

AIDS associated diarrhea, beyond the basics

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Abstract

Gastrointestinal infections due to a variety of bacteria, viruses, fungi and parasites occur frequently in HIV/AIDS patients in whom the immune system is weakened due to progressive depletion and eventual destruction of the Helper T cells (CD4 cells).When the T cell count is<200cells/microL, the HIV infected, now AIDS patient is at risk of opportunistic enteric parasitic infections.

Cryptosporidium parvum, Isospora belli and in the non-opportunistic *Entamoeba histolytica* have been reported as the most commonly identified organisms in patients of AIDS in India and rest of the world . Diarrhea, a common clinical presentation in such cases has been documented in 30-60 per cent of AIDS patients in developed countries and in about 90 per cent of AIDS patients in developing countries. However, may opportunistic infections can be prevented by prophylactic measures and boosting the immune system.

Prevalence of *Borrelia anserina* in *Argas* ticks collected from poultry and poultry farms

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Abstract

Borrelia anserina is a pathogen of high importance in poultry industry which causes fowl spirocheatosis. In this study, the prevalence of *B. anserina* infected *Argus* ticks in poultry birds and farms was studied. A sum of 1500 tick samples from poultry birds and farms were collected and examined for presence of *B. anserina*. Its presence was determined by isolation in BSK-H medium and later the isolates were confirmed by using dark field microscope and indirect immunoflourescence assay techniques. Molecular identification of the isolates was done, additionally, with the help of polymerase chain reaction (PCR) by using the specifically designed primers of *fla* B gene. Out of 750 tick samples collected from poultry birds, 144 (19%) samples showed the presence of *B. anserina*. Whereas, the tick samples collected from the farm premises had prevalence of 131 (17%). The data indicated that the *Argus* ticks had the significant prevalence of *B. anserina* which might be a threat to poultry industry. Furthermore, the data may warrant future studies towards the vector control and/or immunoprophylaxis against *B. anserina* which might indirectly be helpful for the eradication of this threatening disease.

Development of tissue cysts of *Toxoplasma gondii* in mammalian cell cultures

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Abstract

About 30% of the world's population has antibody evidence of exposure to the protozoan parasite, *Toxoplasma gondii*. Congenital transmission from mother to fetus is well known because of the devastating results observed in pregnant women and infected babies. Reactivated toxoplasmosis is another important clinical form of the disease. Reactivated toxoplasmosis is observed in immunosuppressed patients, organ transplantation patients and in patients with toxoplasmic retinitis. Reactivation occurs when the bradyzoites in tissue cysts become activated and stage convert to tachyzoites. These tachyzoites destroy tissue and cause clinical signs. Tissue cysts and bradyzoites are essential for the continued transmission of *Toxoplasma gondii*. Only the bradyzoite stage of *T. gondii* can produce enteroepithelial schizont stages, sexual stages, and oocysts in cats. The host immune response does not drive stage conversion and tissue cyst formation because tissue cysts form spontaneously in cell culture with no immune treatment (antibody or cytokine) needed. Physiological stress will drive tachyzoite to bradyzoite stage conversion and result in increased tissue cyst formation. Sporozoites, tachyzoites, and bradyzoites are all able to produce tissue cysts in vitro when used as the initial inoculum. Most genotypes of *T. gondii* will spontaneously produce tissue cysts in vitro. The slow growing ME49 Type II strain is most often used by researchers. During early tissue cyst formation in vitro organisms with the ultrastructure of tachyzoites and bradyzoites can be seen in the same tissue cyst. Antibodies specific for tachyzoites and bradyzoites also demonstrate that these 2 stages are present at the same time. Tissue cysts can be seen as early as 3 days in cell culture. Tissue cysts that are produced in cell culture and fed to cats will result in the production of oocysts indicating that some of these tissue cysts are biologically equal to those that form in vivo. We have identified many of the factors that cause tachyzoite to bradyzoite stage conversion in *T. gondii*. There are several interesting problems that still need to be researched. These include the development of methods to examine tissue cyst reactivation and bradyzoite to tachyzoite stage conversion. Once developed these systems could be used to study relapse in toxoplasmic encephalitis, retinitis in immunocompetent patients, and transplantation-induced toxoplasmosis.

Development and Standardization of ELISA for the Evaluation of Humoral Immune Response against Measles

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Abstract

Measles is a highly contagious disease caused by measles virus; a *Morbilivirus* belongs to family *Paramyxoviridae*. It is a common cause for childhood morbidity and mortality in developing countries, the disease is characterized by fever, cough, conjunctivitis, coryza and malaise followed by a maculopapular rash. World Health Organization (WHO) initiated Expanded Program on Immunization (EPI) in 1974 and it was started in Pakistan in 1978 to control vaccine preventable diseases in infants and children. In the present study ELISA was developed and standardized in Virology and Immunology Lab of Department of Microbiology, Govt. College University, Faisalabad for monitoring and evaluation of humoral immune response against measles. In first step, antibodies against measles virus were raised in experimental rabbits using live attenuated measles virus of CAM 70 strain (which contain not less than 1000 $CCID_{50}$ in 0.5ml). The same virus was used to coat ELISA plates using carbonate buffer. Other reagents, including negative control, positive control, cut-off control, dilution buffer, washing buffer, stop solution, measles anti-IgG conjugate and TMB substrate solution, were procured from Nova Tech Immunodiagnostica GmbH Germany. The coated ELISA plates were used to evaluate serum antibodies raised in experimental rabbits, negative and positive controls along with samples collected from children vaccinated against measles. The optical density (OD) of serum samples from experimental animals and from vaccinated samples was found to be 0.913±0.021 and 0.986±0.017 at 450 nm wavelength using spectrophotometer, respectively. It was concluded that the indigenously developed ELISA may be used successfully to detect IgG antibodies in serum samples from experimental rabbits as well as from vaccinated children.

Seroprevalence of Measles and Estimation of Vaccine Coverage in Children in District Jhang

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Abstract

Vaccine preventable diseases (VPDs) account for infants and childhood mortality and morbidity across the globe. In the current investigation we determined the overall coverage of measles vaccination under Expanded Program on Immunization (EPI) in District Jhang. A total of 443 houses (647 vaccinated individuals out of 813 children under 10 years of age) were surveyed from September, 2013 to May, 2014 about vaccination and disease history of measles. Out of these, 86 blood samples (66 vaccinated and 20 non-vaccinated) were collected randomly and analyzed for the presence of anti-measles IgG antibodies using an indigenous optimized ELISA in Department of Microbiology, Govt. College University, Faisalabad. Overall vaccination coverage was found to be 79.58% (647 out of 813 children) from vaccination history profiles. Cut-off value of optical density (OD) measured using spectrophotometer at 450nm wavelength. Humoral immune response was detected in 50 out of 66 samples (75.7%) from vaccinated children with mean OD of 0.934±0.017, whereas 14 out of 20 samples (70%) of non vaccinated children also showed anti measles IgG antibodies with mean OD of 1.247±0.012. From the results of present work, it was concluded that overall coverage of EPI against measles in District Jhang was 79.58% which was less than highest values in European countries (94%) as reported by WHO. Furthermore, humoral immune response developed in non-vaccinated children in response to possible natural infection showed a significantly higher anti measles IgG antibodies.

A Novel Rho-Like Protein TbRHP Is Involved in Spindle Formation and Mitosis in Trypanosomes

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Abstract

Background: In animals and fungi Rho subfamily small GTPases are involved in signal transduction, cytoskeletal function and cellular proliferation. These organisms typically possess multiple Rho paralogues and numerous downstream effectors, consistent with the highly complex contributions of Rho proteins to cellular physiology. By contrast, trypanosomatids have a much simpler Rho-signaling system, and the Trypanosoma brucei genome contains only a single divergent Rho-related gene, TbRHP (Tb927.10.6240). Further, only a single RhoGAP-like protein (Tb09.160.4180) is annotated, contrasting with the .70 Rho GAP proteins from Homo sapiens. We wished to establish the function(s) of TbRHP and if Tb09.160.4180 is a potential GAP for this protein.

Methods/Findings: TbRHP represents an evolutionarily restricted member of the Rho GTPase clade and is likely trypanosomatid restricted. TbRHP is expressed in both mammalian and insect dwelling stages of T. brucei and presents with a diffuse cytoplasmic location and is excluded from the nucleus. RNAi ablation of TbRHP results in major cell cycle defects and accumulation of multi-nucleated cells, coinciding with a loss of detectable mitotic spindles. Using yeast two hybrid analysis we find that TbRHP interacts with both Tb11.01.3180 (TbRACK), a homolog of Rho-kinase, and the sole trypanosome RhoGAP protein Tb09.160.4180, which is related to human OCRL.

Conclusions: Despite minimization of the Rho pathway, TbRHP retains an important role in spindle formation, and hence mitosis, in trypanosomes. TbRHP is a partner for TbRACK and an OCRL-related trypanosome Rho-GAP.

Cockroaches and locusts: answer to parasitic infections?

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Abstract

With the worsening trends of drug resistance, there is a need for newer and more powerful anti-parasitic agents. The search for new compounds originating from natural resources is a promising research area. We hypothesized that animals living in polluted environments are potential source of novel anti-parasitic molecules. In support, our studies identified potent antimicrobial properties in the lysates of cockroaches, locusts, Black cobra that intrigued the scientific community. We hope that the discovery of antimicrobial activity in the cockroach brain will stimulate research in finding anti-parasitic agents from unusual sources, and has potential for the development of novel strategies in the control of worm infections.

Sero-prevalence of *Trypanosoma evansi* in Camels from Cholistan, Pakistan

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Abstract

Trypanosomosis adversely affects the health, productivity and working capacity of animals. *Trypanosoma evansi*, the causative agent of surra, is principally transmitted by hematophagous flies (*Tabanus*, *Stomoxys*, *Chrysops*, *Haematopota*, *Lyperosia* and *Atylotus*). A cross sectional study was carried out in three districts of the Cholistan desert, Rahimyar Khan, Bahawalpur and Bahawalnagar, to estimate the sero-prevalence of *T. evansi* amongst camels. From the 1,000 visited animals, 0.7% were positive in Giemsa stained thin smear and 48% were positive in ELISA/*T. evansi*. This study declares Cholistan desert to be a high risk area for *T. evansi*. Studies on Trypanosomosis of various animal species (sheep, goat, camels, buffloes, cattle and donkeys) are underway and various serological, hematological, biochemical, molecular and

immunological parameters are being investigated. *Tabanus striatus* and *Stomoxys Calcitrans* have been identified as major vectors of Surra at Cholistan Desert.

Loop-mediated Isothermal Amplification (LAMP) assay: a simple and cost effective molecular diagnostic tool for the Neglected Tropical Diseases (NTDs)

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Abstract

In developing countries, where the control of diseases is poor, 30-50% of potential productivity (quantity and quality) is lost due to disease. Conventional diagnostic capabilities (microscopy, culture, biochemical testing and serology) are insufficient to effectively monitor the infections. For the efficient control of these infections a continuous monitoring program is required incorporating simple and cost effective molecular diagnostics. In recent years, Loop-mediated isothermal amplification (LAMP) assay has emerged as low and cost effective diagnostic tool for the molecular detection of many animal diseases, especially in developing countries because no costly equipment like thermal cycler is required to perform this assay. The LAMP uses a set of 4-6 primers that target the 6-8 site of a conserved gene. The LAMP assay relies on autocycling strand displacement DNA synthesis performed by *Bst* or *Bsm* DNA polymerase, which leads to an excellent sensitivity. Being a very sensitive test the results can be visualized by seeing turbidity or colour change of solution, thus limiting the analysis on gel. On these grounds it is possible to present working molecular diagnostic technology that would equally work well under field as well as small laboratory setups thus favours point-of-care testing (POCT). The POCT format is acutely demonstrated in neglected tropical diseases (NTDs), where access to reliable diagnostic testing is severely limited and misdiagnosis commonly occurs. Loop-mediated isothermal amplification (LAMP) is an innovative molecular technique that has been validated for point-of-care testing to diagnose malaria *Plasmodium falciparum*, *Plasmodium vivax*, *Toxoplasma gondii, Toxocara canis*, *Toxocara cati, Leishmania donovani, Leishmania infantum*, *Trypanosoma cruzi,* Shistosomiasis, *Echinococcus multilocularis*, *Angiostrongylus cantonensis* Acanthamoeba, Dengue virus, Rabies virus, Highly Pathogenic Avian Influenza, (HPAI) and *Mycobacterium tuberculosis*. We believe that better disease diagnosis through LAMP will ultimately strengthen the molecular capabilities of developing world to combat with NTDs and other public health issues.

A report on the incidence of Tick-borne haemoprotozoan diseases in bovines and Equines in the suburbs of Jhang, Pakistan

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Abstract

Ticks infestation in animals is a global problem. Seasonal predisposition and husbandry practices are the major determinants in the prevalence and propagation of ticks and disease transmission thorough these vectors under the favorable circumstances. A total of 70 sick animals consisting of 56 bovine and 24 horses were presented at the outdoor clinic of College of Veterinary and Animal Sciences, Jhang, Pakistan during May-September 2014, with the major complaint of pyrexia, anorexia, lethargy and gradual emaciation etc. Haematological examination of 56 bovine (Crossbred cattle, *n*=48; Buffalo *n*=8) showed that out of 36 adult cows; 12 (33.33%) animals were found infected with Babesia *spp.* only, while 24 cows (66.66%) were found infected with mixed haemoprotozoan infections. Mixed infections determined were; 4 cows (4/36) harboring Babesia and Theileria *spp.* while the other 20 (55.55%) cows were carrying Babesia and Anaplsma *spp.* together. In buffaloes, 4 animals (50%) were infected with *Babesia bovis* while the remaining 4 animals were carrying co-infections of Babesia and *Anaplsma marginale spp.* Out of 24 adult horses, 12 horses (50%) were found infected by haemoprotozoans. Out of these 12 infected horses, 8 (66.66%) were determined positive for mixed infection of Anaplasma and Babesia *spp.,* while the remaining 4 horses were confirmed infected with only Babesia *spp*. It was concluded that the incidence of tick borne diseases particularly mixed haemoprotozoal infections in cattle, buffaloes and horses was quite high during summer season which necessitates taking prophylactic measures against the ecto-parasites in livestock to prevent tick-borne diseases and to optimize their production potential.

Therapeutic Efficacy of Diminazine diaceturate against Trypanosomiasis in Camels in Jhang, Pakistan

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Abstract

A total of 20 male adult camels were presented at the outdoor clinic of Department of Clinical Sciences, College of Veterinary & Animal Sciences, Jhang, Pakistan during the period of April to September 2014 with the main complaint of intermittent fever, anorexia, ocular mucopurulent discharge, lack of stamina, sluggishness, slothfulness, reluctant to move, progressive

emaciation, edema of the ventrum of the body along with groin region and limbs etc. Out of 20 camels, 15 were found positive for trypanosomiasis after performing wet blood smear examination. Injection Diminazine® (containing Diminazine diaceturate 35 mg/ml, Star Laboratories, Pvt. Ltd. Lahore) was administered as 30 ml intramuscularly twice at an interval of 24 hrs besides being used the antipyretics (Tinospora extract 100 gms daily for 3 days per os). After elapsing 7 days, wet blood smears were again examined. All the treated animals were found negative for trypanosomes. Those began to eat normally and edema disappeared, restored their normal sheen of eyes looking agile and robust. It was concluded and recommended that Diminazine diaceturate could be used with great confidence to treat the trypanosomiasis in camel.

Historical trends show significant warming but non-significantly increased precipitation in arid and semiarid Punjab - Pakistan

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Abstract

Historically, the droughts and monsoons have adversely affected socio-economic and environmental conditions of Pakistan especially of the Punjab province. The objective of this modeling approach is to evaluate climate change patterns by analyzing time series data of climate extremes (minimum and maximum temperatures) data. Thirty years (1981-2010) of climate extremes data were collected from five representative cities across Punjab, i.e., Multan, Bahawalnagar, Faisalabad, Sargodha, and Rawalpindi, Thirteen temperature indices, established by the Expert Team on Climate Change Detection, Monitoring and Indices (ETCCDMI), were calculated using above data and RClimDex software. Mann-Kendall Test and Sen's Slope Estimates were used to detect rare, general, and prevailing climate trends. The results indicated consistent patterns of global warming over most of the province. Overall, extremely warm events have increased and the extremely cold temperature events have decreased for the analysis period. Cool nights have significantly decreased and hot nights have significantly increased. All the cities experienced the most number of cold spells during 1984. Regionally averaged per decade summer days (when temperature was > 25^{o}C) and tropical nights (when temperature was > 20^{o}C) were computed to increase by 5 and 8, respectively.

Opthalmomyiasis externa: An uncommon zoonosis by Sheep botfly, Transmission, Remedy and Control

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Abstract

Oestrus ovis commonly known as Sheep bot fly/Nasal bot fly belongs to the family Oesteridae and are obligate parasites at their larval stages. The larvae of all the members of this family invade the internal organs of their hosts to feed and complete their lifecycle. Sheep are more common host but goats may also be infested if they are kept with affected sheep in herd. Wild ungulates like deers and black bucks are also found to be affected according to some reports. They may also cause accidental myasis in other species including human and pet animals like cats. Although these flies have seasonal occurrence during spring season but once the larvae infest the host, they stay inside the host for months and after consecutive moultings within the host the third instar larvae come out of the host during the next spring season, pupate in the environment and adult flies come out which mate in the environment and again infect the new host. In Pakistan, the region of southern Punjab has also the suitable environmental conditions for propagation of these flies and most of the farmers do not maintain proper cleanliness which makes the environment feasible for the flies to multiply and infest the host. In humans these larvae cause ophthalmomyiasis externa, a pathological condition of external tissues of eye and damage by entering through lacrimal duct. Adult fly caries the larvae and deposit them in the eyes on accidental striking. Although further moultings of the larvae are seized but the damaging effects are severe. Surgical removal of larvae at initial stages is the most fruitful remedy among all the suggestive treatments and control of flies at farm level and proper maintenance of sanitary conditions is the best way to get rid-off this malady.

Parasite Control Strategies

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Abstract

Deworming is the most cost-effective management practice worldwide. It results in healthier animals with sound immune systems, better weight gains that provide more profits. The concept has always been appealing, but it requires emphasis on pasture hygiene, intelligent use of anthelmintics to avoid resistance, knowledge of life cycles and bionomics of various parasites, and then hope that the tremendous losses due to parasitism can be reduced. The primary purpose of parasite control is not to cure sick but to reduce levels of pasture contamination and consequently prevent episodes of illness. There are many parasitic diseases affecting a wide range of hosts and so Strategic Parasite Control is need of the day. Lack of understanding of epidemiology, lack of convenience, poor understanding of Parasites Impact on animals and human beings, Subclinical infections are often overlooked, Effectiveness of today's parasiticides, regional differences in parasite control, Seasonal Transmission Patterns (Summer vs. Winter Parasites), Dynamics of Parasite Transmission, Mathematics of Parasite Infection, multiple hosts, Pasture Contamination, Timing is Everything. You could provide consistent control throughout the grazing season with a single treatment, the real-world alternative is to maintain low parasite populations with multiple, well-timed treatments. The goal is to achieve maximum parasite control with appropriate strategy nationwide.

Bacterial flagellar assembly inhibitors of biofilm formation

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Abstract

Biofilms formation is a major hazardous problem from both clinical and environmental perspective. Flagellum-mediated motility is important for biofilm formation by several gram-negative bacteria. >50 genes are involved in flagellar biosynthesis and function in *Salmonella typhimurium*. The flagella basal body is a representative of Type III protein secretion systems; used by several gram-negative bacterial pathogens to colonize foreign tissues and substrates. The mechanism of flagellar assembly was analyzed in *S. typhimurium*, using bioinformatics analysis to identify conserved structural elements. In this study, FliI a flagellar protein that is needed for flagellar assembly and may be involved in a specialized protein export pathway was

cloned and overexpressed. Using vital dyes, visualization of single and motile was established based on optical microscopy techniques which will extend initial evidence that flagellum-mediated rotation is critical for biofilm formation. The flagellar basal body is a particularly convenient drug target, since the architecture of most its components has been determined near atomic resolution and it is an ancient evolutionarily conserved macromolecular assembly. The knowledge gained will also have implications for elucidation of the mechanistic design principles underlying protein secretion complexes.

Non-Chemical dewormers against Internal Parasites of Livestock-A Review of Scientific Knowledge

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Abstract

Infestation with endoparasites have been become a major threat in ruminants causing server economic losses in livestock sector. The parasites of primary concern are tapeworms, lungworms, liver flukes, and coccidia. Livestock producers mostly relied on anthelmintics to control internal parasites in animals. However, anthelmintic resistance has become a global problem threatening the productivity of ruminants. So there is increasing demond to avoid chemical intervention of deworming. In addition the search for alternatives has become necessary due to the development of organic farming systems and the increased public awareness for drug residues in agricultural products. Many alternative dewormers including Diatomaceous earth, *Quercus ssp., Punica granatum*, *Allium Sativum, Castanea sativa* and *Vitis ssp.* Extract have been tried for parasitic control. However none of these non-chemical options offer immediate help for infected animals. The aim of this paper was to provide information about the effective alternative dewormers against internal parasites, the physiological consequences of herbal administration including possible risks, side-effects and future applicability.

Impact, quality and acceptability of a musculoskeletal screening

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Abstract

Objective: To investigate the impact, quality and acceptability of a musculoskeletal screening clinic provided by physiotherapists for patients referred to the outpatient orthopedic department at a major metropolitan hospital.

Design, setting and participants: Prospective observational trial undertaken between 29 November 2013 and 6 June 2014 at the Northern Hospital of 52 patients with non-urgent musculoskeletal conditions who were assessed by one of two physiotherapists with postgraduate qualifications and subsequently by an orthopedic surgeon.

Main outcome measures: Proportion of new patients referred who could have been managed without needing to see a surgeon; level of agreement between physiotherapists and orthopedic surgeon on diagnoses and management decisions; and levels of satisfaction of patients, referring general practitioners and the orthopedic surgeon with the physiotherapist-led screening initiative.

Results: 45 of 52 selected patients (31 women and 21 men; mean age, 53.3 years) attended their appointment with the physiotherapist; of these, 38 also attended a later appointment with the orthopaedic surgeon. Seven of the 38 patients were listed for surgery, and seven others needed management by the surgeon (injection for three, imaging for four). Almost two-thirds (63%) were appropriate for non-surgical management. The physiotherapists identified the same patient management plans as the surgeon for 74% of the group. Patients and doctors reported high levels of satisfaction with the physiotherapist-led service.

Conclusions: Nearly two-thirds of patients with non-urgent musculoskeletal conditions referred by their GPs to one public outpatient orthopaedic department did not need to see a surgeon at the time of referral, and were appropriately assessed and managed by experienced, qualified physiotherapists.

Arcobacter – “serious hazard to human health”

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Abstract

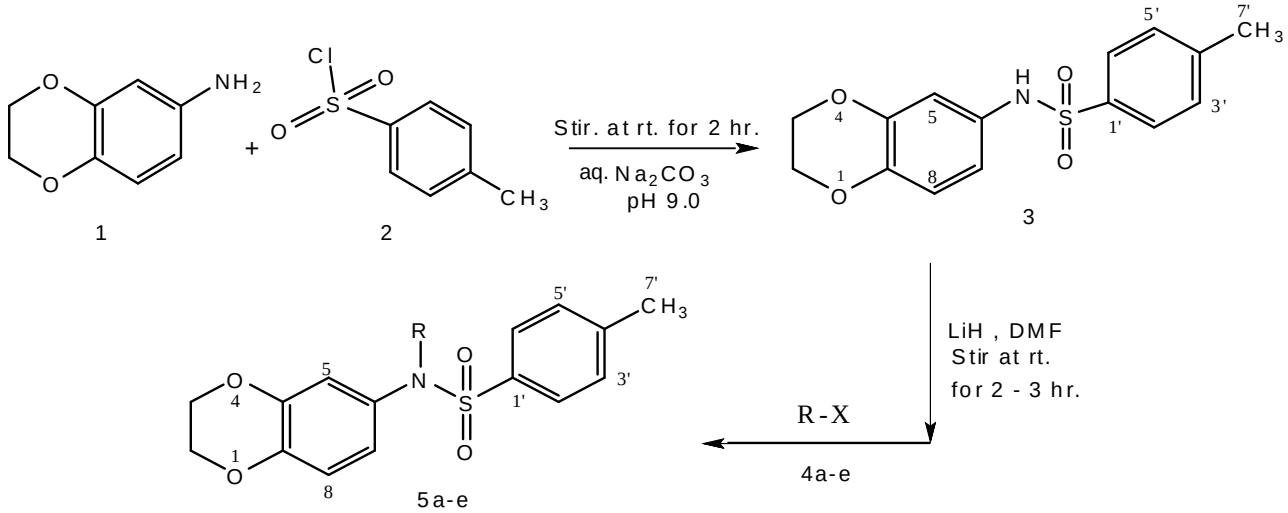
Providing safe food to human is a challenging issue today due to high occurrence of foodborne pathogens. Many of these diseases have the potential to spread through various means over long distances and becoming global problems. Foodborne diseases are a major public health problem in developed and developing countries to varying degrees. Arcobacter is one of the emerging zoonotic pathogen which belongs to family *Campylobacteracae*. Human beings get infected though various sources, among them intake of contaminated foods of animal origin and drinking contaminated water are two major routes. The species of this genus have been isolated from domesticated and non-domesticated animals, animal origin foods including beef, pork, rabbit meat, duck meat, mutton and milk, sea food and water. The presence of *Arcobacter* spp. in these foods has led to its categorization as a “serious hazard to human health”. So far, more than fifteen species of *Arcobacter* have been isolated and identified from diverse environmental niches. Among them, three species viz. A. *butzleri*, A. *skirrowii* and *A. cryaerophilus* are considered as significant human pathogens, which have been found associated with pathological conditions such as bacteraemia, septicemia and diarrhea. Generally, *Arcobacter* infection is underestimated in humans possibly because of unavailability of a standard protocol for its isolation. Many factors such as health status, age, and hypertension may predispose a person to *Arcobacter* infection. Clinical sings noticed in *A. butzleri* infections includes watery diarrhea, abdominal pain, nausea, vomiting and/or sometimes pyrexia. In addition to intestines, *Arcobacter* spp. have the capability to invade other parts of the body. Travelers’ diarrhea (TD) is commonly acquired by visitors travelling to developing countries. Emergence of antimicrobial resistant bacteria of animal and human origin has become a serious issue in terms of failure in treatment of infections, dissemination of resistant clones and exchange of resistance genes among human and animal origin bacteria. These factors may contribute to treatment failure of infections which ultimately may lead to increased morbidity and death ratio. Development of resistance in zoonotic bacteria constitutes a public health risk, primarily through the increased risk of treatment failures. A great attention need to be paid to reduce the risk of Arcobacter to public health.

Synthesis and Pharmacological Evaluation of Some *N*-Substituted Sulfamoyl Derivatives of 3'-Aminobenzo-1,4-dioxacyclohexane

Sumaira Nazir

Abstract

Sulfonamides are biologically active compounds and have vast applications in pharmaceutical industry. A series of sulphonamides have been synthesized from 3'-Aminobenzo-1,4-dioxacyclohexane. First *N*-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-4-methyl benzenesulfonamide (**3**) was synthesized by reacting 2,3-dihydro-1,4-benzodioxin-6-mine (**1**) with 4-methylbenzenesulfonyl chloride (**2**), by using 10% aqueous Na_2CO_3 solution as reaction medium. After that, the parent molecule **3** was used to react with different alkyl/aralkyl halides (**4a-e**) to achieve different target compounds **5a-e**, using polar aprotic solvent; *N*,*N*-dimethyl formamide (DMF) and lithium hydride as a base. The characterization of synthesized compounds was performed by different spectral techniques using IR, ^{1}H-NMR and EIMS. All the synthesized molecules were further evaluated for their pharmacology by screening against certain bacterial strains of Gram-positive and Gram-negative bacteria. The MIC values rendered the molecules valuable antibacterial agents.



AI-2 Quorum sensing inhibition in marine isolates

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Abstract

This study aims to test marine microorganism for their biofilm forming capability using the crystal violet *in vitro* microplate assay. We examined that whether patulin and penicillic acid, two well-known quorum sensing inhibitors (QSI) for human pathogen *Pseudomonas aeruginosa* PAO1, can prevent biofilm formation. This study reports, for the first time, the successful amplification of a putative *luxS* gene homologue in the marine microorganism *H. pacifica* ATCC 27122, as well as a log 3 fold expression of AI-2 during early and mid-exponential growth in marine nutrient broth batch cultures. Degenerated primers were designed based on the *luxS* protein sequence of ten gram-negative, □-, □- and □- proteobacteria, and used for *luxS* gene amplification in *H. pacifica*. AI-2 assays were carried out using the well-established *Vibrio harveyi* BB170 AI-2 bioluminescence assay. Effect of penicillic acid on AI-2 induction of *H. pacifica* showed strong inhibitory effect at non-growth inhibitory concentrations compared to patulin having adverse effect at the highest concentration (25 µM) tested in our study. QSIs effect on biofilm forming capability of marine isolates was isolate specific. Detection of bioluminescence in the autoinducer bioassay and the presence of a putative *luxS* gene orthologue are biochemical and genetic evidence for the production of a signaling molecule(s) and provide a first step in characterizing *H. pacifica* quorum sensing. Our study emphasize that QSI compounds must be selected in the specific system in which they are to function and they cannot easily be transferred from one QS system to another.

Roles of CFA/I, CFA/II and CS2 in biofilm formation by Enterotoxigenic *Escherichia coli*

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Abstract

Enterotoxigenic *Escherichia coli* (ETEC) strains are leading causes of childhood diarrhea in developing countries. Adhesion is the first step in the pathogenesis of ETEC *E. coli* infections and ETEC pili designated colonization factor antigens (CFAs) are believed to be important in the biofim formation, colonization and host cell adhesions. As a first step, we have determined the biofilm capability of enterotoxigenic *E. coli* expressing various types of pili (CFA/I, CFA/II and CS2) and tip mutated piliated CFA/I strains. Further, enzyme-linked immunosorbent assay (ELISA) assay were developed to compare the binding specificity of CFA/I, CFA/II (CS1 - CS3) and CS2 of ETEC *E. coli*, using extracted fimbriae and fimbriated bacteria. CFA/II strain as well as extracted pili exhibited significantly higher binding both in biofilm and ELISA assays compared to non piliated and mutant/wild recombinant strains. This indicates that co-expression of two or more CSs in the same strain is more efficient in increasing adherence compared to those having one only. Significant decrease in binding specificity of CS2 strain with deleted cotD and *CfaE*-R181 tip mutant strain indicated the important contribution of minor tip proteins in adherence assays. In addition no effect was observed on agglutination of bovine erythrocytes in R181-CotD mutant strains of CS2 showed that minor tip protein may not be important as adhesions in these strains. Isolated CFA/I, CFA/II and CS2 pili as well as bacteria expressing particular antigens on their surface bound to several intestinal cell membrane structures and play a significant role in host cell colonization. In summary, our data suggest that pili, their minor subunits are important for biofilm formation and adherence mechanisms. Overall, the functional reactivity of strains co expressing various antigens, particularly minor subunit antigen observed in this study suggest that fewer antibodies may be required to elicit immunity to ETEC expressing a wider array of related pili.

Detection of MRSA in milk and poultry meat: imperative sources of spread to human population

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Abstract

In human, food poisoning is a great problem and *Staphylococcus (S.) aureus* is frequently associated with this. The *S. aureus* is ubiquitous in distribution and is among normal microflora of skin and mucous membranes of animals and human beings. The organism is also major cause of mastitis in dairy animals, different systemic and skin infections in poultry and ultimately severe threat to the exposed human population. Poultry birds are frequently sold at retail with the skin intact, thus, the risk of *S. aureus* transmission to human population from whole raw poultry is even greater. The consumption of contaminated milk by human is also a contributing factor. The emergence of methicillin resistant *S. aureus* (MRSA) maddening the situation even more.

The detection of MRSA in milk and poultry skin was carried out by conventional (isolation, staining and identification through biochemical tests) and molecular (*16s rRNA* sequencing, *spa* typing). For *16s rRNA* sequencing, genus specific primers were used in PCR with subsequent sequencing and comparative studies in MEGA *5.0* with previously submitted sequences of MRSA to GenBank from different areas of Pakistan and world. Protein A is the surface protein which is basically a virulence factor for MRSA. It contains region X which has repetitive sequences, any variation or mutation can be detected by sequence analysis in *Spa* server. For *Spa* typing first the DNA isolation by cTAB method was carried out. The *Spa* gene was amplified by PCR with sequencing of amplified products and checking in *Spa* server.

We were able to confirm the presence of MRSA in milk and poultry skin by conventional and molecular methods. Minor variations in the sequences of isolated MRSA were observed in *16s rRNA* gene and *Spa* typing. Upon phylogenetic analysis, it was revealed that closely related sequences were from Pakistan region. Furthermore, we were able to confirm the presence of MRSA on the skin of exposed personals (poultry shops) through sequence analyses. While tackling with the skin infections in human, the presence of MRSA would be taken in account to prescribe a representative medication with anticipated good results.

Frequency Distribution of Fasciolosis in Domestic Goats (*Capra hircus*) Population of District Sargodha

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Abstract

Fasciolosis, caused by members of genus Fasciola (Platyhelminthes: Trematoda) is the most common and economically important helminth infection of livestock. A cross-sectional survey was conducted to investigate the epidemiological aspects of fasciolosis in domestic goats (CAPRA HIRCUS) of district Sargodha, Punjab, Pakistan. Coprological examination of 2268 goats was performed from all the six tehsils of district Sargodha during a year from October, 2011 to September, 2012; which revealed an overall prevalence of 39.63% (899/2268). Overall prevalence of *Fasciola (F.) hepatica* was 33.15 % (752/2268) and *F. gigantica* 6.48 % (147/2268) (χ^2=70.6325; P>0.01). Tehsil wise prevalence of disease was 40.88% in Bhalwal, 41.82% in Sargodha, 45.83% in Silanwali, 42.86 % in Sahiwal, 34.09% in Shahpur and 33.73% in Kot momin tehsils was recorded. Monthly variation of fasciolosis was also observed and found highest in July (57.69%) and lowest in January (26.92%), Breed-wise distribution of fasciolosis was 46.65% in Beetle, 37.27% in Teddy, 8.85% in Dera Din Pannah and 7.24% in Desi breed (χ^2=15.3415, P= 0.0015). Sex and age categories were found significantly associated (P-value=0.001) with the risk of disease in goat. The distribution of disease in young female, young male, adult female and adult male was 40.98%, 38.13%, 24.88%, and 21.66%. Significant association of various managemental practices with the epidemiology of caprine fasciolosis was also determined during this study. The results of this study may provide some useful data in GI parasite control in general and Fasciola control in goat in specific in the study area.

Antibiotic residues in poultry products

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Abstract

Drug residues in poultry products are still a potential threat to human health in most parts of the developing world. In depth knowledge of the presence of drug residues would be beneficial to safeguard human health. Present study was undertaken to test the presence of antibiotic residues in poultry products in Peshawar city of Pakistan. One hundred samples of fresh (n=50) and frozen (n=50) poultry products from liver, thigh and breast muscle were randomly collected and analyzed. Positive samples were isolated using well diffusion method and tested for the presence of penicillin, ciprofloxacin, amoxicillin and Colistin sulphat residues using Thin Layer Chromatography (TLC). More than half of fresh (53.3%) and frozen samples (42.2%) were confirmed positive for drug residues. Concentration of antibiotic residues was higher in liver and thigh region compared to breast muscles. Among different antibiotics, the presence of penicillin was greater followed by amoxicillin in different meat products tested. Randomly collected eggs (n=100) have shown that more than half of the eggs (60%) were positive for presence of antibiotic residues. Albumins of the eggs were detected to accumulate higher (70%) antibiotic residues compared to yolk. This depict that most of the meat and egg produced in this region was highly contaminated with drug residues and needs serious attention and concrete measures to minimize the incidence and level of drug residues to protect human health.

Hematological and biochemical indices of β-thalassemia patients on deferiprone therapy

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Abstract

The study was designed to determine the hematological and biochemical profiles of thalassemic patients using deferiprone as iron chelating agent. The aim was to find the effect of drug on blood profiles of the patients. Fifty male thalassemic patients using deferiprone since last one

year were enrolled from different thalassemic centers of Punjab (Pakistan). The average ± SD value of CBCs were: RBCs 3.7×106 ± 1×106/µL, WBCs 24.3×103 ± 8.2×103/µL, Hb 8.2 ± 1.3 g/dL, MCHC 30.1 ± 5.2 g/dL, MCV 26.7 ± 3 pg, RDW 45.1% ± 9.6%, platelets 269×103 ± 65×103/µL, ESR 54 ± 9 mm/h after 1 h. The CBCs of thalassemic patients showed significant deviation from normal values ($P < 0.05$). The biochemical parameters were: ALT 95.7 ± 33 U/L, AST 82.7 ± 16.7 U/L, ALP 208 ± 88 U/L, triglyceride 181 ± 22.7 mg/dL, cholesterol 191 ± 35 mg/dL, TP 3.8 ± 1.1 g/dL, serum creatinine 1.5 ± 0.4 mg/dL, urea 3.3 ± 1.5 mg/dL, TB 2.8 ± 0.9 mg/dL, SF 3670 ± 1457 µg/L. There was statistically significant correlation among biochemical parameters ($P < 0.05$). The altered hematological and biochemical parameters may change iron chelation response in the patients. Therefore, demography, CBC, and biochemical parameters should be considered for future plan of transfusion and iron chelation therapy.

Anticancerous potential of *Heterometrous xanthopus* (scorpion) venum

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Abstract

Anti-cancer drugs synthesized synthetically possess life threatening side effects and increased resistance to chemotherapy due its less selective action to the target tumors and its synthetic nature which leads to some serious complications and resistance to other therapeutic agents. The remedy for this problem is to design chemotherapeutic agents based on natural products as it has very selective action towards its target tumor cell receptors to bind and specifically kill that cell without affecting the surrounding normal tissues. The purpose of this study is to identify protein components responsible for anti-cancer activity in the venom of *Heterometrous xanthopus* (Asian black scorpion). The venom was subjected to reverse phase HPLC for proteins and peptides in UV absorbance for proteins for the isolation of separate proteins and then assayed separately for MTT anticancer assay along with one Crude venom fraction and one total protein fraction on hep2 liver cancer cell lines. In a total of seven fractions analyzed, two showed strongly significant inhibition on the cell lines with P values 0.0075 and 0.0093, one showed nearly significant inhibition (P value of 0.0859) while crude venom and venom total protein fractions both showed significant inhibition with P values 0.0389 and 0.0264 respectively. It is concluded from this study that venom of the experimented specie has strong cancerous activity and can be used for drug development against cancer.

Role of microorganisms in growth, production and health of dairy cattle

Shahid Zaman

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Abstract

Pakistan is an agriculture based country, where livestock contribution is about 55.1 per cent to the agriculture value added and 11.6 per cent to the national Gross Domestic Product. Although, Pakistan stands at 4th position in milk production in the world but still it is struggling to bridge a wide gap between the demand and supply of milk. The milk yield per animals in Pakistan is measly low (6 liter/day). Multiple interacting problems have been found such as: effects of nutrition, reproduction, environment and genetics. By several independent investigation, it has been well established that the major constraint in the development of dairy sector of Pakistan is poor availability of nutritive and balance feeding to livestock. This malnutrition consequently has resulted in slow growth rate, low milk yield, and poor reproductive and health performance. According to various experts' analysis, it has been inferred that with proper feeding, the production of existing animals can be increased by 30 to 35%. The ruminants in Pakistan are predominantly maintained on low grade roughage resulting in their poor nutrient utilization and productivity. The feeding pattern that could not be changed, as is total depend on availability, while feed consumption and nutrients utilization could be enhanced. The balance in rumen microbial flora plays a crucial role in feed utilization and can result in better productivity. An anaerobic rumen microorganism mainly contains bacteria, protozoa and fungi degrade ligno-cellulosic feeds. The rumen microbial profile directly depends on the microbial profile of intake feed. This ultimately impacts the productivity of dairy animals. Therefore, the feed microbial flora could be managed by using beneficial microbial supplementation.

Pathological and Molecular Diagnosis of Caseous lymphadenitis a public health threat in Chinkara Deer (*Gazella bennettii*), in Pakistan

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Abstract

Corynebacterium pseudotuberculosis is an important cause of caseous lymphadenitis (CL), a complex, chronic devastating and destructive disease of small ruminants. In present study, postmortem examination of Chinkara deer (n=25) was conducted from April 2012 to February 2014. Pus samples suggestive of CL were collected from the superficial lymph nodes, liver, spleen and lungs during necropsy and subjected to standard microbiological procedures for isolation and molecular analyses of bacterial pathogens. Pus samples collected from carcasses (25) presenting clinical lesions *C. pseudotuberculosis* infection was identified in 19 (76%) carcasses on the basis of culture characteristics. The frequency of *C. pseudotuberculosis* bacterium was increased in older animals when compared to young animals. Grossly, multiple tubercles of variable size having caseous material were observed in liver, lungs, spleen and lymph nodes. Histopathologically, tissue sections from all the visceral organs were extensively plugged with abscess. In present study specific prolineiminopeptidase (PIP) gene of the *C. pseudotuberculosis* was amplified by the Polymerase chain reaction technique (PCR) in 17(25) cases. The efficient and reliable molecular analysis along with necropsy findings in present study can be used as valuable approach for diagnosis of caseous lymphadenitis in small ruminants.

Prevalence and risk factors of Coccidiosis in cattle and buffaloes from Ravi river region, Lahore, Pakistan

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Abstract

The present study was accomplished to determine the prevalence and risk factors of coccidiosis in cattle and buffaloes of Ravi River region, Lahore from October, 2012 to September, 2013. A total of 500 fecal samples (cattle n=250; buffaloes n=250) were randomly collected and coccidial oocysts were identified using Direct Smear Method and Salt Floatation Techniques. Data regarding each sampled animal was entered in a data capture form. The *Eimeria* spp. were identified on the basis of variation in shape, size, texture, color of oocyst wall, polar cap and presence or absence of micropyle of oocysts using taxonomic key. Overall prevalence of coccidiosis in cattle and buffaloes was recorded as 57.2% and 58.8%, respectively.. Seven *Eimeria* species identified from cattle were *E. zuernii* (56.64 %), *E. bovis* (41.25%), *E. ellipsoidalis* (33.56%), *E. canadensis* (26.57%), *E. cylindrical* (21.67%), *E. alabamensis* (17.48%), and *E. subspherica* (10.45%). On the other hand, common *Eimeria* spp. found in buffaloes were *E. bovis* (52.38%), *E. zurnii* (46.93%), *E. canadensis* (31.29%), *E. ellipsoidalis* (23.12%), *E. alabamensis* (15.64%) and *E. cylindrical* (11.56%). Coccidial infection was significantly higher ($p<0.05$) in females compared to males in both species. Prevalence of *Emeria* was significantly higher ($P<0.05$) in <6 m of age cohorts (cattle, 66.66%; buffaloes, 70.31%) as compared to ≥1 year animals(cattle, 44.87%; buffaloes, 48.91%). Peak prevalence was recorded in August. A significant difference ($p<0.05$) in prevalence was observed in stall feeding confined animals compared to animals with grazing having outdoor access. Prevalence had significantly increased ($p<0.05$) in animals with poor and weak body condition than healthy. A significant difference ($P<0.05$) in prevalence was observed in diarrheic animals compared to normal feces. It is concluded that coccidial infestation is severe in both species and bovine coccidiosis is widely distributed in Ravi River region Lahore. It is suggested that an integrated strategies should be implemented for the effective prevention and control of coccidiosis in cattle and buffaloes in this region.

Therapeutic efficacy of furazolidone, amprolium and potentiated sulfonamide against coccidiosis and its effect on hemogram in buffalo calves

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Abstract

This study was aimed to evaluate the therapeutic efficacy of furazolidone, amprolium and trimethoprim/sulfadiazine in buffalo calves naturally infected with coccidiosis and to determine the effect of coccidiosis on various blood parameters. Thirty buffalo calves were randomly divided into five groups designated as A, B, C, D and E comprising six animals each. Calves in groups D and E served as positive control (infected non-treated) and negative control (non-infected non-treated), respectively. The calves in group A were treated with trimethoprim/sulfadiazine at 30mg/kg body weight (BW) whereas animals in group B were given furazolidone at 10mg/kg BW. The members in group C were administered amprolium at 10mg/kg BW. Oocyst per gram (OPG) of each group was determined at day 0 (pre-treatment) and days 7, 14, 28 (post-treatment) by the McMaster Technique. At days 7, 14 and 28 the OPG values decreased significantly ($P<0.05$) in groups A, B and C compared to group D. The efficacy of furazolidone, emporium, and trimethoprim/sulfadiazine was 99, 100, and 99 percent, respectively at the end of the experiment on day 28. Statistically non-significant difference ($P>0.05$) in efficacy was observed among three drugs. No adverse effect of either of the drugs was observed. A significant reduction (*P<0.05*) in total erythrocytic count, hemoglobin concentration, packed cell volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration was observed at day 0 in calves of groups A-C as compared to group D. Whereas, total leukocyte count and differential leukocyte count (except lymphocytes) were significantly increased (*P<0.05*) in calves of groups A, B and C compared to calves in group D. On the contrary, at day 28, all these blood parameters reversed to normal ranges except calves of group D. In conclusion, coccidiosis has deleterious effects on hemogram. Moreover, furazolidone, amprolium, and trimethoprim/sulfadiazine are equally effective to treat coccidiosis in buffalo calves under field conditions and the values of altered hemogram can be reversed to normal using either of these drugs.

Clinico-hematological and biochemical studies on Naturally Infected Camels with *Trypanosomiasis*

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Abstract

Blood borne diseases such as trypanosomiasis have negative impacts on health, production and working efficiency of camels in different camel-rearing areas of the world including Pakistan. In present study blood samples were collected from camels kept at desert condition of cholistan to estimate the prevalence of trypanosomiasis and hemato-biochemical changes in naturally infected cases. Results showed an overall 9.31% prevalence of trypanosomiasis in camels. Various clinical signs such as pyrexia, occasional shivering, inappetance, urticarial swelling, lethargy, going down in condition and edema of pads were observed in few cases. The statistical analysis did not show significant association of age and sex with trypanosomiasis. However, results revealed significantly decreased values of total erythrocyte counts, packed cell volume, hemoglobin concentration, mean corpuscular hemoglobin concentration, serum total proteins and albumin while increased values of mean corpuscular volume was recorded in infected animals as compared to healthy. A significant ($P<0.01$) increased values of total leukocyte count, monocyte, lymphocyte, neutrophils and eosinophils was recorded in infected animals. Moreover, microscopic examination of blood films obtained from naturally infected cases showed presence of parasite and various morphological changes in cells such as stomatocyte, hypochromacia and polychromacia. Significantly increased values of different hepatic enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were also recorded.

Spatial cluster analysis of human cases of Crimean Congo Hemorrhagic Fever reported in Pakistan

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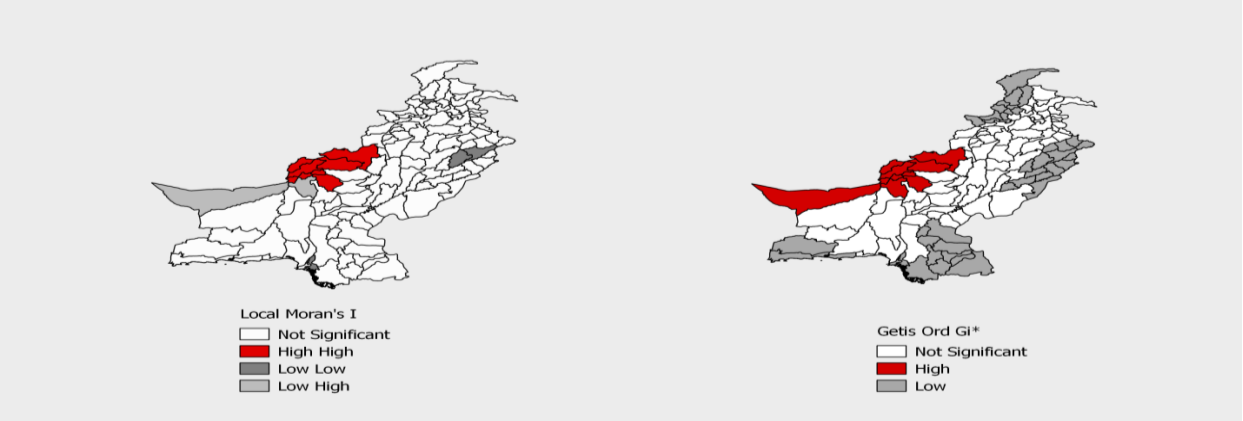
Abstract

Background : Crimean Congo hemorrhagic fever (CCHF) is a tick born viral zoonotic disease that has been notified from almost all regions of Pakistan. The aim of this study was to investigate spatial distribution of CCHF cases reported to National Institue of Health , Islamabad during year 2013. Spatial statisics were applied to detect spatial autocorleation and clusters of the disease based on adjusted cumulative incidence per million population for each district. Results: The data analyses explored a large multi-district cluster of high values in the uplands of Balochistan province near Afghanistan border. The cluster included following districts: Pishin; Qilla Abdullah; Qilla Saifullah; Quetta, Sibi; Zhob; and Ziarat. These districts may be given priority in CCHF surveillance, control programs, and further epidemiological research .



Figure

Map of Pakistan displaying output of Local Moran's I and Getis- Ord Gi* analyses based on district-level adjusted cumulative incidence of Crimean-Congo Hemorrhagic Fever per million population in 2013. Local Moran's I statistic detected **high - high (red) clusters**, low- low (dark gray) clusters, and low-high (light gray) type outliers. Getis- Ord Gi* also identified **hot spots (red)** and cold spots (gray).

Raw milk and its marketing system – A Threat to Human Health.

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Abstract

Dairy sector plays a vital role in providing high quality food for the people of Pakistan. The annual milk production is more than 50990 thousand tons in the year 2013-14. Pakistan ranked 4th largest milk producing country in the world. In Pakistan, 95% milk is sold in raw form and it could pose a potential threat for the consumers. The good animal health and proper hygienic conditions on the farm are important considerations not only for the welfare of animals but also for the quality and wholesomeness of the raw food products for the human consumption. Good herd management demands that efforts should be done to keep the animals free from diseases. Such diseases as tuberculosis, brucellosis and mastatitis. All the animals in the herd must be tested for milk borne diseases regularly. On the other hand, a number of dairy producers are not aware with the zoonotic potential of the most common zoonotic bacterial contaminants in milk include *Samonella spp.,* Listeria spp., Mycobacterium bovis and *Brucella abortus*, *Coxiella burnetii*; *Mycobacterium avium* subspecies paratuberculosis, Campylobactor species; Coliforms, including *E.coli.* Stretegies like screening and culling of *Mycobacterium bovis* and *Brucella abortus* from our dairy herds could help to eliminate the zoonotic potential of these e pathogens.. Likewise, Mycobacterium Paratuberculosis (Johne's disease) vaccination of cattle herds may help to avoid crohn's disease in humans.

Prevalence and significance of hemoparasitic infections in cattle, Comparative efficacy of diminazene and butalex against theileriosis in cow calves of Pakpattan, Pakistan.

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Abstract

The hemoparasitic infections are major threat to the cattle population of the livestock and dairy industry, particularly the cross bred cows are more prone to the hemoparasitic infections. As due to the prevalence of the hemoparasitic infections, the devasting losses to the livestock and dairy sector have been reported throughout world. The hemoparasites have great impact on the economics of livestock department due to decreased production of animals (milk, meat leather etc). The most of the hemoparasites are intracellular, so having the great impact on the hematological profile of the affected animals. The hemolytic anemia is the major clinical manifestations of the hemoparsitic infections in bovines. Keeping in view the importance of the hemoparasitic infections the present study was designed to determine the prevalence and significance of hemoparasites in cattle of district pakpattan, Pakistan. A total of 1450 blood samples were collected in sterilized, disposable vacutainers containing anticoagulant, from cattle of randomly selected villages of district pakpattan. All the samples were processed and examined in Animal Disease Diagnostic Laboratory, pakpattan, Directorate of Animal Disease Reporting and Surveillance, Livestock and Dairy Development Department Punjab, Lahore. The thin smears of all the blood samples were prepared and stained with Giemsa's stain; those were used for screening of the hemoparasites. All the prepared thin smears were observed under oil emersion lens for the identification of hemoparasites. The microscopic examination of the thin blood smears stained with the Giemsa's stain revealed that an overall prevalence of the hemoparasites was 27.31%. Theleiriosis was the most prevalent (12.83%) hemoparasitic infection in cattle followed by the anplasmosis (8.27%) and babesiosis (6.21%), respectively. It was also observed that prevalence of theileriosis was higher in cross bred cow calves as compared to the adult animals. The 18 calves that were found positive for theileriosis were used to determine the efficacy of diminazeze and butalex against the theileriosis. Out of 18 infected calves, 9 were treated with butalax along with the long acting oxytetracycline while the other 9 were treated with diminazine along with long acting oxytetracycline. The efficacy of butalex was determined as 100% while the efficacy of diminazene was found as 88.89%. The butalex was found to be highly effective against the theileriosis in cross bred cow calves as compared to the diminazene.

Prevalence of theileriosis in cattle and buffaloes; comparative efficacy of butalex and diminazene against theileriosis in cattle of Mandibahuddin, Pakistan

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Abstract

The devastating losses to the dairy and livestock industry have been observed due to the prevalence of hemoparasitic infections throughout the over the world. Theileriosis is one of the most prevalent hemoparasitic diseases of bovine particularly the cross breed cows. Theileriosis is a fatal disease of cross breed cows and is caused by *Theileria annulata* and *Theileria parva*; it is transmitted by certain Ixodid ticks of genus *Hyloma*. *Theileria annulata* is considered to be the most pathogenic species of theileria and is widely distributed in many areas of the world. As the bovine theilerial species are intracellular parasites thus, having the great impact on hematological parameters.

The present study was planned to investigate the prevalence of theileriosis among the dairy breeds of cows and buffaloes of district mandibahuddin, Pakistan. In this study, a total of 1400 blood samples (700 from cows and 700 from buffaloes) from 30 different villages of district mandibahuddin were collected in sterilized, disposable vacutainer tubes containing anticoagulant. All the collected blood samples were used to analyze the hematological parameters through hematology analyzer in Animal Disease Diagnostic Laboratory, Mandibahuddin, Directorate of Animal Disease Reporting and Surveillance, Livestock and Dairy Development Department, Punjab, Lahore. The thin smears of all the blood samples were prepared and Giemsa's stain was used for screening of the hemoparasites. All the prepared thin smears were observed under microscope and morphological changes in the erythrocytes were identified. The prevalence of theileriosis among the dairy breeds was investigated as 8.43% (59 out of 700) and as 27.71% (194 out of 700) both in buffaloes and cattle, respectively. Out of 194 infected cows, 97 were treated with butalax along with the long acting oxytetracycline while the other 97 cows were treated with diminazine along with long acting oxytetracycline. The cows treated with the butalex were cured 99% while cows treated with diminazene were cured 93%. There was no significant difference between comparative efficacy of butalax and diminazin for the treatment of theileriosis in cattle was observed, although the butalax is more effective as compared to the diminazine against the theileriosis in cattle. It was also observed that the prevalence of theileriosis was correlated with the tick infestation that was recorded high during hot and humid season.

Clinico-bacterialogical investigation of sub-clinical and clinical mastitis in dairy Goats

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Abstract

Mastitis is one of the common management associated problem of goats. In the present study, mastitis prevalence was studied in Dera Din Pannah, Beetal, Teddy, Desi and non-descriptive goat breeds reared in the areas of District Muzaffargarh. The study was aimed at investigating the pathogenic bacteria of mastitis and their susceptibility to various antibiotics. Milk samples were also collected from goats and subjected to Surf Field Mastitis Test for screening. The positive samples were cultured on blood agar, tryptose agar and MacConkey's agar for bacterial isolation. *Staphylococcus aureus* (53.73%), *Streptococcus agalactiae* (26.86%) and *Streptococcus dysagalactiae* (19.40%) were investigated. Additionally, two thousand milk samples from 1000 lactating goats of different breeds were tested. Out of which 435 (21.70%) were found positive for subclinical and 125 (6.25%) of clinical mastitis while chronic mastitis 64 (3.2%). Breed wise prevalence rate was 26.82% in Beetle, 19.67% in Teddy, 26.315% in Dera Din Pannah, 19.23% in Desi, 18.60% in non-descriptive breeds of dairy goats for subclinical mastitis. Enrofloaxin, Ciprofloaxin, Ampicillin, Gentamycin, Oxytetracycline, Penicillin, Amoxicillin, Norfloxacin, Chloramphenicol and Streptomycin were used for anti-bio-gram profiling against bacterial isolates. Norfloxacin, Enrofloxacin and Ciprofloxacin were found effective *in vitro*.

Clinico-Heamatological and Mutagenic Changes induced by Arsenic and Copper Sulphate in Adult Male Birds

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Abstract

The present experimental study was conducted to determine the toxicological effects induced by oral administration of arsenic and copper sulphate in adult male birds. After acclimatization all the male birds were randomly divided into 7 groups each having four birds. The entire experimental group received arsenic and copper sulphate together in different combination and alone. The duration of this experiment was 30 days. The blood samples were collected from each bird at day 10, 20 and 30 of the experiment. Various clinical signs like decreased feed intake, body weight, ruffled feather, depression, dullness, ocular discharge, open mouth breathing, diarrhea and pale comb were observed at higher levels of arsenic and copper sulphate. In treated birds the values of total erythrocytes counts, leukocyte counts, hemoglobin concentration and mean corpuscular hemoglobin concentration decreased while pack cell volume and mean corpuscular volume increased significantly. Significantly higher values of erythrocytes with micronuclei, blabbed, lobed, vacuolated, notched, broken egg nuclei and cells with nuclear remnants were increased. From the results of this study in can be concluded that arsenic and copper sulphate alone at higher levels and in combination even at lower levels poses serious clinico-hematological and mutagenic effects in avian species

Hemato-biochemical and Mutagenic effects of Triazophos in Fresh water Fish *Labeo rohita (Rohu)*

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Abstract

In present experimental study micronucleus assay and nuclear abnormalities were used as a biomarker to assess the cyto-genotoxic potential of different concentrations of triazophos insecticide in fresh water fish. For this purpose 16 fresh water fish of same age and weight were kept into four equal groups (A-D) having four each. All the fish were kept in aquaria with 75 liter water capacity for six days for acclimatization. Various sub lethal concentrations of organophosphate insecticide triazophos (0.010, 0.015 and 0.20 ppm) were tested. For hematological and cyto-genotoxic studies blood samples were collected from each fish after 48, 72 and 96 h of post treatment. Duplicate thin blood smear was made from fresh blood of each fish. No clinical and behavioral abnormalities were observed in fish kept in control group. However, mild to severe clinical signs such as loss of equilibrium, gasping, opercular movement, increased mucous secretion in gills and restlessness were observed in experimental organisms. Results revealed that total erythrocyte count, pack cell volume, hemoglobin concentration, serum total proteins, mean corpuscular hemoglobin concentration, lymphocyte and monocyte values were significantly decreased while mean corpuscular hemoglobin and leukocyte count was significantly increased. Overall significantly higher frequency of erythrocyte with micronuclei, blebbed nuclei, lobed, notched, vacuolated, heteropicnotic nuclei, binucleated and pear shape erythrocyte was observed. Levels of different enzymes and lipid peroxidation products were significantly elevated in exposed fish. Therefore from the results of this study it can be concluded that triazophos poses adverse clinico-hematological and mutagenic effects in fish.

Assessment of Glucose Transporters in Development of the Mammalian Placenta using Rabbit as Animal Model during Successful Pregnancy

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Abstract

The early-mid pregnancy in mammals is very critical for the continued existence of the growing fetus and most of the embryonic loss occurs during that period. Since Glucose is essential as major energy source for metabolism during the period of extensive organogenesis and embryogenesis in mammals at placentation period during successful pregnancy. Therefore, the most important theme of the current study is to examine the appearance and localization of Glucose Transporters at the process of normal mammalian placentation using rabbit as an experimental model. Glucose transporters (GLUT1 and GLUT3) were proposed as a fuel supplier in functional mammalian placenta for developing fetus during successful pregnancy. Experiments were conducted on the normal placentas, maternal and fetal blood and fetal liver collected from the Japanese white rabbits during days 8-28 of pregnancy. The study was focused on Glucose Transporters GLUTI and GLUT3, proposing as an essential transporter of maternal glucose in mammal placentas. Glucose is essential for fetal growth, since fetus is unavailable for their own produced glucose until late pregnancy. Our results showed that glucose level was elevated in maternal blood as well as fetal blood and liver, accompanied with pregnancy progress. Real-time RT-PCR analysis on the rabbit placentas demonstrated that GLUT1 transporter were prominently elevated at day 18 and maintained before parturition period (day 28). GLUT3 was up- regulated at early placentation period (day 13) and then down-regulated after day 18. Immunohistochemical study confirmed the abundant localization of GLUT1 at labyrinthine region at outer trophoblast layers at day 25, which form blood sinuses filled with maternal nutrient blood. Immunoreactivity of GLUT3 was found in the inner trophoblast layers around the fetal blood vessel at day 25. Elevation of GLUT1 and GLUT3 is well enough to make the availability of glucose to the developing fetus and indicate a possible effect to act on placental vascular system for nutritional transportation. Localization of GLUT1 in outer trophoblast and that of GLUT3 in the inner trophoblast demonstrated that GLUT1 is responsible for transportation of glucose to the maternal-placental circulation whereas GLUT3 for the placental-fetal circulation. Therefore, the present study suggested an essentiality of both transporter for glucose availability to the functional placenta and growing fetus in rabbits.

Based on high expression of Glucose Transporters until day 18 placentation period and localization in the vascular system or in the diverse types of trophoblast, it is evident that they play a fundamental role in the placentation process as well as in fetal growth. Glucose transporters were supposed to work on the transplacental mechanism for fetal nutrient and maintenance of successful rabbit pregnancy. Thus alteration or disturbance in any of transporters during placentation periods would badly affect reproductive condition of the rabbit

placenta, which results in disturbed supply of essential nutrient to the developing fetus or defective vascular development of placenta.

These data can serve as a baseline for future researches, such as maternal hypoglycemia or hyperglycemia that are available experimental rabbit as a useful model, to evaluate various prenatal stressors on the development of placenta and fetus.

REPRODUCTIVE PERFORMANCE OF CROSSBRED COWS IN AZAD KASHMIR

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Abstract

Data of 235 breeding and performance records of 55 crossbred crossbred cows (Jersey x indigenous), maintained at Livestock Development Research Centre (LDRC) Muzaffarabad, Azad Kashmir, Pakistan over a period of 23 years, from 1989-2002 was analyzed and studied for different reproductive traits and an effort was made to estimate the magnitude of various environmental sources of variation on these reproductive traits. The least squares mean for age at first service and age at first calving in crossbred cows in present study was 676.54+13.25 and 968.79+2.41 days. The analysis of variance revealed a non-significant effect of year and season of birth on age at first service and age at first calving. The least squares means for services per conception, postpartum oestrous interval, service period calving interval and dry period was 1.5 65.32+34.33 days, 93.38+21.61 days, 368.31+20.65 and 151.03+65.64 days. Analysis of variance revealed a non-significant effect of year and season of calving on Post-partum oestrous interval and service period. Dry period was influenced by the year of calving but a non-significant effect of season of calving on dry period was noticed in this study. The effect of year of calving on calving interval was non-significant, while the effect of season of calving on calving interval was significant. Parity had non-significant effect on all the reproductive traits.

Antibiotic resistance in *Campylobacter jejuni* isolated from turkey flocks in Germany

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Abstract

The development of antibiotic resistance among *Campylobacter* isolates recovered from poultry has increased dramatically. Monitoring the progress of this resistance becomes a growing public health issue. Uses of molecular techniques to study resistance mechanisms are available and also essential for monitoring programs. This study provides sufficient data about the current status of the antimicrobial susceptibility to 76*C. jejuni* isolated from 67 turkey farms in different region in Germany. The use of a rapid and a simple molecular assay proves useful option for clinical diagnosis or epidemiological studies for rapid detection of developed resistance in *C. jejuni* to frequently applied antimicrobial agents in poultry production.

The methods are used for the detection of resistance and the judgement varies between the laboratories. To correctly monitor the development of the antimicrobial resistance in an area, for comparison between laboratories quality assurance and ring-trials are necessary.

Campylobacter as a zoonotic pathogen is exposed to antibiotics used in both animal production and human medicine. For clinical therapy of campylobacteriosis, erythromycin (a macrolide) is considered to be the drug of choice, but fluoroquinolons e.g., ciprofloxacin are also frequently applied because of their broad spectrum of activity against enteric pathogens. Alternative drugs including tetracycline were also applied in cases of systemic infection. These drugs are also used in poultry production for treatment of several infections.

As increasing antimicrobial resistance is an alarming trend that limiting the number of therapeutic options and making an empirical treatment more difficult. Therefore a coordinated monitoring system for detection of resistance prevalence is mandatory.

Socio-Economic Impact of Parasitic Infestations on Livestock Production in Developing Countries

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Abstract

Livestock are important in supporting the livelihoods of poor farmers, consumers, traders and labourers throughout the developing world. Many qualitative statements can be found in the literature in this regard. Parasitic infestations are endemic worldwide constituting greatly as a single worldwide cause of illness, disease and loss of productivity in food animals and cause substantial economic losses. Poor hygienic conditions, poverty, illiteracy, lack of access to clean drinking water, infected range conditions and hot-humid tropical climate are the important factors contributing to the most of parasitic infestation in developing countries. Many parasitic infestation can in fact be transmitted between vertebrates (animals and man) and 'about 20 species are of public health importance causing severe or fatal infestation. The effect of the infestation is determined by a combination of factors amongst which the varying susceptibility level of the host species, the pathogenicity of the parasite species, the host/parasite interaction, and the ineffective dose are the most important ones. The economic losses are closely associated with the extent to which the pathogenic effect of parasitic infestation influences the production of the individual animal. In many parts of Africa and Asia parasitic infestation are responsible for enormous economic losses, hampering especially rural development programmes and reducing the pace of economic growth. Estimates of financial loss are based on mortalities in young animals and the failure of adult livestock to reach potential normal mature weights (weight loss). These are assumed to vary according to the severity of infestation. The consequences of parasitic diseases of livestock and of their control are part of a complex involving many socio-economic factors and therefore consequences of disease should not be measured without considering the other related factors. These facts are based on different reports written by experts. Consideration should be given to the need for and the value of studies on the socio-economic impact of parasitic diseases and, in turn, of the impact of socio-economic factors on parasitic infestations and their control. For poor livestock keepers, who pays and who benefits is a crucial question when it comes to planning and targeting control and eradication efforts for the parasitic infestation in livestock that really support their household

livelihood. Finally, suggestions are made as to the feasibility, scope and design of such studies and based upon that suggested routine procedures adopted by the farmers for controlling the parasitism for the farmers prevailing conditions. There is an urgent need to put in place control measures for all types of parasitic infestation in Africa and Asia. This target could be potentially achieved by a package approach of control measures for parasitic infestation as an important component of overall farm activity and it can be boosted by farmer education program and participatory research in developing countries.

Utilization of E-Resources For Surveillance of Parasitic infestations in Pakistan

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Abstract

Surveillance systems are of great importance for monitoring and controlling parasitic infections and infestations. Developing countries are facing acute problems in maintaining public health and they have poor or even no surveillance systems for disease control. E-resources provide sufficient means for effective surveillance and control of diseases due to parasites. Developed countries have setup strong and effective surveillance systems using software programmes and e-surveillance. By using internet and other e-resources effective e-surveillance system would not help in control of parasitic infestation nation-wise but also help in making policy decisions those would lead to improved health status of the nation. The salient feature include fast track information tracking, national database formation, history records, effective check-up and prompt solution, rapid information flow and setting up quarantine facilities.

Mechanical transmission of blood pathogens by biting insects

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Abstract

Biting insects such as tabanids and stomoxes are present worldwide in all kinds of landscapes and climates. In humans and animals, these insects are not only blood feeders and annoying pest, they are also acting as mechanical vectors of a number of pathogens including bacteria, viruses and parasites. Performing as small syringes, they can take infected blood from a first host, and, following an interrupted bloodmeal, inoculate a small amount of blood remaining on the mouthparts (0.05-10 nanoliters) into nearby host a few seconds or minutes later, at short distance. Due to the short survival of pathogens in the insect mouthparts (10-40 minutes), mechanical vectors are generally considered as intra-herd transmitters. To assess the importance and main parameters of mechanical transmission, we performed a series of experiments on mechanical transmission of African trypanosomes by Atylotus sp. (Diptera: Tabanidae). The main parameters of the model were either quantified (parasitaemia, insect burden, daily prevalence) or estimated (unknown parameters), and a mathematical model was developed. Within 3 weeks of exposure to insect bites, the incidence of the infection was above 60% which clearly demonstrated the efficiency of mechanical transmission. Number of insects and rate of pathogens in the blood were the main parameters of transmission which proved to occur when the pathogenemia is above 100.000 infective doses per ml of blood. Stable flies are another mechanical vector, suspected to be responsible of the transmission of Trypanosoma sp., Anaplasma, Bovine Leukemia Virus, Besnoitia etc. Importantly, Stomoxys would not only act as immediate transmitter like other biting flies, but they are also suspected of delayed transmission by regurgitation of blood from crop or gut. Because stomoxes are able to feed at various time intervals (6-48 hours), they are suspected to transmit pathogens some hours after being contaminated. Thus, they would be able to transmit a disease from a group of animals to another group of animals, when they share a pasture or a water point, or, if they live in close areas. Stomoxes would act both as intra-group and inter-group transmitters, which would considerably impact their role in the epidemiology of a number of diseases. Mathematical models could be developed for tabanids and stomoxes and adapted to various pathogens, providing their specific parameters be established in experimental conditions. Because mechanical vectors are present everywhere, they may contribute to the transmission of any pathogen when the pathogenemia is above 100.000 infective doses per ml of blood. Based on a better knowledge of their role and their biology, new means of control of tabanids and stomoxes are currently under study to specifically attract these insects to toxic targets.

Immunological aspects in the detection of certain parasitic infections of veterinary importance

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Abstract

Parasitic infections cause diseases and death both in human and animals which ultimately leads to economic loss of the country. Traditional diagnostic methods identify a pathogen based on its phenotype. The correct assessment of a clinical isolate takes more time. The diagnosis of a parasitic infection is usually made by the direct demonstration of the adult parasite or developmental stage, in blood, faecal samples or biopsy material.

Parasitic infections are conventionally diagnosed by microscopy, which is considered as the "Gold standard", as it is simple and does not involve the purchase and maintenance of expensive equipments. However, various problems are associated with microscopy as a diagnostic tool. For example, some parasites are morphologically similar or very small and difficult to stain and detect. Consequently, well trained microscopists are essential for accurate identification and this holds true for many parasites. Furthermore, culture of parasites may be necessary in some cases. Specialized media and laboratory facilities are required for culture. Besides, there is a relatively long period before results are obtained. Diagnosis by microscopy is also extremely labour intensive, especially when a large number of samples need to be examined in a relatively short time, such as during epidemiological studies.

Parasitological methods do not succeed if the density of parasites in the specimen is below the sensitivity of the method employed or if the parasite cannot be demonstrated in the host as is the case in occult infections such as cystic ecchnococcosis, cysticercosis, sarcosystosis etc. In such cases indirect methods have to be employed. Faster and simpler methods of diagnosis is of great advantage.

To overcome some of the difficulties encountered during microscopy of parasitic diagnosis, serological diagnostic methods have been developed. However, some of the limitations associated with serology are inability to differentiate between current and previous parasitic infections. Further, serological tests have limited value while examining individuals from endemic areas with high circulating antibodies. In addition, the cost of producing specific purified antigens is generally very high and consequently crude antigen preparations are often used, resulting in reduced specificity and sensitivity.

Highly specific antigens require intensive efforts to cultivate protozoans and helminthes. Specificity is still not satisfactory in majority of the tests and cross reactions still interfere with the precise interpretation of serologic results. The need for better preparation of antigens by biochemical and molecular biological methods still exists. The monoclonal antibody technique may help to meet the demand for purified fractions or even pure antigens to improve serodiagnostic methods.

Immunodiagnosis of parasitic infections can be made by detection or identification of an antigen or detection of specific antibody in serum so as to determine whether host has been exposed to a specific antigen. However, host immune responses to parasitic infections measured by a variety of conventional immunological tests may provide little or no correlation with protective immunity. This could be due to responses elicited to infection which are irrelevant to the continued well being of the parasite with no protection to the host. The responses to antigens may not be critical to parasite-survival or may even be the result of immune evasion by the parasite. Protective immunity should be based on measurement of the development, growth, reproduction and survival of parasites and can be made by the determination of in-vivo expression of resistance, in vitro analysis of effectors to kill parasites or manipulations of host and parasite in experimental systems.

The development in serodiagnostic methods, however, which are easy and cheap to use in countries with a population at high risk in contracting parasitic infections, has been neglected. The value of serology for clinical diagnosis depends on the interaction of the specific parasite and the host. Parasites do not only stimulate the humoral immune response as part of the immunological defense mechanisms but they are also capable of evading the immune response to exposed epitopes. Each serological assay has advantages or disadvantages in regard to sensitivity, specificity or cost effectiveness. The combination of at least two different test systems will increase the diagnostic reliability. That is why molecular biology technique is the first and foremost choice.

Genetic analysis is a step forward for identifying the target and thereby confirming the diagnosis. In epidemiological term the organism involved in the outbreak is clonally related at genome level and share virulence factor, biochemical traits and genomic variation resulting in the existence of a subtype. DNA based diagnostic rely upon the detection of the genome at nucleotide sequence level. They not only can confirm the presence of a particular pathogen but also dissects its genome to show its type and strains. The molecular tests have virtually revolutionized the basic understanding on pathogen with respect to its origin, virulence and

biochemical characteristics. Thanks to the recombinant DNA and PCR technologies, which along with other disciplines are responsible for the rapid evolution of molecular tests.

Advancements in parasite genomics and proteomics have opened up new frontiers of knowledge on parasite biology and improved diagnosis and control measures taken against these pathogens. The tools of molecular biology are increasingly becoming relevant to parasitology. Research in parasitology has been greatly influenced by the advancements in biotechnological tools. The sequencing of the complete genomes of parasitic helminths and protozoa is allowing great advances in studying the biology and improving diagnosis and control of parasites. Unique DNA sequences provide very high levels of specificity for the diagnosis and identification of parasite species and strains and polymerase chain reaction (PCR), a unique biotechnological tool allows extremely high levels of sensitivity.

New techniques, such as the use of uniquely designed molecular beacons and DNA microarrays will eventually allow rapid screening for specific parasite genotypes and assist in diagnostic and epidemiological studies of veterinary parasites. The ability to use genome data to clone and sequence genes which when expressed provides antigens for vaccines and receptors and enzymes for mechanism-based chemotherapy screening.

This keynote address will focus on Immunological Aspects in the detection of certain Parasitic Infections of Veterinary importance.

Biological and integrated control of dengue vector using different *Mesocyclops* from Punjab Pakistan

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Current study was evaluated for the biological control of *Aedes aegypti* larvae using *Mesocyclops* collected from different water bodies. The main objective was to control *Ae. aegypti* larvae using environment friendly method with no toxic effect on human and other animals. Single species culture of different species of *Mesocyclops* was prepared by using pure stock culture of *Chilomonas* and *Paramecium* in laboratory. Predatory capacities of the three identified species *M. aspericornis, M. pehpiensis and M. ogunnus* was evaluated in laboratory at different larval densities using different size containers. The mean predatory capacity/rate of female and male *M. aspericornis, M. pehpiensis and M. ogunnus* in 200 ml water was 83, 69 and 63% and 62, 60 and 50% at 10 larval densities post 24 hours exposure respectively. In general predatory capacity increased with decreased larval density. Moreover, predatory capacity of different species of *Mesocyclops* decreased with increasing volume and size of the containers against different larval density (25-250). In addition best predatory ratio was 1:20 with fixed number (100) of first instars in 200 ml container post 24 hours exposures. However, predatory capacity increased by increasing number of copepods. Moreover, predatory capacity/rate of various *Mesocyclops* against 1st instars *Aedes* larvae decreased in the presence of alternative food. Predatory effect was enhanced when *M. aspericornis, M. leukarti and M. ogunnus* were integrated with only 0.1ppm *Bti* WDG where 100-80% larval mortality was observed 02-10 weeks post exposure in small containers. In general, the three identified species from Punjab has high potential for biological control of *Aedes* larvae. Overall *Mesocyclops aspericornis* is an effective predator as compared to the other two species for the control of dengue vector from Lahore, Pakistan.

Zoonotic trematode infections and their Control

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Abstract

Potentially every dimension of man's environment is a source of parasitic infection – The ground he walks, eg: Ancylostoma larvae – cutaneous larva migrans – The water he drinks Eg: Entamoeba – amoebiasis, Giardia - Giardiosis etc. The degree of risk to which he is exposed depends on geographic, climatic and socio-economic factors.

Water gets polluted in many ways – Faeces of man, domestic and wild animals (Entamoeba & Giardia sps), Infection from water, By drinking water (Ascaris-Visceral larval migrants Dracunculus), When the enters in it (Schistosoma-Cercarial dermatitis), Through IH developing in (Diphyllobothriosis, Artifechinostomosis, Opisthorchosis, Malaria, Filariosis). By consuming infected aquatic plants as well as through irrigated crops/vegetables etc. (Fasciolopsis-water chestnuts, Fasciolosis-vegetation).

Pollution of the environment is associated with Faecal pollution of the water supply, the soil and the vegetation, especially in market gardens etc.

In polluted water reservoirs and streams, various parasites and vectors of parasites may develop and persist. The parasitological problems associated with sewage have become important in almost every part of the world.

Soil pollution is primarily by eggs and larvae of different parasites (helminths and protozoan cysts) is a scourge of some rural and urban areas (Ascaris, Toxocara-Visceral Larva Migrans, Entamoeba-amoebiosis, Toxoplasma-Toxoplasmosis). Soil pollution is hazardous where night soil or inadequate treated sewage sludge is used for manuring crops, especially plants consumed raw. (Cysticercosis / Neuro Cysticercosis).

Our knowledge about the control of such large-scale pathogen dissemination is still limited.

The effect of air pollution and the influence of synanthropic vegetation in rural and urban areas on the incidence of parasites and parasitic infections are practically unknown.

A WHO expert committee on microbiological aspects of food hygiene grouped food borne parasites into two categories.

Parasites of which the infective stages occur naturally in food (meat, fish, molluscs, etc) Eg: Taenia, Diphyllobothrium, fish borne Trematodes and Toxoplasma.

Parasites that are derived from the environment (soil, water etc) through animals or food handlers and whose infective stages occur as contaminants in food etc., Eg: Echinococcus, Fasciolopsis, Ascaris, Dracunculus, Toxoplasma, Glardia, Entamoeba, etc. (WHO, 1969).

Zoonotic parasitic infections naturally transmitted between animals and man assumes increasing importance in the modern world. In part, this is due to economic development, increased contact of man with rural or sylvatic environments. By tourism and increased leisure time and the increase in domestic pets in urban and suburban areas are a measure of this. In part, it is due to the increasing urbanization and the need for companion animals. In part, it is due to factors inimical to economic development, such as the migration of rural operations or refugees to cities, political unrest and natural disasters. Finally it is due to modifications of the environment wrought by man. The large quantities of animal wastes from animal breeding establishment (pigs, poultry, cattle, etc) dairies, abattoirs and carcass-disposal constitute another health hazard that must be taken into account. Environment factors such as soil, water, food, air and vector are important parameters to help in the transmission of parasites. A large number of parasites are capable of producing clinical manifestations in animals and human beings in their larval or adult forms. Of the various trematode infections intestinal, Amphistomate, Echinostomate, Heterophyid, Hepatic, Pulmonary, Blood flukes etc., are considered to be more important trematodes (flukes) of zoonotic importance.

Polymerase chain reaction and restriction fragment length polymorphism based diagnosis of trypanosomiasis in horses in Lahore region of Punjab, Pakistan

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Abstract

Trypanosomiasis, also known as trypanosomosis, is an important protozoal disease with a diverse range of susceptible host including human. In the present study, a pan-trypanosome polymerase chain reaction (PCR) along with restriction fragment length polymorphism (RFLP) was optimized to characterize the important trypanosome species of horses and to replace multiple PCR tests with a single PCR assay. Blood samples from two hundred and thirty (230) horses, manifesting clinical picture of trypanosomiasis, were subjected to screening for trypanosomes through microhaematocrit centrifuge technique (MHCT), simple PCR, semi-nested PCR and RFLP. Microscopic examination of Giemsa stained blood smears revealed 18 (8%) horses infected with trypanosomiasis. Simple and semi-nested PCR amplified the DNA from 48 blood samples (21%). RFLP analysis of the semi-nested products, using Msp1 and Eco571 enzymes, negated the presence of *T. congolense* and *T. vivax* in the positive samples and revealed that the animals might be suffering from *T. evansi* or *T. brucei* infection as the enzyme used cannot differentiate between these two species. It is inferred that PCR is more sensitive than the microscopic examination and the pan-trypanosome PCR assay is suitable for carrying out laboratory diagnosis of field samples and epidemiological studies. Further studies on the possibilities of use of other restriction enzymes may help to improve the species specificity of the PCR-RFLP assay.

Prevalence of resistance strains of *Staphylococcus aureus* in throat samples is more frequent in healthy Nigerian students than local inhabitants of Lahore, Pakistan.

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Abstract

Staphylococcus aureus is a frequent cause of infections that are associated with considerable morbidity and even mortality. Healthy carriers of *Staphylococcus aureus* strains have an important role in the dissemination of this bacterium. The prevalence and strain type can also vary in members of different communities. Objective: Present study was designed to compare the prevalence of *S. aureus*, their antibiotic resistance and strain type in healthy Pakistani and immigrant students from Nigeria. Moreover, the impact of different risk factors, on isolation frequency of S. aureus from throat was studied.

A total of 450 samples were collected from inhabitants of Pakistani (n= 350) and Nigerian community (n= 100). The isolation frequency was found to be higher in Nigerian community. Moreover, the gender-wise comparison showed its higher incidence in males. Among risk factors, smoking, gym/spa/public bath usage and skin infections were found to play an important role in the prevalence of *S. aureus*. All the strains of local community were sensitive to Vancomycin, Linezolid, Gentamycin and Clindamycin, but the strains from Nigerian community showed different levels of resistance against these antibiotics. Antimicrobial profiles indicate that Nigerian and local population have probably different strains of bacteria. Collectively, these data indicate that different communities have different fauna and strain type of the same spp. of bacteria.

Doramectin for the treatment of adult-onset generalized demodicosis in Calves

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Abstract

The efficacy of doramectin for the treatment of clinical generalized demodicosis was assessed in two calves. Two non-descript calves of 3-4 months age with adult-onset generalized demodicosis that had failed to respond to application of amitraz solution were presented at Veterinary Medical Teaching Hospital, Department of Clinical Sciences, Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University, Rawalpindi. The disease was diagnosed on the basis of physical examination along with multiple deep skin scrapings. The calves were then treated with five shots of undiluted doramectin (Dectomax®, Pfizer Animal Health Pakistan) at a dosage of 0.6 mg/kg of body weight, SQ, at interval of one week. No other parasiticidal agent given along doramectin topically or systemically. Miticidal efficacy of doramectin was evaluated through resolution of clinical signs and reduction in mite (Demodex bovis) count. Clinical examination and multiple skin scrapings were performed each time at the time of treatment. Skin scrapings were performed at approximately the same sites at every examination. On day 45, no mite was detected indicated 100% parasiticidal activity of doramectin against Demodex bovis. Skin condition significantly improved as a result of count reduction of mite. Follow-up information was collected through telephonic call on monthly basis for 06 months. Calves were considered cured from demodicosis after 06 months of discontinuation of doramectin administration. In conclusion, weekly administration of doramectin at a dosage of 0.6 mg/kg, SQ, was found effective in the treatment of adult-onset generalized demodicosis in non-descript calves.

Probiotic and cholesterol reduction potential of *enterococcus spp.* isolated from horses

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Abstract

Probiotics are viable microorganisms that promote health benefits upon consumption. These have been recommended as dietary adjuncts to hypercholesterolemia subjects. The objective of the present study was to evaluate the probiotic and cholesterol reducing potential of our four lab isolates labelled as (P, R, X, and Y) isolated from horse faeces. These strains were selected on the basis of initial screening for lactic acid production and cholesterol reducing ability. These were identified on morphological, biochemical, physiological and molecular basis for their probiotic properties and evaluated for in vitro study. Cholesterol reducing potential of one strain (*Enteococcus ratti*) was also evaluated in vivo.

All the isolates were non-spore former, gram positive cocci. They were catalase negative and could utilize glucose both in aerobic and anaerobic environment. All the isolates gave positive test for Esculin bile salt test and were salt tolerant up to 8%. On the basis of 16rRNA gene sequencing, the strains were identified as belonging to genus Enterococcus and strain (R) was identified as *Enterococcus ratti*.

Among probiotic properties the selected isolates were found bile salts tolerant, bile salt hydrolase producer, thermo-tolerant (35oC-50oC) and were able to grow at wide pH range of (2-8). On media manipulation, the isolates could grow in the presence of lactose, glucose but maximum growth was observed in the presence of fructose. Moreover, isolates were found to possess cell surface hydrophobicity, show attachment with intestinal epithelial cells and survival in the presence of lysozyme. Cholesterol reduction potential of *Enterococcus ratti* was maximum among the selected strains and it was used in in vivo trial. *E. ratti* treatment resulted in significant reduction in triglycerides (TG) and low density lipoprotein–cholesterol (20%) and rise in high density lipoprotein-cholesterol (28%). No toxicological effect could be noticed one week post feeding using liver profile and haematological picture. Further extensive long term feeding studies are suggested on *Enteococcus ratti* so as to include it in the list of probiotic strains.

Therapeutic efficacies of *Fumaria parviflora*, C*aeselpinia crista* and oxyclozanide against fasciolosis in sheep in Rawalakot-Azad Jammu & Kashmir (Pakistan)

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Abstract

Chemotherapeutic trial was conducted to check the efficacies of *Fumaria parviflora*, *Caeselpinia crista* and Oxyclozanide against Fasciolosis in sheep. Eighty sheep naturally infected with Fasciolosis were randomly divided in to four groups viz A, B, C & D. Group A & B were further divided in to three sub groups viz A1, A2, A3 and B1, B2, B3 comprised of 10 animals each. Group C and D were also incorporated with 10 animals in each group. Group A (A1, A2, A3) was treated with dried leaves of *Fumaria parviflora* (Shahterah) while Group B (B1, B2, B3) was treated with finely grinded seeds of *Caeselpinia crista* (Karanjwa). Group C was treated with Oxyclozanide (Zanil-ICI Pakistan pvt, Ltd), while group D was kept as infected untreated control. The efficacies of the different anthelmintics were recorded on 3rd, 7th, 18th, 21st and 28th days post treatment. The efficacy of Oxyclozanide was found 100% on 18th day post treatment. *Fumaria parviflora* was slightly more effective compared to *Caeselpinia crista*. It was concluded that Oxyclozanide was highly effective anthelmintic ($p<0.01$) against Ovine Fasciolosis.

Exploring *in vitro* anthelmintic activity of the essential oils from *Boenninghausenia albiflora* (Hook.) reichb. ex heynkh, of Pakistan

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Abstract

A study was conducted to evaluate in vitro anthelmintic activity of essential oils from root, stem and leaves of Boenninghausenia albiflora. Adult motility assay was employed for this purpose using *Haemoncus contortus* adult worms. Essential oils were used in three concentrations viz. 10, 35 and 50 µL per 10 ml in PBS plus 10µL of Tween 20 (Tween 20 acted as carrier/emulsifier). Levamisole was used as a positive control @ 0.55 mg/ml. A significant difference was found in anthelmintic activity of different concentrations of each EO, LC50 was also determined, which was 31.70 and 50.25 µL for leaves+stem and roots respectively. The dose dependant effect by EOs from different plant parts was found to be greater for stem, maximum R2 value for stem was 0.996. Loss of motility and change in the color of the dead worms was taken to be the indicator parameter for the damage/death caused by EOs. The investigation for time dependent mortality of adult *H. contortus* revealed that EOs were more effective than Levamisole that after 3:50 hr 100% mortality was achieved by application of all the three types of EOs @ 50 µL whereas in case of Levamisole (0.55 mg/ml) 100% mortality was achieved after 4:00 hr. It can be concluded that all the three EOs in this study have anthelmintic properties and can exhibit better control than Levamisole.

Epidemiological studies of Trypanosomiasis in camels of cholistan

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Abstract

Trypanosomiasis is a vector born disease caused by different species of Trypanosoma. *Trypanosoma evansi* infections are very common in Pakistan. It is a great source of mortality and morbidity in animals especially in desert areas of Pakistan including Cholistan and Tharparkar. The current study was designed to evaluate existing status of Trypanosomiasis in camels of Cholistan region of Punjab. Total, 332 samples were taken from different Tobas of Cholistan. The confirmation of positive samples was made through thin smear, formal gel, packed cell volume, micrhametocrit and PCR techniques. TBR1 and TBR2 primers were used in PCR analysis and target size of product was 164bp. Results of current study revealed that *T. evansi* infections is common in camels of Cholistan region. Different diagnostic techniques are to detect parasite from animals and results are as follows: 1.8% samples were positive by thin smear, 3.6% by PCV, 1.8% by microhaematocrit, 9.6% by formal gel and 5.1% by PCR. All the thin smear positive cases were 100 % positive in PCR analysis but all the PCR positive cases were not 100% positive on thin smear microscopy, which means that PCR is more sensitive and specific test for diagnosis of T. evansi infections. According to month wise data of cases reported, highest rate of infection was found in month of September (20%). According to another parameter of this study, males and sub adults found to be least affected by *T. evansi* as compared to females and adults. In the vector part of this study, the only potential vector found in this area was *Tabanus striatus*. Maximus abundance of flies was seen in August. Along with *T striatus*, there is possibility of other vector(s) in this area to be associated with this disease.

Molecular characterization of Trypanosoma species from Cholistani camel, equines, cattle, goat and sheep by using species specific PCR

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Abstract

Trypanosoma is the blood-dwelling protozoan parasite that cause a disease known as Trypanosomiasis which is common in livestock species like cattle, sheep, goats, horses and camels. Basically this disease spread through a vector known as tsetse fly commonly but rarely by other biting insects which feed on infected animal. The objective of current study is to identify the existing trypanosoma species in animals of Cholistan area of Pakistan and to estimate most recent rate of infection in different groups of animals. This study is conducted blood samples harvested from camels, equines (donkeys), cattle, goat and sheep in and around the Cholistan belt from different localities of Cholistan and Bahawalpur Distric. The animals of all age and sex groups are included in study. Total 310 samples are collected from January 2014 to August 2014 and out of these 310 samples 60 samples belong to camel, 50 from donkey, 50 from cattle, 100 from goat and 50 from sheep are included in this study. Blood samples are collected from the jugular vein of animals and further divided into three parts. The first part is disseminated in EDTA coated tubes, second part in serum separating tube and third part in cryopreservative tubes with cryopreservative agent. These blood samples were kept in ice and in liquid nitrogen during sampling in field. Thin smears are made from these blood samples and microscopy is done after staining through Giemsa stain. The blood samples are further subjected to parasitological diagnosis (micro-haematocrit method, MHCT) and for the extraction of trypanosomal DNA. Similarly, the serum isolated from second part of blood sample is used for formol gel test. PCR conditions are optimized for screening of all above mentioned blood samples and specie specific primers like TBR, TRYP 4 and ROTAT are used for optimization studies. DNA extractions from all blood samples is made and right now PCR of these samples is in progress.

Molecular and Serological prevalence of *Anaplasma species* in Cattle and Human population of District Kohat

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Abstract

A study was carried out to investigate the molecular and serological prevalence of Anaplasma species in District Kohat, Khyber Pakhtunkhwa, Pakistan. For this purpose, a total of 200 samples each, were randomly collected from various cattle and human population. The animal samples were screened for the presence of *Anaplasma marginale,* while the human population was screened for the presence of A. phagocytophilum. A questionnaire based study was conducted based on the parameters such as Age, breed, sex, farming system, feeding regime and the presence of ticks in animals sampled. For human samples, parameters such as age, sex, occupation, fever and contact of the people with ticks/ animals were taken into consideration. For serological investigation, an Indirect ELISA technique was used, while a Real-Time PCR was used for amplification and quantification of *Anaplasma marginale* specie-specific primer sequences in samples collected from animals selected for sampling. Results revealed an overall 16.5% prevalence of *Anaplasma marginale* in animal samples by both the diagnostic techniques. In case of human samples, the prevalence of Anaplasma phagocytophilum was recorded to be 7.5% by applying a conventional PCR technique. Our findings suggested that *Anaplasma phagocytophilum* is prevalent in Pakistan.

Prevalence and pathology of bovine coccidiosis in district Faisalabad, Pakistan

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Abstract

Cattle and buffalo farming in Pakistan plays important role in the economy of the people. Little is known about the prevalence and pathology of bovine coccidiosis in these species. Present study was conducted to determine the prevalence of Eimeria infection at a local dairy farm located in vicinity of district Faisalabad. For this purpose faecal samples were collected from 541 animals including cattle (379) and buffaloes (162) after the death of five animals at this farm. All the faecal samples were examined after 1-2 h of collection using flotation techniques. The results revealed an overall 2.9% prevalence of coccidial oocysts in cattle and buffaloes. There was non significant difference in prevalence of coccidial oocysts in both species including cattle (2.3%) and buffaloes (4.3%). No significant difference was also observed in prevalence of coccidial oocysts on the basis of sex and different age groups. During postmortem examination of dead animals cattle (n=3) and buffalo calves (n=2) no gross lesions were observed in the thoracic viscera however, from cecum to colon part of the large intestine exhibited sever hemorrhages and necrotic ulcers. Transverse zebra stripings over the mucosal surface of the large intestine (colon) of all the dead animals were the characteristic features. Desquamation of epithelial lining, mononuclear cell infiltration in the lamina propria, healing mucosal scars with sever hemorrhages were observed during histological examination of intestine. Moreover, massive numbers of coccidial cells were nesting in the lamina propria of all infected animals.

Histopathological and serum biochemical changes by sub-chronic doses of Triazophos in quail

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Abstract

The objective of this study was to evaluate the possible histopathological and some serum biochemical effects of frequently used triazophos (O, O-diethyl O-1phenyl-1 H-1, 2, 4- triazol-3-yl phosphorothioate) an organophosphate insecticide in avian species. For this purpose a total of 60 adult Japanese quail were randomly allocated to five equal groups (A-E) having 12 birds. All the birds in experimental groups were orally administered triazophos @ 0, 2, 4, 6 and 8 mg/kg/day for 48 days. The blood and morbid tissues were collected at day 16, 32 and 48 of post treatment. Grossly, kidneys, lungs, spleen and heart of birds received higher concentration of triazophos (8 mg/kg bw) were swollen and congested. No significant change was observed in relative weight of bursa, trachea and proventriculus. However, the relative weight of spleen, kidneys, lungs and heart was significantly increased and decreased as compared to control group. Serum urea, creatinine, cardiac enzymes (CPK and CK-MB) were significantly increased while total proteins decreased in treated birds. Histologically, mild congestion, hypertrophied glomeruli, detachment of renal tubules, tubular necrosis, atrophied Bowman's spaces and pyknotic nuclei of epithelial cells in tissue sections of kidneys were observed. Light microscopic analysis of heart tissues showed degenerating cardiac muscle fibers along with loss of transverse striations. Bursa of Fabricius in treated birds showed severe cytoplasmic vacuolation. Congestion in lungs and spleen was also observed at higher concentrations of insecticide. The present study revealed for the first time that triazophos causes histo-architectural and serum biochemical changes in birds in proportion to dose and duration.

Helminthosis of rural poultry in Quetta, Pakistan

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Abstract

Helminth parasites were studied in 120 rural chickens in Quetta, showing overall infection to be 75.8%. Prevalence was higher in winter (81.6%) than in summer (70%). Five cestodes were found, i.e., *Raillietina tetragona* (48.3%), followed by *Choanotaenia spp.* (20%), *R. echinobothrida* (19.2%), Cotugnia spp. (1.7%) and *R. cesticillus* (0.8%). The nematode, *Ascaridia galli* was more prevalent (10.8%) than *Allodapa suctoria* (0.8%). Infection of one host with single species of helminth was seen at most occasions (53.3%), followed by double, triple and quadruple infections (20, 1.7 and 0.8%), respectively. Mixed infection with both nematodes and cestodes was seen in 5.8% of chickens. In conclusion, anthelmintic treatments should be done routinely in the rural poultry.

Anthelmintic and antitick activity of *Calotropis procera*, *Syzygium cumini* And *Ziziphus jujuba*

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Abstract

The activity of water based extract from leaves of *Calotropis procera*, *Syzygium cumini* and Ziziphus jujuba as an anthelmintic and antitick was evaluated. For evaluation of anthelmintic activity, in vitro egg hatch test (EHT) and adult motility assay were conducted on eggs and adult worms of Haemonchus contortus while in vivo anthelmintic activity was determined in sheep naturally infected with H. contortus. Likewise, acaricidal activity was evaluated against *Rhipicephalus microplus* by using modified larval immersion test (syringe method). In EHT, LC50 of plant extract was found to be 41.05 % while LC50 of Oxfendazole was found to be 4.1406%. Time dependant response was observed in case of adult motility assay. Activity of extract increased with the passage of time. LC50 (95% CI) at 2 h post treatment was 176.03% which was reduced to 4.744% after 12 h post treatment. In syringe test, LC50 collected after 24 h was 3641.108% totally different when compared with the LC50 of extract after 6 days 1576.55%. This significant difference between LC50 values at 24 h and 6 days indicate that extract was more toxic after 6 days of treatment. Moderate activity was found in fecal egg count reduction test. 2 ml/kg b. wt of extract induced only 37.03 % reduction at the end of experiment. Higher doses of extract were found to induce 27.38% and 40.78 % reduction respectively. Based on results it is concluded that plants used in this study may be subjected to individual evaluation both in vivo and in vitro. Moreover, the assumption of synergistic effect of phytochemicals of three plants is tested by repeating the in vivo experiments and also using different solvent fractions of the plant extract in some other study.

Hydratid cyst in cattle: a case report

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Abstract

The basic aim of this study was to document the case study of Cystic echinococcosis (CE) i.e a chronic zoonotic disease caused by the cyclophyllidean cestode *Echinococcus granulosus* in cattle. Six year old sahiwal cattle with the history of chronic nonproductive cough, decreased rumination, mild constipation was brought to the civil veterinary hospital Toba Tek Singh. Upon physical examination dry muzzle with frothy salivation and stingy serous nasal discharge was found. Animal died suddenly at night after one day of clinical examination. Upon postmortem examination, multiple variables sized cystic lesions scattered majorly on lungs, followed by liver and spleen. The lesions were 1.5 to 6cm in diameter, inflated and enclosed by fibrous capsule. The case study serves as a quick reference source on hydatidosis in the country and basis for future studies.

Prevalence of goat warble fly infestation (*Przhevalskiana silenus*) in district Chakwal, Pakistan

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Abstract

Goats are mainly raised for meat purpose in most areas of Punjab, Pakistan. To find out the prevalence of goat warbles fly infestation (GWFI) a survey was conducted in district Chakwal. A total of 850 goat examined in the slaughter house for GWFI. Among the examined animal 345 (40.58%) were infested with goat warble fly. Highest prevalence of GWFI was recorded in the month of December, whereas, the lowest infestation was recorded in August in slaughter house. The numbers of nodules present in infested animals ranged from2 to 61 and recorded from dorsal surface. Prevalence of GWFI was greater in males than in females. More over it was observed that the geography of the study area favors the onset of fly activity and enhances the prevalence of the disease.

Brain-eating amoebae: invasion of the central nervous system by emerging free-living Protists

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Abstract

Free-living amoebae (Acanthamoeba, Balamuthia, Naegleria) can cause central nervous system infections that almost always result in death. However, the pathogenesis and pathophysiology remains unclear. Haematogenous spread is a key step in the development of meningo-encephalitis, but it is not clear how circulating amoebae invade the brain to produce disease. Here, we summarize the current understanding of amoebae penetration of the central nervous system. We describe parasite factors and immune-mediated mechanisms involved in parasite attack of the central nervous system, leading to neuropathogenesis. Advances in understanding the molecular mechanisms of parasite penetration of the brain offer unprecedented opportunities for the development of novel therapeutics.

Fascioliasis prevalence and gross pathology in sheep in and around Quetta District, Pakistan.

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Abstract

The economic impact of helminth diseases on livestock encopasses morbidity losses, mortality losses, enhanced susceptibility to bacterial and viral diseases and losses from high cost of drugs and veterinary care. This study was conducted to determine the prevalence of fascioliasis in sheep in Quetta district from January to june 2007. Fifty liver samples along with gall bladder and bile ducts were collected from sheep. Prevalence of liver fluke infestation in sheep was recorded as 12% in young and adult and 14% in aged sheep. Out of these positive samples Fasciola hepatica was found 50% and *Fasciola gigantica* 10% in case of young whereas in adult 20% with *Fasciola hepatica* and 14%with *Fasciola gigantica* and in aged sheep the prevalence was recorded as 33.33% and 13.33% with fasciola hepatica and *Fasciola gigantica* respectively. Variable degree of gross lesions were noted in affected livers included cholangitis (63.15%),biliary obstruction (57.89%), fibrosis (68.42%), hyperplasia (60%), hemorrhages (47.36%), enlargement(52.63%), fibrinous exudates (63.15%) and oedema(57.0%) in sheep during the study period.

Prevalence of Lymnea snails, their rate of infection with intermediate stages of liver fluke and their correlation with meteorological conditions in Mirpur Azad Jammu and Kashmir

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Abstract

The study was designed to know about the prevalence of the Lymnea snails , identification of infected and non infected snails with intermediate stages of liver fluke and to recommend suitable copper sulphate dilution i. e. 1mg /66 and 1mg /100 as molluscicide under laboratory and experimental pond conditions Prevalence of Lymnaea species was found to be 42.36 % and infection of Lymnaea snails with intermediate stages of F. Hepatica ranged between 37 .50 % and 68 .75 % in five habitats studied during the period from october-2009 to march-2010.Copper sulphate dilution 1mg / 1oo ml (10 PPM) was found to be effective as molluscicide and safe for other aquatic fauna and vegetation. For the present study total of four thousand snails were collected from five places in and around Mirpur Azad Kashmir , which included different genera namely ; Lymnaea , Gyraulus, Physa , Bulinus , and oncomelania . The %centage being, 42.36, 19.11,12.29 ,12.54 and 13.7 respectively . However out of 4000 snails only 1233 (30.8%) were found alive during the whole of thestudy time. The variation in the number and species may be due to different environment . The study was under taken during the period from october-2009 to march-2010 . It was found that alive Lymnaea spp. in october were 30.8 % in november, 16 % , in december, 15.78%, in january february were , 28.46 % and in march 64.02 % . The difference may be due to the different ecological conditions. It was found that from October to March , the prevalence of alive snails in mirpur fish pond was 11 % in October and no snails were found in other months as the fish pond became dry . Due to non availability of water the snails were not found.

Control Strategies of Hemoparasites in Pakistan

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Abstract

Control of hemoparasites is an indispensible requirement in reducing the vector borne infections in dairy animals. Pakistan is an agricultural country and has tremendous livestock potential therefore hempoarasites impart direct influence on national economics. In Pakistan, periodic application of acaricides has been the most common strategy in controlling hemoparasites. Other managmental practices i.e. use of tick proof building, separate housing of animals and quarantine practices also play a contributing role. Local and cross bred dairy animals are naturally immune to ticks and flies. Pasture spelling and live bait technology are not very much evident in Pakistan. All of the above mentioned methods have disadvantages and none has proven to be advantageous. In country like Pakistan, the continuous resource unavailability, hot and humid weather and presence of multiple strains are the major constrains in developing the hemoparasite eradication programme. Over the last decade, plenty of work has been done in other countries for the develpment of vaccination against hemoparasites. Selection of specific specie varies from country to country and is highly dependent on the effect of gene knock down on tick mortality, feeding and fertility. Development of vaccination against hemoparasites is the need of hour in Pakistan. There is also a need of using traps, targets and bait technology as an alternate of aerial and ground spraying for more effective hemoparasite control.

Threat of arsenic to livestock as insecticides, pesticides and sheep dips

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Abstract

Arsenic is used as pesticide, herbicide, fungicide, cotton desiccant, cattle and sheep dip, major source of arsenic poisoning. Exposure to inorganic arsenic causes the cancer in bladder, lung and skin, cardiovascular disease, developmental and reproductive disorders. The safe level of arsenic in water for livestock is upto 25µg/L. In Pakistan, level of arsenic in drinking water has reached upto 906-1000µg/L due to continuous contamination drinking water via insecticides on crops as well as anthropogenic activities. It is also used as anticoccidial and growth promoter in feed of poultry and pigs. In developed countries, arsenic has been banned in poultry feed due to carcinogenic actions. Arsenic can accumulate in the liver, kidneys and muscle tissue of animals. It also accumulates in the eggs and meet of poultry. Arsenic is also very toxic to animals and affects the gastrointestinal tract and the cardiovascular system. Symptoms of arsenic poisoning in animals include watery diarrhea, severe colic, dehydration, cardiovascular collapse and hyperkeratosis of skin. Arsenic is threat to livestock due to continuous rise in water as well as human due to contamination the food chain in Pakistan.

Role of *Staphylococcus aureus* in mastitis and its treatment in lactating buffaloes

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Abstract

A present study was carried for the measurement of pattern of antibiotics commonly used for prescription of *Staphylococcus aureus* mastitis treatment in Pakistan. Only Lactating dairy buffaloes were included while pregnant, heifer and dry period buffaloes were excluded from study. Four hundred and seventy two (472) clinical and subclinical mastitis milk samples from five hundred (500) buffaloes were collected from different livestock farms from pre-urban area of Lahore on basis of California Mastitis Test (CMT) and Surf Field Mastitis Test (SFMT). These positive milk samples were transported to Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore for isolation and identification of *Staphylococcus aureus* by conventional method. The quarter wise prevalence of mastitis was 23.6%. Among these 472 samples, three hundred and fifty seven (357) isolates of Staphylococci were obtained. All these, three hundred and fifty seven (357) isolates of Staphylococcui were Gram positive and catalase positive. Among these two hundred and sixty seven (267) were positive and ninety (90) were coagulase negative while staphytect plus test confirmed 267 isolates as *Staphylococcus aureus*. The occurrence of coagulase positive *Staphylococcus aureus* from 472 samples was 56.56%. The following antibiotics ampicillin, amoxicillin, gentamycin, chloramphenicol, tetracycline, ciprofloxacin, enrofloxacin and cotrimaxazole were used for measurement of antibiotics sensitivity. Antibiotic sensitivity of all coagulase positive two hundred and sixty seven (267) isolates of *Staphylococcus aureus* were done by disk diffusion method according to guideline of Clinical Laboratory Standard Institute (CLSI). The sensitivity of antibiotics was measured in percentage as ampicillin, amoxicillin, gentamycin, chloramphenicol, tetracycline, ciprofloxacin, enrofloxacin and cotrimaxazole 79.21%, 83.50%, 73.20%, 83%, 95.30%, 84.45%, 93% and 96.50% respectively. Cotrimaxazole and tetracycline is more suitable drug for treatment of *Staphylococccus aureus* mastitis.

Gross morphological and histological examination of cecal tonsils of aseel chicken (*Gallus gallus domesticus*)

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Abstract

The Aseel chicken is famous as a game bird all over the world. Due to its unique aggressive behavior and meat value, it is especially bred by bird lover communities in different areas of Pakistan as backyard poultry farming. It is well known that the lymphoid tissues play an important role in the defense against various pathogens. The cecal tonsils are known as lymphoid organ of birds .Information regarding morpho-histology of the cecal tonsils of native chickens is limited; therefore, the current study was designed to understand the morpho-histology of the organ. A total of 150 healthy Aseel chickens of either sex were divided into three age groups each comprising of fifty birds with equal number of male and female, namely, A (2 months), B (4 months) and C (6 months). The samples were collected after slaughtering of the birds and gross and microscopic examination was performed. Statistically, no significant difference was found in the length and width of lymphatic nodules of the organ between both the sexes but significant difference ($P<0.001$) was found among different age groups. Cecal tonsils were found to be immunologically active and showed regression of lymphatic nodular distribution area with advancement of age.

Warble fly infestation in small and large ruminants of Punjab, Pakistan

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Abstract

Pakistan is an agricultural country and livestock sector contributes more than 54 percent in agriculture sector. Currently, the livestock population is facing many health problems which not only upset the health of animals but also affect the animal products like leather. Warble fly infestation is common problem in ruminants. The present project was designed to study the prevalence of Warble fly infestation in various regions of Punjab which ultimately reduces the economic value of skins and hides. The skins and hides of the animals were examined at various abattoirs, hide markets and tanneries of Lahore, Kasur, Faisalabad and Multan. A total of 17459 skins of goat were examined out of which 213(1.22 %) had lesions of Warble fly infestation while 1334 skins of sheep were examined and 2(0.1%) showed the lesions of the infestation. In case of cattle, a total of 2878 hides were examined and 105(3.6 %) had lesions of Warble fly infestation. The percentage of infestation of Warble fly larvae was comparatively more in cattle followed by goat and sheep. In buffalo hides, the warble fly larval infestation was not seen as reported in earlier studies. It is hypothesized that black skin color and comparatively less hair might distract flies laying eggs on the buffaloes. Further investigations are needed to elaborate this finding. Perforations in the skins/hides were mostly seen in prime area hence rendering the skins and hides of low economic value. The disease can be controlled by the use of prophylactic treatment and anthelmintics.

Assessment of glucose transporters in development of the mammalian placenta using rabbit as animal model during successful pregnancy

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The early~mid pregnancy in mammals is very critical for the continued existence of the growing fetus and most of the embryonic loss occurs during that period. Since Glucose is essential as major energy source for metabolism during the period of extensive organogenesis and embryogenesis in mammals at placentation period during successful pregnancy. Therefore, the most important theme of the current study is to examine the appearance and localization of Glucose Transporters at the process of normal mammalian placentation using rabbit as an experimental model. Glucose transporters (GLUT1 and GLUT3) were proposed as a fuel supplier in functional mammalian placenta for developing fetus during successful pregnancy. Experiments were conducted on the normal placentas, maternal and fetal blood and fetal liver collected from the Japanese white rabbits during days 8-28 of pregnancy. The study was focused on Glucose Transporters GLUTI and GLUT3, proposing as an essential transporter of maternal glucose in mammal placentas. Glucose is essential for fetal growth, since fetus is unavailable for their own produced glucose until late pregnancy. Our results showed that glucose level was elevated in maternal blood as well as fetal blood and liver, accompanied with pregnancy progress. Real-time RT-PCR analysis on the rabbit placentas demonstrated that GLUT1 transporter were prominently elevated at day 18 and maintained before parturition period (day 28). GLUT3 was up- regulated at early placentation period (day 13) and then down-regulated after day 18. Immunohistochemical study confirmed the abundant localization of GLUT1 at labyrinthine region at outer trophoblast layers at day 25, which form blood sinuses filled with maternal nutrient blood. Immunoreactivity of GLUT3 was found in the inner trophoblast layers around the fetal blood vessel at day 25. Elevation of GLUT1 and GLUT3 is well enough to make the availability of glucose to the developing fetus and indicate a possible effect to act on placental vascular system for nutritional transportation. Localization of GLUT1 in outer trophoblast and that of GLUT3 in the inner trophoblast demonstrated that GLUT1 is responsible for transportation of glucose to the maternal-placental circulation whereas GLUT3 for the placental-fetal circulation. Therefore, the present study suggested an essentiality of both transporter for glucose availability to the functional placenta and growing fetus in rabbits. Based on high expression of Glucose Transporters until day 18 placentation period and localization in the vascular system or in the diverse types of trophoblast, it is evident that they play a fundamental role in the placentation process as well as in fetal growth. Glucose transporters were supposed to work on the transplacental mechanism for fetal nutrient and maintenance of successful rabbit pregnancy. Thus alteration or disturbance in any of transporters during placentation periods would badly affect reproductive condition of the rabbit placenta, which results in disturbed supply of essential nutrient to the developing fetus or defective vascular development of placenta. This data can serve as a baseline for future researches, such as maternal hypoglycemia or hyperglycemia that are available experimental rabbit as a useful model, to evaluate various prenatal stressors on the development of placenta and fetus.

Prevalence of *Theileria parva* in Large Ruminants through Conventional and Molecular Techniques in Lakki Marwat and Peshawar

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Abstract

Theileriosis is one of the most important tick borne diseases of cattle, buffaloes, sheep and goats. *Theileria parva* causes high mortality in animals and recovered cattle become long lasting carriers of the parasite. *Rhipicephalus appendiculatus* is the most important vector for *Theileria parva*. During the present study, two animal species (cattle and buffalo) were selected. A total of 800 samples were collected from different areas of district Lakki Marwat and district Peshawar. All the samples were processed through three different techniques including Conventional Microscopy, Indirect ELISA and Real-time PCR for diagnosis of *Theileria parva* at Veterinary Research Institute Peshawar. The overall prevalence for theileriosis was 27.75% through Real-time PCR followed by Indirect ELISA (24.62%) and Microscopy (11.37%) indicating that PCR gives the accurate results. Transect wise high prevalence for *Theileria parva* was observed in Lakki Marwat with 33.5% for Real-time PCR, significantly different at $P<0.05$ from Peshawar. Cattle showed 100% more positive results as compared to buffalo. All the techniques showed highest prevalence of *Theileria parva* for female population as compared to the male population. Age wise prevalence was not significantly different for both the species. Prevalence was high in animals < 1 year age. It was found that PCR is more sensitive and specific diagnostic tool for diagnosis of *Theileria parva.*

Comparative analysis of Ind. ELISA and RT PCR for the detection of *A. marginale* in Dist. Peshawar and Lakki Marwat

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Abstract

Anaplasmosis is a pandemic disease of livestock. In the present study the specificity and sensitivity of Ind. ELISA and RT PCR was analysed in the samples collected from Dist. Peshawar and Lakki Marwat. A total of 900 samples were collected from three species, Cattle, Buffalo and Sheep from two transects Peshawar and Lakki Marwat for determination of rate of *Anaplasma marginale* through Ind. ELISA and RT PCR. Over all rate for Anaplasmosis were 37.7% (339/900) for all three techniques, where as techniques wise the rate of A. marginale was 28.7% and 34.8 % through Ind. ELISA and Real-time RT PCR, respectively. Majority of the Cattle (41.6 %) were found positive for *A. marginale* as compared to 23.7% and 16.2% in Sheep and Buffalo respectively. Transects were significantly different at P <0.05 in term of rate of *A. marginale*. Transect wise distribution of anaplasmosis was higher in Lakki Marwat (41.5%) as compared to Peshawar (30%). Gender wise rate was higher in female through Ind. ELISA (57.96%) and RT PCR (64.33%) as compared to male individuals having 16.27% and 23.25% rate, respectively. A significant difference was found in seasonality of tick infestation, with the high infestations in summer as compare to winter.

Single nucleotide polymorphisms in *LAG-3* gene can potentially minimize the risk of mastitis development in dairy cattle

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Abstract

Mastitis is the most common inflammatory disease of dairy cattle characterized by physical and chemical changes in the milk and pathological changes in the mammary gland. Mastitis is the most costly disease of lactating animals affecting animal health and welfare, and is important due to its public health concerns. Somatic cell count (SCC) is a useful indicator of udder healthand has highly positive genetic correlation (r_g=0.84) with clinical mastitis. Numerous therapeutic, prophylactic and management strategies are being used as control measures for bovine mastitis; however, a widely proposed strategy is based on improving the host genetics through marker assisted selection. Lymphocyte-activation gene-3 (*LAG-3*) is located on bovine chromosome 5 and plays crucial role in inflammatory condition. The genetic effects of single nucleotide polymorphisms (SNPs) in *LAG-3* gene were investigated on some serum cytokines and mastitis indicator traits in a population of 268 Chinese Holstein cattle. Pooled DNA sequencing revealed three novel SNPs including one(SNP 1) in exon 4 and two (SNP 2 and SNP 3) in 3' flanking region of *LAG-3*.Fixedeffect model considering the effects of SNPs, parity, herd, season and year of calving was used by general linear model (GLM) procedure of SAS 9.1. Genotypic frequencies of these SNPs in the population were in Hardy–Weinberg Equilibrium (HWE) (P>0.05). SNP in exon 4 was missense that was predicted to cause amino acid substitution from threonine to proline.The results of association study showed that SNP2 was significantly associated with SCC, whereas SNP3 was significantly associated with SCC, SCS and IL-10 (P<0.05). The combination of SNP2 and SNP3 showed significant effect on SCC and SCS (P<0.05). As for mRNA expression analysis, the homozygous wild type genotype in SNP3showed higher expression level and were significantly different from the heterozygous genotype (P<0.05). The results imply that *LAG-3*gene can be considered as useful candidate gene, and the identified polymorphisms could be potentiallystrong genetic markers to select dairy cattle for genetic resistance against mastitis. Thus improving the host genetics eventually can minimize the public health hazards associated with mastitis.

Key words: *LAG-3* gene, single nucleotide polymorphism, mRNA expression, mastitis resistance, public health hazards

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Pakistan Science Foundation